A One Health systems approach to the epidemiology, management, and regulatory control of bovine brucellosis at the human-cattle-farm interface in Gauteng, South Africa

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Declaration

I, **Krpasha Govindasamy** declare that the research work reported in this dissertation is my own, except where otherwise indicated and acknowledged. It is submitted for the degree of Doctor of Philosophy in Veterinary Medicine at the University of Pretoria, Pretoria. This thesis has not, either in whole or in part been submitted for a degree and or diploma to any other university and or publication.

Signature:  Date: 08 February 2021

Dedication

Dedicated to those who have cried out from neglect,

"…*I am poured out like water, and all my bones are out of joint: my heart is melted in the midst of my bowels. My strength is dried up like a potsherd; and my tongue cleaveth to my jaws; and thou hast brought me into the dust of death*…" Psalm 22: 14–15

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Abstract

A One Health systems approach to the epidemiology, management, and regulatory control of bovine brucellosis at the human-cattle-farm interface in Gauteng, South Africa

by

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Background and Introduction

Human brucellosis, a neglected zoonotic disease of global public health importance, can be prevented by controlling the disease in livestock hosts. In South Africa (SA) there has been an increasing number of reported bovine brucellosis outbreaks with a concomitant lack of increasing numbers of human brucellosis cases.

Objective and aim

The objective of study was to determine the risk factors of bovine brucellosis as well as the epidemiology of brucellosis in cattle handlers and veterinary field officials working at the human-cattle-farm interface in Gauteng province, by undertaking an interdisciplinary field investigation under the precept of "One Health". We aimed to understand the increase of reported numbers of bovine brucellosis outbreaks and concomitant lack of increasing numbers of human brucellosis cases.

Method

A narrative review of South African literature on brucellosis was firstly conducted. We then analysed a dataset of bovine brucellosis laboratory test results from 2013-2018. A case-control study was conducted to identify herd management risk factors and symptoms of bovine brucellosis in the province. All herds in Gauteng that participated in the programme between 2014–2016 were eligible for this study. Farms were categorised as either case—when two or

more cattle tested seropositive, or control, following routine regulatory screening using the Rose Bengal test (RBT), and confirmation of reactors with the complement fixation (CFT) test. Finally, a cross-sectional study of cattle handlers on case farms were tested for brucellosis using four commercially available serological tests: the RBT and IgM ELISA, the IgG ELISA, and an immunocapture agglutination (BrucellaCapt) test. A subset of cattle handlers on control farms and veterinary officials from the three State Vet Areas of the province were also tested. Seroprevalence was measured according to each test. Furthermore, seroprevalence is reported for five mutually exclusive combinations of test results, indicative of infection evolution from short to long, in this group of persons. These combinations were: (i) RBT positive AND IgM ELISA positive AND IgG ELISA negative, (ii) RBT negative AND IgM ELISA positive AND IgG ELISA positive, (iii) RBT positive AND IgM ELISA positive AND IgG ELISA positive, (iv) RBT positive AND IgM ELISA negative AND IgG ELISA positive, and (v) RBT negative AND IgM ELISA negative AND IgG ELISA positive. Seropositive reactors on the BrucellaCapt test were allocated to the group defined by the outcomes of the RBT, IgM ELISA and IgG ELISA. Risk factors and symptoms associated with infection of short and long evolution as well as inactive/resolved infection or exposure were explored using univariate and multi-level multivariable logistic regression. Knowledge of brucellosis and health seeking response to brucellosis-like symptoms in this group were also described.

Results

From 1928-2016, 32 articles were published on human or bovine brucellosis in SA. Bovine brucellosis outbreaks were detected from 1906 in the Johannesburg area of SA and the first case of human brucellosis, reported in 1924, was caused by *B. abortus*. Since 1959, only one further serological survey in people, conducted in 2001 was reported. The cattle prevalence for bovine brucellosis reported, decreased from 19.6% in 1934 to 5.6% in 1980. In 1990, the national herd prevalence was reported to be 14.7%. Since 1990, there has been no further report on a national or provincial estimate of herd or cattle prevalence for bovine brucellosis.

Analysis of bovine brucellosis laboratory test reports from 2013-2018, for Gauteng province, revealed no significant change in prevalence of *Brucella* reactor herds (mean=22.1%) or within-herd seroprevalence (mean=7.4%). However, Randfontein and Germiston State Vet Areas had significantly ($p<0.05$) higher odds of reactor herds than the Pretoria State Vet Area. Reactor herds were also associated with increased herd size ($p<0.001$). Additionally, Germiston and Randfontein both had within-herd prevalence count ratios 1.5 times greater than

the Pretoria State Vet Area ($p<0.001$) and larger herd sizes were associated with lower within-herd prevalence ($p<0.001$).

Herd management factors associated ($p<0.05$) with being *a Brucella* infected herd were: being a government-sponsored farm, beef vs. dairy herd, open vs closed herd and the presence of antelope on the farm. Seroprevalence amongst farm workers on case farms (n=30 farms) ranged from 4.0% (BrucellaCapt) to 16.7% (IgG ELISA), compared to control farms (n=11 farms), where this seroprevalence ranged from 1.9% (BrucellaCapt) to 5.7% (IgG ELISA). Overall, 5.7% (13/230) of persons tested were seropositive to the RBT and IgM ELISA and IgG ELISA tests and 3.9% (9/230) were seropositive to all four serological tests. Farm workers on control farms presented with antibody profiles of short to longer evolution, compared to a more spread-out profile of infection evolution amongst farm workers on control farms. The difference in seroprevalence amongst farm workers between case and control farms for all the test combinations was not significant. However, seroprevalence amongst veterinary officials was significantly greater compared to farm workers on case farm for the RBT+ IgM- IgG+ outcome (OR=11.1, 95% CI: 2.5 – 49.9, p=0.002) and for the RBT- IgM- IgG+ outcome (OR=6.3, 95%CI: 2.3-17.3, $p<0.001$).

Univariate analysis of symptoms associated with infection of short evolution (RBT, IgM and IgG ELISA seropositive), long evolution (IgM ELISA seronegative and RBT and IgG ELISA seropositive) and inactive/resolved infection or exposure (RBT and IgM seronegative and IgG seropositive), showed weak evidence of an association between reported generalized aching and infection of short duration (OR=4.8, 95%CI: 0.4-27.9, p=0.103), and strong evidence for an association between reported joint pain and infection of long duration (OR=5.1, 95%CI: 0.9-33.3, p=0.030). Mixed effects multivariable logistic models fit to identify risk factors associated with infection of short evolution (RBT, IgM ELISA and IgG ELISA seropositive), long evolution (RBT and IgG ELISA seropositive and IgM seronegative) and for likely inactive or resolved infection (RBT and IgM ELISA seronegative and IgG ELISA seropositive) identified an association between the handling of afterbirth or placenta (OR=8.9, 95% CI: 1.0-81.1, p=0.052) and strong evidence for an association between slaughter of cattle (OR=5.3, 95% CI: 1.4-19.6, p=0.013) and infection of a short evolution. Evidence of a weak association was found between infections of a long evolution and veterinary officials compared to farm workers exposed to seropositive herds (OR=4.1, 95%CI: 0.2-8.1, p=0.049). However, there was strong evidence of an association between inactive/resolved infection or exposure and veterinary officials compared to those exposed to seropositive herds (OR=7.0, 95%CI: 2.4-

20.2, p<0.001), whilst handling of afterbirth or placenta was associated with non-reactors in this group (OR=3.9, 95%CI: 1.3-11.3, p=0.012).

Only 20.7% (42/203) of cattle handlers knew that *B. abortus* can cause abortions in cattle, can cause calves to be born weak and can also be in a herd without causing abortions. Furthermore, whilst 36.9% (75/203) knew that bovine brucellosis can cause disease in people, only 16.3% (33/203) reported knowing the human symptoms of disease. In contrast 63% (17/27) of veterinary officials knew the symptoms of bovine brucellosis and 100% knew it to be a zoonotic disease, but only 89% (24/27) knew the symptoms of human disease. Despite having greater awareness of the zoonotic nature of bovine brucellosis and human symptoms of the disease, only 22.2% (6/27) of veterinary officials would opt to visit a clinic, doctor or hospital in response to self-experienced brucellosis like symptoms, compared to 74.9% (152/203) of cattle handlers ($p < 0.001$). Furthermore, 53% (8/15) of BrucellaCapt seropositive persons reported to either pray, self-medicate or ignore brucellosis like symptoms experienced instead of visiting a clinic, doctor or hospital. This may indicate a proportion of undetected and untreated clinical cases of brucellosis amongst this group.

Conclusion

Human brucellosis has been a public health concern in SA from as early as 1924. Analysis of laboratory test reports for bovine brucellosis between 2013-2018, indicated no progression toward eliminating the disease from cattle herds in the province. The presence of significant risk factors and symptoms associated with infection of short and long evolution and poor health seeking behaviour in response to brucellosis-like symptoms among farm workers and veterinary officials with these antibody profiles, strongly suggest the presence of undetected cases of human brucellosis on cattle farms.

This study provides a methodology for exploring the epidemiology of brucellosis at the human-cattle-farm interface from a One Health perspective. Variables associated with seropositivity in cattle handlers and *Brucella* infected cattle herds in this study suggest a complex interaction of human, herd, socio-economic, epidemiological, and sub-national disease regulatory systems. We recommend a systems-thinking approach and use of the One Health model to better manage this identified complexity to reduce bovine brucellosis and prevent human brucellosis in South Africa.

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Chapter One: General Introduction

This chapter gives an overview of the global prioritization of brucellosis followed by short review of *Brucella* spp. and the importance of *B. abortus*. Lastly, the recent advocacy for a One Health approach to brucellosis control is highlighted. A background to the current study is then provided followed by a brief overview of each chapter of the thesis.

Global public health importance of brucellosis

Since 1948, global organised momentum to achieve public health has been informed by member states of the World Health Organisation (WHO). More recently, the twelve Millennium Development Goals (MDGs) followed by the United Nations Sustainable Development Goals (SDGs) (https://www.sdgfund.org/mdgs-sdgs) have been agreed on to direct progression of global health. The shift from the MDGs to the SDGs takes into consideration the inter relatedness of human, animal and environmental health and the importance of these dynamic relationships to achieving the SDGs (Bangert et al., 2017). The goal of "good health and wellbeing", SDG 3, has nine targets indicators. Of these, Target 3, stated as "*End the epidemics of AIDS, tuberculosis, malaria, and neglected tropical diseases and combat hepatitis, water-borne diseases, and other communicable diseases*" (Bangert et al., 2017), has direct relevance to the control of brucellosis, a bacterial zoonotic disease.

Brucellosis was considered to be of global health and economic concern by the WHO since 1948, because of its association with human suffering, decreasing ability to work, and decreased production in the affected host livestock population (Mableson et al., 2014). Brucellosis was categorized as a neglected zoonotic tropical diseases (WHO, 2005) and more recently reclassified as a "forgotten neglected zoonotic tropical disease" after it was removed, along with tuberculosis and anthrax, from the original eight zoonotic diseases of the seventeen prioritized neglected tropical diseases identified in World Health Assembly (WHA) Resolution WHA66.12 of the WHO in 2013 (Mableson et al., 2014, WHO, 2014). This change in definition, was due to the deficiency of "tools" that, according to the WHO, are necessary for better control methods (Mableson et al., 2014). However, it should be noted that in the original WHO report (WHO, 2007), no explanation was given to justify why existing tools are deficient.

Despite the global shift in prioritization of brucellosis, the true incidence and burden of the disease is unknown but low and middle income countries are more affected than to

developed countries (McDermott et al., 2013). The disease has been associated with poverty in Africa, and is suggested to be a barrier to socio-economic development on the continent (Grace et al., 2012). Franc et al. (2018) argue further that the economic and public health repercussions of brucellosis present barriers to achieving the SDGs. Little is known about brucellosis in humans in Africa (Pappas et al., 2006b, Dean et al., 2012b, Rubach et al., 2013, Boukary et al., 2014, WHO, 2014, Ducrotoy et al., 2017) and country data on human brucellosis in sub-Saharan Africa is sparse (Dean et al., 2012b, Ducrotoy et al., 2017, Ducrotoy et al., 2014). This includes South Africa (SA), where currently there is a noticeable gap in the literature reporting on the prevalence of human brucellosis in the country (Wojno et al., 2016).

Brucella and the evolutionary importance of *B. abortus*

Brucellosis is a zoonotic disease caused by Gram negative facultative intracellular bacteria of the genus *Brucella.* These bacteria are known for their ability to infect both phagocytic and non-phagocytic cells, and for expressing virulence elements that trigger a redirection of intracellular trafficking to the endoplasmic reticulum, where lysosome fusion is evaded allowing for intracellular reproduction occurs, expansion and transmission of the bacteria to other host cells (Doganay and Aygen, 2003). The smooth lipopolysaccharide coating of the bacteria play a critical role in evading the immune response and may be involved in inhibiting the programmed cell death of the host cell (Franco et al., 2007a). These bacterial survival mechanisms can therefore result in chronic infection in hosts.

Despite ongoing controversy on the taxonomy of *Brucella* spp. bacteria considered to be within this group can be classified as zoonotic or non-zoonotic (Moreno, 2021). Each of the species reported to cause human disease is associated with a preferred host or reservoir (Al Dahouk et al., 2013, Suárez-Esquivel et al., 2017) (

Table 1.1).

Table 1.1: Zoonotic *Brucella* species host preference (adapted from Al Dahouk, 2013)

Brucella species	Biovars	Animal Host
B. abortus	1 – 7, 9	Cattle, Camel, Bison, Buffalo, Elk, Yak
B. melitensis	1 – 3	Sheep, Goats, Cattle, Camel
B. suis	1, 3, 4	Horses, Pigs, Wild boars, Caribou, Reindeer

It has been estimated that human infection with *Brucella* requires exposure to as few as 10 – 100 bacteria (Kaden et al., 2018, Kahl-McDonagh et al., 2007), although no explanation is given as to how this infectious dose was determined or the variation in infectious dose between different *Brucella* species. Despite this missing evidence, *Brucella* is recognised as "moderately easy to disseminate; resulting in moderate morbidity rates and low mortality rates; and requiring specific enhancements of Centre of Disease Control's diagnostic capacity and enhanced disease surveillance" (CDC, 2021). *Brucella* species are therefore classified as a Class B terrorist agents (CDC, 2021). *B. abortus* was found to have an infective dose less than that of *B. melitensis,* which resulted in chronic infections in challenge studies using BALB/c mice, as compared to *B. melitensis* infection which cleared more rapidly (Kahl-McDonagh et al., 2007).

B. abortus is the second most common known cause of human brucellosis, after *B. melitensis* (OIE, 1987, OIE, 2008a). It is a non-sporulating, non-encapsulated, facultative intracellular coccus, coccobacillus or short rod that causes bovine brucellosis, a highly contagious disease of cattle (OIE, 2008b). In cattle, infection results in reproductive disorders such as abortions in the third trimester, retained placenta, epididymitis, orchitis and sometimes arthritis. The bacteria are shed in the milk or uterine discharges (OIE, 1987). Herd symptoms vary across breeding systems and management (Ducrotoy et al., 2017) and usually detected as weak or still born calves, a drop in milk production, extended inter-calving periods, sterility in bulls and hygromas, or abortion storms in naïve animals introduced into an infected herd, resulting in significant production losses to the farmer (Crawford and Hidalgo, 1977, Crawford, 1990, Akakpo et al., 2010). To counteract the spread and lower the rate of infection in herds, two live attenuated vaccine strains have been registered for use in cattle (S19 and RB51). (OIE, 2016). Transmission to humans can occur directly with exposure to infected reproductive material or indirectly through the consumption of infected unpasteurized dairy products (Doganay and Aygen, 2003, Corbel et. al., 2006), accidental inoculation with the vaccine (OIE, 2016), laboratory exposure during culture and isolation of the bacteria (Corbel et. al., 2006), or through a covert act of biological warfare aimed at long term disability in the victims and social disruption in the long term (Pappas et al., 2006a).

Recent literature report the incubation period for brucellosis in people to range from one to five weeks (Doganay and Aygen, 2003), however, incubation time for *B. abortus* has been documented to vary from 1 week to 7 months (Spink, 1956) or longer (Dalrymple-Champneys, 1960). Human brucellosis has been categorised as asymptomatic or symptomatic,

with an acute or insidious onset (Doganay and Aygen, 2003). However, despite these categories and various recent attempts to classify the disease as "acute", "subacute" or "chronic", according to duration, severity of symptoms or the presence of biomarkers (Dean et al., 2012a), the distinction between acute and chronic disease is arbitrary and varies across the literature (Young, 1995). Brucellosis can have a short evolution or long evolution, with or without complications. In most cases patients present with fever which may be accompanied by malaise, anorexia, extreme physical weakness, or emotional exhaustion. If not correctly treated, may persist for weeks or months, sometimes resembling the "chronic fatigue syndrome", with psychological sequelae (Corbel et. al., 2006). The disease can affect any organ system, with hepatomegaly and splenomegaly being common findings. Clinical signs, although non-specific, include relapsing fevers, chills, sweating, joint pain and depression, (Glynn, 2008). Complications include sacroiliitis, orchitis, epididymitis, neurobrucellosis, and endocarditis which may result in death (Dean et al., 2012a).

There is no vaccine for humans to prevent infection, but the disease, if detected can be treated with a combination of antibiotics (Glynn, 2008), taking into consideration that vaccine RB51 is rifampicin-resistant strain and vaccine Rev 1 is streptomycin-resistant (OIE, 2016). A combination of a course of antibiotics prescribed by a medical doctor is needed over a duration of at least six weeks. Relapses are common with inappropriate treatment or with late initiation of treatment (Franco et al., 2007b). Untreated cases of brucellosis result in a complicated disease and subsequent loss of life years from persistent disability and time lost from daily activities (Dean et al., 2012a, Madkour, 2012, Glynn, 2008). The disability weight for brucellosis has been estimated to be 0.2 (Roth et al., 2003) and later proposed to be 0.150 for "chronic" brucellosis and 0.190 for "acute" brucellosis (Dean et al., 2012a). More recently in a WHO report of estimates for the global burden of foodborne diseases, brucellosis was reported to have a disability weight ranging from 0.079 to 0.2 for various manifestations of the disease (WHO, 2015). These disability weights are in the same range as disability resulting from diseases such as anaemia as a consequence of malaria or schistosomiasis (GHDx, 2017).

Historical aspects of bovine brucellosis as a zoonotic disease and the emergence of national eradication programmes

B. abortus was first discovered in 1897, by Bernhard L.F. Bang, a Danish veterinarian (MacNeal and Kerr, 1910). He identified and named the causative intracellular bacillus, *Bacillus abortus* (Evans, 1947, Madkour, 2012, MacNeal and Kerr, 1910). The disease in cattle was known as Bang's disease but commonly referred to as 'contagious abortion' (Crawford and Hidalgo, 1977, Bishop, 1994). For a period the disease was of interest only to the veterinary profession, dairy farmers and meat producers (Madkour, 2012). Interest was mainly due to economic losses resulting from the disease, accumulated through increases in cow infertility, abortions, weak calves (Crawford and Hidalgo, 1977, Anon, 1943, Crawford, 1990), death of term-calves, the birth and subsequent death of calves within the first week, a drop in milk production in the first year and resultant reduction in weight gain of calves born (Crawford, 1990, Olsen and Tatum, 2010).

Then, in 1918, twenty one years after Bang's discovery, Alice Evans identified the microbiological relatedness of *Micrococcus melitensis*, the causative agent of Undulant Fever in British soldiers stationed on Malta Island, and *Bacillus abortus*, as both being bacilli and eliciting the same response to the serological agglutination test (Evans, 1918). She identified *Bacillus abortus* in cow's milk and suggested the possibility of zoonotic disease, as was the case in Malta, where *Micrococcus melitensis*, in goats was transmitted to the soldiers through the consumption of unpasteurized milk from these goats (Evans, 1947). She proposed the reclassification of *Micrococcus melitensis* and *Bacillus abortus*, as *Bacterium micrococcus* and *Bacterium abortus*, respectively and suggested the new name of 'Brucellosis' for Malta Fever, after Dr David Bruce who had identified *Micrococcus melitensis* as the cause of 'Malta Fever' on 26th December 1886 (Madkour, 2012, Wyatt, 2009). The genus *Bacterium* was changed again two years later, by Meyer and Shaw, to *Brucella* (Moreno, 2021). The clinical connection between Contagious Abortion and Undulant Fever soon followed, with several authors isolating *B. abortus* from human cases. Amongst these was J.T. Duncan (Duncan, 1924, Duncan, 1928), of the London School of Hygiene and Tropical Medicine, who isolated *B. abortus*, from the blood of a patient returning from Southern Africa in 1924. This was eighteen years after the first reported case of bovine brucellosis in SA in 1906 (Henning, 1949, Drimmelen, 1949).

Global evidence of the zoonotic and economic importance of bovine brucellosis resulted in national bovine brucellosis eradication schemes emerging (Crawford and Hidalgo,

1977, Evans, 1947, OIE, 2008a), as a means of preventing human brucellosis and supporting optimal herd production. SA was amongst those countries that initiated a national bovine brucellosis eradication scheme, supported by legislation and regulated by veterinary state services in response to the economic and zoonotic threat of the disease in cattle (Bosman, 1980, Drimmelen, 1949, OIE, 1987).

Such schemes at the time were being successfully implemented in developed countries, and relied on a well-coordinated and managed programme to determine the level and distribution of brucellosis, and to reduce cattle and herd infection levels through vaccination with strain 19 and subsequent cattle test and slaughter programmes (Alton, 1977, Becton, 1977, Cunningham, 1977, McKeown, 1977, Michael, 1977, Morgan, 1977). Since the inception of bovine brucellosis eradication schemes, the USA (Olsen and Tatum, 2010), Sweden, Finland, Denmark, the United Kingdom (excluding Northern Ireland), Germany, Luxembourg, Belgium, Netherlands, Austria, Switzerland, Norway, France (Pappas et al., 2006b), Malta (Wyatt, 2013) and Australia (DOA, 2019) have achieved a bovine brucellosis-free status. This is not the situation in low and middle income countries or developing countries (Arturo del Rio, 1977, Aznar et al., 2014, DAFF, 2016, Mohan et al., 1996, OIE, 1987), where brucellosis in livestock is endemic and human brucellosis persists as a neglected zoonotic disease (McDermott et al., 2013, Rubach et al., 2013). SA is currently amongst countries considered to be endemic for brucellosis, despite having a national bovine brucellosis eradication programme since 1979 (Bosman, 1980).

Advocacy for a One Health approach to the complexity of bovine brucellosis surveillance and control

A growing body of recent literature has drawn attention to the role of complexity in the failure or ineffectiveness of neglected zoonotic tropical disease control programmes (Berezowski et al., 2019, Pearce and Merletti, 2006, Peters, 2014, Scott and Hofmeyer, 2007, Waltner-Toews, 2001). Complexity has been described to be a result of "inter-relationship, inter-action and inter-connectivity of elements within a system and between a system and its environment" by Chan et al. (2001) and is recognised to be a feature of complex adaptive systems (Chan, 2001), where a system is defined, according to the Oxford English Dictionary as, "a set of things working together as parts of a mechanism or an interconnecting network; a complex whole".

One Health is a recent re-emergent understanding of the inter-connectivity, inter-relationship and inter-action of human, animal and ecosystem components, forming a system of health or disease. Rüegg et al. (2017) considers health from the perspective of the One Health framework, to be an effect of complex biological and social system interrelatedness involving the interaction of multiple actors and processes over time, at local, national and global levels (Rüegg et al., 2017). This framework forms the basis of global advocacy to promote the collaborative efforts between the medical and veterinary disciplines to prevent and control endemic and emerging brucellosis (Bardosh, 2016, Godfroid et al., 2013, Godfroid et al., 2011, Plumb et al., 2013, Zinsstag et al., 2005, Zinsstag et al., 2011). The economic benefit of this approach to reduce human disease has been studied in Mongolia (Roth et al., 2003, Zinsstag et al., 2007) and is further evident from multiple WHO reports highlighting that the One Health approach might control brucellosis in Africa alleviating poverty (WHO, 2012, WHO, 2014, WHO, 2005) and helping to progress toward sustainable development (WHO, 2017). However, despite the recognition of the complexity related to controlling brucellosis and the acceptance of One Health as a possible approach to brucellosis in Africa, no One Health study of brucellosis has been conducted in SA.

Background to study

In contrast to countries that have successfully implemented bovine brucellosis eradication programmes, bovine brucellosis in SA remains unresolved. This is despite the introduction of *B. abortus* Strain 19 and RB51 into the country for vaccination of cattle in 1970 and 2002 respectively (Bosman, 1980, Davey, 2014, Frean et al., 2018). Furthermore, bovine brucellosis was listed as a controlled animal disease in the Regulations of the Animal Disease Act 35 of 1984 (Republic of South Africa, 1984) and the Bovine Brucellosis Scheme (Republic of South Africa, 1988) was gazetted in December 1988 (Republic of South Africa, 1988) to promote the eradication of bovine brucellosis for the improvement of human and animal health.

The national bovine brucellosis eradication scheme, operational since 1980 (DAFF, 2016) followed a similar approach as that of successful bovine brucellosis eradication programmes in countries now declared bovine brucellosis free (DOA, 2019, Becton, 1977, Brinley Morgan and Richards, 1974, Fritsohi, 1964, McKeown, 1977, Michael, 1977, Morgan, 1977, Thomsen, 1957). Yet, despite the existence of the scheme, an increasing number of outbreaks have been reported in SA, with more than 250 (range: 263-416) a year since 2003, and 78 outbreaks reported in the first two months of 2014 (Davey, 2014). Moreover, an

increasing trend of bovine brucellosis in the country has been reported by the National Department of Agriculture, Forestry and Fisheries Veterinary Services, to have begun with the decentralization of the bovine brucellosis control programme from national to provincial management in 1994 (DAFF, 2016). However, to date no study has been conducted to identify herd management risk factors associated with bovine brucellosis in the country.

Furthermore, recent published medical literature on human brucellosis in SA, suggested that *B. abortus* and not *B. melitensis*, was the most common cause of the disease (Frean et al., 2018). Attention has also been drawn to the lack of recent studies on the estimated burden of the disease (Frean et al., 2019) and the level of misdiagnosis, under-detection and under-reporting of human brucellosis cases in SA (Wojno et al., 2016). These papers emphasize that human brucellosis is still a disease of concern in the country and seem to suggest that the solution is a revision of the existing bovine brucellosis regulatory, control and eradication policy for the successful prevention of human disease (Frean et al., 2019). However, these papers do not provide a review of the clinical importance of human brucellosis in South Africa in correlation to the epidemiology of bovine brucellosis in the country, nor is there a review of the progression of veterinary regulatory control efforts in the cattle population and the effect of this on the prevalence of human brucellosis in the country.

It is, however, accepted that human brucellosis cases are correlated with the presence of disease in livestock, especially in those people that live in close contact or are occupationally exposed to infected animals (Pappas et al., 2006b, Pappas, 2010, Ducrotoy et al., 2017). An increase in the reported number of occupationally associated human brucellosis cases could therefore be expected (Wojno et al., 2016), in light of the reported increasing prevalence of bovine brucellosis (DAFF, 2016). Currently the level of exposure to *Brucella* and associated risk factors, knowledge and health seeking behaviour, amongst cattle handlers, including veterinary officials, in SA, is unknown, despite these occupational groups forming the critical functional field component of the bovine brucellosis control system.

Recent published information on the spatial distribution of *Brucella* infected herds indicates a clustering in the north-east of South Africa, in and around Gauteng province (Frean et al., 2019, RUVASA, 2018a, RUVASA, 2018b, Pistorious, 2016). Yet, since 1994, there has been no published report on the trend and spatial distribution trend of bovine brucellosis reactors on cattle farms in any province, even though data have been routinely collected for reporting purposes by provincial veterinary services for the duration of the programme. This is despite literature consistently reporting that variation in the spatial distribution and prevalence

of livestock brucellosis in a region, country or provincial area has much to do with the geographical and ecosystem features, including the presence of wildlife, that support different types of livestock farming practises (Dean et al., 2012b, Godfroid, 2017, Pappas et al., 2006b).

Research problem

An increase of bovine brucellosis outbreaks in SA have been reported, yet this was not accompanied by an increase of human brucellosis cases. In this study we consider four possible explanations for this scenario.

Firstly, human brucellosis due to *B. abortus* from cattle has never been a medical condition of concern in SA implying that an increase in bovine brucellosis outbreaks will not lead to an increase in human brucellosis cases reported. This theory cannot be tested due to a gap in recent SA literature on prevalence studies of human brucellosis in the country and no recent review of the historical medical importance of brucellosis.

Secondly, fewer cases of human brucellosis may be explained by lower rates of transmission to people, which would be plausible if there was a decrease in *Brucella* reactor herds and reactor cattle within a herd. However, despite a national veterinary regulated bovine brucellosis eradication scheme from 1979, there are no published reports on progress toward disease eradication from any province in SA to test this hypothesis.

Thirdly, *Brucella* seropositivity amongst cattle handlers exposed to reactor herds could be circumstantial evidence to suspect under detection or under reporting of human brucellosis cases. However, no study has been conducted to rule out transmission of *Brucella* amongst cattle handlers working on *Brucella* infected cattle farms in any province of SA.

Finally, the lack of increase in human cases may also be explained by infected cattle handlers not presenting to a medical facility in response to brucellosis-like symptoms. Currently there is no information on cattle handler knowledge of human and cattle symptoms of brucellosis and health seeking responses to brucellosis-like symptoms amongst this group.

Overall objective, approach, aim and anticipated impact of the study

The overall objective of study was to determine the risk factors of bovine brucellosis as well as the epidemiology of brucellosis in cattle handlers and veterinary field officials working at the human-cattle-farm interface in Gauteng province, by undertaking an

interdisciplinary field investigation under the precept of "One Health". We aimed to understand the increase of reported numbers of bovine brucellosis outbreaks and concomitant lack of increase of numbers of human brucellosis cases.

Findings from this study were intended to inform and support the revision of the existing national bovine brucellosis control policy and assist the Gauteng veterinary service in adopting a One Health approach to the provincial bovine brucellosis regulatory control strategy.

To achieve the overall objective this study focussed on five specific objectives that are addressed in the following chapters:

Objective 1: Review published literature, from 1900 to present, on human and bovine brucellosis in SA and summarize the main clinical symptoms of human brucellosis reported by SA medical practitioners.

Objective 2: Determine the prevalence and distribution of reactor herds in Gauteng, and the proportion of reactor cattle per herd test in the province over six-years (2013–2018). Identify factors associated with herd reactor status and within-herd prevalence.

Objective 3: Identify herd management risk factors associated with bovine brucellosis and measure *Brucella* seroprevalence at various stages of infection evolution expressed amongst cattle handlers on *Brucella*-infected cattle farms.

Objective 4: Determine cattle handlers' knowledge of brucellosis and health seeking response to brucellosis-like symptoms and identify risk factors associated with selected stages of infection evolution in this group.

Objective 5: Conceptualize a systems model of brucellosis at the human-cattle-farm interface in Gauteng to explain the increase of reported numbers of bovine brucellosis outbreaks and concomitant lack of increase of numbers of human brucellosis cases using the findings from studies conducted to meet objectives 2-4.

Framing the human-cattle-farm interface system in Gauteng

In this study we approached bovine brucellosis control using a One Health framework. That is, we assumed an inter-relationship between cattle, human and environmental health. Cattle herds participating in the Gauteng provincial veterinary services bovine brucellosis control programme were within the scope of this study. In Gauteng, cattle are nested within

herds. Cattle handlers and herds are nested within farms. Farms are nested within farm parcels (neighbouring farms). Farm parcels are nested within health districts which are nested within State Veterinary Areas (State Vet Areas). State Vet Areas are nested within provinces, which are nested within South Africa. South Africa is nested within the WHO and OIE as a member state. The WHO, OIE and FAO are nested within an overarching One Health framework and the UN SDGs.

In Gauteng, a State Vet manages all the cattle herds within a State Vet Area. Each State Vet Area employs animal health technicians (AHTs). AHTs are responsible for collecting blood samples from cattle for bovine brucellosis testing and for vaccinating herds against brucellosis with RB51. Provincial Veterinary Services and Provincial Health services operate as separate silos unless there is a zoonotic outbreak of public health importance.

Overview of thesis chapters

Chapter 2

In order to understand the medical importance of human brucellosis associated with bovine brucellosis in SA history, a narrative review of published literature on human and bovine brucellosis in SA, was conducted. Chapter 2 assimilated these findings as an article aimed to increase practitioner awareness of brucellosis by presenting evidence of the historical importance of the disease in SA from the published literature. Furthermore, clinical findings were reviewed in the context of the most pertinent challenges that clinicians face in the detection, treatment and management of brucellosis in the current SA context. The article was accepted and published in the South African Medical Journal.

Chapter 3

Chapter 3 addresses the lack of published information on the progress of bovine brucellosis eradication. The aim of this chapter was to assess trends in the prevalence and distribution of reactor herds in Gauteng and the proportion of reactor cattle per herd test in the province over six-years (2013–2018). We analysed laboratory test results of all cattle herds that participated in the Gauteng Provincial Veterinary Services' eradication scheme between 2013–2018. Herd reactor status and within-herd seroprevalence were modelled using mixed-effects logistic and negative binomial regression models, respectively.

Chapter 4

In order to address the gap in known herd management risk factors for bovine brucellosis and the zoonotic risk of exposure to cattle handlers in Gauteng province, Chapter 4 reports on identified herd management risk factors associated with the persistence of bovine brucellosis, *Brucella* seroprevalence and the various stages of infection evolution expressed amongst cattle handlers on *Brucella*-infected cattle farms. We conducted a case-control study on cattle farms participating in the bovine brucellosis control programme in Gauteng province. All herds in Gauteng that participated in the programme between 2014–2016 were eligible for this study. Farms were categorised as either case—when two or more cattle tested seropositive, to increase the specificity of a herd diagnosis of brucellosis and select herds presenting greater risk for cattle handler exposure —or control, following routine regulatory screening using the Rose Bengal test (RBT), and confirmation of reactors with the complement fixation (CFT) test. All cattle handlers on case farms were tested for brucellosis using four commercially available serological tests: the RBT and IgM ELISA, the IgG ELISA, and an immunocapture agglutination (BrucellaCapt) test. A subset of cattle handlers on control farms and veterinary officials from the three State Vet Areas of the province were also tested. A structured questionnaire on herd management practises and cattle symptoms was administered to herd managers.

Seroprevalence was calculated for cattle handlers on case farms, control farms and veterinary officials, according to (1) RBT (2) IgG ELISA and (3) BrucellaCapt serological tests. Furthermore, seroprevalence is reported for five mutually exclusive combinations of test results, indicative of infection evolution from short to long, in this group of persons. These combinations were: (i) RBT positive AND IgM ELISA positive AND IgG ELISA negative, (ii) RBT negative AND IgM ELISA positive AND IgG ELISA positive, (iii) RBT positive AND IgM ELISA positive AND IgG ELISA positive, (iv) RBT positive AND IgM ELISA negative AND IgG ELISA positive, and (v) RBT negative AND IgM ELISA negative AND IgG ELISA positive. Seropositive reactors on the BrucellaCapt test were allocated to the group defined by the outcomes of the RBT, IgM ELISA and IgG ELISA.

Univariate analyses were conducted to identify herd management factors and symptoms associated with case herds. Herd management factors with two categories were tested using the 2-sided Fisher test, and the Chi^2 test was used to analyse factors with more than two categories. Variables associated with case herds, at significance $p < 0.2$ in the univariate analyses, were included in a multivariable logistic regression model. Backward stepwise

selection was used to identify significant ($p < 0.05$) factors. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. Analyses were conducted in STATA 14 (StataCorp, College Station, TX, U.S.A.).

Chapter 5

The final study aims to address the existing gap in our understanding of firstly, cattle handlers' knowledge of brucellosis and health seeking response to brucellosis-like symptoms and secondly, the risk factors associated with selected stages of *Brucella* infection evolution in this group. A cross sectional survey of cattle handlers, exposed to confirmed *Brucella* seropositive and seronegative cattle herds, and a subset of provincial veterinary officials, was conducted using face-to-face structured questionnaires, between March and November 2016. The questionnaire captured information on participants knowledge of brucellosis and risk factors for exposure to *Brucella*.

Descriptive statistics were done in Microsoft® Excel®. Univariate analyses were conducted in STATA 14®, for outcomes (1) RBT and IgM ELISA, (2) IgG ELISA and (3) BrucellaCapt seropositivity amongst farm workers and veterinary officials (N=230).). Univariate associations between each variable and the outcomes were assessed using Fisher's exact test. Variables with $p<0.20$ were selected for inclusion into the multivariable logistic regression models. Three separate mixed effects logistic regression models were fit to identify risk factors for possible (1) infections of a short evolution (outcome: RBT and IgM ELISA seropositive), (2) infections of a long evolution (outcome: IgG ELISA seropositive) and (3) undiagnosed clinical infections of both short and long evolution (outcome: BrucellaCapt seropositive). Farm was included as a random effect in all three models. Veterinary officials were clustered into three groups, according to the State Vet Area they serviced. Each cluster was allocated a unique number and added to the Farm variable. On verification of the herd status, five herds (with 53 cattle handlers enrolled) were reclassified as control herds. Herd status was therefore included as an additional predictor in the models. Variables with $p>0.05$ in the models, were systematically removed by backward elimination (Kleinbaum and Klein, 2010, Tabachnick and Fidell, 2001).

Chapter 6: Discussion

Findings from the previous chapters are integrated in this final chapter. The main findings of the thesis are integrated using systems thinking as a methodology to describe a hypothetical system of bovine brucellosis persistence at the human-cattle-farm interface in Gauteng and to identify areas for further research.

The thesis concludes with a set of recommendations derived from each of the studies.

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Chapter 2: A review of human brucellosis in South Africa: clinical symptoms, medical diagnosis and treatment implications for general practitioners*

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Abstract

Brucellosis is recognized as a neglected zoonotic tropical disease of global health and economic importance. Medical practitioner unawareness of the disease is reported to contribute to the overall neglect. In South Africa (SA) human brucellosis is a notifiable medical condition and bovine brucellosis is a controlled animal disease. The overall aim of this paper is to increase medical practitioner capacity to detect, diagnose and treat brucellosis within the SA context. A brief review of literature on human brucellosis in SA is presented together with a discussion of current issues related to medical detection, treatment and management of brucellosis, applicable to the South African context.

Introduction

Human brucellosis is a neglected zoonotic tropical disease, caused by facultative intracellular gram-negative *Brucella* bacteria that are transmitted directly or indirectly from animals to people (McDermott and Arimi, 2002). Several species of *Brucella* have been implicated in zoonotic infections (Moreno, 2021). Of these, *B. abortus, B. suis, B. melitensis* and *B. canis*, have domestic animals (cattle, pigs, goats and sheep, and dogs) as preferred hosts.

The incubation period for brucellosis in people is reported to range from between one to five weeks. Infection is associated with a humoral response. *Brucella* antibody expression in an infection of a short evolution (first week after exposure/inoculation (Al Dahouk and Nöckler, 2011)) is typified by a predominance of IgM as opposed to infection of long evolution in which IgM decreases and IgG (and IgA) increases and eventually predominates over IgM peaking at about four weeks after inoculation. Infection of longer evolution is also typified by the presence of non-agglutinating antibodies that increase over agglutinating antibodies (IgM and IgG) as the duration of infection increases (Al Dahouk and Nöckler, 2011, Díaz et al., 2011). Clinically, brucellosis has been referred to as asymptomatic or symptomatic disease, with symptomatic disease noted to have either an acute or an insidious onset (Doganay and Aygen, 2003, Glynn, 2008). Doganay et. al. (2003) further divided the symptomatic stage of

disease according to the duration and severity of the symptoms as “acute”: lasting up to eight weeks, “sub-acute”: lasting between eight weeks to a year, or “chronic” disease which lasts more than a year. These classes aim to categorize the symptoms of disease but importantly, these classes of symptoms are not strictly equivalent to antibody expression over the evolution of infection.

The disease has also been described as either uncomplicated or focal, where focal implies localization of the bacterium in an organ system resulting in symptoms related to that system (Bosilkovski, 2016). Such localized infection has also been referred to as a “complication” of brucellosis (Doganay et. al., 2003). Complications of any kind are more likely to occur, if infection is not detected and treated correctly, irrespective of onset or symptomatic stage of disease (Corbel et al., 2006). Common complications of brucellosis are: sacroiliitis, orchitis and epididymitis, whilst neurobrucellosis and endocarditis are rarer but may result in death when it occurs (Dean et al., 2012a). Any organ system may be affected, revealing the multisystem nature of this disease (Mantur, 2006, Franco et al., 2007a, Buzgan et al., 2010).

Human brucellosis is a disease of global importance disproportionately affecting low- and middle-income countries impacting poorer and more marginalized people (Franc et al., 2018, Mableson et al., 2014, Michael and Madon, 2017, WHO, 2014). Re-emerging endemic foci in countries that controlled the disease in livestock have been reported (Pappas et al., 2006), indicative of intermittent or imperfect serological surveys and imperfect control. Little is known about brucellosis in humans in Africa (Pappas et al., 2006). Country data on human brucellosis in Sub Saharan Africa, is sparse and the true burden of the disease in this region is unknown (Dean et al., 2012, Ducrotoy et al., 2017, Pappas et al., 2006).

In South Africa (SA), bovine brucellosis is a controlled animal disease (DAFF, 2016, Frean et al., 2019) and human brucellosis is a notifiable medical condition. However currently there is no surveillance program for human brucellosis in SA (Frean et al., 2019). Two cases of brucellosis in humans were reported in 2016, from the Western Cape (Wojno et al., 2016) and Mpumalanga respectively (Frean et al., 2019). This followed a published report of *B. abortus* infective endocarditis of a prosthetic valve in a patient from KwaZulu-Natal (Mahomed et al., 2015). The latter paper references an article published by Schrire in 1962 (Schrire, 1962), which reports the national incidence of human brucellosis to be less than 0.2 per 100 000 population. The paucity of reported cases of brucellosis since Schrire’s article is used to support the conclusion that the incidence of human brucellosis in SA is low, and it is

suggested that the low number of human cases reported is indicative of effective vaccination against brucellosis in livestock (Mahomed et al., 2015).

More recent literature emphasizes the problem of under-diagnosis and under-reporting of human brucellosis in SA, highlighting medical practitioners' unawareness of the disease (Wojno et al., 2016). Furthermore, Frean et al, 2019 (Frean et al., 2019) draw attention to the re-prioritization of *B. abortus* as a public health risk in South Africa and the measures being taken by government veterinary services to reduce the risk. Strong recommendation is made for clinician awareness, involvement, and vigilance in these papers.

This paper therefore aims to increase practitioner awareness of brucellosis by presenting evidence of the historical importance of the disease in SA from published literature. Clinical findings will be reviewed within the context of the most pertinent challenges clinicians will face in the detection, treatment and management of brucellosis in the present context of SA.

History of human brucellosis in SA

In Southern Africa, the first reported human case of brucellosis recorded was in 1924 and was caused by *B. abortus* (Duncan, 1924, Duncan, 1928). Outbreaks of abortions in cattle herds, first detected in 1906, were confirmed to be a result of *B. abortus* infection in 1913. These occurred in the Johannesburg area of the Transvaal province (Bishop, 1994). There is speculation that *B. abortus* has been endemic in the area for a long time following a recent paleo pathological finding that proposes *Brucella* to have been the cause of disease in the late Pliocene hominin species *Australopithecus africanus* (Stw 431) in the Sterkfontein caves complex, which is found in the Transvaal province, approximately 2.4 to 2.8 million years ago (D'Anastasio et al., 2009), This is however hypothetical, since vertebral affection is not pathognomonic of brucellosis.

The history of prioritization of human brucellosis in Southern Africa dates back to 1919, when "Malta Fever" (caused by *B. melitensis*), was included as a notifiable human disease in the Public Health Act,1919 of the Union of South Africa. Human brucellosis, caused by *B. abortus*, was recognized to be public health risk in South Africa (Duncan, 1924, Duncan, 1928) ten years after the discovery of the zoonotic nature of the bacterium (Evans, 1947). This conclusion was based on substantial evidence of undulant fever cases in man attributed to *Brucella* spp. that did not share the morphological or culture conditions of *B. melitensis* and

was not associated with direct or indirect contact with goats, but instead with indirect or direct contact with cattle (Duncan, 1928). Human cases of undulant fever, caused by *B. abortus* were notified as "Malta Fever" and were detected and reported from all provinces of the Union of South Africa (except Natal) from 1928 - 1980 (Duncan, 1928, Campbell et al., 1937, Barnetson, 1939, Zoutendyk, 1958, Schrire, 1962, Mauff, 1980).

In 1938, clusters of cases were identified in the Transvaal province (Barnetson, 1939). The endemic state in the North Eastern Transvaal and South West Africa, was highlighted in 1958 (Ipp, 1958) and human brucellosis was reported to be a disease more common in South Africa than was generally believed. In 1959, brucellosis in humans was recognized as a problem in Krugersdorp and Transvaal (Lewis, 1959). The endemicity of this region was further supported by Shire, 1962 (Schrire, 1962) who identified the North and East Transvaal, Witwatersrand and Swaziland as areas representing 66.4% (77/116) of cases reported between 1956 and 1959. Furthermore, evidence of a risk to the public through the consumption of contaminated milk was identified in the Witwatersrand (Lewin et al., 1948) and Northern Highveld (Erasmus and Floor, 1988) regions of the Transvaal in 1948 and 1962.

The importance of brucellosis as a disease in humans seems to have diminished significantly by 1980, with a publication from Mauff (1980) (Mauff, 1980) reporting seven cases of acute brucellosis within a nine month interval, five of which were associated with a new abattoir plant in Johannesburg, as an unusual event. The last reported annual incidence rates from an analysis of the Department of Health notifications, covering the period between 1977 and 1984, was 0.1 and 0.3 per 100 000 population (Frean et al., 2019).

However, interest in *Brucella*, post 1980, continued as research into its use as a biological weapon in South Africa (Gould and Folb, 1990). In this covert government program *B. melitensis* and *B. abortus* are mentioned as being on the list of pathogens available for sale by the Roodeplaat Research Laboratory. On the list, *B. abortus* is identified as "terminating pregnancy in cows" (Gould and Folb, 1990). During this period there was a paucity of published articles on human brucellosis.

Prevention of brucellosis caused by *B. abortus*

Brucella abortus, the cause of bovine brucellosis, is considered one of the major zoonotic species causing human brucellosis in South Africa (Frean et al., 2019). *B. abortus* occurs in cattle and may also occur in horses, pigs, sheep, goats, bactrian camels, dromedary camels, water buffalo and yaks (Crawford and Hidalgo, 1977) It has also been occasionally

reported to occur in wildlife species such as the African buffalo, hippopotamus, zebra, eland and impala (OIE, 2008b, Godfroid et al., 2011).

Brucellosis infected cattle are characterised by one or more of the following symptoms: abortion, retained placenta, stillbirths, poor weight gain, orchitis, epididymitis and hygromas (Bishop, 1994). In cattle *B. abortus* causes abortions usually in the third trimester. Bacterial concentrations within the placenta and foetal tissues can be as high as $10^9 – 10^{10}$ colony forming units (CFUs)/g and therefore is the main source of transmission to humans or uninfected bovine through aerosolized or direct mucosal contact. Infection of the reproductive system does not always lead to abortion, but can persist in a herd without any overt clinical symptoms except for the birth of weak or nonviable calves and a reduction of milk yield (Crawford and Hidalgo, 1977, Olsen and Tatum, 2010).

Therefore, direct contact with infected reproductive material or uterine discharge or indirect contact through the ingestion of bacteria shed in the milk, are the main routes of transmission of *B. abortus* to humans and to other cattle. Further sources of infection have been reported to be a contaminated environment especially if it is wet and muddy or contact with equipment used for milking or artificial insemination (Bishop, 1994).

Global evidence of the zoonotic and economic importance of bovine brucellosis resulted in the emergence of national bovine brucellosis eradication schemes (Crawford and Hidalgo, 1977, Evans, 1947, OIE, 2008a). South Africa was amongst those countries that initiated such a scheme, supported by legislature, and regulated by veterinary state services in response to the economic and zoonotic threat of the disease in cattle (Bosman, 1980, Drimmelen, 1949, OIE, 1987). Such schemes at the time were being successfully implemented in developed countries and relied on a well-coordinated and managed veterinary services program to reduce cattle and herd infection levels through vaccination with S19 and subsequent cattle test and slaughter programs (Alton, 1977, Becton, 1977, Cunningham, 1977, McKeown, 1977, Michael, 1977, Morgan, 1977). Since the inception of bovine brucellosis eradication schemes, the USA (Olsen and Tatum, 2010), Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Ireland, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia Sweden, Switzerland (EU, 2017), and Australia (DOA, 2019) have achieved a bovine brucellosis-free status.

Vaccination of cattle with attenuated live vaccines against *B. abortus* is a critical component of bovine brucellosis eradication or control programs (Dorneles et al., 2015). Two

attenuated vaccines, S19 and RB51, have been registered for use in national bovine brucellosis eradication programs (OIE, 2008b). Whilst there are no known antibiotic resistant properties of S19, the attenuated live rough strain, RB51 is a rifampicin resistant attenuated strain of the smooth *B. abortus* biovar 1 S2308 strain (Dorneles et al., 2015, OIE, 2016) that has been shown to cause infection in occupationally exposed persons at an estimated rate of 2 unintentional needle stick injuries for every 1 000 inoculations performed (Ashford et al., 2004), and has resulted in several outbreaks affecting consumers of milk in the United States (CDC, 2019, Negrón et al., 2019, Sfeir, 2018). Routine serological tests are unable to detect infection with RB51 (CDC, 1998, CDC, 2019), presenting a diagnostic challenge to clinicians suspecting brucellosis.

In South Africa, control of bovine brucellosis began with compulsory vaccination of cattle with S19. Testing for bovine brucellosis for maintenance and export purposes were being conducted from 1913 at the Onderstepoort laboratory in Pretoria, Gauteng (Drimmelen, 1949). Further organization of control activities began in 1978 with the introduction of the bovine brucellosis eradication scheme, that was first announced in 1968, but began to be effective after 1976 (Bosman, 1980). This scheme was officially nationally ratified and promulgated in 1989 and was aimed at preventing and controlling brucellosis in cattle, which would in turn reduce brucellosis in humans as well as increase cattle herd productivity.

SA as a country has since undergone a political shift from an apartheid government to a democratic government over the century spanning the initial discovery of *B. melitensis* and *B. abortus* (Evans, 1947, Madkour, 2012). The political shift resulted in the decentralization of veterinary services in 1994 leading to management of the bovine brucellosis eradication program falling within the mandate of the nine provincial veterinary services (DAFF, 2016) to ensure an extension of veterinary services to the previously marginalized group of non-white cattle farmers. Currently a revision of the bovine brucellosis eradication scheme of 1980 is proposed to change from a voluntary testing of cattle to compulsory testing of all cattle in SA (DAFF, 2016, Frean et al., 2019). Vaccination of cattle herds and test and slaughter of infected cattle will still form critical components of the strategy. Occupationally exposed persons to *Brucella* infected cattle herds and those that routinely vaccinate, test or slaughter infected cattle are therefore presently at risk of brucellosis in SA.

Detection and diagnosis of brucellosis

Difficulty in detecting and diagnosing brucellosis is well described in literature (Al Dahouk and Nöckler, 2011, Al Dahouk et al., 2013, Franco et al., 2007b, Gazapo et al., 1989, Gupte and Kaur, 2016, Hasibi et al., 2013) and is still a major constraint to the early and accurate detection of brucellosis world-wide (Al Dahouk and Nöckler, 2011, Al Dahouk et al., 2013). Difficulty is primarily due to brucellosis being marked by non-specific symptoms that are common to other infectious diseases, such as malaria, tuberculosis, and influenza (Al Dahouk and Nöckler, 2011). Secondly, fever is not always associated with a detectable bacteraemia reducing the sensitivity of isolation and culture of the bacteria from blood or tissues despite the presence of symptoms. Thirdly, an infected person may have mounted an immune response detectable using serological tests before the appearance of symptoms, but tests may also be negative in the early stages of the disease. Furthermore, brucellosis patients successfully treated or that have recovered without treatment may remain seropositive for several months or years, making differentiation between patients with active disease, and patients with past disease but presenting with brucellosis like symptoms, difficult (Al Dahouk and Nöckler, 2011, Al Dahouk et al., 2013, Gazapo et al., 1989). To address this diagnostic complication, literature recommend that the population prevalence of brucellosis in healthy individuals in the area, be measured to determine a reliable cut-off value for serological tests used by clinicians to diagnose brucellosis in endemic regions (Al Dahouk and Nöckler, 2011). Despite these complications, clinicians have relied on available serological tests to support the diagnosis of brucellosis, to initiate treatment and follow the progression of disease (Ducrotoy and Bardosh, 2017, Díaz et al., 2011, Ducrotoy et al., 2017).

Clinicians in SA experienced similar difficulties in detecting and diagnosing brucellosis. At the time there were no protocols for the standardization of *Brucella* suspensions and clinicians depended on dissociated *Brucella* suspensions. Clinicians recognised that available serological agglutination tests could not differentiate between *B. melitensis, B. abortus and B. abortus (intermediate type)* (Campbell et al., 1937). The low sensitivity of culture to confirm brucellosis was also a concern. This is illustrated in reports of patients that tested seropositive for brucellosis whilst presenting with subacute endocarditis, but were culture negative for *Brucella* and culture positive *Streptococcus viridans* (Campbell et al., 1937).

The most reported challenge to clinicians in that time was interpreting serological test results and determining appropriate titre cut-offs to confirm a diagnosis of brucellosis in

patients with fever of unknown origin, where malaria, typhoid, paratyphoid and tuberculosis were already ruled out (Campbell et al., 1937, Barnetson, 1939). Campbell et al, in 1937 made use of live suspensions of *B. melitensis* and the Rhodesian strain of *B. abortus* as antigen for the agglutination tests and used a minimum titre of 1:400 to diagnose brucellosis in these patients with fever of unknown origin. They reported a 4.84% prevalence (32/661). The authors regarded a titre of 1:100 to 1:200 (9.36%) as probable cases or cases that had brucellosis (Campbell et al., 1937). This titre is higher than that used in a seroprevalence survey conducted by Barnetson from 1936 to 1938 to determine the frequency of *Brucella* agglutinins in the Union of South Africa (Barnetson, 1939). In this study 1 900 blood samples routinely submitted to the South African Institute for Medical Research to test for Typhoid Fever, were tested for antibodies to *B. abortus* and *B. melitensis* antigens using a titre of 1:50 as indicative of brucellosis (Barnetson, 1939). Using this titre, the incidence of brucellosis was reported to be 2.5% (40/1577) for the country during this period. However, interpreting these data is difficult because no protocols for the standardization of *Brucella* suspension were available at that time.

Use was made of the indirect Coombs tests over a three-year period to determine the frequency of *Brucella* antibodies in 2 393 patients (Zoutendyk, 1958). This test detects non-agglutinating antibodies to *Brucella* and was therefore considered a more sensitive test to detect past or present infection. Patients that were tested were provisionally diagnosed with one of the following: arthritis, acute rheumatism, brucellosis, pyrexia of unknown origin, backache, pneumonitis, anaemia, adenitis, hepatosplenomegaly, hepatitis, tuberculosis and included a proportion in which no diagnosis was given. Twenty one percent of these patients were seropositive to the indirect Coombs test compared to 5% amongst 300 randomly selected controls made up of blood donors and ante-natal patients.

An explanation for brucellosis titres in healthy persons in South Africa in the 1960s, was that these persons were exposed to "non-virulent *Brucella* antigen" (Van Drimmelen, 1963), a form of *Brucella* that did not cause disease, and therefore positive titres did not necessarily come from active infection. This led to a de-prioritization of the possibility of disease especially amongst occupationally exposed persons, such as farmers, abattoir workers and veterinarians that showed serological titre levels without clinical symptoms of disease (Van Drimmelen, 1963, Mauff, 1980). In contrast, other literature recognized that farmers and veterinarians frequently exposed to *Brucella* tend to display a hypersensitivity reaction that causes symptoms typical of acute brucellosis (Henderson and Hill, 1972, Ashford et al., 2004). Furthermore recent international studies suggest that the absence of clinical symptoms in the

presence of positive serology can be indicative of patients that have subclinical (asymptomatic) infection, frequently found in veterinarians, farmers and abattoir workers (Franco et al., 2007a). To date there is no evidence to suggest that the presence of antibodies confers a consistent immunity in these occupational groups. However, it is known that infection with *Brucella* spp. is dose dependent (Kahl-McDonagh et al., 2007) and evidence of disease with a long evolution ("chronic"), with a temporary absence of clinical symptoms in the presence of a serological response have been documented (Spink, 1951, Henderson and Hill, 1972, Mantur, 2006, Lewis, 1959), supporting the hypothesis that these occupational groups are at still at risk of brucellosis despite presenting as asymptomatic.

Currently in SA, the most commonly used serological tests are the Coombs anti-*Brucella* test, the serum agglutination test (SAT), the Rose Bengal test (RBT), complement fixation and the enzyme-linked immunosorbent assay (ELISA) (Frean et al., 2019), with the Coombs anti-*Brucella* test being regarded as the most specific to diagnose brucellosis.

The RBT has been reported to have a relatively lowered specificity in endemic areas with respect to detecting clinical disease (Al Dahouk et al., 2013), and a low sensitivity to detect chronic and complicated brucellosis cases in which there is an increase of non-agglutinating antibodies (Al Dahouk et al., 2013, Araj, 2010). Furthermore, RBT has been reported to have a lowered sensitivity in the presence of strongly positive sera due to the presence of prozones (Muma et al., 2009). However, when used according to the methodology presented by Diaz et. al., (2011), there is compelling evidence showing that the RBT test was not affected by prozones, blocking and non-agglutinating antibodies. In this study RBT performance was compared with several serological tests on sera of confirmed cases of brucellosis proved by bacterial isolation and sera of persons with no recent contact or brucellosis symptoms and was found to be sensitive in short ("acute") and long ("chronic") evolution brucellosis cases (Díaz et al., 2011), making it an appealing test in middle-and-low income endemic settings, due to the affordability, ease of use conducted and adaptability to test serum dilutions (Diaz et. al., 2011).

The ELISA IgG has also been reported to be a very sensitive serological test to detect antibodies of the IgG class , which are predominately found in long evolution ("chronic") phase of brucellosis and has been reported useful for detecting focal, complicated and chronic (long evolution) disease (Araj, 2010). Using a commercial kit in an endemic area, Hasibi et al. (2013) found a cut-off of 10IU/ml to be the most sensitive and specific. In comparison, using a home-made kit, Peeridogaheh et. al., (2013) found a cut-off of 10.78IU/ml produce the best sensitivity

and specificity in an endemic area. These papers (Hasibi et al., 2013, Peeridogaheh et al., 2013), illustrate the variability of cut-offs due to the origin of the test as well as the differences between local prevalence levels of brucellosis.

However, the BrucellaCapt, a single step immunocapture assay, is the recommended test to detect relapses of brucellosis or the disease in the long evolution of infection ("chronic" stage), because of its ability to detect non-agglutinating (blocking) or incomplete antibodies, which are dominant during this stage of infection (Al Dahouk et al., 2013). The BrucellaCapt also detects IgM, IgG, and IgA antibodies. It is commercially available, both cost effective and rapid and is reported to have a sensitivity of 99.2% and a specificity of 96%, on samples determined positive by the Coomb's test (OrduñA et al., 2000). Furthermore, BrucellaCapt titres indicate the activity of infection regardless of the stage of disease, decreasing slowly after relapse and more distinctly after treatment (Al Dahouk et al., 2013).

All these tests were developed to diagnose brucellosis in non-endemic countries and needs an adjustment of the cut-off titre to detect clinical cases if used in an endemic area (Peeridogaheh et al., 2013, Franco et al., 2007a).

Clinical symptoms

A multitude of symptoms affecting every body system, associated with culture positive or a seropositive reaction to *Brucella* antigens, were reported by SA clinicians from 1935 onwards (Robinson, 1935, Campbell et al., 1937, Ipp, 1958, Lewis, 1959, Schrire, 1962, Henderson and Hill, 1972, Sacks et al., 1976, Mahomed et al., 2015). Fever and chills, long drawn pyrexia, continuous fever of six weeks, fever of some months' duration, low pyrexia and pyrexia of unknown origin and sweating have been associated with brucellosis (Robinson, 1935, Campbell et al., 1937, Zoutendyk, 1958, Robinson and Metcalfe, 1976).

Lesions of the skin were described in veterinarians, cattle handlers that removed placenta, farmers and abattoir workers. These presented with erythematous granulomatous lesions or a skin rash lasting 4 - 8hrs (Robinson, 1935) progressing to a nodular rash lasting 3 to 4 days, usually on forearm, which sometimes caused gross thickening of the skin. This manifestation of brucellosis was termed erythematous brucellosis by Robinson et al. in 1935 (Robinson, 1935, Schrire, 1962)

Signs of musculoskeletal involvement included arthralgia; pain in joints; arthritis of knee and ankle, described in an Angolan native mine worker and wife of medical doctor from

Canada whilst severe shoulder pain was found in a farmer (Campbell et al., 1937, Lewis, 1959). Sacroiliitis and backache were described in a mine worker and a male European (18yrs) son of a town dairy owner (Ipp, 1958, Sacks et al., 1976), whilst a 65 year old female presented with *Brucella* spondylitis accompanied with radiculitis, which was referred to as "sciatic neuritis" (Ipp, 1958, Sacks et al., 1976). Other musculoskeletal symptoms described in brucellosis cases included peripheral arthritis, osteomyelitis, muscle wasting and palmer erythema (Robinson, 1935, Ipp, 1958, Lewis, 1959).

Hepatomegaly, cirrhosis of the liver, hepatosplenomegaly and hepatitis was also a common finding of brucellosis patients. This was sometimes associated with Spider naevi on chest. (Robinson, 1935, Ipp, 1958, Zoutendyk, 1958, Lewis, 1959). Other respiratory symptoms included pleural effusions, pneumonias, pneumonitis and bronchopneumonia (Campbell et al., 1937). Hilar adenopathy with a non-productive cough and focal pneumonitis was described in laboratory workers (Schrire, 1962). Endocarditis involving the aortic valve was described in 1937 (Campbell et al., 1937), and more recently in 2015 (Mahomed et al., 2015).

Peripheral neuropathies, chorea, meningoencephalitis, cranial nerve involvement, headache, malaise as well as psychiatric manifestations, depression, anxiety and neurosis were described, indicating nervous system involvement (Zoutendyk, 1958, Schrire, 1962, Sacks et al., 1976). Much of these psychiatric symptoms were associated with a diagnosis of “chronic brucellosis” with an insidious onset, recorded for seventeen South African patients (Sacks et al., 1976). These patients were referred by general practitioners to specialists at the Departments of Medicine and Microbiology of the University of the Orange Free State and tested seropositive to *Brucella* with high titres on repeated serological examinations. The seventeen patients did not present with fever, but rather with the symptoms presented in Table 2.1.

Table 2.1: Frequency of symptoms and clinical signs associated with chronic brucellosis (adapted from (Sacks et al., 1976))

Symptoms and clinical signs in 17 patients with chronic brucellosis of insidious onset	
Symptom	Number (%)
Tiredness	16 (94.1)
Fatigue	16 (94.1)

Arthralgia	14 (82.3)
Depression	12 (70.6)
Muscular pain	11 (64.7)
Abdominal pain	7 (41.2)
Headache	7 (41.2)
Sweating	4 (23.5)
Anxiety	3(17.7)
Agitated pain	2 (11.8)
Clinical sign	Number (%)
Lymphadenopathy	9 (52.9)
Cervical adenopathy	6 (35.3)
Axillary adenopathy	6 (35.3)
Inguinal adenopathy	2 (11.8)
Splenomegaly	7 (41.2)
Hepatomegaly	4 (23.5)
Pyrexia	4 (23.5)
Clinical joint involvement	1 (5.9)
Tiredness - physiological decreased ability of an organism or one of its parts to function because of prolonged exertion which causes toxic decomposition in the muscle and nerve.	
Fatigue - feeling of weariness but continuing normal activity. The weariness may be physical or mental in nature.	

Treatment and management

Late initiation of treatment of brucellosis is reported to increase the risk of relapse and treatment failure (Franco et al., 2007b). Currently the recommended treatment regimen for brucellosis patients in South Africa is as described in the tables below.

Table 2.2: Recommended treatment regimens for brucellosis (reproduced from (Frean et al., 2019))

Form of brucellosis infection		Recommended antibiotic regimen	Duration
Uncomplicated	Adults		
		Doxycycline	6 weeks

		plus streptomycin or gentamicin	1 - 2 weeks
		OR	
		doxycycline plus rifampicin	6 weeks
	Children		
	< 8yr	Cotrimoxazole plus rifampicin	4 - 6 weeks
	≥ 8yr	Doxycycline plus rifampicin	
Focal	Adults		
	Spondylitis	Doxycycline	12 weeks
		plus streptomycin or gentamicin	2 weeks
		OR	
		doxycycline plus rifampicin	12 weeks
		OR	
		Doxycycline plus ciprofloxacin	12 weeks
	Neurobrucellosis	Doxycycline plus rifampicin plus (ceftriaxone OR cotrimoxazole)	Prolonged until CSF normalises
	Endocarditis	Doxycycline plus rifampicin plus streptomycin or gentamicin	6 weeks to 6 months depending on clinical response
		Surgery if indicated	
	Children		
	< 8yr	Cotrimoxazole	6 weeks at least
		plus streptomycin or gentamicin	2 weeks
	≥ 8yr	Doxycycline	6 weeks at least
		plus streptomycin or gentamicin	2 weeks
		Rifampicin can be added to either regimen	6 weeks at least
Brucellosis in pregnancy		Rifampicin	6 weeks
		with/without cotrimoxazole (avoid in last week before delivery: risk of kernicterus)	
Complex focal, relapsed or refractory infection, or antibiotic toxicity/resistance		Consider adding quinolone or cotrimoxazole as second line to doxycycline or rifampicin; triple therapy has better cure rates	

Table 2.3: Recommended dosages for brucellosis treatment (reproduced from (Frean et al., 2019))

Antibiotic	Dosage	
Cotrimoxazole	Trimethoprim 10 mg/kg/d (max. 480 mg/d)	In 2 doses/d
	Sulfamethoxazole 50 mg/kg/d (max. 2g/d)	
Doxycycline	2-4 mg/kg/d (max. 200 mg/d)	In 2 doses/d
Rifampicin	15 - 20 mg/kg/d (max. 2 g/d)	In 1 or 2 doses/d
Gentamicin	5 mg/kg/d	
Streptomycin	20-40 mg/kg/d (max. 1 g/d)	In 2 doses/d
Ciprofloxacin	1 g/d	In 2 doses/d
Ofloxacin	400 mg/d	In 2 doses/d

Conclusion and Recommendations

Brucellosis caused by *B. abortus* has been an important medical condition for over a century in SA. In recent years however, there has been a paucity of medical literature describing the incidence of human brucellosis in SA. Even though an active bovine brucellosis control program to prevent human brucellosis exists in the country, occupationally exposed persons to *Brucella* infected cattle herds are still at risk of brucellosis. The public is also at risk of brucellosis through consumption of dairy products contaminated with the field or vaccine strain of *B. abortus.* Evidence of clinical symptoms associated with such exposure in South Africa has been discussed in this paper.

It is recommended that an occupational history including contact with an infected cattle herd/s be considered by general practitioners when managing fevers or symptoms, as described in this paper, of unknown origin. Treatment regimens should be adjusted for persons occupationally exposed to RB51. Communication between clinicians and veterinarians are recommended to strengthen risk mitigation strategies for individual brucellosis patients as part of the management strategy. This has been shown to be integral in the formulation of targeted mitigation and risk reduction strategies that are carried out by public health or government veterinary services (Wyatt, 2013, Hughes and Hughes, 1969).

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Chapter 3: Evaluation of progressive area elimination of bovine brucellosis in Gauteng, 2013-2018, using laboratory test reports

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Abstract

Bovine brucellosis is a zoonotic disease of global public health and economic importance. In South Africa, a voluntary national bovine brucellosis eradication scheme was implemented by State Veterinary Services in 1979. However, to date there is no published information on eradication in any area of the country.

This study analysed laboratory test results of all cattle herds that participated in the Gauteng Provincial Veterinary Services' eradication scheme between 2013–2018. Herd reactor status and within-herd seroprevalence were modelled using mixed-effects logistic and negative binomial regression models, respectively.

In Gauteng, between 2013–2018, no significant change in prevalence of reactor herds or within-herd seroprevalence was found. However, Randfontein (OR=1.6; 95% CI: 1.2-2.1; $p<0.001$) and Germiston State Vet Areas (OR=1.9; 95% CI: 1.5-2.5, $p=0.008$) had higher odds of reactor herds than the Pretoria State Vet Area. Reactor herds were also associated with increased herd size ($p<0.001$). Additionally, Germiston and Randfontein both had within-herd prevalence count ratios 1.5 times greater than the Pretoria State Vet Area ($p<0.001$) and larger herd sizes were associated with lower within-herd prevalence ($p<0.001$).

In conclusion, analysis of the prevalence of bovine brucellosis reactor herds and within-herd seroprevalence in State Vet Areas in Gauteng, using routine laboratory test results revealed no significant progress toward elimination. A vaccination strategy targeting small to medium size herds in Gauteng combined with compulsory test and slaughter of reactors in larger herds may be considered. Progress toward elimination should be communicated to farmers, to enable them to manage the disease in their herds, participate in surveillance and support provincial changes in the control strategy from vaccination to test and slaughter.

Introduction

Brucellosis is a neglected zoonotic disease of global health and economic importance impacting livestock, wildlife and people (Corbel et al. 2006). It is reported to cause a debilitating, oftentimes prolonged disease in humans, characterised by non-specific signs such as fever, sweating depression, weight loss, anorexia, arthralgia, generalized aches and fatigue, leading to ongoing expenditure for treatment and a chronic inability to work (Robinson 2003). *Brucella abortus* is reported by the OIE (OIE, 2016) to be the second most common zoonotic *Brucella* spp. after *B. melitensis,* and occurs in cattle. It is transmitted directly or indirectly to people through contact with uterine discharges of infected animals or the ingestion of unpasteurised dairy products (OIE, 2016).

The successful prevention of human brucellosis from *B. abortus* has been attributed to effective national bovine brucellosis regulatory programmes (Olsen and Tatum, 2010). As early as 1977, these programmes utilized a strategy of progressive area elimination with geographic zoning and the application of regulatory control activities within these zones, dependent upon the cattle and herd incidence of disease (Morgan, 1977, Michael, 1977, McKeown, 1977, Cunningham, 1977, Becton, 1977, Arturo del Rio, 1977, Alton, 1977). This strategy utilizes vaccination and surveillance of cattle herds in demarcated areas to reduce and monitor the reduction of cattle and herd reactor prevalence to less than 2% and less than 5%, respectively. Once these thresholds are reached, compulsory test and slaughter of cattle reactors is initiated in the area. As part of the regulatory control activities, regular cross-sectional surveys are conducted to monitor the progress of elimination.

Despite this approach's success in eradicating bovine brucellosis in the United States, several countries in Western Europe, the European Union and Australia, it has not resulted in similar outcomes in low-and-middle income countries. The reasons for this are multifaceted; they include competing health problems that demand political attention (WHO, 2014), the cost of running the program (Olsen and Tatum, 2010), a lack of epidemiological data to justify the programme (Pappas et al., 2006), the level of organization of Veterinary Services and their ability to establish a census, tag animals, and the possibilities to control animal movements in extensive systems (Blasco and Molina-Flores, 2011). Additionally, farmers' resistance to participate in a compulsory eradication programme presents another barrier to successful implementation. This was noted early on by Cunningham (1977) who highlighted that conducting compulsory test and slaughter activities in areas where the overall cattle reactor prevalence was not low enough, resulted in farming becoming unfeasible for farmers who

opted to go out of business rather than go through the process of eliminating the disease from their herds, for the sake of the national programme (Cunningham, 1977).

Poor control of bovine brucellosis in low- and middle-income countries is problematic since marginalized communities in these countries are the most affected physically and economically by brucellosis (WHO, 2005). In these countries, the initiation or continuation of national bovine brucellosis elimination programmes is supported only after considering epidemiological evidence and burdens of disease associated with brucellosis prevalence (Plumb et al., 2013). Such information is reported to establish trust in policy and regulatory decisions to change bovine brucellosis prevention, management or control strategies (OIE, 2008b, OIE, 2008a, Plumb et al., 2013). Therefore, it is recommended by the World Organisation for Animal Health (OIE) that in circumstances where there is a lack of information on the incidence of disease, data generated by existing control programmes or health schemes, laboratory records or data on the epidemiology of disease can be used by veterinary or medical authorities, to protect human and animal health (OIE, 2008a, Plumb et al., 2013).

However, the limitations of using laboratory data for epidemiologic inference has been noted from as early as 1949. In 1949, Drimmelen commented that serological tests carried out to maintain clean herds or for export requirements, and only occasionally to confirm suspect symptoms were not a representative sample of cattle sera (Drimmelen, 1949). Furthermore, the published literature criticizes the use of laboratory investigation records because of the inadequate and poor quality data routinely collected through this system (Artois et al., 2012). Another important limitation to governmental serological surveillance is the inability to differentiate between antibodies induced by different *Brucella* spp. in the host (Godfroid et al., 2013).

However, in countries that cannot afford to undertake national cross-sectional surveys, useful insight into the distribution and occurrence of animal brucellosis has been published from laboratory data analysis. For example, Mwebe et al. (2011) used laboratory data to identify differences in brucellosis seroprevalence between districts in Uganda from samples submitted to three different laboratories (Mwebe et al., 2011). In this study, the seroprevalence reflected within districts reflect a ten-year period of prevalence. Although it does indicate a trend of within-herd seroprevalence in these districts, it shows that brucellosis testing coverage was extended over 43 districts in Uganda. A similar methodology was used to estimate the prevalence of brucellosis in cattle in Zimbabwe over a five-year period (Vhoko et al., 2018).

These and other similar studies, such as that conducted by Madzingira et al. (2020) in Namibia are useful in understanding the frequency of samples submitted for *Brucella* testing over time and the overall proportion of reactors according to districts or species, but they give no insight into the brucellosis burden individual herds may be suffering from. Knowledge of the proportion of reactors in the herd is essential to understand the economic implication of compulsory test and slaughter to the individual cattle farmer, especially subsistence farmers of smaller herds.

In South Africa (SA), laboratory testing for bovine brucellosis started at the Onderstepoort Veterinary Institute (OVI) in 1914 for diagnostic and export purposes (Drimmelen, 1949). In 1976, a national scheme for the eradication of bovine brucellosis was put into effect. This scheme adopted four actions: (1) the standardization of serological diagnostic tests for bovine brucellosis, namely the milk ring test (MRT) for herd screening, Rose Bengal Test (RBT) for testing individual cattle with the Complement Fixation Test (CFT) as a confirmatory test on RBT reactors; the abolition of charges for laboratory tests; (3) compulsory branding of all reactors with a "C" on the right side of the neck; (4) voluntary accreditation of bovine brucellosis-free herds (Bosman, 1980). Implementation of the actions recommended in the scheme resulted in a reported drop in brucellosis incidence in cattle to 15% in 1977 (Schutte et al., 1977) to 6% in 1979 (Bosman, 1980). In 1979 the scheme was made official (Bosman, 1980), expanding on the four actions adopted in the 1976 scheme. It was declared to be based on six fundamental actions which, in addition to the earlier four, included compulsory vaccination and "the declaration of eradication areas in which testing and slaughter of reactors will be compulsory*"* (Bosman, 1980).

Since the scheme's launch in 1979, only three references were found regarding the national prevalence of herd and cattle brucellosis. The first, in 1983, reported an annual herd prevalence of 33.2% and cattle prevalence of 3.22% for South Africa (Department of Agriculture, 1983). Four years later, the second report stated a decline in both the annual herd and cattle prevalence to 29.8% and 1.92%, respectively, although the report also states that the herd prevalence varied across the country, from 0.8% in some regions to 48.7% in others (Directorate of Veterinary Services, 1987). The national herd prevalence for SA in 1990 was 14.7% (McDermott and Arimi, 2002); however, these reports do not indicate the spatial distribution of herd and cattle reactor prevalence across the country. It is also unclear whether these results reflected the unique individual herds tested or an aggregation of herd tests,

including herds repeatedly tested throughout the year. Furthermore, these figures do not give insight into the variation of within-herd prevalence for brucellosis.

There has been no further published information on the trend or spatial distribution of bovine brucellosis in provinces or municipal areas in South Africa since 1990. Furthermore, the within-herd prevalence of bovine brucellosis has not been investigated yet in the SA. However, in a discussion paper reviewing the bovine brucellosis situation in SA, the national Department of Agriculture reported that a gradual increase of the disease had been observed (DAFF, 2016b). Therefore, this study aimed to assess trends in the prevalence and distribution of reactor herds in Gauteng and the proportion of reactor cattle per herd test in the province over six-years (2013–2018) using data derived from laboratory test results.

Materials and Methods

The study was conducted in Gauteng province, one of the nine provinces of South Africa, and within the historically recognised endemic area for bovine brucellosis (Drimmelen, 1949). Gauteng is divided into three State Veterinary Areas, namely the Pretoria, Germiston and Randfontein State Vet Areas. State Vet Areas are further subdivided into municipal districts. Municipal offices in districts are responsible for public health services. These districts' borders have undergone two stages of re-demarcation between 2000–2016, resulting in the original three districts making up the Pretoria State Vet Area, merging into one Metro.

The province has an estimated cattle head census of 444 151, of which 51.8% is distributed within the Germiston State Vet Area and 36.5 % and 11.6% within the Pretoria and Randfontein State Vet Areas, respectively (Gauteng Department of Agriculture and Rural Development Census Report 2016). The distribution of *Brucella* infected herds detected from 1999–2018 in the province is scattered throughout districts within the three State Vet Areas (Gauteng Department of Agriculture and Rural Development Epidemiology Report 2019) (Figure 3.1).

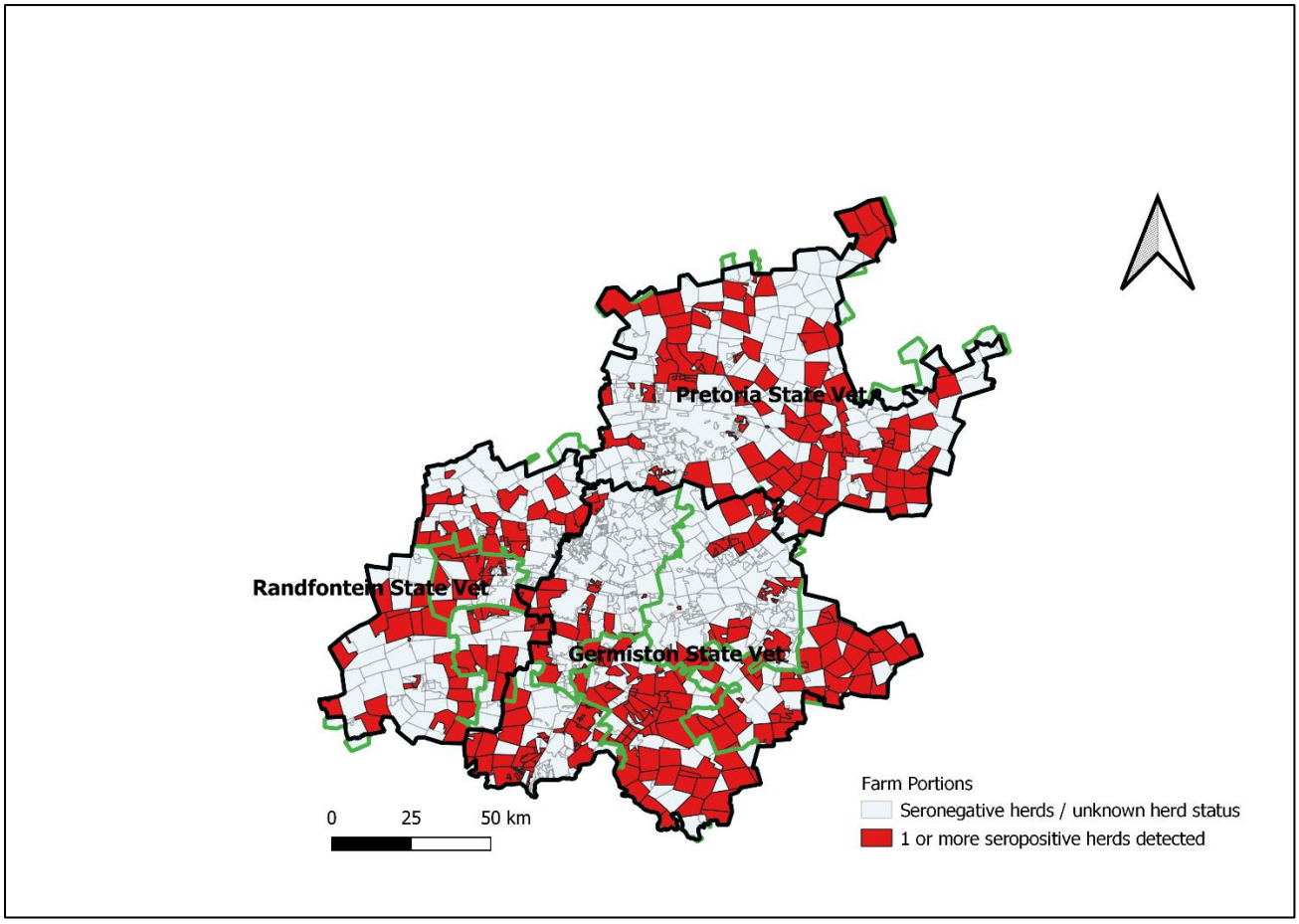

Figure 3.1. Distribution of farm parcels with one or more *Brucella* reactor herds within districts (delineated in green) and State Vet Areas (delineated in black), 1999–2018, Gauteng

The voluntary testing of cattle herds for brucellosis by cattle farmers prescribed in the scheme, at the time of this study, is a passive surveillance system (DAFF, 2016b). However, bovine brucellosis is a controlled animal disease in South Africa (DAFF, 2016a), therefore any herd that tested positive for brucellosis that did not volunteer to participate in the scheme (e.g. diagnosis by a private vet), automatically entered the scheme. Consequently, the laboratory report dataset included all herds in which a cattle reactor was detected but not the total cattle herds at risk in the province.

Individual cow blood samples were collected in dry red-topped serum collection tubes and marked by the animal health technician (AHT). The identity of each cow was captured on the sample collection form by the AHT. The batch of samples was submitted to the Onderstepoort Veterinary Research laboratory (OVR), where it was allocated a unique laboratory number. Each sample was then screened for *B. abortus* antibodies with the Rose Bengal test (RBT) serological test. Serum that reacted on the RBT was retested with the

complement fixation test (CFT) to confirm seropositivity to *B. abortus*. Tests were conducted according to OIE standards.

Test results for each cow were captured on the original sample collection form prescribed by the national Veterinary services (DAFF, 2016a), and a copy of the completed form was made for data capture at the provincial epidemiology branch of the Veterinary Services, whilst the originals were filed at the respective State Vet Offices. Copies of the herd test results were routinely batched in preparation for collection by an AHT at the end of the month. The AHT then delivered the batch of copies to the administrative clerk for the Gauteng Veterinary Services' epidemiology branch, who captured it into a Microsoft Access® database.

Each entry in the dataset was allocated the unique laboratory report number, and captured information from a single herd test. Information was captured to primarily monitor the number of herds tested, herd status - defined as positive if one or more cattle reacted to CFT ≥60 IU/ml, and the total number of cattle testing CFT ≥60 IU/ml positive within a herd. CFT ≥60 IU/ml is considered the threshold to rule out possible false positives due to S19 vaccine reactors (DAFF, 2016a). The initiative to capture herd information electronically was taken by managers of the Epidemiology branch in Gauteng, even though it was not a prescribed indicator for monitoring and evaluation purposed. Other variables captured in the dataset included the herd owners' name; farm name; total number of cattle tested, which is used as the proxy for herd size; the date of blood collection and laboratory report; name of the veterinary official collecting the samples; State Vet Area; district area.

Six variables from the raw dataset were selected for analysis: (1) State Vet Area, (2) District, (3) Herd Size categorized into quartiles, (4) Serum sample sender, (5) Year of herd test, and (6) Herd Status, where one or more reactors (CFT ≥60 IU/ml) is regarded as a positive herd.

Only herds with greater than one animal tested were included in the study, as observations of only one cow tested (n = 268), were assumed to be an individual animal diagnostic test instead of a herd test. Herd sizes greater than 2,500 were regarded as outliers (n = 1). Outliers and observations with missing values in the "District" variable (n = 31) were removed from the dataset.

The laboratory dataset analysis was conducted using R Version 3.6.2. (2019-12-12) Copyright (C) 2019 The R Foundation for Statistical Computing. R packages: dplyr and

ggplot2 were used for descriptive statistics and Stata 14 (StataCorp, College Station, TX, U.S.A.) for the regression models. Significance was assessed at $p < 0.05$.

Proportions of reactor cattle and proportion of reactor herds are presented by province by districts within State Vet Areas for the five-year period. These proportions, representing crude annual prevalence are calculated as (1) the number of cattle reactors divided by the total number tested, for cattle prevalence and (2) the number of reactor herds divided by the total herd tests for that year, for herd prevalence. A reactor herd is defined as a herd with one or more animals testing seropositive (CFT ≥60 IU/ml).

A mixed-effects logistic regression model was fitted to explain the prevalence of reactor herds (one or more CFT > 60 IU/ml cattle in a herd) with herd status as the dependent variable and with State Vet Area, herd size quartile and year as fixed effects and district as a random effect. Odds Ratios for significant variables are presented.

A mixed-effects negative binomial regression model was fit for cattle reactors, with the count of cattle reactors within a herd as the dependent variable and with State Vet Area, year and herd size quartile as predictor variables, district as a random effect and herd size as the exposure variable which effectively models the within-herd prevalence as the dependent variable rather than the count of reactors. Count ratios are reported.

Results

Provincial annual cattle and herd prevalence

From 2013 to 2018, the Gauteng Provincial Veterinary Services conducted 4,395 herd tests comprising 359,026 cattle tests. The mean annual herd prevalence for the six-year period was 22.1% (range: 11.0% in 2016 to 32.4% in 2014; std dev: 6.9). The mean annual cattle prevalence and mean within-herd prevalence for the period was 1.4% (range: 0.4%, in 2016 to 2.3% in 2018, std dev:0.6) and 7.4% (range: 6.1% in 2016 to 9.0% in 2018, std dev:1.1) respectively (Table 3.1).

Table 3.1: Provincial proportions of CFT seropositive cattle, herds and within-herd reactors, Gauteng, 2013–2018

Year	No. of Herd Tests	No. of Reactor * Herds	No. of Cattle Tests	No. of Reactor ** Cattle	Proportion of Reactor Herds (%)	Proportion of Reactor Cattle (%)	Average % CFT positive cattle within Reactor Herds (%)
2013	777	160	49,421	750	20.6	1.52	7.8
2014	611	198	46,012	847	32.4	1.84	7.1
2015	613	149	43,456	536	24.3	1.23	6.5
2016	907	100	99,280	382	11.0	0.38	6.1
2017	637	134	55,429	697	21.0	1.26	8.0
2018	850	195	65,428	1469	22.9	2.25	9.0

Herd prevalence in State Vet Areas and Districts

Between 2013 and 2018, variation in the annual herd prevalence between State Vet Areas is apparent, with Randfontein having the highest herd prevalence in 2014 (Fig. 3.2). Variation is also apparent in herd prevalence between Districts within State Vet Areas over the study period (Fig. 3.3).

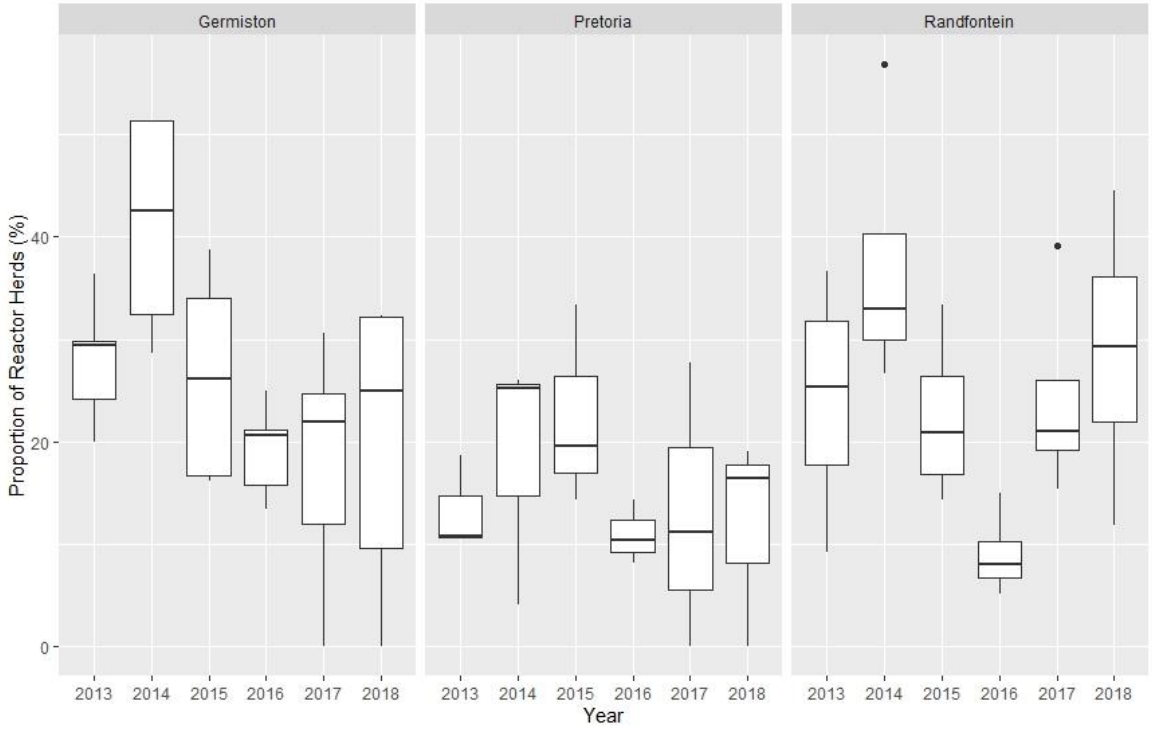

Figure 3.2: Variation in *Brucella* reactor herd prevalence, 2013 – 2018 by State Veterinary Areas within Gauteng

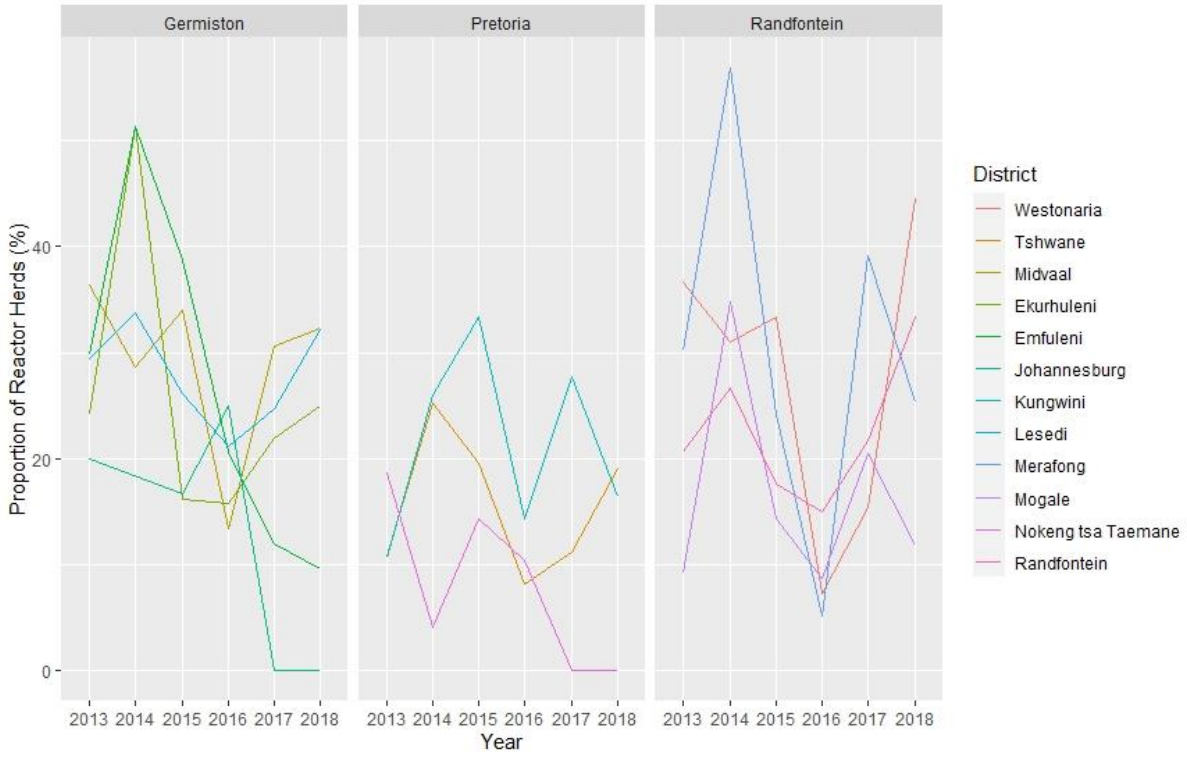

Figure 3.3. *Brucella* reactor herd prevalence by districts within State Veterinary Areas, Gauteng 2013–2018

Cattle prevalence in State Vet Areas and Districts

Similar to reactor herd prevalence, the annual prevalence of reactor cattle appeared to vary between State Vet Areas (Figure 3.4 below). Figure 3.5 illustrates the variation of reactor cattle prevalence between districts.

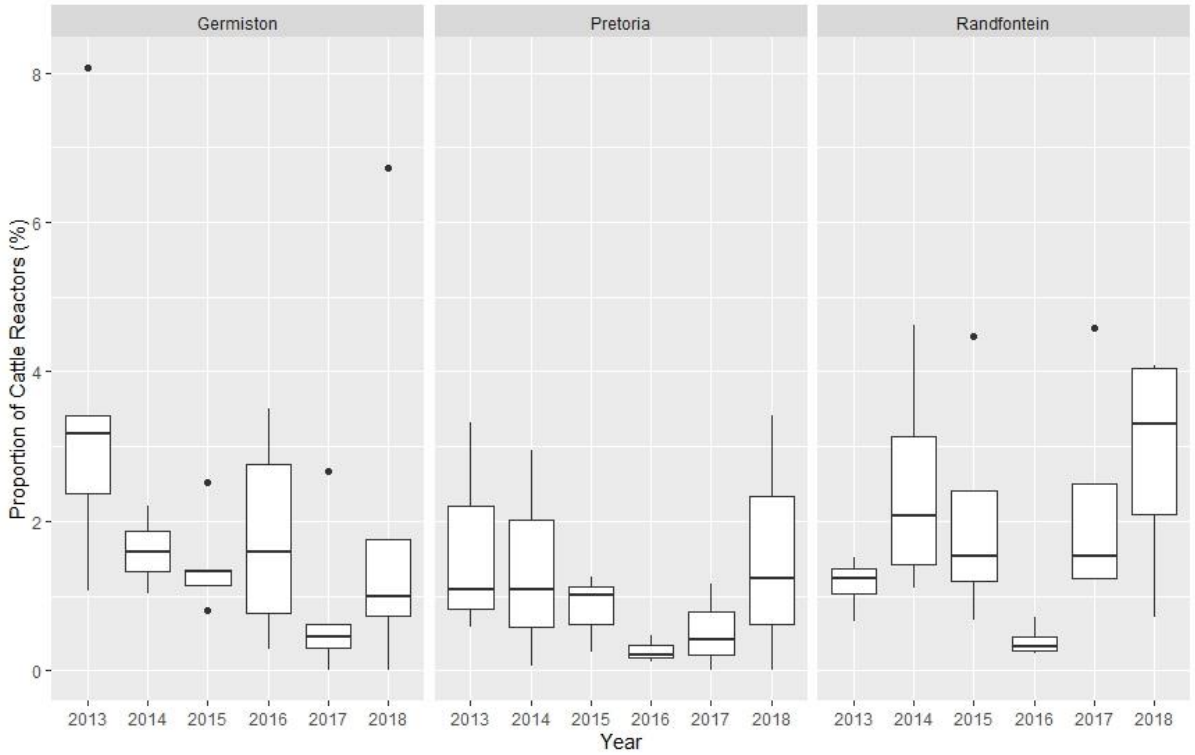

Figure 3.4. Variation in reactor cattle prevalence, 2013–2018 by State Veterinary Areas within Gauteng

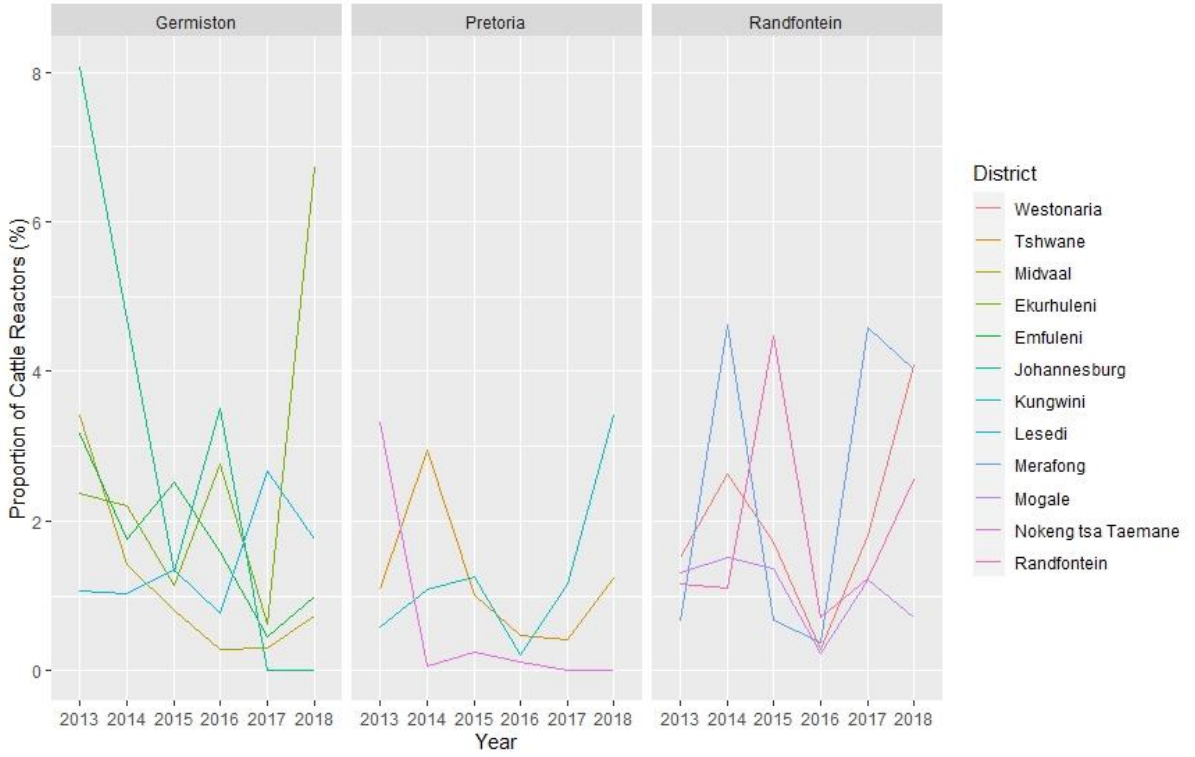

Figure 3.5: Prevalence of reactor cattle by districts within State Veterinary Areas, Gauteng 2013–2018

Herd reactor model

The mixed-effects logistic regression model (Table 3.2), fitted for reactor herds, with State Vet Area, year and herd size as predictor variables and district as a random effect indicated that district was not significant (LR test vs logistic model chibar^2 = 1.85, p>= chibar^2 = 0.09).

Table 3.2: Mixed effects logistic regression model fit for *Brucella* cattle herd reactors, Gauteng 2013-2018:

Variable	Category	Seropositive Herds	%	Total Herd Tests	Odds Ratio (95% CI)	p-value
Year	2013 (reference)	160	17.1	(777)	1	
	2014	198	21.2	(611)	1.7 (1.4- 2.2)	<0.001
	2015	149	15.9	(613)	1.2 (0.9 - 1.6)	0.161
	2016	100	10.7	(907)	0.4 (0.3 - 0.5)	<0.001
	2017	134	14.3	(637)	1.0 (0.7 - 1.3)	0.786
	2018	195	20.8	(850)	1.1 (0.9 - 1.4)	0.305
Herd size	[2 - 12] (reference)	126	13.5	(1102)	1	
	[13 - 27]	233	24.9	(1102)	2.3 (1.8 - 2.9)	< 0.001
	[28 - 91]	254	27.1	(1101)	2.5 (2.0 - 3.2)	< 0.001
	[> 91]	323	34.5	(1090)	3.7 (2.9 - 4.7)	< 0.001
State Vet Area	Pretoria (reference)	277	16.4	(1689)	1	
	Randfontein	275	21.5	(1278)	1.6 (1.2 - 2.1)	0.001
	Germiston	384	26.9	(1428)	1.9 (1.5 - 2.5)	<0.001

Herds in Randfontein (OR=1.6; 95% CI: 1.2-2.1; p=0.001) and Germiston State Vet Areas (OR=1.9; 95% CI: 1.5-2.5) were more likely to be seropositive than those in the Pretoria State Vet Area when controlling for herd size and the year of testing. Furthermore, the odds of a herd testing positive increased with increasing quartiles of herd size, with herd sizes of 13–27 cattle (OR=2.3; 95% CI: 1.8-2.9), 28-91 cattle (OR=2.5; 95% CI: 2.0-3.2) and greater than 91 cattle (OR=3.7; 95% CI: 2.9-4.7) all being significant (p<0.001) compared to herd sizes of 2-12 cattle. Apart from an apparent increase in 2014 and decrease in 2016, the odds of herds testing positive did not change significantly. over the study period.

Within-herd reactor model

In the mixed effect negative binomial regression model (Table 3.3), district as a random effect was not significant (LR test vs logistic model chibar^2 = 1.85, p>= chibar^2 = 0.09).

Table 3.3: Negative binomial regression model fit for within-herd *Brucella* seroprevalence, Gauteng, 2013-2018:

Variable	Category	Seropositive Cattle	%	(Total Cattle Tested)	Count Ratio (95% CI)	p-value
Year	2013 (reference)	750	0.2	(49,421)	1	
	2014	847	0.2	(46,012)	1.3 (1.0- 1.9)	0.082
	2015	536	0.1	(43,456)	0.9 (0.6 - 1.2)	0.514
	2016	382	0.0	(99,280)	0.4 (0.3 - 0.5)	< 0.001
	2017	697	0.1	(55,429)	0.9 (0.7 - 1.3)	0.641
	2018	1469	0.2	(65,428)	1.3 (1.0 - 1.8)	0.080
Herd size	[2 - 12] (reference)	223	13.5	(6,502)	1	
	[13 - 27]	723	24.9	(26,579)	0.9 (0.6 - 1.2)	0.338
	[28 - 91]	1132	27.1	(66,157)	0.5 (0.4 - 0.7)	< 0.001
	[> 91]	2603	34.5	(259,788)	0.3 (0.3 - 0.4)	< 0.001
State Vet Areas	Pretoria (reference)	1227	16.4	(111,129)	1	
	Randfontein	1346	21.5	(83,913)	1.5 (1.2- 1.9)	<0.001
	Germiston	2108	26.9	(163,984)	1.5 (1.2 - 1.9)	<0.001

Furthermore, alpha was 7.6 (95% CI: 7.0–8.3), indicating significant overdispersion and the suitability of the negative binomial model. The model suggests that there has not been a significant decrease or increase in within-herd seroprevalence from 2013–2018. The variation between State Vet Areas was significant, with Randfontein and Germiston having count ratios 50% greater than the Pretoria State Vet Area. Furthermore, the model indicates a significant decrease in within-herd seroprevalence as herd size increases, with herd sizes of 28 –91 having a count ratio (CR) of 0.5 (95% CI: 0.4-0.7; p < 0.001) and herd sizes of greater than 91 cattle having a CR of 0.3 (95% CI: 0.3-0.4; p < 0.001) compared to herd sizes of 2–12 cattle.

Discussion

No significant overall change in herd prevalence or within-herd seroprevalence of bovine brucellosis was found over the study period in Gauteng province, except for an artefactual decrease in 2016 which is addressed below. This study found significant variation in the number of bovine brucellosis reactor herds between State Vet Areas. Furthermore, an association was detected between increasing herd size and the occurrence of seropositive herds.

However, as herd size increased, the within-herd seroprevalence in these reactor herds was found to decrease.

The association between large herd size and the seropositive status of herds is well documented (McDermott and Arimi, 2002, Makita et al., 2011). However, to our knowledge, this is the first study to find an inverse relationship between herd size and within-herd seroprevalence of bovine brucellosis from a laboratory dataset. The limitation of this dataset is that it only represents those herds that were part of the existing bovine brucellosis control program which is based on a passive surveillance system. Therefore, it is possible that the dataset is not representative of smaller infected herds managed by farmers who do not suspect and therefore do not test the herd for brucellosis, resulting in this finding. This finding is in contradiction to a finding of no relationship between herd size and within-herd seroprevalence in a multistage sample cross-sectional study conducted by Makita et al. (2011) in Kampala, Uganda. However, in that study, the sample size of seropositive herds was only 11, and it is possible that the effect was missed (Makita et al., 2011).

In this study, Randfontein and Germiston State Vet Areas had greater odds of having reactor herds and having higher within-herd seroprevalence counts than the Pretoria State Vet Area when controlling for herd size and the year of testing. The finding of variability in herd and cattle prevalence between districts is similar to findings of a cross sectional survey for bovine brucellosis conducted in KwaZulu-Natal across 33 different magisterial districts (Hesterberg et al., 2008). In the Kwa Zulu Natal study, the seroprevalence ranged from 0 to 15.6 % between magisterial districts, with 19 of the 33 magisterial districts having no observed serological reactors. In contrast to that study, no State Vet Area in Gauteng had an annual cattle and herd reactor rate of less than 2% and less than 5%, respectively. These are the epidemiologic thresholds that have been used in successful bovine brucellosis eradication programme to initiate compulsory testing of cattle (Morgan, 1977, Michael, 1977, McKeown, 1977, Cunningham, 1977, Becton, 1977, Arturo del Rio, 1977, Alton, 1977).

This suggests that in Gauteng, cattle vaccination in all districts should be compulsory, and test and slaughter voluntary until herd and cattle rates are reduced. However, from interpreting both the fitted regression models, the variation between State Vet Areas can be better explained by the uneven distribution of herd sizes between State Vet Areas and the relationship between decreasing within-herd seroprevalence and increasing herd size, suggesting that vaccinating smaller herds to reduce the within-herd seroprevalence and slaughtering out reactors in larger herds, might be a feasible strategy.

The mean annual bovine brucellosis cattle and within-herd crude prevalence for the six-year period were 1.4% and 7.4%, respectively. The last estimate of cattle prevalence for the Gauteng area, was in 1949, and was reported to be 14.6% (555/3791) (Drimmelen, 1949). This is much higher than the crude cattle prevalence (1.4%) calculated for this study period suggesting that progress has been made with controlling the disease at the level of cattle. When compared to the range of within herd seroprevalence for the sub-Saharan African region, estimated to be 16.2% (95% CI: 10.2%–25.7%) found by Mangen et al. (2002), our study's finding (7.4%) fell below the reported range. In the Mangen et al. (2002) meta-analysis, the authors also estimated that the mean within-herd seroprevalence was 2.5 times greater than the overall animal seroprevalence (Mangen et al., 2002). This is lower than the present study's finding of a 5.3 times greater within-herd seroprevalence than the overall cattle reactor prevalence for the province. The difference between the two study areas may be explained by differences in the distribution of herd sizes and the variation of within-herd seroprevalence between large and small herds. In Gauteng, it is also possible that repeated testing of larger herds lowers the area cattle reactor seroprevalence, whilst the presence of greater numbers of small herds increases the mean within-herd seroprevalence for the area. A more recent study conducted in Namibia, where the authors also used laboratory data to calculate the seroprevalence of brucellosis, an overall animal prevalence of 0.5% (244/49,718) was found (Madzingira et al., 2020). Additionally, an earlier study conducted that the same region, *Brucella* cattle prevalence ranged from 0%–1.94% (Magwedere et al., 2011), which is similar to our finding of a 1.4% cattle prevalence in Gauteng. Yet, despite this similarity neither study conducted in Namibia reports the within-herd seroprevalence for the area, making it difficult to compare the burden of cattle brucellosis to farmers in Gauteng to farmers in Namibia.

The mean annual bovine brucellosis crude herd prevalence for the six-year period was 22.1%; this is higher than the cattle herd prevalence reported for Namibia (9.26%) in the study conducted by Madizingira et al. (2020) but lower than the 30.1% herd prevalence reported from Zimbabwe (Vhoko et al., 2018). Both of these studies used laboratory data sets for analysis. Many factors such as differences in farm production and management systems, cattle movement (Mangen et al., 2002) and effectiveness of bovine brucellosis control programs (Nicoletti, 2010) may contribute to the variation between reactor herd prevalence across different areas.

Despite the reasonable estimates of herd and cattle prevalence from this study, it is uncertain how reflective this is of the true incidence of cattle and herd reactors in Gauteng due

to an inability to identify unique herds from the available dataset. Without a unique herd identifier per record, it was impossible to link the test result to the herd record or the paper record filed at the relevant State Veterinary office, making it impossible to immediately track or trace the disease progression or duration of infection within a herd. In addition, owner details and farm details were not unique per herd, due to the veterinary official capturing the details of the person handling the cattle on the farm into the sample submission form, instead of the herd owners' name and contact details. This meant that from the submission form, which was also the template for the laboratory report, there was no way to identify herds by owners. In the dataset one herd could be associated with the owner's name or any of the workers on the farm who were there handling the cattle on the day of testing Illegible handwriting and data-capturer mistakes lent further uncertainty to some farm names and owner details. Furthermore, there was no variable within the dataset to indicate if the test was conducted for accreditation, maintenance or diagnostic purposes, which is a barrier to determining the reactor rate within these categories. It was also assumed that the total number of cattle tested was a reasonable proxy for herd size, despite not knowing the category for testing.

In addition to these limitations, the interruption of routine surveillance practises and changes in testing strategies of the Provincial Veterinary Services affected the reliability of interpretations of true cattle and herd reactor rates. The marked decrease in cattle and herd reactors in 2016 coincided with the Provincial Veterinary Services census survey and a change in program targets for the number of cattle tested for brucellosis (personal observation). The low prevalence in 2016 should therefore be considered an artefact.

Conclusion

Despite the recent report of an increasing trend of bovine brucellosis in the country (DAFF, 2016b), analysis of routine laboratory test results did not show a significant change in cattle or herd reactor prevalence between 2013 and 2018. This may indicate that there has not been real progress toward bovine brucellosis elimination during the study period. However, further investigation is needed, given the limitations of this dataset, in order to make inferences on the true prevalence of bovine brucellosis in the province.

Subject to uncertainties regarding data quality, routine laboratory test results can be used to provide an indication of bovine brucellosis reactor herds and within-herd seroprevalence in demarcated areas in the absence of data derived from cross-sectional surveys.

Moreover, analysis of this dataset may be used to identify areas at district level with high cattle and herd reactor rates and within-herd prevalence rates for further investigation or support in the planning and implementation of bovine brucellosis regulatory activities. However, indications of the absence of disease or low prevalence, calculated using laboratory test results data might only be related to the insufficient sampling of some areas or farms.

Recommendations

Based on this study's findings, we firstly recommend planned regular cross-sectional surveys using random sampling to check the real prevalence and the representativeness of the laboratory data sample. Secondly, mass vaccination targeting cattle in small to medium size herds with RB51 and vaccination of replacement heifers in these herds with a combination of S19-RB51, as suggested by Saez et al. (2014), in Gauteng combined with compulsory test and slaughter of reactors in larger herds when the within-herd prevalence is economically feasible to the farmer. Laboratory data derived from implementing this strategy should be collated, analysed, and communicated regularly to farmers and veterinary officials. This information can then be used to justify changes in bovine brucellosis control strategy toward elimination of the disease in demarcated areas when the within-herd prevalence is at an economically acceptable level. Progress toward elimination should be communicated to farmers, to enable them to manage the disease in their herds, participate in surveillance and support provincial changes in the control strategy from vaccination to test and slaughter.

To improve data quality and usefulness, each herd participating in the control programme should have a unique herd identifier that is routinely captured on sample submission forms. To facilitate farmer compliance and stakeholder engagement, we recommend that reports on the progress of bovine brucellosis elimination by district areas per State Vet Area be regularly and routinely shared with district public health units responsible for strengthening the detection and response to zoonotic diseases of public health importance. Furthermore, it is recommended that farmers and their family and workers be tested for brucellosis, since demonstration of human brucellosis is a strong driver for implementing control measures in cattle herds.

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Chapter 4: Bovine brucellosis in Gauteng, South Africa: Seroprevalence amongst cattle handlers, and variables associated with seropositive cattle herds, 2014-2016

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Abstract

Brucellosis is a neglected zoonotic disease of global importance that can be prevented in humans by eliminating *Brucella* spp. from livestock hosts. In South Africa (SA) bovine brucellosis is an endemic controlled animal disease. However, the prevalence of cattle handler exposure to *Brucella* on cattle farms, is unknown and quantitative evidence of management factors and cattle symptoms associated with infected cattle herds are unavailable for the country.

This case-control study was conducted on cattle farms participating in the bovine brucellosis control programme in Gauteng province. It aimed to measure *Brucella* seroprevalence and understand the evolution of infection amongst cattle handlers and determine herd management factors and cattle symptoms associated with bovine brucellosis on these farms.

All herds in Gauteng that participated in the voluntary bovine brucellosis control programme between 2014–2016 were eligible for this study. Farms were categorised as either case—when two or more cattle tested seropositive, to increase the specificity of a herd diagnosis of brucellosis and select herds presenting greater risk for cattle handler exposure — or control, following routine regulatory screening using the Rose Bengal test (RBT), and confirmation of reactors with the complement fixation (CFT) test. All cattle handlers on case farms were tested for brucellosis using four commercially available serological tests: the RBT and IgM ELISA used in series, the IgG ELISA, and an immunocapture agglutination (BrucellaCapt) test. A subset of cattle handlers on control farms and veterinary officials from

the three State Vet Areas of the province were also tested. A structured questionnaire on herd management practises and cattle symptoms was administered to herd managers.

Seroprevalence amongst farm workers on case farms (n=30 farms) ranged from 4.0% (BrucellaCapt) to 16.7% (IgG ELISA), compared to control farms (n=11 farms), where this seroprevalence ranged from 1.9% (BrucellaCapt) to 5.7% (IgG ELISA). Overall, 5.7% (13/230) of persons tested were seropositive to the RBT and IgM ELISA and IgG ELISA tests and 3.9% (9/230) were seropositive to all four serological tests. The difference in seroprevalence amongst farm workers between case and control farms for all the test combinations was not significant. However, seroprevalence amongst veterinary officials was significantly greater compared to farm workers on case farm for the RBT+ IgM- IgG+ outcome (OR=11.1, 95% CI: 2.5 – 49.9, p=0.002) and for the RBT- IgM- IgG+ outcome (OR=6.3, 95%CI: 2.3-17.3, p<0.001).

Herd management factors associated with being an infected farm in the multivariable regression model were: being a government-sponsored farm (OR 4.0; 95%CI: 1.4-11.3; p=0.009), beef vs. dairy herd (OR 7.9; 95%CI:1.4-44.9; p=0.020), open vs closed herd (OR 3.3; 95%CI: 1.1-10.4; p=0.038) and the presence of antelope on the farm (OR 29.4; 95%CI:4.0-218.2; p=0.001). Farmers of case herds were also significantly more likely to report brucellosis-like symptoms having occurred in cattle handlers on the farm or in him/herself (OR=3.4; 95%CI:1.3-8.7; p=0.006).

This One Health study contributed new evidence of cattle handler and veterinary official exposure to *Brucella* on cattle farms participating in the bovine brucellosis control programme of Gauteng, resulting in antibody profiles typical of infection ranging from a short to long evolution. We strongly recommend that cattle handlers and veterinary officials presenting with brucellosis-like symptoms to a doctor, be screened for brucellosis and that medical doctors establish the background *Brucella* seroprevalence in healthy people in Gauteng to enable adjusting cut-off values for brucellosis serological tests to differentiate between clinical disease and asymptomatic infection or exposure. Furthermore, we recommend screening of farm workers, veterinary officials exposed to cattle herds for early detection of infection with *Brucella* using serial dilutions of the RBT test. Risk factors and symptoms associated with herd infection were identified. These may be useful to inform brucellosis control strategies and awareness campaigns targeting cattle handlers.

Introduction

Brucellosis is a neglected zoonotic bacterial disease impacting public health and global agricultural development (WHO, 2012, WHO, 2017, WHO, 2005, Akakpo et al., 2010). *Brucella abortus* causes bovine brucellosis (Corbel et al., 2006, OIE, 2016) and is the second most common zoonotic *Brucella* sp., after *B. melitensis*. It can be transmitted directly or indirectly to people through contact with uterine discharges of infected animals or the ingestion of unpasteurised dairy products. The preferred host is cattle but may also occur wildlife species such as, eland and impala (OIE, 2008, Godfroid et al., 2011). To date, the most effective method to prevent human brucellosis is to eliminate the infection from livestock (Robinson, 2003, OIE, 2016).

Having one or more of the following symptoms is characteristic of cattle infected with brucellosis: abortion, retained placenta, stillbirths, poor weight gain, orchitis, epididymitis and hygromas (Bishop, 1994). In cattle, *B. abortus* usually causes abortions in the third trimester due to necrotising placentitis (OIE, 2016). Exposure to these tissues is the primary source of transmission to humans or uninfected bovine, which occurs through aerosolized or direct mucosal contact (Olsen and Tatum, 2010). However, infection of the reproductive system does not always lead to abortion, but it can persist in a herd without any overt clinical symptoms, other than the birth of weak or nonviable calves and a reduction of milk yield (Crawford and Hidalgo, 1977a, Olsen and Tatum, 2010). Localisation of the bacterium occurs in male reproductive tissue, joints and bones, and within the mammary glands, resulting in sterility, hygromas and mastitis, respectively (Olsen and Tatum, 2010). Other infection sources include contaminated environments, especially if it is wet and muddy, and equipment used for milking or artificial insemination (Bishop, 1994). *In utero* infection or milk and colostrum can also be sources of disease transmission to the new-born calf (Nicoletti, 1989). Infection spread by bulls during natural service is reported to be rare (Nicoletti, 1989, Olsen and Tatum, 2010).

Since some symptoms of bovine brucellosis are covert, once the disease has established itself in the herd it is difficult to detect and, therefore, difficult to control (Crawford et al., 1990, Crawford and Hidalgo, 1977a). Two main factors contribute to this situation. Firstly, the disease has a highly variable incubation period of several months to at least 2 years, and up to 9 years (Bishop, 1994, Nicoletti, 2010), depending on the time at which infection occurs. Secondly, the host's immunological response affects detection of the disease, with 2.5-9% of infected heifers born from seropositive cows remaining seronegative on conventional serological tests for at least 18 months (Bishop, 1994, Neta et al., 2010). These challenges

necessitate extended surveillance and control activities to eliminate brucellosis from a herd (Crawford, 2018). It takes a minimum of two years after the documented absence of reactors (DAFF, 2016, Cunningham, 1977) to declare a herd free, and may take several decades to declare a country free from brucellosis. The duration of successful eradication programs varies greatly between countries, ranging from 23 years in New Zealand and 29 years in Australia (Zhang et al., 2018) up to 100 years in Malta (Wyatt, 2013).

Symptoms of brucellosis in humans are just as non-specific as in animals. However, unlike abortions in the cattle herd, the main symptom of acute infection in humans is a recurring febrile illness, difficult to distinguish from other febrile illnesses (Ducrotoy, Bertu et al., 2017a). Other symptoms include malaise, anorexia, muscular weakness, joint pain, back pain, and depression. The disease can also result in bone and testicular abscesses, endocarditis, and neurological complications (Dean, Crump et al., 2012). Persons suffering from infection of a long evolution ("chronic") are reported to experience chronic disability and time lost from daily activities (Dean, Crump et al., 2012). There is no vaccine against the disease for humans (Corbel et al., 2006), and successful treatment of the disease depends on early detection and initiation of the correct combination of antibiotics (Doganay and Aygen, 2003, Dean et al., 2012a).

For similar biological reasons, detecting brucellosis in humans is as tricky as detecting the disease in cattle. Al Dahouk et al., (2011) reviewed the difficulties in diagnosis of human brucellosis through culture and molecular methods which justify the use of serological tests (Al Dahouk et al., 2013, Al-Dahouk et al., 2003, Corbel et al., 2006). However, these authors also point out the difficulties in clinical interpretation of serological test results in patients living in *Brucella* endemic areas.

In addition to the diagnostic challenge of brucellosis driving the neglect of the disease, a paucity of recent quantitative evidence confirming the interrelationship between the prevalence of the animal disease and human disease, contributes to decreasing prioritization of the disease by government and policymakers (Plumb et al., 2013, Michael and Madon, 2017). Previously, it was accepted that the incidence of human brucellosis correlates with the incidence of brucellosis in livestock (McDermott and Arimi, 2002, Zinsstag et al., 2005). However, more recent reviews recognize that this may not always be the case and can depend on multiple variables, including proximity to the herd, eating, and cultural habits (Ducrotoy et al., 2017).

Quantitative evidence of human exposure to *Brucella* spp. linked to seropositive cattle is possible from integrated epidemiological studies on animal and human brucellosis. However, these studies are difficult mainly because zoonotic disease detection and responses remain siloed (Bardosh, 2016, Godfroid et al., 2013a, Godfroid et al., 2014, Plumb et al., 2013, Zinsstag et al., 2015). As a result, brucellosis data for humans and animals are usually presented separately, not epidemiologically linked by time or location (Zinsstag et al., 2020), or datasets are incomplete because only animal or human data are available (Pappas et al., 2006, Dean et al., 2012b, Hull and Schumaker, 2018). In South Africa (SA), despite bovine brucellosis being a controlled animal disease and human brucellosis a notifiable medical condition, to date there is no published record of a multidisciplinary epidemiologic study of brucellosis, conducted by veterinary officials in collaboration with medical doctors. Furthermore, despite the long history of bovine brucellosis in Gauteng province (Govindasamy, 2020), little is known about herd management factors or cattle symptoms associated with seropositive cattle herds.

This study aimed to measure *Brucella* seroprevalence and understand the evolution of infection amongst workers in *Brucella*-infected cattle farms and to determine herd management factors and cattle symptoms associated with herd-level infection in the province.

Materials and Methods

Ethical approval

The Research Ethics Committee, Faculty of Health Sciences, University of Pretoria (74/2015) and the Animal Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (V011–16) granted ethical approval for the study. This case-control study was conducted in SA's smallest province, Gauteng, which covers 18 176km^2. The province is divided into three State Veterinary Areas, each covering one or more health districts. Herds are typically clustered into farm parcels within State Vet Areas. Figure 4.1 illustrates the distribution of farm parcels with case and control herds participating in this study, within State Vet Areas. One or more herds can occur within a farm parcel. If both a case and control herd occurred within a farm parcel, the parcel was coded as having one or more case herds. Therefore, farm parcels marked as one or more control herds, had no detected case herds in the parcel.

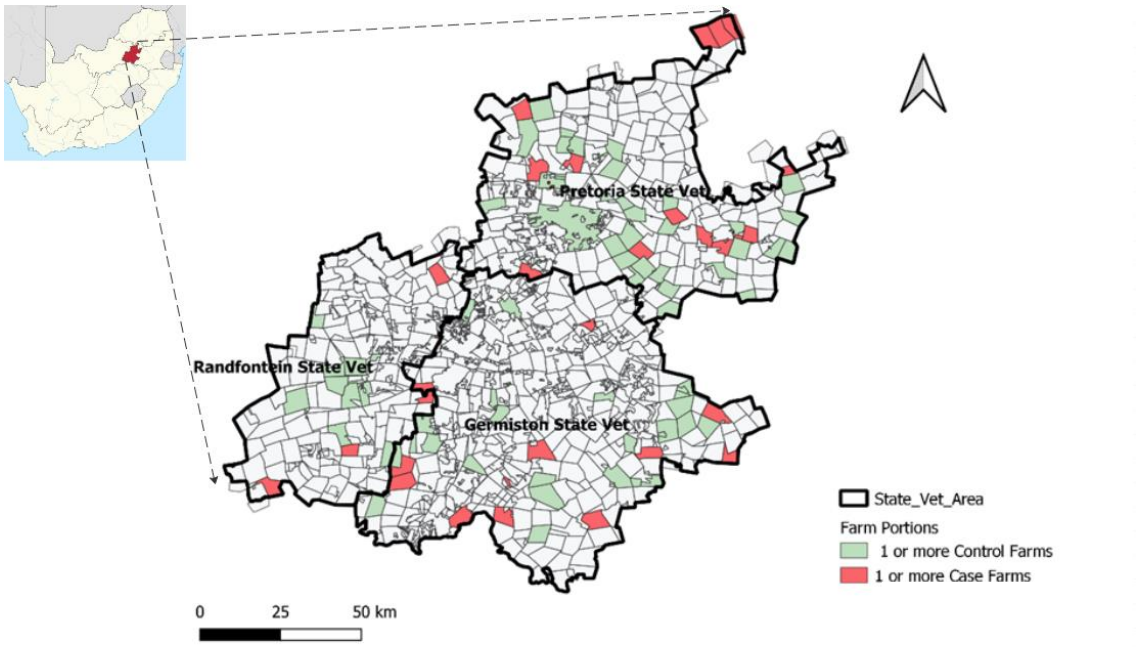

Figure 4.6: Location of Gauteng province in SA and distribution of study case (*Brucella*-infected) and control cattle herds by farm parcels within State Vet Areas in Gauteng province, 2014–2016

All herds participating in the Provincial Veterinary Services voluntary bovine brucellosis control programme between 2014–2016 were eligible for this study. The bovine brucellosis control programme is a passive surveillance system in which farmers volunteer to have their herds tested. However, if the herd tests positive the farmer must comply with the veterinary regulations to control bovine brucellosis.

After routine veterinary regulatory testing of the herd using the Rose Bengal test (RBT) and confirmation of reactors with the complement fixation (CFT) test (OIE, 2008), farms were categorised as either case or control. Based on the laboratory test records, a cattle herd with two or more serological cattle reactors on the RBT and confirmatory CFT with a reaction of greater than 60 IU/ml, between 2014-2016, was classified as a case herd. The 60 IU/ml threshold for the CFT was selected to rule out the S19 vaccine reactors according to the national veterinary guidelines (DAFF, 2016). The case definition 'two or more cattle reactors in a herd' was chosen to increase the specificity of a herd diagnosis of brucellosis and select herds presenting greater risk for cattle handler exposure. A cattle herd with a laboratory-confirmed seronegative test between 2014-2016 and no history of a seropositive herd test during 1990 to 2014, was regarded as a control herd. Verification of case and control classifications was done

by cross-checking case herd records, reported by the State Veterinarians, with the Provincial Veterinary Services' Animal Health directorate in the annual Animal Health reports. Selection of case and control herds was limited to the period between 2014–2016 due to the available budget for testing farm workers on the farm. Farm managers of all originally identified case herds were contacted telephonically, and all those volunteering to participate in the study (n=41) were recruited. For controls, all available controls that could be contacted in the limited available time (n=92) were included.

All farm workers(n=150) on case farms (n=30), a subset of farm workers (n=53) on control farms (n=11), and veterinary officials (n=27) servicing all three State Vet Areas were sampled for testing. On farms where farm workers were tested, farm managers or owners of herds were administered a structured herd management questionnaire (Appendix 1) face-to-face. The questionnaire collected data on herd management factors, and cattle herd and human symptoms of brucellosis detected as abnormal by the farmer in the year before the last herd test result. The same questionnaire was administered telephonically to the remaining herd managers of the control farms where no testing of farm workers took place.

The seroprevalence study on farm workers was conducted on farm sites between March and November 2016. A multidisciplinary team comprising a veterinarian, medical doctor and animal health technician visited each farm. The animal health technician served as the translator and was pre-trained on administering the questionnaire (Appendix 1). The veterinarian administered the herd management questionnaire to the farm manager, while the medical doctor collected blood samples from the study participants. Veterinary officials were sampled at the veterinary offices on appointed days for each State Vet Area. 5 mL of blood from each participant was drawn into two tubes: (1) clot activator without serum separation (dry tubes) and (2) EDTA anticoagulant tube Blood samples were transported on ice to the National Institute for Communicable Diseases, Centre for Emerging Zoonotic and Parasitic Diseases Unit, by the medical doctor for further processing, immediately following the farm visit. At the unit, samples were refrigerated (2-8 ºC) until they were processed. Processing was done within a week of receipt.

Human samples were tested using commercially available kits for the RBT, IgM Enzyme Linked Immunosorbent Assay (ELISA), IgG ELISA (Foz et al., 1985, Vircell, 2016a, Vircell, 2016b) and BrucellaCapt immunocapture serological test (OrduñA et al., 2000) (Vircell, 2016c, Vircell, 2019) according to the manufacturers' instructions and results were interpreted according to the kit guidelines.

For the RBT test, all reagents were brought to room temperature and the antigen suspension carefully shaken. 40µl of sample, 40µl of the positive and negative control were dispensed onto the individual circles of the test kit cards. One drop of the Rose Bengal-stained *Brucella* suspension was added close to the sample or control being analysed. The kit provided 5ml of an acid-suspension of inactivated *Brucella abortus* antigen stained with Rose Bengal, containing phenol (concentration < 1%). Both drops were mixed until all circle surfaces were covered. The card was carefully shaken for 4 minutes, followed by reading of the wells for the presence or absence of agglutination.

For the IgG ELISA, 100µl of serum diluent was added to each well. 5µl of each sample, 5µl of positive and 5µl negative controls, with optical density (O.D.) of positive and negative controls being > 0.9 and < 0.55 respectively, and 5µl of cut off control was added to the corresponding wells and shaken on a plate shaker for 2 minutes. The plate was then incubated for 45 minutes at 37 ± 1ºC for 30 minutes, after which it was excess liquid was aspirated from all wells and the wells washed 5 times with 0.3ml of washing solution per well. Remaining liquid was drained away and 100µl of substrate solution immediately added into each well, after which the place was incubated at room temperature for 20 minutes. After this period, 50µl of stopping solution was added into all wells. Spectrophotometer readings at 450/620 nm were taken within 1 hour of stopping. The mean O.D. for the cut off control was [(< 0.7 x (positive control O.D.) + > 1.5 x (negative control O.D.) / 2]. The antibody index was calculated as [(sample O.D. / cut of serum mean O.D.) x 10]. Samples were classified as negative, equivocal, or positive if the antibody index was < 9, 9-11, and > 11, respectively. The IgM ELISA was conducted, and results interpreted in a similar manner as the IgG ELISA, except for the initial preparation of the wells, which required 25µl of human IgG sorbent to be added to each well to get rid of excess IgG antibodies or rheumatoid factor.

The BrucellaCapt test was carried out as follows: all reagents were brought to room temperature before use. 50µl of serum diluent was added into well A, after which 50µl of serum diluent was added into all wells (A – H). 5µl of each serum, negative and positive control were added to well A. Doubling dilutions with 50µl of each well was made from A to H. 50µl of the provided bacterial suspension (well homogenized by prior vigorous shaking) was added into all wells. Wells were sealed with adherent tape and incubated for 24hrs at 37ºC in a chamber. Titre results were read after this and interpreted as follows: Row A – 1:40, Row B – 1:80, Row C – 1:160, Row D – 1:320, Row E – 1:640, Row F – 1:1280, Row G – 1:2560, Row H – 1:5120.

Subjects with insufficient blood for the RBT (n=2) were excluded from the analysis. All samples were tested with the RBT, IgG ELISA and BrucellaCapt tests. Samples that were seropositive on the ELISA IgG were tested further using the IgM ELISA. Samples seronegative on the IgG ELISA, but seropositive using the RBT, were also subjected to an IgM ELISA test. This selective testing of samples using the IgM ELISA was due a limited budget. The purpose was to detect the presence or absence of *Brucella* IgM antibodies in these selected samples to better understand the evolutionary stage of infection in the farm workers and veterinary officials. Stages of infection were considered along a continuum from a very short evolution of infection (IgM seropositive, IgG seronegative), reported to last approximately a week after exposure/inoculation with *Brucella* spp. (Al Dahouk and Nöckler, 2011), to a long evolution of infection (IgM seronegative, IgG seropositive, possible presence of blocking or non-agglutinating antibodies). As such, each seropositive person fell into one of five mutually exclusive groups depending on the outcome of a combination of tests: (i) RBT positive AND IgM ELISA positive AND IgG ELISA negative (indicative of a very short evolution infection), (ii) RBT negative AND IgM ELISA positive AND IgG ELISA positive, (iii) RBT positive AND IgM ELISA positive AND IgG ELISA positive (indicative of a short evolution infection), (iv) RBT positive AND IgM ELISA negative AND IgG ELISA positive (indicative of a long evolution infection), and (v) RBT negative AND IgM ELISA negative AND IgG ELISA positive (indicative of inactive or resolved infection). Seropositive reactors on the BrucellaCapt test were allocated to the group defined by the outcomes of the RBT, IgM ELISA and IgG ELISA.

Subjects with test results for the IgG ELISA that were classed as equivocal (n=3) were removed from the analysis. Titres were determined using the BrucellaCapt test. A titre of greater or equal to 1:320, was considered positive.

Questionnaire responses and test results from human participants were captured into an ACCESS 2013 (Microsoft suite 2013) relational database, using a unique herd identifier to link test results from farm workers to the herd they were in contact with. The farm managers' questionnaire response shared this unique number. Definitions and classification of selected variables are shown below (Table 4.1).

Table 4.2: Description of selected herd management risk factors

Risk Factors	**Description**
Brucella vaccination (RB51)	The herd has a history of vaccination preceding the herd test result
Open Herd	Cattle (heifers, cows or bulls) are bought in or are a part of a communal herd (multiple owners) as opposed to a herd that uses its own replacement heifers, bulls or only AI.
Government Sponsored	Farmers have received a grant to farm or access to purchase cattle, buy or rent land as part of governmental redress of apartheid policies to support previously disadvantaged persons to farm.
Herd Type	Dairy (Fresian / Jersey) Beef (Bonsmara / Brahman / Nguni) Mixed (Beef breed/s and dairy breed/s)
Handling Facilities	An assessment of handling facility quality by the farmer/manager
Brucellosis in Neighbouring Herds	Neighbouring farmers reporting the brucellosis status of their herds to the manager/owner being interviewed.

Seroprevalence was calculated for farm workers on case farms, control farms and veterinary officials, according to (1) RBT (2) RBT and IgM ELISA (3) IgG ELISA and (4) BrucellaCapt serological tests, as an indication of recent infection (1 and 2) with the RBT – IgM ELISA combination aiming to increase specificity for detecting recent infection, chronic infection (3) and complicated, persistent, relapsing brucellosis (4). Univariate analyses were conducted to identify herd management factors and symptoms associated with case herds. Herd management factors with two categories were tested using the 2-sided Fisher test, and the Chi^2 test was used to analyse factors with more than two categories. Variables associated with case herds, at significance $p < 0.2$ in the univariate analyses, were included in a multivariable logistic regression model. Backward stepwise selection was used to identify significant ($p < 0.05$) factors. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. Analyses were conducted in STATA 14 (StataCorp, College Station, TX, U.S.A.).

Results

Descriptive analysis

In total, 133 cattle herds (Figure 4.2) were recruited into the study, of which 30 met the definition of a case farm and 103 were control farms. The average herd size on case farms and control farms was 196 (median:120; IQR:71-238) and 150 (median:100; IQR: 43-218) cattle.

In total, 230 individuals were tested, ranging in age from 16–75 (median:38; IQR: 32-49). Twenty-seven veterinary officials servicing *Brucella*-infected and non-infected herds were tested, and a total of 203 farm workers were tested on the 31 case farms (n = 150) and 11 of the control farms (n=53), respectively. In this study, farm workers occurred in groups of median size of 4 persons (IQR:3-7; range: 1–16).

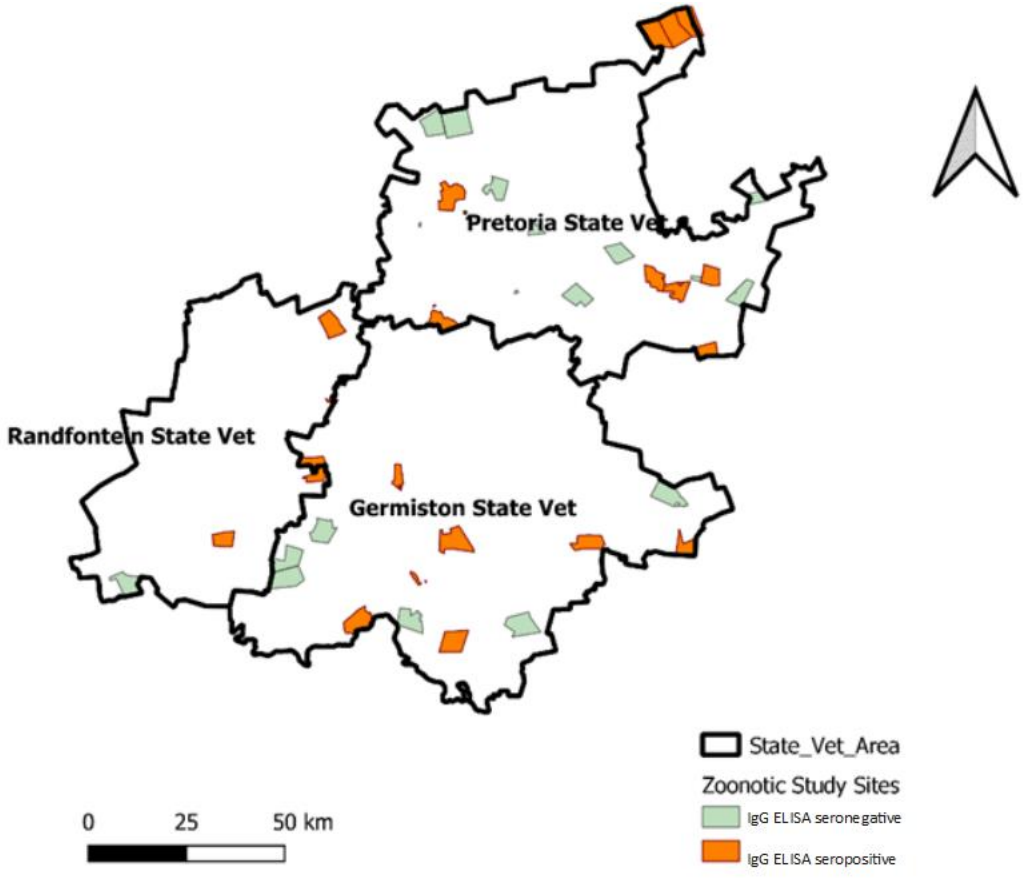

Figure 4.7: Distribution of farm parcels included in the study with *Brucella* IgG ELISA seropositive and negative farm workers, Gauteng, 2016

Seroprevalence amongst farm workers on case farms (n=30 farms) ranged from 4.0% (BrucellaCapt) to 16.7% (IgG ELISA), compared to control farms (n=11 farms), where this seroprevalence ranged from 1.9% (BrucellaCapt) to 5.7% (IgG ELISA) (Table 4.2). Overall, 5.7% (13/230) of persons tested were seropositive to the RBT and IgM ELISA and IgG ELISA tests and 3.9% (9/230) were seropositive to all four serological tests. Farm workers on control farms presented with infection of short to longer evolution, compared to a more spread-out infection evolution amongst farm workers on control farms (Table 4.3).

The difference in seroprevalence amongst farm workers between case and control farms for all the test combinations was not significant. However, seroprevalence amongst veterinary officials was significantly greater compared to farm workers on case farm for the RBT+ IgM- IgG+ outcome (OR=11.1, 95% CI: 2.5 – 49.9, p=0.002) and for the RBT- IgM- IgG+ outcome (OR=6.3, 95%CI: 2.3-17.3, p<0.001).

Table 4.3: Brucella seroprevalence amongst farm workers on case and control farms and veterinary officials according to different serological tests, Gauteng, 2016

	Farm Workers on Control Farms		**Farm Workers on Case Farms**		**Veterinary Officials**		**Total**	
Serological Test	**(n = 53)**		**(n = 150)**		**(n =27)**		**(N =230)**	
	Seropositive	%	Seropositive	%	Seropositive	%	**Total**	**%**
RBT	2	3.8	13	8.7	8	29.6	23	10.1
IgG ELISA	3	5.7	25	16.7	20	74.1	48	20.9
BrucellaCapt	1	1.9	6	4.0	8	29.6	15	6.5

Table 4.4: Brucella seropositivity among farm workers and veterinary officials (N = 230) on cattle farms in Gauteng, according to combinations of serological tests to indicate prevalence across the evolution of infection

		Farm Workers on Control Farms		**Farm Workers on Case Farms**		**Veterinary Officials**		**Total**	
	Combination of serological Test Results	**(n = 53)**		**(n = 150)**		**(n =27)**		**(N =230)**	
		Seropositive	%	Seropositive	%	Seropositive	%	Total	%
Evolution of infection: Very short (± 1 wk)----------> Long	(i) RBT+ IgM+ IgG-	1	1.9	1	0.7	0	0	2	0.9
	(ii) RBT- IgM+ IgG+	2	3.8	2	1.3	3	11.1	7	3
	(iii) RBT+ IgM+ IgG+	1	1.9	9	6	3	11.1	13	5.7
	(iii b) RBT+ IgM+ IgG+ BrucellaCapt +	1	1.9	5	3.3	3	11.1	9	3.9
	(iv)RBT+ IgM- IgG+	0	0	3	2	5	18.5	8	3.5
	(iv)RBT+ IgM- IgG+ BrucellaCapt +	0	0	0	0	5	18.5	5	2.2
	(v) RBT- IgM- IgG+	0	0	11	7.3	9	33.3	20	8.7
	(v b) RBT- IgM- IgG+ BrucellaCapt	0	0	1	0.7	0	0	1	0.4

Univariate and multivariable analysis

Open herd management was identified as significant on the univariate analysis (p=0.032). However, *Brucella* testing of cattle before introduction into the herd and vaccination (RB51) of cattle introduced into the herd were both not significant (p=1.0). Open herd management and several factors associated (p<0.2) with herd *Brucella* infection status in the univariate analysis (Table 3) were selected for inclusion in the multivariable model.

In the final model (Table 3), being a government-sponsored farm (OR 4.0; 95%CI: 1.4-11.3; p=0.009), beef vs. dairy herd (OR 7.9; 95%CI:1.4-44.9; p=0.020), open vs closed herd (OR 3.3; 95%CI: 1.1-10.4; p=0.038) and the presence of antelope on the farm (OR 29.4; 95%CI:4.0-218.2; p=0.001) were significantly associated with herd *Brucella* infection status. The Hosmer-Lemeshow goodness of fit test indicated adequate fit (χ^2=14.0, p=0.300).

Table 4.5: Univariate and multivariable analysis results of herd management factors associated with herd *Brucella* infection status in cattle herds in Gauteng, 2014 – 2016

Variable and level	Univariate analysis					Multivariable analysis		
	Case Farms (n = 30)		Control Farms (n = 103)		p - value			
	n	%	n	%		Odds ratio	95% CI	p-value
Government Sponsored					0.009			
No	18	60	87	84.5		1†	–	–
Yes	12	40	16	15.5		4.0	1.4 - 11.3	0.009
State Veterinary Area					0.751			
Pretoria (base)	15	50	50	48.5				
Randfontein	6	20	27	26.2				
Germiston	9	30	26	25.2				
Herd Type					0.021			
Dairy (base)	2	6.7	32	31.1		1†	–	–
Beef	23	76.7	54	52.4		7.9	1.4 - 44.9	0.020
Mixed	5	16.7	17	16.5		4.0	0.5 - 30.6	0.187
Presence of antelope					<0.001			
No	23	76.7	101	98.1		1†	–	–
Yes	7	23.3	2	1.9		29.4	4.0 - 218.2	0.001
Brucella vaccination (S19/RB51)					0.404			
Yes	27	90	84	81.6				
No	3	10	19	18.4				
Open Herd					0.032	1†	–	–
No	6	20	44	42.7		3.3	1.1 - 10.4	0.038
Yes	24	80	59	57.3				
Herd Size (Quartiles)					0.093			
[3-37] (base)	6	20	24	23.3				
[38-88]	3	10	28	27.2				
[89-200]	13	43.3	24	23.3				

[>200]	8	26.7	27	26.2		
Calving					0.3304	
Separated	7	25	25	35.2		
Together	21	75	46	64.8		
Bull					0.275	
Use bull from own herd	21	70	77	74.8		
Use bull from another herd	5	16.7	6	5.8		
Use bull & AI	3	10	14	13.6		
Use AI only	1	3.3	6	5.8		
Farm fenced in					0.818	
No	9	30	28	27.2		
Yes	21	70	75	72.8		
Handling facilities					0.419	
None (base)	2	6.7	7	6.8		
Poor	8	26.7	23	22.3		
Good	16	53.3	44	42.7		
Excellent	4	13.3	29	28.2		
Brucellosis in neighbouring herds					0.046	
No	25	83.3	98	95.1		
Yes	5	16.7	5	4.9		

Symptoms of bovine brucellosis

In the univariate analysis of herd symptoms (Table 4.4), abortions (OR=5.1; 95%CI:2.0-13.3; p < 0.001), weak calves in the herd (OR=8.0; 95%CI:2.6-24.4; p < 0.001), reduction in number of calves born (OR=9.0; 95%CI:2.1-43.6; p < 0.001), a reduction in conception rate (OR=3.9; 95%CI:0.8-18.3; p=0.046) and hygromas in cattle (p=0.011) were more likely to have been reported by farmers of *Brucella* infected herds than those on in control farms.

In addition to these cattle and herd symptoms, farmers of case herds were significantly more likely to report brucellosis-like symptoms having occurred in farm workers on the farm or in him/herself (OR=3.4; 95%CI:1.3-8.7; p=0.006).

Table 4.6: Univariate analysis results of farmer-reported cattle and human symptoms associated with herd *Brucella* infection status herds in Gauteng 2014 – 2016

Variable and level	**Univariate analysis**				
	Case Farms (n = 30)		**Control Farms (n = 103)**		**p - value**
	n	**%**	**N**	**%**	
Abortions					<0.001
No	13	43.3	82	79.6	
Yes	17	56.7	21	20.4	
Retained placentas					0.156

	No	23	76.7	90	87.4	
	Yes	7	23.3	13	12.6	
Weak calves in herd						<0.001
	No	17	56.7	94	91.2	
	Yes	13	43.3	9	8.7	
Reduction in number of calves born						<0.001
	No	22	73.3	99	96.1	
	Yes	8	26.7	4	3.9	
Reduction in milk yield						1
	No	27	90	92	89.3	
	Yes	3	10	11	10.7	
Reduction in conception rate			0			0.046
	No	25	83.3	98	95.1	
	Yes	5	16.7	5	4.9	
Hygromas in cattle						0.011
	No	27	90	103	100	
	Yes	3	10	0	0	
Farmer reported brucellosis-like symptoms in farm workers or self						0.006
	No	11	36.7	68	66.0	
	Yes	19	63.3	35	34.0	

Discussion

This study presents new evidence of cattle handler and veterinary official exposure to *Brucella* on cattle farms participating in the bovine brucellosis control programme of Gauteng, resulting in antibody profiles typical of infection ranging from a short to long evolution. The difference in seroprevalence amongst farm workers between case and control farms for all the test combinations was not significant. However, seroprevalence amongst veterinary officials was significantly greater compared to farm workers on case farm for the RBT+ IgM- IgG+ outcome ($p=0.002$) and for the RBT- IgM- IgG+ outcome ($p<0.001$).

Brucella seroprevalence in veterinary officials and assistants, has previously been explained by greater exposure to infected reproductive material, accidental exposure to *Brucella* vaccine strains, through needle stick injuries and noncompliance with use of protective clothing (Patil et al., 2013, Kutlu et al., 2014, Pereira et al., 2020). In our study, all seropositive veterinary officials were AHTs. The most likely source of exposure for AHTs is accidental needle stick injury during vaccination of cattle herds with S19. Vaccination against and testing of cattle herds for brucellosis are amongst the main activities of AHTs in the province and assistance to veterinarians performing deliveries in cattle is limited. Furthermore, use of protective clothing during sampling and vaccination is sporadic (personal

communication, 2016). AHTs will be performing a greater number of vaccinations more frequently than cattle handlers exposed to a single herd. This may explain the difference in seroprevalence between these groups.

In this study, we found that the pattern of *Brucella* antibody expression in the group tested ranged from profiles associated with infection of short evolution, typified by a predominance of IgM, to infection of long evolution in which IgM decreases and IgG (and IgA) increases and eventually predominates over IgM. We also found a class of long evolution categorised by the presence of IgG and low levels of non-agglutinating antibodies (RBT negative and BrucellaCapt negative). The antibody profile amongst this group of seropositive farm workers and veterinary officials, indicate that participants were are at different stages in the evolution of infection. It is currently unknown if there are specific risk factors or symptoms significantly associated with these stages in the evolution of infection in this group of people. Further investigation will be needed to clarify this.

Discrepancies between seroprevalence measured using the RBT, IgG ELISA and BrucellaCapt test for screening cattle handlers at the human-cattle-farm interface is not unexpected, since test sensitivity is associated with the class of circulating antibody at the time of testing (Al Dahouk et al., 2013, Al-Dahouk et al., 2003) and is correlated with the cut-off used to distinguish between clinical brucellosis and exposure to *Brucella*. In an endemic area, cut-offs of commercial tests need to be adjusted according to the seroprevalence of *Brucella* exposure in the healthy population (Franco et al., 2007). A cut off of 1/320 is recommended for the serum agglutination test in endemic areas (Franco et al., 2007). In this study the cut off for the BrucellaCapt test was 1/320, with reactors below this titre being regarded as negative. Our findings suggest that the IgG ELISA is not well adjusted to differentiate between low levels of IgG antibody circulating in exposed farm workers and veterinary officials and potential undetected clinical cases of brucellosis in this group. When considering the high sensitivity and specificity of RBT as described in Diaz et al. (2011) and comparing seroprevalence according to the RBT test with that of the BrucellaCapt test used in this study, the RBT when used on its own or in combination with IgM ELISA or IgG ELISA, was found to be more sensitive than the BrucellaCapt test. However, no serial dilutions were conducted for the RBT test in this study as compared to the Diaz et al. (2011) study, which may be indicating that the RBT is sensitive to titres less than 1:320. The implication being, that if RBT is to be used in the clinical setting, serial dilutions are recommended, and a suitable cut-off should be determined to differentiate between disease and asymptomatic infection. Findings

from this study, however, illustrate that at least 2.2% to 3.9%, if we consider the combination of tests to be most specific or 6.5%, if we consider only the BrucellaCapt results, of those tested, had titres high enough to be considered clinical cases. The clinical implication is that delayed diagnosis and treatment is associated with increased risk of complicated focal brucellosis (Rubach et al., 2013), treatment failure and relapses (Doganay and Aygen, 2003).

Differences in seroprevalence between cattle handlers and veterinary officials of this study, compared to cattle handlers in other African countries can be explained by differences in exposure due to different herd management systems across countries or study designs. Seroprevalence amongst villagers in Togo and small-scale farmers in Tanzania according to the RBT test was 0.44% and 5.5% respectively (Dean et al., 2013, Swai and Schoonman, 2009). This was lower than seroprevalences in cattle handlers on case farms (8.7%), control farms (3.8%) found in our study using the RBT test alone (Table 1). In contrast 10.1% of cattle handlers tested in Ghana (Tasiame et al., 2016) and 10.4% of farm-workers, abattoir workers and veterinarians in Ethiopia screened using the RBT (Kassahun et al., 2006, Ducrotoy et al., 2017) were higher than the seroprevalence found in cattle handlers on both the case and control farms in this study, but still lower than that found in the veterinary officials (29.6%). However, it was equal to the overall prevalence of cattle handlers and veterinarians (10.1% in Table 1)

Two tests were used to test seroprevalence amongst villagers in Togo. Variation of seroprevalence ranged from 0.44% using the RBT alone to 0.73% on the IgG ELISA. The higher seroprevalence amongst cattle handlers, according to the RBT and IgG ELISA found in this study compared to the study conducted in Togo (Dean et al., 2013) may be explained by the fact that in this study, majority of cattle handlers were exposed to known serologically confirmed *Brucella* seropositive cattle farms. This may be explained by the fact that our study our study specifically selected cattle handlers exposed to *Brucella* infected herds in contrast to the randomized cross-sectional study design used in the Togo study.

This is the first study in Gauteng province to identify herd management risk factors and cattle symptoms associated with *Brucella* seropositive cattle herds. Furthermore, it is the first study in SA to identify an association between brucellosis-like symptoms in cattle handlers exposed to seropositive cattle herds serviced by the provincial veterinary services.

Beef herds, government funded project herds or herds that in contact with antelope were associated with *Brucella* infected herds. Government-funded herds being a risk factor suggests that socio-political variables have an indirect effect on herd health, lending credibility to the

complex nature of bovine brucellosis control. Such complexity has been discussed in detail by (Beauvais et al., 2015, Waltner-Toews, 2001). Furthermore, the association found between government-sponsored farms and case herds is not consistent across SA, as illustrated in findings from a recent study conducted in KwaZulu-Natal, South Africa (Nogwebela, 2018), where government-sponsored herds were less likely to be infected. This may be due to the variation in provincial government programmes and in how farms were selected for government funding, or possibly the separation between the agriculture and veterinary state functions in Gauteng, resulting in the distribution of cattle of unknown *Brucella* status to farmers.

The finding of abortions associated with case herds is expected (Evans, 1947, Crawford and Hidalgo, 1977b, Ray, 1977, Olsen and Tatum, 2010) and commonly reported in sub-Saharan Africa (Anka et al., 2014, Makita et al., 2011, Matope et al., 2011, Muma et al., 2007). A reduction in number of calves born, likewise, is a commonly reported herd symptom associated with chronic brucellosis in a herd (Duboz et al., 2018). This is most likely due to the combination of a reduction in conception rate and an increase in abortions in the herd. This finding is consistent with bovine brucellosis being an endemic disease in South Africa and its long history in Gauteng province (Govindasamy, 2020).

The reasons for the strong association between the presence of antelope and *Brucella* herd infection are unknown, although it should be interpreted with caution due to the relatively small number of herds with antelope. It is possible that there were other, unmeasured management or environmental factors associated with the presence of antelope which may be related to likelihood of *Brucella* infection. Further investigation is needed to identify the species of antelope most associated with reactor cattle herds in Gauteng. However, the risk of transmission of *B. abortus* between infected wild ungulates and livestock is well documented (Godfroid et al., 2013b, Olsen, 2010), and the presence of a possible wildlife reservoir may hinder efforts at eradication. Findings from this study support the need for further research into the potential role of wildlife in the maintenance of *B. abortus* on cattle farms in Gauteng and elsewhere.

A limitation of the study is that inferences cannot be generalized to the population of cattle handlers or cattle herds beyond those that participated in the provincial bovine brucellosis control programme. Furthermore, since this was a voluntary study, the selected herds and human population investigated reflect the farmers who participated. Only cattle handlers and veterinary officials present on testing day were included. This excludes those cattle handlers

that may not have been present due to ill health or other work commitments. Furthermore, the study design did not include follow up testing of seropositive cattle handlers. Therefore, it was not possible to differentiate between asymptomatic infection, active infection, or previous resolved infection.

Conclusion and Recommendations

This One Health pilot study found evidence of cattle handler exposure to *Brucella* on cattle farms participating in the provincial veterinary services' bovine brucellosis control programme as well as significant herd risk factors and symptoms.

Variation of seroprevalence amongst cattle handlers according to serological test is consistent with the picture in a brucellosis endemic area. This study suggests the possibility of undetected and untreated cases of brucellosis amongst cattle handlers, including veterinary officials, in the province. Therefore, as suggested by Mantur (2006) for a brucellosis endemic area, we recommend that medical practitioners routinely screen farm workers and family members, and veterinary officials exposed to cattle herds for early detection of infection with *Brucella* using serial dilutions of the RBT test as recommended by Diaz et. al., (2011). In addition, ongoing training to cattle handlers is recommended to increase awareness of the zoonotic occupational risk of brucellosis as well as human symptoms of the disease. Further investigation into the health-seeking behaviour in response to brucellosis-like symptoms amongst RBT and BrucellaCapt seropositive cattle handlers is needed to rule out undetected chronic or relapsing brucellosis. Furthermore, However, commercial screening tests recommended cut-offs need to be adjusted to differentiate between clinical disease and asymptomatic infection in the province.

Interpreted as a whole, findings from this study corroborate a complex One Health model of human, cattle and socio-political interrelatedness with respect to bovine brucellosis at the human-cattle-farm interface. Therefore, a One Health approach is recommended for use by government veterinary, agriculture, and health departments to further to investigate the clinical significance of seropositive cattle handlers, to mitigate the identified herd risk factors and to calculate the economic and socio-economic impact of bovine brucellosis on farms in Gauteng.

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Chapter 5: Knowledge of brucellosis, health-seeking behaviour and risk factors for *Brucella* seropositivity amongst workers on cattle farms in Gauteng, South Africa

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Abstract

Brucellosis, a zoonotic disease of global importance, is under-detected and underreported in sub-Saharan Africa. In South Africa, there is little evidence of brucellosis amongst workers on cattle farms, and knowledge of brucellosis in this group is unknown. Furthermore, there is a lack of quantitative evidence of risk factors associated with *Brucella* seropositivity amongst workers on cattle farms.

A cross sectional survey of farm workers exposed to confirmed *Brucella* seropositive and seronegative cattle herds, and a subset of provincial veterinary officials, was conducted using face-to-face structured questionnaires, between March and November 2016. The questionnaire captured information on participants knowledge of brucellosis and risk factors for exposure to *Brucella*. All participants were also screened for brucellosis using commercial RBT, IgM and IgG ELISA tests, and the BrucellaCapt, an immunocapture agglutination test.

Cattle handler knowledge of brucellosis symptoms in cattle was low with 20.7% (42/203) aware that *B. abortus* can cause abortions in cattle, can cause calves to be born weak and can also be in a herd without causing abortions. Whilst 36.9% (75/203) knew that bovine brucellosis can cause disease in people, only 16.3% (33/203) reported knowing the human symptoms of disease. In contrast 63% (17/27) of veterinary officials knew the symptoms of bovine brucellosis and 100% knew it to be a zoonotic disease, but only 89% (24/27) knew the symptoms of human disease. Despite having greater awareness of the zoonotic nature of bovine brucellosis and human symptoms of the disease, only 22.2% (6/27) of veterinary officials would opt to visit a clinic, doctor or hospital in response to self-experienced brucellosis like

symptoms, compared to 74.9% (152/203) of farm workers ($p < 0.001$). We also found that 53% (7/15) of BrucellaCapt seropositive people did not visit a clinic in response to brucellosis-like symptoms which may be undetected cases of brucellosis.

Finally, we found weak evidence of an association between infection of a short evolution (RBT, IgM ELISA and IgG ELISA seropositive) and the handling of afterbirth or placenta (OR=8.9, 95% CI: 1.0-81.1, p=0.052), but strong evidence of an association to the slaughter of cattle (OR=5.3, 95% CI: 1.4-19.6, p=0.013). Weak evidence of an association between infection of a long evolution (RBT and IgG ELISA seropositive and IgM ELISA seronegative) and veterinary officials compared to farm workers exposed to seropositive herds was found (OR=4.1, 95%CI: 0.2-8.1, p=0.049), although the small number of events of this outcome increases the uncertainty of this confidence interval. However, there was strong evidence of an association between inactive/resolved infection or exposure and veterinary officials compared to those exposed to seropositive herds (OR=7.0, 95%CI: 2.4-20.2, p<0.001), whilst handling of afterbirth or placenta was associated with non-reactors in this group (OR=3.9, 95%CI: 1.3-11.3, p=0.012). This is to be expected given that the above finding that the handling of placenta and after birth was associated with people who were RBT, IgM and IgG ELISA seropositive (short evolution infection).

This study identified a gap in cattle handler knowledge of animal and human symptoms of brucellosis. Findings suggest a proportion of undetected clinical cases of brucellosis amongst workers on cattle farms in Gauteng. Furthermore, risk factors for *Brucella* seropositivity amongst persons occupationally exposed to cattle farms, were identified. Increased veterinary and public health awareness programmes for detecting and diagnosing brucellosis in farm workers and veterinary officials exposed to cattle herds in Gauteng are recommended.

Introduction

Brucellosis is a neglected zoonotic disease of global health and economic importance (WHO, 2012, WHO, 2014, WHO, 2005). Symptoms of brucellosis in humans are non-specific and are difficult to distinguish from those of other febrile illnesses (Ducrotoy, Bertu et al. 2017a). They include malaise, anorexia, recurrent fever, muscular weakness, joint pain, back pain and depression. The disease can also result in bone and testicular abscesses, endocarditis and neurological complications (Dean, Crump et al. 2012). Persons suffering from chronic brucellosis experience a loss of life years from persistent disability and time lost from daily activities (Dean, Crump et al. 2012).

Early detection of human brucellosis and initiation of the correct combination of antibiotics (Doganay and Aygen, 2003, Dean et al., 2012a) are needed for successful treatment. There is no vaccine against the disease for humans (Corbel et al., 2006). Brucellosis is a difficult disease to diagnose through culture and molecular methods (Al Dahouk and Nöckler, 2011). Serological tests are more sensitive than culture or molecular methods to detect exposure to *Brucella* (Al Dahouk et al., 2013, Al-Dahouk et al., 2003, Corbel et al., 2006) and therefore are important in detecting zoonotic transmission of the pathogen.

Prevention of brucellosis in humans through public health intervention has been aimed at reducing the indirect transmission of *Brucella* bacteria through contaminated milk (Wyatt, 2013). However, reduction of infection in the animal host is usually by vaccination and test and slaughter activities conducted by government veterinary officials control (Nicoletti, 2010). However, a lack of resource allocation to animal disease control programmes challenge the effectiveness of such programmes in low and middle income countries (Godfroid, Al Dahouk et al. 2013, McDermott et al., 2013).

A lack of knowledge of the zoonotic risk of brucellosis amongst people occupationally exposed to brucellosis has been identified as a further barrier to the control of brucellosis and the continued spread of the disease amongst livestock (Mufinda et al., 2015, Kansiime et al., 2014). Furthermore, it has also been associated with ineffective precautionary behaviour needed to reduce self-exposure to contact with infected aborted material or infected cows at calving (Zinsstag, Roth et al. 2005) and to inappropriate response to brucellosis-like symptoms, such as self-medication with an antibiotic or at the suggestion of a pharmacist (Doganay and Aygen, 2003), resulting in under detection and under diagnosis or difficulties in treating clinical brucellosis by a clinician (Benon et al., 2018).

There is little data on the prevalence of brucellosis in people in Africa (Dean et al., 2012). The limited seroprevalence studies of brucellosis in humans in sub-Saharan Africa have targeted patients with fevers of unknown origin (Animut et al., 2009, Bouley et al., 2012), abattoir workers (Megersa et al., 2011) and the farming community and veterinarians (Dean et al., 2013, Swai and Schoonman, 2009, Schelling et al., 2003, Kassahun et al., 2006). However, no such study has been conducted in southern Africa yet.

Literature supports the use of the Rose Bengal Test (RBT) in developing countries (Díaz et al., 2011) due to the affordability, ease of conducting the test and adaptability to test serum dilutions. It can detect IgM, IgG and IgA and was found to be highly sensitivity in short ("acute") and long ("chronic") evolution brucellosis cases when the test is optimised to have a pH capable of agglutinating blocking IgA antibodies and removing prozones. RBT was found

to also be highly specific in the sera of persons with no contact with *Brucella* and its ability to detect IgM, IgG and IgA was comparable to that of the BrucellaCapt test (Díaz et al., 2011).

However, it is often recommended that a complementary test be used with commercially available (Vircell, 2016b) RBT tests when attempting to differentiate between an infection of short or long evolution, since infections of a long evolution may be indicative of focal brucellosis or a relapse of disease. This stage is typified by an absence of IgM, an increase of IgG, IgA, and non-agglutinating antibodies (Díaz et al., 2011, Al Dahouk and Nöckler, 2011).

The ELISA IgG is recognised to be a very sensitive serological test to detect antibodies of the IgG class, which are predominately found in long evolution brucellosis cases, however available IgG ELISA tests may have variable cut-offs due to the manufacturer differences and differences in brucellosis prevalence across areas and populations. An example of this can be found in the different cut-off reported in Hasibi et el. (2013) and Peeridogaheh et al. (2013).

The BrucellaCapt, a single step immunocapture assay, has been recommended to detect relapses of brucellosis or the disease in the long evolution of infection ("chronic" stage), because of its ability to detect non-agglutinating (blocking) or incomplete antibodies, which are dominant during this stage of infection (Al Dahouk et al., 2013). The BrucellaCapt, like the RBT, also detects IgM, IgG, and IgA antibodies. It is commercially available, both cost effective and rapid and is reported to have a sensitivity of 99.2% and a specificity of 96%, on samples determined positive by the Coomb's test (OrduñA et al., 2000). Furthermore, BrucellaCapt titres indicate the activity of infection regardless of the stage of disease, decreasing slowly after relapse and more distinctly after treatment (Al Dahouk et al., 2013).

However, all these tests were developed to diagnose brucellosis in non-endemic countries, and it is necessary to adjust the recommended cut-off titre to detect clinical cases if used in an endemic area (Franco et al., 2007, Peeridogaheh et al., 2013,).

In South Africa (SA), human brucellosis is a notifiable medical condition and bovine brucellosis is a controlled animal disease (Frean et al., 2019). However, human brucellosis is suspected to be under detected and underdiagnosed in SA (Wojno et al., 2016). Transmission of *Brucella* to workers on farms that have brucellosis infected cattle herds is a known historical occupational hazard in the Gauteng region of SA (Govindasamy, 2020, Frean et al., 2019), yet to date, no study has been conducted on cattle farms in SA to understand farm workers or veterinary officials' knowledge of brucellosis, or to identify risk factors for exposure or potentially undiagnosed clinical infection, amongst these workers on farms in Gauteng.

The objectives of this study were firstly to understand knowledge of brucellosis and health seeking response to brucellosis-like symptoms amongst workers on cattle farms, and secondly to identify risk factors for *Brucella* seropositivity in this group.

Materials and methods

Ethical considerations

Ethical approval for the study was granted by the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria (74/2015) and the Animal Ethics committee of the University of Pretoria (V011-16). All persons were informed about the objectives of the study and counselled prior to consent on the significance of a positive test result by the medical doctor on the team. All participants were telephonically informed of their result. Reactors were revisited and further counselled on the interpretation and implication of their seropositive result. Each reactor was also given a referral letter for their doctor's attention. This letter gave background on the study, a brief review of brucellosis and suggestions for follow-up, confirmation of disease and management of brucellosis patients to ensure the doctor was sufficiently capacitated to manage the patient.

Study area and participants

The study was conducted in Gauteng, the smallest of South Africa's nine provinces with an area of 18 176km^2. Selection criteria for eligibility to participate in this study was occupational contact with cattle herds participating in the provincial state veterinary services' bovine brucellosis control programme between 2014 and 2016.

Study design

This study was designed as a cross-sectional study of workers on *Brucella*-positive cattle farms. A two-stage non-probabilistic sampling strategy was used to select participants. In the first stage cattle farms participating in the provincial bovine brucellosis control programme between 2014 and 2016, were selected: herds with 2 or more cattle testing seropositive on RBT and confirmatory CFT ≥ 60IU/ml were prioritized and purposively selected to increase the probability of detecting recent exposure to *Brucella* amongst workers on cattle farms. All farm workers present on a farm on the day of testing were included in the sample (n=203). The study was conducted on the farm sites between March and November 2016. In addition to the farm workers, a subset of veterinary officials (n=27) was included in the sample. These officials service provide different services to farmers participating in the

provincial bovine brucellosis control programme. Veterinary officials participating in this study was a sample of those who routinely collect blood samples and vaccinate cattle herds (n=12/19), vaccinate cattle without collecting blood samples (n=13/15), provide advisory services to cattle farmers without performing vaccinations or testing (n=2/4), perform diagnostic and clinical services on individual cattle (4/15). Only those veterinary officials volunteering to participate and who were available on the allocated day for testing were included in this study. Table 5.1 summarises the study participants and the terminology that will be used interchangeably throughout the thesis.

Table 5.7:Groups of workers exposed to cattle farms, participating in the *Brucella* sero-survey in Gauteng, 2016

Group	Study participants	n (people in each group)	Exposure	Number of herds represented
1	Farm workers	53	*Brucella* seronegative cattle herd	11
2		150	*Brucella* seropositive cattle herd	30
3	Veterinary officials (state vet (n=1/3), community state vets (n=3/12), AHTs (n=23/38)	27	*Brucella* seronegative and seropositive cattle herds	herds participating in bovine brucellosis control programme (nb. not necessarily in contact with the herds participating in this study)

Structured questionnaires were used to collect information on risk factors for cattle handler and veterinary officials' exposure, knowledge and health seeking response to brucellosis-like symptoms brucellosis (Appendix 2). The questionnaire was piloted on farm workers on two farms and questions clarified from feedback gained during the pilot. Participants in the pilot study were included in the sample. All farm workers were screened on the farms using commercially available kits for the RBT ®, IgG ELISA ®, IgM ELISA ® (Vircell, 2016a, Foz et al., 1985) and BrucellaCapt ® test (Vircell, 2016b, Vircell, 2019, OrduñA et al., 2000) according to the manufacturers' instructions and results were interpreted according to the kit guidelines.

For the RBT test, all reagents were brought to room temperature and the antigen suspension carefully shaken. 40µl of sample, 40µl of the positive and negative control were dispensed onto the individual circles of the test kit cards. One drop of the Rose Bengal-stained *Brucella* suspension was added close to the sample or control being analysed. The kit provided 5ml of an acid-suspension of inactivated *Brucella abortus* antigen stained with Rose Bengal,

containing phenol (concentration < 1%). Both drops were mixed until all circle surfaces were covered. The card was carefully shaken for 4 minutes, followed by reading of the wells for the presence or absence of agglutination.

For the IgG ELISA, 100µl of serum diluent was added to each well. 5µl of each sample, 5µl of positive and 5µl negative controls, with optical density (O.D.) of positive and negative controls being > 0.9 and < 0.55 respectively, and 5µl of cut off control was added to the corresponding wells and shaken on a plate shaker for 2 minutes. The plate was then incubated for 45 minutes at 37 ± 1ºC for 30 minutes, after which it was excess liquid was aspirated from all wells and the wells washed 5 times with 0.3ml of washing solution per well. Remaining liquid was drained away and 100µl of substrate solution immediately added into each well, after which the place was incubated at room temperature for 20 minutes. After this period, 50µl of stopping solution was added into all wells. Spectrophotometer readings at 450/620 nm were taken within 1 hour of stopping. The mean O.D. for the cut off control was [(< 0.7 x (positive control O.D.) + > 1.5 x (negative control O.D.) / 2]. The antibody index was calculated as [(sample O.D. / cut of serum mean O.D.) x 10]. Samples were classified as negative, equivocal, or positive if the antibody index was < 9, 9-11, and > 11, respectively. The IgM ELISA was conducted, and results interpreted in a similar manner as the IgG ELISA, except for the initial preparation of the wells, which required 25µl of human IgG sorbent to be added to each well to get rid of excess IgG antibodies or rheumatoid factor.

The BrucellaCapt test was carried out as follows: all reagents were brought to room temperature before use. 50µl of serum diluent was added into well A, after which 50µl of serum diluent was added into all wells (A – H). 5µl of each serum, negative and positive control were added to well A. Doubling dilutions with 50µl of each well was made from A to H. 50µl of the provided bacterial suspension (well homogenized by prior vigorous shaking) was added into all wells. Wells were sealed with adherent tape and incubated for 24hrs at 37ºC in a chamber. Titre results were read after this and interpreted as follows: Row A – 1:40, Row B – 1:80, Row C – 1:160, Row D – 1:320, Row E – 1:640, Row F – 1:1280, Row G – 1:2560, Row H – 1:5120.

Subjects with insufficient blood for the RBT (n=2) were excluded from the analysis. All samples were tested with the RBT, IgG ELISA and BrucellaCapt tests. Samples that were seropositive on the ELISA IgG were tested further using the IgM ELISA. Samples seronegative on the IgG ELISA, but seropositive using the RBT, were also subjected to an IgM ELISA test. This selective testing of samples using the IgM ELISA was due a limited budget. The purpose was to detect the presence or absence of *Brucella* IgM antibodies in these selected samples to

better understand the evolutionary stage of infection in the farm workers and veterinary officials. Stages of infection were considered along a continuum from a short evolution of infection (IgM seropositive, IgG seronegative) to a long evolution of infection (IgM seronegative, IgG seropositive, possible presence of blocking or non-agglutinating antibodies). As such, each seropositive person fell into one of five mutually exclusive groups depending on the outcome of a combination of tests: (i) RBT positive AND IgM ELISA positive AND IgG ELISA negative, (ii) RBT negative AND IgM ELISA positive AND IgG ELISA positive, (iii) RBT positive AND IgM ELISA positive AND IgG ELISA positive, (iv) RBT positive AND IgM ELISA negative AND IgG ELISA positive, and (v) RBT negative AND IgM ELISA negative AND IgG ELISA positive. Seropositive reactors on the BrucellaCapt test were allocated to the group defined by the outcomes of the RBT, IgM ELISA and IgG ELISA.

Subjects with test results for the IgG ELISA that were classed as equivocal (n=3) were removed from the analysis. Titres were determined using the BrucellaCapt test. A titre of greater or equal to 1:320, was considered positive.

The RBT and IgM ELISA were used in series to increase the specificity of RBT to detect *Brucella* IgM, as an indication of infection of a short evolution. To detect *Brucella* IgG, an indication of a possibly longer evolution, we used the IgG ELISA test. The BrucellaCapt test was used, with the recommended cut-off titre of 1:320, for the detection of possible clinical brucellosis with either a short or long evolution All tests were done according to the manufacturer's guidelines.

Data and sample collection

A multidisciplinary team comprising a veterinarian, medical doctor and animal health technician visited each farm. The animal health technician served as the translator, if and when needed, and was therefore pre-trained on the administration of the questionnaire (Appendix 2). The veterinarian administered the questionnaire whilst the medical doctor collected blood samples from the study participants. The sampling of veterinary officials took place at the veterinary offices on appointed days for each State Vet Area. Five millilitres of blood from each participant was drawn into two tubes: (1) clot activator without serum separation and (2) EDTA anticoagulant tube. Blood samples were transported on ice, respecting the biosecurity regulations for human samples transport, to the National Institute for Communicable Diseases, Centre for Emerging Zoonotic and Parasitic Diseases Unit by the medical doctor following the farm visit, for further processing.

Data management

Completed questionnaires were captured into the electronic form function of Microsoft® Access® (2013). Laboratory results were captured into the appropriate record, by matching the unique identifiers of the samples.

Data analysis

Descriptive statistics were done in Microsoft® Excel®. Univariate analyses were conducted in STATA 14®, for outcomes (1) RBT positive AND IgM ELISA positive AND IgG ELISA positive, (2) RBT positive AND IgM ELISA negative AND IgG ELISA positive, and (3) RBT negative AND IgM ELISA negative AND IgG ELISA positive and (4) BrucellaCapt seropositivity amongst farm workers and veterinary officials (N=230).

Univariate associations between each variable and the outcomes were assessed using Fisher's exact test. Variables with $p<0.20$ were selected for inclusion into the multivariable logistic regression models. Three separate mixed effects logistic regression models were fit to identify risk factors for increasing evolution of infection: (1) RBT positive AND IgM ELISA positive AND IgG ELISA positive (short evolution), (2) RBT positive AND IgM ELISA negative AND IgG ELISA positive (long evolution), and most likely inactive or resolved infection but indicative of exposure to *Brucella* spp (3) RBT negative AND IgM ELISA negative AND IgG ELISA (Exposure / inactive or resolved infection).

Farm was included as a random effect in all three models. Veterinary officials were clustered into three groups, according to the State Vet Area they serviced. Each cluster was allocated a unique number and added to the Farm variable. On verification of the herd status, five herds (with 53 farm workers enrolled) were reclassified as control herds. Herd status was therefore included as an additional predictor in the models. Variables with $p>0.05$ in the models, were systematically removed by backward elimination (Kleinbaum and Klein, 2010, Tabachnick and Fidell, 2001).

Results

A total of 230 individuals were tested, of which 65% (150/230) were farm workers exposed to *Brucella* infected cattle herds (30 farms), 23% (53/230) were farm workers exposed to *Brucella* seronegative herds (11 farms). The remaining 12% were state veterinary officials

who are routinely exposed to both seropositive and seronegative *Brucella* cattle herds although not necessarily on the farms where farm workers were tested.

Using tests individually, seroprevalence ranged from 3.9% (BrucellaCapt) to 16.3% (IgG ELISA) amongst farm workers on case farms (n=30 farms). On control farms (n=11 farms), seroprevalence in farm workers ranged from 1.8% (BrucellaCapt) to 5.5% (IgG ELISA). Amongst veterinary officials, seroprevalence ranged from 26.6% using either the RBT or the BrucellaCapt, to 74.1% (IgG ELISA).

Antibody profiles amongst those tested ranged from an infection of a very short evolution to long evolution cases with BrucellaCapt seropositive cases approximating RBT positive and IgM ELISA positive and IgG ELISA positive reactors, RBT positive and IgM ELISA negative and IgG ELISA positive reactors (Figure 5.1.).

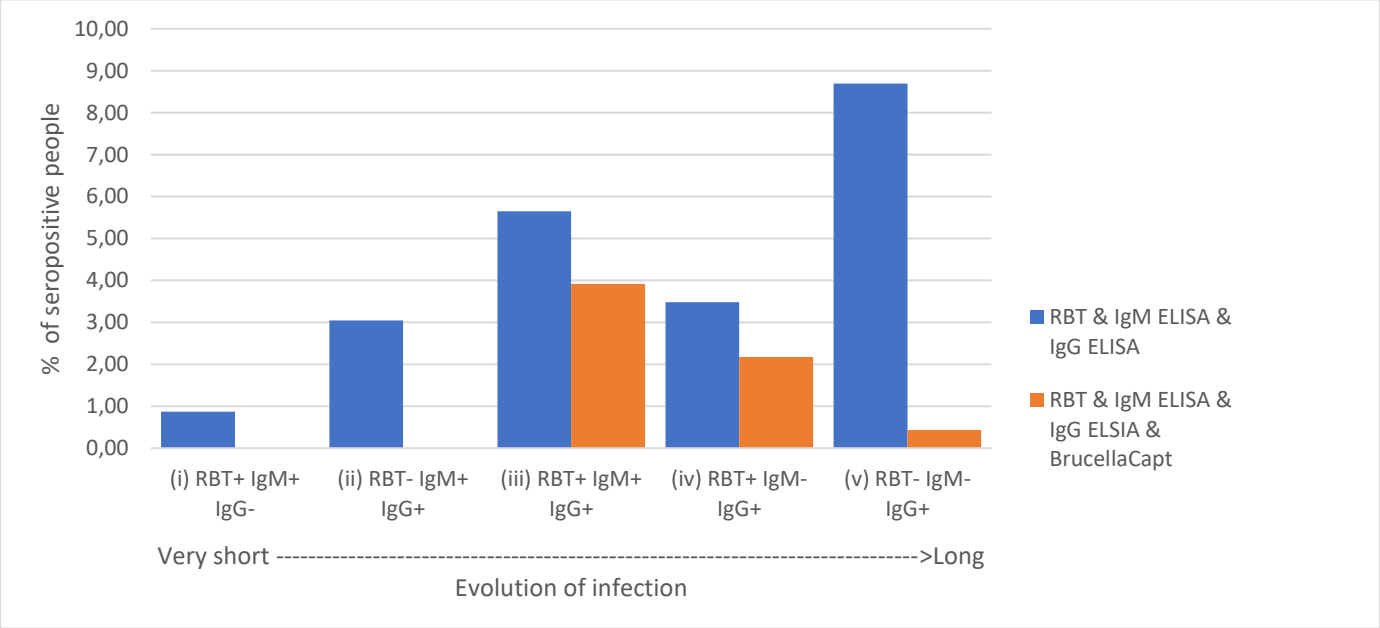

Figure 5.8: % of *Brucella* seropositive cattle workers and veterinary officials (N=230) on cattle farms (N=41) per serological test combination, Gauteng, 2016

Symptoms reported by BrucellaCapt seropositive persons (n=15) were distributed across titres and infection of both short and long evolution, with more symptoms being reported by those who had an antibody profile indicative of a long evolution infection (Table 5.6).

Table 5.2: Distribution of BrucellaCapt titres and reported symptoms (within previous 6 months) over infection evolution (n=15)

Case	BrucellaCapt Titre	Short evolution (RBT, IgM, IgG ELISA seropositive)	Long evolution (RBT, IgG ELISA seropositive & IgM ELISA seronegative)	Exposure / inactive or resolved infection (RBT, IgM ELISA seronegative & IgG ELISA seropositive)
1	1:320	no symptoms		
2	1:320	no symptoms		
3	1:320	no symptoms		
4	1:320	joint pain		
5	1:320	fever		
6	1:320		fatigue	
7	1:320			no symptoms
8	1:640	no symptoms		
9	1:640	no symptoms		
10	1:640		joint pain	
11	1:640		fever	
12	1:640		headache	
13	1:640		fatigue	
14	1:1280	headache		
15	1:2560	fever		

The majority of BrucellaCapt seropositive study participants (87%) either did not visit a clinic in response to brucellosis-like symptoms, or they attended a medical facility but were not asked their occupational history by the attending doctor (Table 5.7).

Table 5.3: Possible undetected brucellosis cases amongst BrucellaCapt seropositive study participants (n=15)

BrucellaCaptTitres	Self-medicate/Do nothing/pray	Visit clinic/GP/hospital	
		No Occupational History taken by attending medical doctor	Occupational History taken by attending medical doctor
1:320	4	2	1
1:640	3	2	1
1:1280	1	0	0
1:2560	0	1	0
Total	8 (53%)	5 (33%)	2 (13%)

Knowledge of brucellosis and health seeking behaviour to brucellosis-like symptoms

Cattle handler knowledge of brucellosis symptoms in cattle was low with 20.7% (42/203) aware that *B. abortus* can cause abortions in cattle, can cause calves to be born weak and can also be in a herd without causing abortions. Whilst 36.9% (75/203) knew that bovine brucellosis can cause disease in people, only 16.3% (33/203) reported knowing the human symptoms of disease. In contrast 63% (17/27) of veterinary officials knew the symptoms of bovine brucellosis and 100% knew it to be a zoonotic disease, but only 89% (24/27) knew the symptoms of human disease. There was a significant difference in wanting more information on brucellosis, between farm workerson case farms (OR=2.5, 95% CI: 1.5-5.3, p=0.019) and

veterinary officials (OR=7.3, 95% CI: 2.6-20.7, p<0.001) vs farm workers on control farms. Despite having greater awareness of the zoonotic nature of bovine brucellosis and human symptoms of the disease as well as wanting information more thanfarm workers, only 22.2% (6/27) of veterinary officials would opt to visit a clinic, doctor or hospital in response to self-experienced brucellosis like symptoms, compared to 74.9% (152/203) of farm workers (p < 0.001). We also found that 53% (7/15) of BrucellaCapt seropositive people did not visit a clinic in response to brucellosis-like symptoms which may reflect undetected cases of brucellosis. Further findings are summarised in Table 5.3.

Table 5.4: Distribution of responses to knowledge questions, amongst workers on cattle farms in, Gauteng, 2016

	Farm workers exposed to *Brucella* seropositive cattle herds (n=150)		Farm workers exposed to *Brucella* seronegative cattle herds (n=53)		Veterinary officials exposed to *Brucella* seropositive and seronegative herds (n=27)		Total (N=230)	
	n	%	n	%	n	%	n	%
"Do you know the symptoms of brucellosis in cattle?"								
No	112	74.7	41	77.4	10	37.0	163	70.9
Yes	38	25.3	12	22.6	17	63.0	67	29.1
Verified Yes	30	20	12	22.6	17	63.0		
"Do you know the symptoms of brucellosis in people?"								
No	129	86.0	41	77.4	3	11.1	173	75.2
Yes	21	14.0	12	22.6	24	88.9	57	24.8
"Do you understand how brucellosis causes a drop in profit?"								
No	115	76.7	39	73.6	0	0.0	154	67.0
Yes	35	23.3	14	26.4	27	100.0	76	33.0
"Do you know how brucellosis can reduce your ability to work?"								
No	120	80.0	40	75.5	6	22.2	166	72.2
Yes	30	20.0	13	24.5	21	77.8	64	27.8
"Drinking raw milk is safe and healthy"								
Don't know	14	9.3		0,0		0	14	6.1
No	62	41.3	13	24.5	25	92.6	100	43.5
Yes	74	49.3	40	75.5	2	7.4	116	50.4
"Brucellosis can cause abortions in cattle"								
Don't know	45	30.0	10	18.9		0.0	55	23.9
No	42	28.0	26	49.1	1	3.7	69	30.0
Yes	63	42.0	17	32.1	26	96.3	106	46.1
"Brucellosis can cause calves to be born weak"								
Don't know	49	32.7	10	18.9	1	3.7	60	26.1
No	48	32.0	29	54.7	6	22.2	83	36.1
Yes	53	35.3	14	26.4	20	74.1	87	37.8
"Brucellosis can be in the herd and not cause abortions"								
Don't know	50	33.3	10	18.9	2	7.4	54	23.5
No	67	44.7	30	56.6	2	7.4	99	43.0
Yes	33	22.0	13	24.5	23	85.2	69	30.0

"Brucellosis can cause disease in people"								
Don't know	45	30.0	11	20.8		0.0	49	21.3
No	44	29.3	28	52.8		0.0	72	31.3
Yes	61	40.7	14	26.4	27	100.0	102	44.3
Health seeking response to self-experienced brucellosis-like symptoms								
Ignore symptoms, self-medicate, pray	42	28.0	9	17.0	21	77.8	72	31.3
Visit clinic, doctor or hospital	108	72.0	44	83.0	6	22.2	158	68.7
What would you do if you observe a foetus in the field?								
Report to farm manager or veterinarian	103	68.7	14	26.4	26	96.3	143	62.2
Dispose of the foetus and do nothing more	47	31.3	39	73.6	1	3.7	87	37.8
Would you like more information on brucellosis?								
No	95	63.3	43	81.1	10	37.0	148	64.3
Yes	55	36.7	10	18.9	17	63.0	82	35.7

Univariate analysis: reported brucellosis-like symptoms associated with evolution of *Brucella* infection

Univariate analysis of symptoms associated with infection of short evolution (RBT, IgM and IgG ELISA seropositive), long evolution (IgM ELISA seronegative and RBT and IgG ELISA seropositive) and likely inactive infection (RBT and IgM seronegative and IgG seropositive), identified a weak association between reported generalized aching and infection of short duration (OR=4.8, 95%CI: 0.4-27.9, p=0.103), and a strong association between reported joint pain and infection of long duration (OR=5.1, 95%CI: 0.9-33.3, p=0.030). The distribution of symptoms across these stages of infection evolution and the associated significance, is shown in Table 5.3.

Table 5.5: Univariate analysis of *Brucella* antibody expression along the evolution of infection and brucellosis-like symptoms reported by farm workers and veterinary officials within the 6 months prior to the study, Gauteng, 2016

Symptoms within previous 6 months	**Study participants (N=230)**	**RBT, IgM & IgG ELISA seropositive**			**RBT & IgG ELISA seropositive & IgM ELISA seronegative**			**RBT & IgM ELISA seronegative & IgG seropositive**		
		N	**%**	**p - value**	**N**	**%**	**p - value**	**N**	**%**	**p - value**
Generalized aching				0.103*			1			0.212
No	220	11	5.0		8	3.6		18	8.2	
Yes	10	2	20.0		0	0		2	20.0	
Joint pain				1			0.030*			1
No	170	10	5.9		3	1.8		15	8.8	
Yes	60	3	5.0		5	8.3		5	8.3	
Fever				0.466			0.357			0.755
No	191	10	5.2		8	4.2		16	8.4	
Yes	39	3	7.7		0	0		4	10.3	
Sweating				0.698			0.362			1
No	194	12	6.2		8	4.1		17	8.8	
Yes	36	1	2.8		0	0		3	8.3	
Night-Sweating				0.475			0.357			1
No	190	12	6.3		8	4.2		17	8.9	
Yes	40	1	2.5		0	0		3	7.5	
Fatigue				0.434			0.614			0.747

No	194	10	5.2		6	3.1		18	9.3	
Yes	36	3	8.3		2	5.6		2	5.6	
Headache				0.538			0.109			1
No	161	8	5.0		8	5.0		14	8.7	
Yes	69	5	7.2		0	0		6	8.7	
Anorexia				1			0.303			1
No	220	13	5.9		7	3.2		20	9.1	
Yes	10	0	0		1	10.0		0	0	
Weight loss				1			1			0.637
No	214	12	5.6		8	3.7		18	8.4	
Yes	16	1	6.3		0	0		2	12.5	

Univariate analysis and multivariable analysis

Short evolution (RBT, IgM, IgG seropositive)

Univariate analysis of factors associated with infection of a short evolution identified worker group, handling of afterbirth or placenta, vaccinating cattle with RB51/S19 and slaughter of cattle, for inclusion into the multivariable logistic regression model at significance $p < 0.2$ (Table 5.4). The handling of afterbirth or placenta was marginally significant (OR=8.9, 95% CI: 1.0-81.1, p=0.052) and slaughter of cattle significant (OR=5.3, 95% CI: 1.4-19.6, p=0.013) in the mixed effects logistic regression model fit for (RBT, IgM ELISA and IgG ELISA) seropositivity amongst persons tested. The random effect of clustering at farm level was not significant (p=0.2137).

Table 5.6: Univariate and multivariable analysis of factors associated with *Brucella* infection of a short evolution (RBT, IgM ELISA and IgG ELISA seropositivity) amongst farm worker and veterinary officials in Gauteng, 2016

Variable and level	**Study participants (N=230)**	**Univariate analysis**			**Multivariable analysis**		
		RBT, IgM & IgG ELISA seropositive		**p - value**			
		N	%		**Odds ratio**	**95% CI**	**p-value**
Worker group				0.089*			
Farm Workers exposed to *Brucella* non-reactor herds (reference)	53	1	1.9				
Farm Workers exposed to *Brucella* reactor herds	150	9	6				
Veterinary officials	27	3	11.1				
Duration of occupation				0.286			
≤2 y	63	1	1.6				
2 - 6 y	59	4	6.8				
6 - 14 y	54	5	9.3				
>14 y	54	3	5.6				
Brucellosis-like symptoms				0.567			
None	100	7	7				
1 or more	130	6	4.6				
Health seeking behaviour				0.551			
Self-medicate/do nothing/pray	72	5	6.9				
Visit doctor/clinic	158	8	5.1				
Drink Unpasteurized milk				1			

No	111	6	5.4				
Yes	119	7	5.9				
Handle cows at calving				0.236			
No	73	2	2.7				
Yes	157	11	7				
Handle placenta or afterbirth				0.020*			
No	87	1	1.1		1†	–	–
Yes	143	12	8.4		8.9	1.0 - 81.1	0.052
Handle newborn calves				1			
No	71	4	5.6				
Yes	159	9	5.6				
Vaccinate cattle (S19/RB51)				0.151*			
No	103	3	2.9				
Yes	127	10	7.9				
Milk cows				0.561			
No	144	7	4.9				
Yes	86	6	7.0				
Slaughter cattle				0.007 *			
No	169	5	3.0		1†	–	–
Yes	61	8	13.1		5.3	1.4 - 19.6	0.013

Long evolution (RBT positive AND IgM ELISA negative AND IgG ELISA seropositive)

Farm workers exposed to case herds and veterinary officials compared to those exposed to seronegative herds in the Exposure Group variable, increasing duration of occupational exposure, and handling new-born calves were associated with infection of a long evolution ($p<0.02$) in the univariate analysis and were included in the mixed effect multivariable logistic regression model.

In the mixed effects multivariable logistic regression model (Table 5.5), Veterinary officials compared to farm workers exposed to seropositive herds (OR=4.1, 95%CI: 0.2-8.1, p=0.049), was identified as marginally significant, although the small number of events of this outcome increases the uncertainty of this confidence interval. The random effect of clustering at farm level was significant (LR test vs. logistic model: chibar2=4.68, p=0.015). The Wald Chi^2 statistic for the mixed effects model (3.88) was also marginally significant (p=0.049). The clustering identifies that 3/5 veterinary officials in this group, were from the Germiston State Vet area.

Table 5.7: Univariate and multivariable analysis of factors associated with RBT positive AND IgM ELISA negative AND IgG ELISA *Brucella* IgG ELISA seropositivity amongst farm workers and veterinary officials in Gauteng, 2016

Variable and level	**Study participants (N=230)**	**Univariate analysis**		**Multivariable analysis**
		RBT positive & IgM ELISA negative & IgG	**p - value**	

		ELISA seropositive					
		n	%		Odds ratio	95% CI	p-value
Exposure Group				0.001 *			
Farm Workers exposed to *Brucella* non-reactor herds	53	0	0		–	–	–
Farm Workers exposed to *Brucella* reactor herds	150	3	2.0		1†	–	–
Veterinary officials	27	5	18.5		4.1	0.2-8.1	<0.049
Duration of occupation				0.017 *			
≤2 y	63	0	0				
2 - 6 y	58	1	1.7				
6 - 14 y	52	2	3.8				
>14 y	49	5	10.2				
Brucellosis-like symptoms				0.142			
None	100	1	1.0				
1 or more	130	7	5.4				
Health seeking behaviour				0.262			
Self-medicate/do nothing/pray	72	4	5.6				
Visit doctor/clinic	158	4	2.5				
Drink Unpasteurized milk				0.003			
No	111	8	7.2				
Yes	119	0	0				
Handle cows at calving				0.268			
No	73	4	5.5				
Yes	157	4	2.5				
Handle placenta or afterbirth				0.481			
No	87	4	4.6				
Yes	143	4	2.8				
Handle newborn calves				0.111 *			
No	71	5	7.0				
Yes	159	3	1.9				
Vaccinate cattle (S19/RB51)				0.302			
No	103	2	1.9				
Yes	127	6	4.7				
Milk cows				0.713			
No	144	6	4.2				
Yes	86	2	2.3				
Slaughter cattle				0.440			
No	169	5	3.0				
Yes	61	3	4.9				

Exposure / inactive or resolved infection (RBT and IgM ELISA seronegative and IgG ELISA seropositive)

In the univariate analysis of factors associated with likely exposure / inactive or resolved infection (RBT and IgM ELISA seronegative and IgG ELISA seropositive), there was evidence of an association between the outcome and self-medicating, praying or ignoring brucellosis-like symptoms in this group compared to those who seek out medical attention in response to symptoms. Seropositive people in this group were associated with not being engaged in the following risk activities: handling cattle at calving, handling afterbirth or

placenta, handling new-born calves and milking cows. Altogether seven variables (p<0.20) were identified for inclusion into the multivariable model (Table 5.9).

The only variable remaining associated with seropositivity in this group was veterinary officials compared to those exposed to seropositive herds in the Exposure Group Veterinary (OR=7.0, 95%CI: 2.4-20.2, p<0.001), whilst there was strong evidence of an association between the handling of afterbirth or placenta and seronegative people in this group (OR=3.9, 95%CI: 1.3-11.3, p=0.012). This is to be expected given that the above finding that the handling of placenta and after birth was associated with people who were RBT, IgM and IgG ELISA seropositive (short evolution infection) (Table 5.4).

Table 5.8: Univariate and multivariable analysis of factors associated with RBT and IgM ELISA seronegative and IgG ELISA seropositive reactors amongst farm workers and veterinary officials in Gauteng, 2016

Variable and level	**Study participants (N=230)**	**Univariate analysis**			**Multivariable analysis**		
		RBT and IgM ELISA seronegative and IgG ELISA seropositive		**p - value**			
		n	**%**		**Odds ratio**	**95% CI**	**p-value**
Exposure Group				< 0.001*			
Farm Workers exposed to *Brucella* non-reactor herds	53	0	0		–	–	–
Farm Workers exposed to *Brucella* reactor herds	150	11	7.3		1†	–	–
Veterinary officials	27	9	33.3		7.0	2.4-20.3	<0.001
Duration of occupation				0.005*			
≤2 y	61	2	3.3				
2 - 6 y	56	3	5.4				
6 - 14 y	49	5	10.2				
>14 y	44	10	22.7				
Brucellosis-like symptoms				0.486			
None	100	7	6.0				
1 or more	130	13	10.0				
Health seeking behaviour				0.010*			
Self-medicate/do nothing/pray	72	12	16.7				
Visit doctor/clinic	158	8	5.1				
Drink Unpasteurized milk				1			
No	111	10	9.0				
Yes	119	10	8.4				
Handle cows at calving				0.010*			
No	73	12	16.4				
Yes	157	8	5.1				
Handle placenta or afterbirth				0.003*			
No	87	14	16.1		3.9	1.3-11.3	0.012
Yes	143	6	4.2		1†	–	–
Handle newborn calves				0.005*			
No	71	12	16.9				
Yes	159	8	5.0				
Vaccinate cattle (S19/RB51)				0.239			
No	103	6	5.8				

Yes	127	14	11.0		
Milk cows				0.031*	
No	144	17	11.8		
Yes	86	3	3.5		
Slaughter cattle				0.603	
No	169	16	9.5		
Yes	61	4	6.6		

Discussion

This study identified a gap in cattle handler knowledge of brucellosis symptoms in cattle and people and identified symptoms and risk factors associated with infection of short and long evolution and likely inactive/resolved infection or exposure.

Overall cattle handler knowledge of brucellosis symptoms in cattle (29.1%) was similar to a recent global pooled awareness estimate (28.4%) for knowledge of animal symptoms of brucellosis (Zhang et al., 2019), and marginally higher than the one found amongst cattle keepers (22.6%) in the Eastern Cape (E. Cape) of SA (Cloete et al., 2019). In contrast, cattle handler knowledge of brucellosis symptoms in people in this study (25%), was much lower than the global statistic (41%) (Zhang et al., 2019) but higher than the one (12.7%) found in the E. Cape study (Cloete et al., 2019). Differences between the global and local proportions of awareness of human brucellosis symptoms, may be attributed to both SA studies selecting workers cattle farms. A significant source of knowledge for this group are the veterinary officials (Cloete et al., 2019) whose main task is to increase cattle keepers' knowledge of the livestock disease.. The difference between cattle handler knowledge of human symptoms of brucellosis in the E. Cape study and this study, may be partially explained by greater awareness amongst veterinary officials (88.9%) who formed part of this study group as opposed to only farm workers (16.3%) in this study.

The significant difference in wanting more information on brucellosis, between farm workers on case farms, veterinary officials and farm workers on control farms was unexpected and needs to be investigated further. A possible explanation may be that veterinary officials perceived themselves to be at greater risk than farm workers or began to believe themselves to be susceptible to brucellosis. Such belief is a key construct in the health belief model of health seeking behaviour (Babazadeh et al., 2019), triggering a drive for more information. It is also likely that exposure to the questionnaire made them realise that despite knowing brucellosis to be a zoonotic disease, they did not know the symptoms of human disease which has a direct effect on their own health, well-being and occupational safety.

Farm workers and cattle keepers' health seeking behaviour in response to brucellosis-like symptoms also varied between provinces. In the E. Cape, 93.2% of farm workers and cattle keepers' reported that they would go to a clinic in response to brucellosis-like symptoms experienced (Cloete et al., 2019), as opposed to 68.7% in this study. The difference between farm workers and veterinary officials' attitudes toward experiencing brucellosis like symptoms in themselves with only 22% of veterinary officials seeking out medical care in response to brucellosis-like symptoms, is a finding of concern and needs further investigation by occupational health and safety officers.

The importance of seeking out medical care in these occupational groups is highlighted by finding that 7/15 of those that tested seropositive on the BrucellaCapt would not seek out medical care in response to brucellosis-like symptoms. It has been documented that brucellosis cases delay presenting to a medical facility from the onset of symptoms with a median delay time of 90 days (Kunda et al., 2007). Such delays increase the likelihood of complicated brucellosis, treatment failure and chronic brucellosis (Doganay and Aygen, 2003). These findings may also suggest lack of awareness amongst medical clinicians of the occupational risk of brucellosis to farm workers and veterinary officials, which has been highlighted as a matter of concern in SA (Wojno et al., 2016, Frean et al., 2019).

In this study, different risk factors were found to be associated with different serological tests combinations selected to detect infection of short and long evolution. We identified that the handling of afterbirth or placenta to be marginally significant and slaughter of cattle significantly associated with infection of a short evolution whilst infection of a long evolution was marginally significantly associated with being a veterinary official compared to farm workers. However, the small number of events of seropositivity indicative of a long evolution infection increases the uncertainty of this confidence interval. However, veterinary officials compared to farm workers were significantly associated with inactive/resolved infection or exposure. Farm workers without this serological outcome were significantly associated with afterbirth or placenta. This is to be expected given that the above finding that the handling of placenta and after birth was associated with people who were RBT, IgM and IgG ELISA seropositive (short evolution infection).

These findings may be suggesting that those farm workers engaged in the slaughter of cattle were more recently exposed as opposed to those who routinely handle afterbirth or placenta. Alternatively, for slaughter of cattle there could be a recall bias as people refer to the last months rather to the last years and therefore it may not appear to be a risk factor for IgG.

Regardless, this finding indicates the importance of selecting appropriate screening tests in endemic areas.

Veterinary officials are more regularly and frequently exposed to RB51 and S19 vaccination, as this is a fundamental bovine brucellosis control activity. Accidental exposure to RB51 through needlestick injury has been implicated as one of the main causes of brucellosis in veterinarians and their assistants (Kutlu et al., 2014, Pereira et al., 2020). Occupational risk to abattoir workers (Swai and Schoonman, 2009) and veterinarians has been well documented (Lewis, 1959, Schrire, 1962, Robinson and Metcalfe, 1976, Sacks et al., 1976, Gummow, 2003, Dean et al., 2012). In this study, all the veterinary officials that tested seropositive were para-veterinarians, also known as Animal Health Technicians, employed by government to perform selected veterinary services. Transmission of *Brucella* at the cattle-human-interface to officials in this context can occur through accidental self-inoculation whilst vaccinating cattle with S19 or RB51 vaccine, both of which are attenuated strains of *B. abortus* (OIE, 2008). It may also occur during the collection of blood or milk samples for routine regulatory herd testing from farms participating in the provincial state veterinary services' bovine brucellosis control programme between 2014 and 2016. Furthermore, at least 50% of AHTs reported assisting cattle with dystocia (unpublished government data), which may present a further route of transmission and exposure. Further investigation is needed to determine and mitigate the role of these variables in AHT exposure to *Brucella* on cattle farms.

The presence of significant risk factors and symptoms associated with infection of short and long evolution and poor health seeking behaviour in response to brucellosis-like symptoms among farm workers and veterinary officials with these antibody profiles, strongly suggest the presence of undetected cases of human brucellosis on cattle farms.

Conclusion and Recommendations

Evidence of cattle handler exposure to *Brucella* on cattle farms participating in the bovine brucellosis control programme in Gauteng varies depending on the serological screening test used. However, when tests results were combined to illuminate the evolution of infection in this group, significant risk factors and symptoms were found to be associated with infection of short and long evolution. This in addition to the finding of poor health seeking behaviour in response to brucellosis-like symptoms among farm workers and veterinary officials with these antibody profiles, strongly suggest the presence of undetected cases of human brucellosis on cattle farms.

It is therefore recommended people exposed to cattle herds in Gauteng be routinely screened for brucellosis using the RBT test as described in Diaz et al. (2011) to facilitate an early detection and response to brucellosis in these occupationally exposed persons and their families. In brief RBT should be used on plain serum and, if positive, RBT on serum dilutions up to 1/32. Dilutions should be contrasted with clinical symptoms (if any). It is also recommended that medical practitioners in SA be made aware of the clinical symptoms of both short and long evolution brucellosis (Chapter 2) and the risk of brucellosis amongst persons occupationally exposed to cattle herds in Gauteng province. Awareness programmes to increase knowledge of human and cattle symptoms of brucellosis are recommended to be part of the routine veterinary regulatory service to these farms. Occupational health and safety measures to protect the health of veterinary officials should be implemented and monitored.

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Chapter 6: General Discussion

Introduction

The overall aim of this study was to understand the reported increase of numbers of bovine brucellosis outbreaks and concomitant lack of increasing numbers of human brucellosis cases by explicating the epidemiology, management, and regulatory control of bovine brucellosis at the human-cattle-farm interface in Gauteng province using a One Health framework. In this final chapter, we present and integrate the main findings of the thesis chapters using a method of systems thinking to describe a hypothetical system of bovine brucellosis persistence at the human-cattle-farm interface in Gauteng and to identify areas for further research.

'Systems thinking' as a discipline is aimed at understanding how things are related to each other, their patterns of relationship within a whole entity and how these translate into emergent behaviours (Kay, 2008, Bishai et al., 2014). Systems thinking provides a means to work with complexity and to understand it (Kay, 2008, Rwashana et al., 2014). Complexity is characterized as defying linear logic, having unpredictable outcomes, emergent behaviour (Kingsley and Taylor, 2017) and exhibiting "self-organisation and feedback loops, wherein the effect is its own cause" (Kay, 2008) making it impossible to distinguish causal order (Bishai et al., 2014). .

System components and interrelationships

Chapter 2

Bovine brucellosis outbreaks were detected from 1906 in the Gauteng area, and the first case of human brucellosis, reported in 1924, was caused by *B. abortus*. Clinical symptoms of acute, chronic, uncomplicated and focal brucellosis in people living in SA was reported in the literature. From 1924 to 1959, studies of human brucellosis seroprevalence were conducted on patients by medical practitioners, whilst studies of bovine brucellosis prevalence began to be reported within this period but seemed to end in 1990. From our review of the available literature, it became clear, that in SA the bovine brucellosis eradication scheme (introduced in 1979) followed the prioritization of human brucellosis as a disease of public health importance in the country. Furthermore, it is plausible that there is a feedback loop between government

prioritization of human brucellosis and the detection, diagnosis and reporting of the disease by medical practitioners (Figure 6.1).

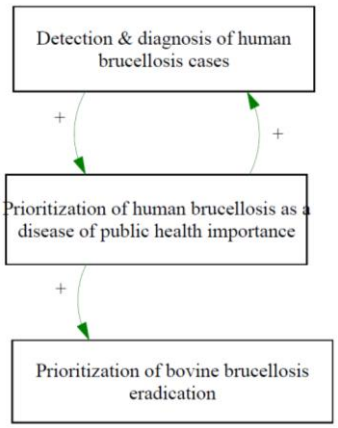

Figure 6.1: Positive reinforcing feedback loop between human brucellosis cases detected and national prioritization of brucellosis

Elements of this feedback relationship have been identified as being central to the neglect of human brucellosis. It is likely that currently clinicians in SA find early detection, diagnosis and management of human brucellosis to be challenging, leading to under diagnosis (Franco et al., 2007b, Franco et al., 2007a). This in turn leads to difficulties in establishing population specific parameters to interpret diagnostic tests (Franco et al., 2007b, Mantur, 2006, Wojno et al., 2016) and research gaps in understanding the clinical epidemiology of the disease in farm workers and veterinary officials responsible for bovine brucellosis control on cattle farms. A de-prioritization of the disease by public health practitioners follows the lack of evidence (Figure 1). This hypothesis is supported by the two following logical links: without clinical information on the frequency, distribution, symptoms and duration of human brucellosis, the public health and economic impact of the disease cannot be determined (Plumb et al., 2013, Vallat and Plumb, 2013); the lack of public health and impact evidence drives the decline in prioritization and resource allocation for the eradication of brucellosis in livestock (McDermott and Arimi, 2002, WHO, 2014).

Chapter 3

Analysis of bovine brucellosis laboratory test reports from 2013-2018, for Gauteng province, revealed no significant change in prevalence of *Brucella* reactor herds (mean=22.1%)

or within-herd seroprevalence (mean=7.4%) over the 6-year period. However, Randfontein (OR=1.6; 95% CI: 1.2-2.1; p<0.001) and Germiston State Vet Areas (OR=1.9; 95% CI: 1.5-2.5, p=0.008) had higher odds of reactor herds than the Pretoria State Vet Area. Reactor herds were also associated with increased herd size (p<0.001). Additionally, Germiston and Randfontein both had within-herd prevalence count ratios 1.5 times greater than the Pretoria State Vet Area (p<0.001) and larger herd sizes were associated with lower within-herd prevalence (p<0.001).

These findings contrasted with the report of increasing incidence of outbreaks of bovine brucellosis in the country. Notwithstanding the possibility that the national increase may truly be due to outbreaks occurring in other provinces in the country, this study has revealed a weakness in management of bovine brucellosis control data, making it difficult to verify the reported increase in incidence of outbreaks. During this aspect of the study, it became clearer that regulatory services focussed on detecting *Brucella* reactor herds and cattle (blue arrows in Figure 2) and not on the evidence of a decrease in reactor herds or cattle within demarcated areas, as a trigger to make strategic changes to the eradication strategy (red arrow in Figure 6.2).

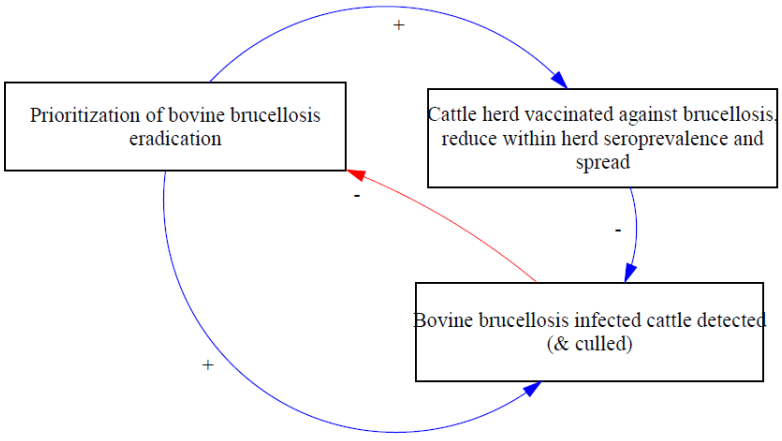

Figure 6.2: Feedback loop between the number of *Brucella* reactor herds and cattle detected and prioritization of bovine brucellosis eradication

The blue arrows in Figure 2 are typical of a linear, reductionist approach to brucellosis control strategies. In essence, bovine brucellosis control is perceived to be a simple system as opposed to a complex system, where a simple system is known to function according to

deterministic principles (Kay, 2008). When such rationale predominates, policy makers and programme managers rely on the output of deterministic models of human-animal transmission or on stochastic computer-simulated econometric models to inform bovine brucellosis control strategies (Amoson, 1990, Zinsstag et al., 2005), to identify feasible interventions and performance targets or focus on the strategic reduction of uncertainty related to risks identified.

Despite the value of these methods to identify plausible causal pathways for disease reduction or risk factors for mitigation, literature suggests that these models promote interventions based on the assumption that it will result in a predictable measurable effect. Pre-determined targets are then set by organisations to monitor and evaluate the effectiveness of control and eradication programmes (Michael and Madon, 2017, Booth and Clements, 2018, Berezowski et al., 2019, Waltner-Toews, 2001). Furthermore, policies informed by this way of thinking, influence the collection and reporting of information and enables the prioritization and resource allocation for the control of brucellosis in livestock (McDermott and Arimi, 2002, WHO, 2014). However, this approach to disease reduction is not so effective with a complex system of disease. With bovine brucellosis control this is due to complexity introduced by the human factor and the chain of dependencies, reactions and decisions to report or not from the farm workers, farmers, veterinarians, state veterinarians and government. This socio-ecosystem in which bovine brucellosis control is nested, being complex in nature, will, according to the systems thinking paradigm, adapt and self-organise itself toward the most stable state, even if that state is a state of disease (Kay, 2008). Without ongoing representative quantitative evidence of change in the incidence of reactor herds and cattle, risk factors and interventions done to control disease, it is not possible to monitor progress toward bovine brucellosis eradication or adjust the priority of the disease (Figure 2) to make changes to the control programme strategy. Nevertheless general theories of systems thinking that allow the representation of elements interconnected by complex and dynamic relationships (Duboz et al., 2018) helps to compensate for the weaknesses highlighted in epidemiological modelling.

Chapter 4

In Chapter 4 we identified that government-sponsored farms, open herd management (i.e. replacement cattle are bought in from herds other than the herd resident on the farm) vs. managing the herd as a closed herd, and the presence of antelope on the farm, were more likely to be *Brucella* infected. Furthermore, seroprevalence amongst farm workers and veterinary officials varied according to test and the *Brucella* status of the herd. Seroprevalence amongst farm workers on case farms (n=30 farms) ranged from 4.0% (BrucellaCapt) to 16.7% (IgG

ELISA), compared to control farms (n=11 farms), where this seroprevalence ranged from 1.9% (BrucellaCapt) to 5.7% (IgG ELISA). Overall, 5.7% (13/230) of persons tested were seropositive to the RBT and IgM ELISA and IgG ELISA tests and 3.9% (9/230) were seropositive to all four serological tests. We found that the pattern of *Brucella* antibody expression in those that tested seropositive on one or more of the tests, ranged from profiles associated with infection of short evolution, typified by a predominance of IgM, to infection of long evolution in which IgM decreases and IgG (and IgA) increases and eventually predominates over IgM. We also found a class of long evolution categorised by the presence of IgG and low levels of non-agglutinating antibodies (RBT negative and BrucellaCapt negative). The antibody profile amongst this group of seropositive farm workers and veterinary officials, indicate that participants were are at different stages in the evolution of infection. Farm workers on control farms presented with antibody profiles of short to longer evolution, compared to a more spread-out profile of infection evolution amongst farm workers on control farms. The difference in seroprevalence amongst farm workers between case and control farms for all the test combinations was not significant. However, seroprevalence amongst veterinary officials was significantly greater compared to farm workers on case farm for infection of longer evolution. These findings suggest that farm workers on control farms may be exposed to *Brucella* in routes other than contact with the cattle herd. Further investigation was needed to clarify whether specific risk factors or symptoms was associated with these stages in the evolution of infection in the farm workers and veterinary officials tested. This was addressed in Chapter 5.

A causal loop diagram integrating significant risk factors and symptoms associated with seropositive cattle herds illustrate a possible complex adaptive system for bovine brucellosis at the human-cattle-farm interface in Gauteng (Figure 6.3). Orange arrows illustrate possible relationships between variables found in the current study. Symptoms were observed in the herd in the year prior to the last herd test. Two causal feedback loops, between case herds and abortions in the herd or brucellosis-like symptoms in farm workers on a *Brucella*-infected farm, are possible in this dataset. Both may plausibly contribute to maintaining ongoing infection in cattle herds increasing within-herd seroprevalence. It is widely accepted that brucellosis in people increases absenteeism and may result in debilitating conditions (Corbel et. al., 2006). This may be a contributing factor to poor management or control of brucellosis in these cattle herds. Another important feedback loop was identified to be the association between a *Brucella* infected herd and being managed as an open herd, i.e. cattle are bought in from other herds.

The movement of cattle of unknown brucellosis status into a herd that is participating in the control programme poses a risk to the herd, but at least the brucellosis status of the introduced cattle can be determined. There is, however, always the possibility that farmers are trading undetected *Brucella* infected cattle between open herds.

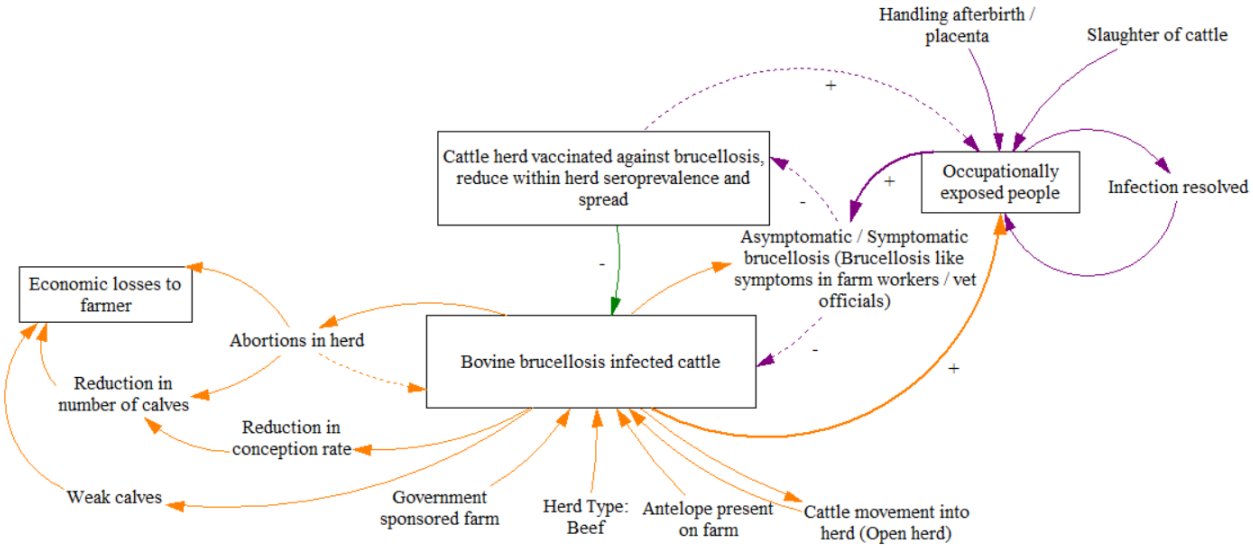

Figure 6.3: Causal Loop diagram mapping predictors and effects of *Brucella* seropositive cattle herds, Gauteng, 2014-2016 (Dashed orange line represents the effect of abortions in maintaining herd infection. Dashed purple line indicates the negative effect that ill health in workers on cattle farms may have on detecting *Brucella* infected cattle.)

In Gauteng, government sponsored farms are associated with *Brucella* reactor herds. This finding lends credibility to the complexity argument for bovine brucellosis control in the province due the likely interaction of socio-political variables (Waltner-Toews, 2001). The sponsoring of farms was part of the redress of apartheid practices and was aimed at redistribution of land to previously disadvantaged groups to promote agriculture in the country. Policies for land redress and government support for emerging farmers occurs at levels far higher than the human-cattle-farm interface. This finding suggests that bovine brucellosis control may be a multi-level issue (Kay, 2008) in Gauteng, also indicative of a complex problem. Did these farmers receive enough awareness and training with regards to zoonotic disease such as brucellosis management? Or did the sponsoring with multiple visits of the

veterinary officials lead to an increase of the risk of disease transmission as technicians may visit more than one farm in the same day. The following chapter tries to explore this complexity.

Chapter 5

In chapter 5, we identified a weak significant association between reported generalized aching and infection of short duration (p=0.103), and a significant association between reported joint pain and infection of long duration (p=0.030). Finally, we identified that the handling of afterbirth or placenta to be marginally significant (p=0.052) and slaughter of cattle (p=0.013) significantly associated with infection of a short evolution (RBT, IgM ELISA and IgG ELISA seropositive), whilst being a veterinary officials compared to farm workers exposed to seropositive herds was identified as marginally significant (p=0.49), although the small number of events of this outcome increases the uncertainty of this confidence interval for infection of a long evolution. Seropositivity in the inactive/resolved infection or exposed group was associated with being veterinary officials compared to those exposed to seropositive herds in the Exposure Group Veterinary (p<0.001), whilst handling of afterbirth or placenta was significantly associated with non-reactors of this group (OR=3.9, 95%CI: 1.3-11.3, p=0.012). This is to be expected given that the above finding that the handling of placenta and after birth was associated with people who were RBT, IgM and IgG ELISA seropositive (short evolution infection).

Farm workers have a poor knowledge of bovine brucellosis and its symptoms and furthermore, they barely know its zoonotic aspect and even less the human symptoms of disease. In contrast 63% (17/27) of veterinary officials knew the symptoms of bovine brucellosis and 100% knew it to be a zoonotic disease, but only 89% (24/27) knew the symptoms of human disease. Despite having greater awareness of the zoonotic nature of bovine brucellosis and human symptoms of the disease, only 22.2% (6/27) of veterinary officials would opt to visit a clinic, doctor, or hospital in response to self-experienced brucellosis like symptoms, compared to 74.9% (152/203) of farm workers (p < 0.001) (purple arrow in Figure 6.4). The study also found that 53% (7/15) of BrucellaCapt seropositive occupationally exposed persons did not visit a clinic in response to brucellosis-like symptoms (depicted as a pink arrow in Figure 6.4). The presence of significant risk factors and symptoms associated with infection of short and long evolution and poor health seeking behaviour in response to brucellosis-like symptoms among farm workers and veterinary officials with these antibody profiles, strongly suggest the presence of undetected cases of human brucellosis on cattle farms.

Understanding human decision making and behaviour in response to disease risk and disease is a field beyond the scope of veterinary epidemiology but should be part of the brucellosis risk analysis as an interdisciplinary approach of this disease. The importance of human decision making to brucellosis prevention has been an area of ongoing research. Babazadeh et al. (2019) explored cognitive factors amongst brucellosis patients associated with brucellosis preventative behaviours in 2019, using the theoretical framework of the empowerment model to study behaviour. The model includes the constructs of knowledge, attitude, self-efficacy and self-esteem. Knowledge, attitude and self-efficacy were identified as being significantly associated with brucellosis preventative behaviours (Babazadeh et al., 2019). Perceived susceptibility, a construct in the health belief model (a model explaining health seeking behaviour), was also found to be associated with brucellosis preventative behaviours (Babaei et al., 2016). Furthermore, neuropsychiatric evaluation of brucellosis patients revealed not only overt and neurological manifestations, but also depression, and impairment in mental control, logical memory and visual reproduction, all of these being cognitive functions (Shehata et al., 2010). In our study, we considered only knowledge (skills, facts and information acquired by a person through education or experience) of people occupationally exposed to cattle farms. We did not examine the positive or negative evaluation of behaviour by individuals (attitude), or the personal belief of ability to carry out the recommended plan of action successfully (self-efficacy), the individual's feeling of being valued (self-esteem) or perceived susceptibility (belief). Furthermore, we did not investigate neurological or psychiatric symptoms amongst farm workers or veterinary officials. Further investigation of the role of these variables in human brucellosis amongst farm workers and veterinary officials may provide a more comprehensive systems understanding (purple and red arrows in Figure 4) of brucellosis at the human-cattle-farm interface.

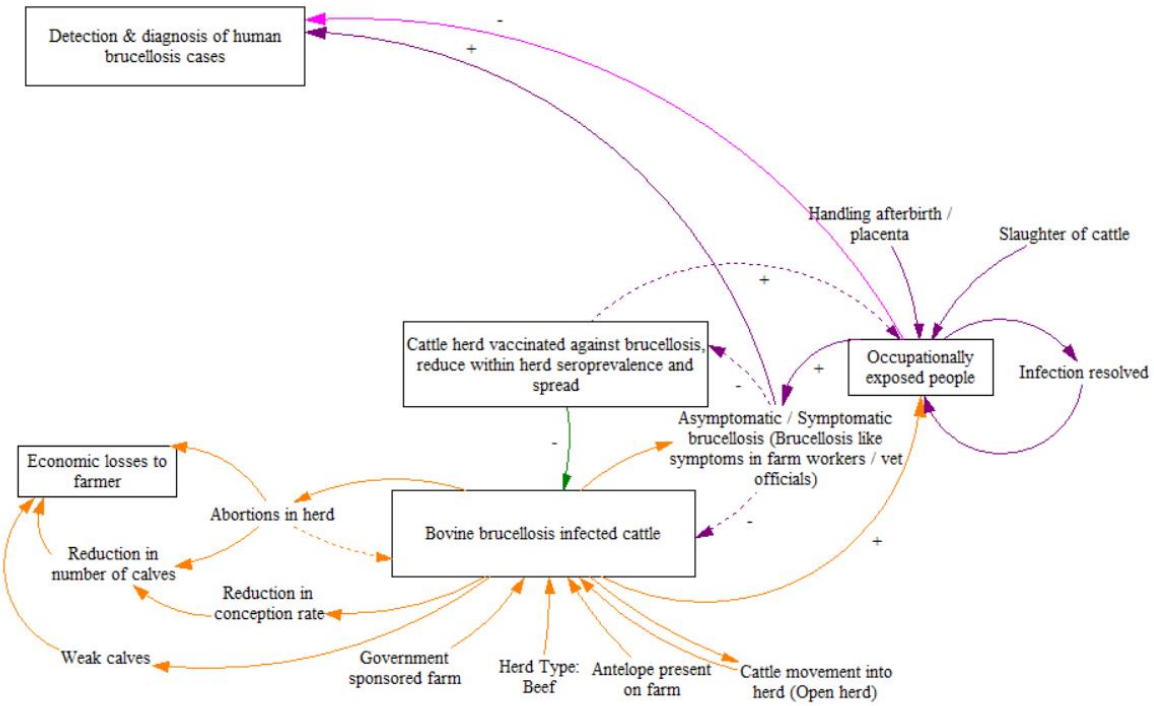

Figure 6.4: Health seeking behaviour of farm workers and veterinary officials depicted in One Health causal loop diagram

In our study, the awareness and health seeking response in farm workers and veterinary officials to brucellosis-like symptoms presents a barrier to detection and treatment of brucellosis. Doganay and Aygen (2003) emphasised the association between a lowered rate of positive blood culture and difficulties in diagnosing brucellosis in patients with fever that self-medicated or took an antibiotic on their own initiative or at the suggestion of a pharmacist (Doganay and Aygen, 2003). In addition, a feedback relationship has been postulated between a lack of knowledge of the zoonotic risk of brucellosis amongst occupationally exposed people, and difficulties in the implementation of bovine brucellosis prophylaxis and control plans (Kansiime et al., 2014). This results in the continued spread of the disease amongst livestock, further exacerbating the risk to those occupationally exposed.

A systemic description of *B. abortus* persistence at the human-cattle-farm interface in Gauteng from a One Health perspective

Combining the system components from each chapter (Figure 1 to Figure 4), a possible explanation for the non-correlation between the reported increase in numbers of bovine

brucellosis outbreaks and no increase in human brucellosis cases, can be hypothesized from the complete systemic diagram below (Figure 6.5).

B. abortus is difficult to detect with existing tests (Chapter 1). Bovine brucellosis has been in Gauteng since 1906 and has been a known cause of human brucellosis in SA, since 1924 (Chapter 2). Despite a national bovine brucellosis eradication scheme (Chapter 2), Gauteng province showed no significant change in prevalence of *Brucella* reactor herds or within-herd seroprevalence from 2013 to 2018 (Chapter 3).

There has been an increase in the number of emerging cattle farmers in SA post-1994, who have needed government support to establish their farming enterprise. In Gauteng, these government-sponsored farms were more likely to be reactor herds (Chapter 4). Furthermore, the presence of antelope on a farm was identified as a risk factor for being a reactor herd. These two risk factors suggest that socio-political and environmental variables may be playing a role in the persistence of bovine brucellosis in the province (Chapter 4). Finally, beef herds were more affected than dairy herds and herds managed as open herds, allowing movement of cattle into the herd were more likely to be infected (Chapter 4).

Bovine brucellosis seemed to manifest more as chronic herd infection in Gauteng (Chapter 4) with farmers experienced the effect as weak calves or a drop in the number of calves born in the year prior to the herd test. Abortions were also reported in this period and were associated with reactor herds vs non-reactor herds, which may be partly accountable for the drop in calving rate.

It is likely that government supported farmers as well as existing farmers are reluctant to slaughter infected cattle perhaps due to a perceived loss of income as opposed to selling weaker offspring from infected cows or selling low producers. It is also quite likely that government sponsored emerging farmers are owners of smaller herds and are experiencing a higher within-herd prevalence of brucellosis compared to owners of larger herds (Chapter 3). It is also possible that these farmers are practising an open herd management style, being financially dependent on continuing trading cattle of, irrespective of an unknown brucellosis status, to build up their herd (Chapter 4). This practise may be increasing the within-herd prevalence. A higher within-herd prevalence may make quarantine and slaughter unfeasible to farmers of smaller herds. It is possible that farmers respond to this conundrum by supporting bovine brucellosis control efforts to access free distribution of RB51 due to the perceived protection this affords a herd. These perceptions may create a situation of non-compliance to

herd quarantine, testing, branding and slaughter of infected cattle - activities needed for effective control of the disease in the herd. However, an urgent need by farmers and other economic stakeholders to increase herd productivity may result in pressure being put on state veterinary services to supply vaccination to reduce infection in the herd. A trigger for greater distribution of RB51, would be an increase in the number of bovine brucellosis outbreaks reported, which is also reflective of a possible situation of non-compliance to veterinary regulatory activities, or the non-implementation of these activities.

The zoonotic potential of *B. abortus* may be playing a fundamental role in survival of the pathogen, through the effect of disease human behaviour. Firstly, as described above, the productivity of the herd is affected to such a point that it is not economically feasible for the farmer to cull the infected animals. Should herd production increase and control measures be taken to eradicate *B. abortus*, those persons responsible for testing, vaccinating or slaughter are infected with the pathogen. When this is not detected, diagnosed and treated, these persons are decapacitated to follow through with regular routine testing, vaccination or slaughter of infected cattle, needed to eradicate the disease from the herd. This also reflects the problem of human resources in the province, as in a perfect world each sick leave should be replaced by an alternative.

Farmers of reactor herds reported brucellosis-like symptoms in their farm workers significantly more than farmers of non-reactor herds. Brucellosis symptoms cause increased absenteeism, psychological disorders and physical disorders (Chapter 1 & 2) all of which could pose a barrier to implementing regulatory control measures according to a routine programme which presupposes a mentally and physically healthy individual. Furthermore, symptoms of brucellosis in humans are non-specific (Chapter 2). A lack awareness of the zoonotic risk of bovine brucellosis and symptoms of disease in cattle or people may lead to infected persons ignoring early symptoms of disease and delay in seeking out medical treatment (Chapter 5). The attitude of occupationally exposed persons, especially veterinary officials, to symptoms experienced may be that symptoms are not considered serious enough to incur the medical costs or time away from work. *B. abortus* can thus evade medical detection in this way as well as within an infected persons' body. With decreasing ability of occupationally exposed persons to control the disease in cattle, *B. abortus* stays within herds or spreads silently through herds, surviving generation after generation of cattle and cattle farmers in SA.

Although the presence of one or more brucellosis-like symptoms were not significantly associated any stage in the evolution of infection, the observation of brucellosis-like symptoms

amongst farm workers, made by farmers of reactor herds should not be overlooked. Farmers were asked to recall symptoms prior to the final herd test, whilst farm workers (and veterinary officials) were asked if they had experienced any symptoms within the six months prior to the interview to reduce recall bias. Seropositive persons may have experienced symptoms before then. Furthermore, evidence of generalized aching and joint pain being associated with infection of short and long duration specifically (Chapter 5) and reported symptoms associated with titres of these that tested BrucellaCapt seropositive (Chapter 5) suggests the presence of brucellosis amongst farm workers and veterinary officials.

Furthermore, 53% (8/15) of BrucellaCapt seropositive persons reported to either pray, self-medicate or ignore brucellosis like symptoms experienced instead of visiting a clinic, doctor or hospital. This may indicate a proportion of undetected and untreated clinical cases of brucellosis amongst this group. Another unexpected finding was that different risk factors were associated with the different stages of infection evolution, hinting at the complex and multifactorial nature of the disease.

Overall, this suggests a complex feedback system organised around the survival of *B. abortus* in cattle. Furthermore, there may be a maintenance cycle between domestic cattle and antelope, ensuring a source of cattle reinfection and *B. abortus* proliferation should the opportunity present itself. If considered from the perspective of *B. abortus*, this is a well organised sustainable system. If we consider that *B. abortus* infection in people may be contributing to a diminishing health seeking behaviour in this host, the overall effect of this system would be increasing reports of bovine brucellosis but little increase in cases of human brucellosis (Figure 5). According to the system construct this discord between bovine and human brucellosis reported will drive diminishing prioritization of bovine brucellosis control, resulting in a stable endemic state of brucellosis in a country, with ongoing financial and health burdens.

The implication of this being the possible mechanism underlying the low number of human brucellosis cases reported, is that the future of the animal health system in Gauteng is not sustainable. This is because of the veterinary animal health system's dependence on the health of farm workers and veterinary officials, who are responsible for more than bovine brucellosis control. These occupationally exposed persons are on the forefront of detection and response to emergent zoonotic diseases of public health importance, as well as animal disease of economic importance. The future of animal health depends on the health of these persons. Productivity follows health, in both people and animals. In striving for sustainable management

of animal health in Gauteng, it therefore seems feasible to invest in early detection, treatment and management of brucellosis in occupationally exposed persons.

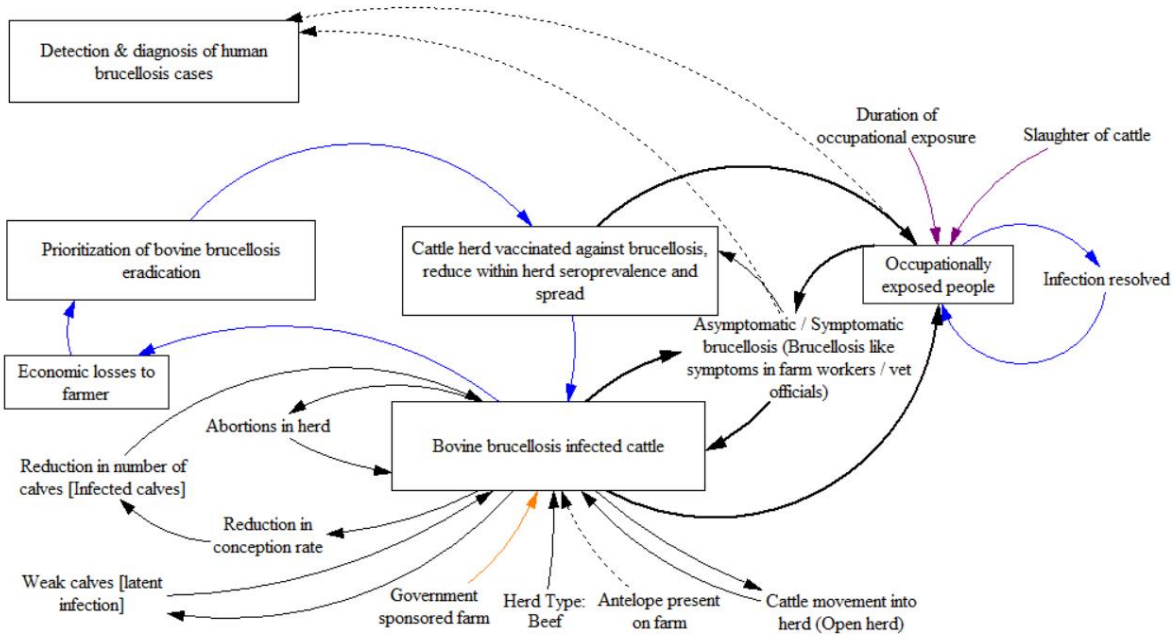

Figure 6.5: *B. abortus* persistence and survival (black arrows) within the human-cattle-farm interface system

A One Health systems approach to the epidemiology, management and regulatory control of bovine brucellosis at the human-cattle-farm interface: a framework for transdisciplinary research

Gaps identified as a result of this study can also be illuminated using the constructed systems description from the previous sections (Figures 1 -4). Due to the complexity of bovine brucellosis control, a more complete understanding requires interdisciplinary research. Figure 6.6 depicts the contribution that different fields of research can make to gaining a better understanding of brucellosis based on field specific research methodology resulting in quantitative evidence. Potential research questions are posed for each discipline, represented in Figure 6 as different coloured arrows.

Medical professionals (clinicians and psychologists): Pink arrows:

- What is an appropriate cut-off for commercially available serological tests for brucellosis, to detect clinical disease?

- What drives brucellosis preventative and health seeking behaviour in farm workers and veterinary officials?
- What is the progressive course of disease in *Brucella* seropositive farm workers and veterinary officials?

Public health epidemiologists: Purple arrows

- What are the risk factors for human brucellosis at the human-cattle-farm interface?
- What is the quantitative effect of brucellosis amongst seropositive farm workers and veterinary officials in terms of person-days lost working?
- What is the seroprevalence of brucellosis in the general population in SA?

Veterinary epidemiologists: Orange arrows

- What are the herd management and cattle risk factors for bovine brucellosis persistence at the human-cattle-farm interface and how do these change in response to a vaccination programme vs. test and slaughter vs. vaccination with test and slaughter?
- Evaluation and analysis of farmer data recording and management systems for bovine brucellosis prevention and control in their herd
- Social network analysis of markets and trading of unknown *Brucella* status beef cattle in Gauteng

Social scientists / economists: green arrows

- What are the economic costs vs benefits to the farmer by participating in a bovine brucellosis control programme?
- How will evidence of bovine brucellosis eradication progress affect the economics of animal disease control and animal health programmes?
- What are cattle traders' perceptions of the public health risk of bovine brucellosis?

Social scientists / policy and governance: blue arrows

- What is the effect of evidence of brucellosis on changes in endemic zoonotic disease control policy?
- How effective are zoonotic disease control policies that are not based on recent accurate data of disease?

Social scientists / social science: blue arrows

- Is there a relationship between risk knowledge and overconfidence or mistreatment of symptoms and the worsening of psychological health due to *B. abortus* infection?

- Mapping outrage factors and behaviour towards brucellosis risk: from personal risk (mis-) control to loss of control of the veterinary and human public health situation.

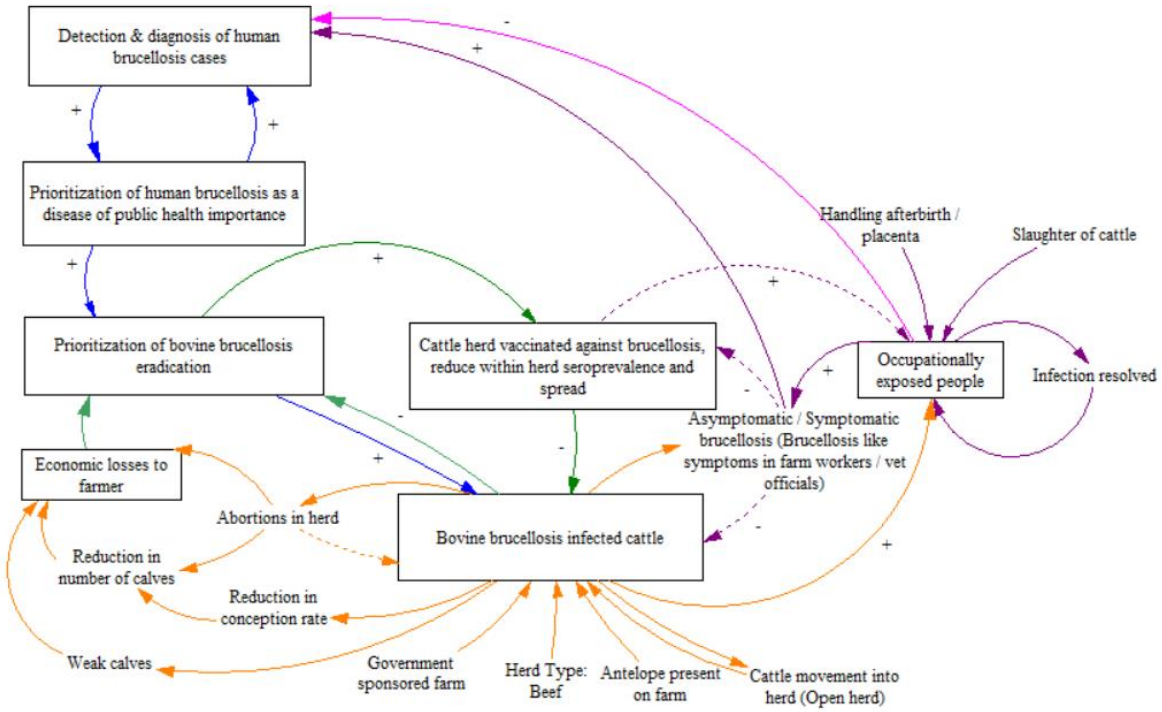

Figure 6:6: A systems approach to the epidemiology, management and regulatory control of bovine brucellosis at the human-cattle-farm interface in Gauteng, SA

One Health as a concept and methodology is based on the understanding that there are significant benefits arising from a breakdown of barriers between disciplinary silos. These benefits extend beyond the effect on cattle and human health at the human-cattle-farm interface, to economic benefits shared by the human and animal health sector, public and private (farmers) sector. These benefits that can be quantified economically as the amount of cost that would be averted in the absence of disease (Roth et. al., 2003). In South Africa, majority of the cost to prevent human brucellosis has been incurred by the public and private animal health sector. However, findings from this study suggest a hidden cost to the health

sector, public and private sector resulting from undiagnosed brucellosis in people. These costs result from having to incur the cost of medication or hospitalisation and working days lost due to the disabling effect of brucellosis, increasing the risk of poverty in the affected households (Roth et. al, 2013).

The added value of a One Health approach to bovine brucellosis in SA stems firstly from an understanding that costs of an intervention, such as mass vaccination of cattle to reduce the within-herd prevalence or compensation of farmers engaging in test and slaughter to eradicate bovine brucellosis from their herds (Chapter 3), can be proportionately shared across the sectors that benefit from the control of bovine brucellosis (Roth et. al., 2013). Secondly, a One Health approach assumes a culture of transparency, harmonization within and between sectors resulting in the timeous sharing of data and information between the animal and human health sectors and public and private (farmers) sectors. This is necessary for early detection and response to both bovine and human brucellosis. Information on the distribution and prevalence of bovine brucellosis (Chapter 3), increases the medical sector's understanding of the risk of human brucellosis, whilst information on the prevalence and effects of human brucellosis increases the private and public sector's awareness of the need to prioritize bovine brucellosis eradication and increase food safety measures. Finally, a One Health approach has been reported to have significant social psychological and cognitive benefits (personal communication during Transdisciplinary Workshop held at Future Africa, Pretoria, 2020) due to multiple disciplines and expertise working together toward a common goal for the common benefit of all. With this in mind, we recommend a One Health systems approach to the epidemiology, management, and regulatory control of bovine brucellosis at the human-cattle-farm interface in South Africa.

Study limitations

This study considered only cattle herds (and associated persons) that participated in the provincial veterinary services' bovine brucellosis control programme. As such, it is not representative of all persons occupationally exposed to cattle herd or all cattle herds in Gauteng, SA or beyond. Furthermore, participation was voluntary and at the convenience of the participants. This may have resulted in farm workers or veterinary officials not being included in the sample due to them not being available on the day of testing or an over representation of persons who had a greater interest in the study than those who did not participate. Furthermore, it was most unlikely to have sampled a cattle handler on a farm or a veterinary official, if that person had acute or subacute brucellosis, as this condition may have resulted in the person not

being available on the day of sampling. This therefore biases the results to represent those that are currently asymptomatic, which can either be recovered, or in the subclinical or convalescent form of chronic brucellosis.

Only one sample per cattle handler was collected, therefore, it is very difficult to interpret if there was progressive disease in those that were seropositive to the tests used. In addition, the method of inquiry, being in the form of structured questionnaires, focussed on clinical symptoms associated with clinical forms of brucellosis and did not allow for more symptoms, or syndromes that farm workers and veterinary officials might have experienced (such as cognitive impairment), being documented. A more open form of enquiry might have elicited the experience of symptoms more associated with chronic and subclinical brucellosis, such as those more related to the psychological symptoms experienced by those that were refractory to treatment or chronic brucellosis cases.

Questions to farm managers and persons screened for brucellosis were based on participant recall of events following a herd diagnosis of brucellosis. Despite trying to keep the time frame within a two-year period, participants were likely to have experienced recall bias. This was most evident with farm managers, who remembered experiencing a change in herd productivity, but could not remember "the exact numbers". The study was dependent on farmers' observation of herd symptoms. We did not pursue an examination or evaluation of data recorded by the farmer, due to some farmers having little to no data recording and management practises, the non-standardization of herd management and production data recording systems and the restricted time for the study not allowing for a perusal of existing farmer herd data. Despite the advantages that an interdisciplinary study has, with regard to being able to administer questionnaires focussed on different fields of study simultaneously during a single field visit, participation involved the entire farm workforce, which halted work output for the duration of the visit.

With regard to the sample frame of cattle herds to be included in the study, the electronic recording of laboratory test results database could not provide an accurate sampling frame of cattle farmers and herds in the province. Therefore, a cross-sectional study to determine the true cattle and herd brucellosis prevalence or to assess the representativeness of the laboratory-based surveillance trends could not be properly done. Management of bovine brucellosis control data was partly electronic, but mostly paper based in files located in three different locations across the province. Due to time constraints, it was not possible to capture all the relevant data to construct a sample frame from the filed information. Furthermore, there

was reluctance amongst some state veterinarians and some of the AHTs to share information, resulting in significant time delays due to negotiating the sharing of data and information.

Lastly, despite this study being focussed on bovine brucellosis control from a One Health perspective, we did not consider the following important aspects of bovine brucellosis control: vaccination coverage, reactor herd quarantine time, frequency of herd tests, and time from detection of reactor cattle to slaughter.

Conclusion

Human brucellosis has been a public health concern in SA from as early as 1924. Analysis of laboratory test reports for bovine brucellosis between 2013-2018, indicated no progression toward eliminating the disease from cattle herds in the province. In this study, herd management risk factors associated with *Brucella* infected herds were identified. There was evidence of cattle handler exposure to *Brucella* on cattle farms in Gauteng using the RBT, IgM ELISA, IgG ELISA and BrucellaCapt as screening tests. Knowledge, and health seeking behaviour of farm workers and veterinary officials, to brucellosis were explored and we found strong evidence suggesting a proportion of undiagnosed cases of brucellosis in this group.

Variables associated with seropositivity in persons occupationally exposed to *Brucella* infected cattle herds in this study suggest a complex interaction of human, herd, socio-economic, epidemiological and sub-national disease regulatory systems. This study has integrated quantitative evidence of variables associated with bovine brucellosis and *Brucella* seropositivity in farm workers and veterinary officials into a One Health systems framework to provide a methodology for exploring the epidemiology, management and regulatory control of bovine brucellosis at the human-cattle-farm interface. We recommend a systems-thinking approach and use of this One Health framework to better manage the identified complexity to reduce bovine brucellosis in the province and prevent human brucellosis in South Africa.

This thesis contributes to the field of performance of veterinary services, occupational health, preventative veterinary medicine, public health epidemiology and the emerging field of "One Health", by illuminating the complexity that is encountered when undertaking field research into a controlled endemic neglected zoonotic disease. This study can therefore be used to inform the review and/or formulation of the bovine brucellosis public health and surveillance policies targeted specifically for the South African situation. It also contributes to informing training programmes for para-veterinarians and farm workers in SA.

This project has already impacted on the provincial stakeholders of the bovine brucellosis eradication programme, through a One Health Zoonotic disease awareness day, held in 2016 for which new awareness material was designed and distributed to farmers, veterinary officials and public health officers (APPENDICES 5a-5c) and a basic epidemiology training course for veterinary epidemiologists aimed at the detection and joint response with the medical and public health profession to an outbreak of zoonotic brucellosis, held in August 2019. Findings from this study were presented as oral presentations at two international congresses and six national congresses and has been used to create awareness amongst undergraduate medical students on the role of the doctor in the detection and diagnosis of brucellosis in high risk occupational groups. It serves as an example of interdisciplinary collaborative research and learning to collectively move towards the elimination and eradication of bovine brucellosis in SA. It also serves to guide the transdisciplinary approach to the detection and response to emerging, re-emerging and endemic zoonotic diseases in Southern Africa towards a transdisciplinary approach.

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APPENDICES

APPENDIX 1: Bovine brucellosis herd management case-control questionnaire

Management & Epidemiology of Bovine Brucellosis in Gauteng, 2014- 2016

INFORMED CONSENT

Dear Participant

I am a PhD student from the School of Veterinary Tropical Diseases, University of Pretoria. You are invited to volunteer to participate in this survey:

Management & Epidemiology of Bovine Brucellosis in Gauteng, 2014-2016

This letter gives information to help you to decide if you want to take part in this study. Before you agree you should fully understand what is involved. If you do not understand the information or have any other questions, do not hesitate to ask us. You should not agree to take part unless you are completely happy about what we expect of you.

The purpose of this survey is to determine:

(1) The risk of your herd getting or maintaining Brucellosis
(2) The risk of you/your co-workers/your employees getting or having Brucellosis
(3) Your current knowledge and understanding of Brucellosis

Your participation will help us to:

(1) Identify the most important risk factors to control in order to reduce Brucellosis in your herd, and/or prevent your herd being infected in the future.
(2) Determine the extent of Brucellosis in people.
(3) Target education/awareness campaigns to capacitate you/your co-workers your family and other farmers/farmworkers to detect, respond to, and most IMPORATANTLY to PREVENT Brucellosis in your animals and the people you know.

I am going to ask you some questions in order to complete a questionnaire as well as request a blood sample from you. We will let you know the result of your brucella blood test and if it is positive, you will receive a referral letter from the medical practitioner of the research team for further medical management. The questionnaire will take approximately 15 minutes to complete.

The questionnaire will be kept in a safe place to ensure confidentiality. Your identity and the name of your farm will not be used in any analysis or reports. I will be available to help you with the questionnaire or to fill it in on your behalf.

In order to determine if Brucellosis is infecting people, we do a test on the blood of a person to see if his/her body has detected and responded to the brucellosis bacteria by looking for the presence of antibodies to the brucellosis bacteria. The person drawing your blood is a qualified medical doctor. There are two tests that will be done on you blood: a serological test for Brucellosis and Leptospirosis. Leptospirosis is another bacterium that you can get from handling animals, and we want to make sure that if you did feel sick, it was not due to these two organisms.

Your participation in this study is voluntary. Compensation will not be given for participation and there are no foreseeable risks and no direct personal benefit for the study to the participants. You can refuse to participate or stop at any time without giving any reason. The information you give us remains anonymous. Once you have given the questionnaire back to us, you cannot recall your consent. You will not be identified as a participant in any publication that comes from this study.

Note: The implication of completing the questionnaire is that informed consent has been obtained from you. Thus any information derived from your form (which will be totally anonymous) may be used for e.g. publication, by the researchers. We sincerely appreciate your help.

The Research Ethics committee of the University of Pretoria granted ethics approval for this study.

Yours truly,

_____

Dr. K. Govindasamy (BVSc, MPH)

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

FOR OFFICE USE ONLY:

(To be filled out & registered on Case Control Database by Investigator before distribution to Field Technician):

(O.2) Farm Study Status (please mark with X):

Case Farm (2 or more serologically positive cattle in herd on confirmatory CFT where vaccination is unlikely to be the cause)	
Control Farm (A herd where all cattle tested negative within 6 months of a case herd (see above definition) within the same State Vet Area & in the absence of any clinical signs of brucellosis within the herd during those 6 months)	

(O.1) Unique Record #

(O.3) Reference Date
(Date of herd test)

(O.4) Laboratory #

D_____

(O.5) Where cattle vaccinated within the 2 year period before the Reference Date (O.3) Y/N?

(O.6) FARM DETAILS (From CAS/laboratory sample submission form)

O.6.1	FARM NAME + FARM ID			
O.6.2	STREET			
O.6.3	SUBURB			
O.6.4	DISTRICT			
O.6.5	STATE VET AREA			
O.6.6	PROVINCE			
O.6.7	POSTAL CODE			
O.6.8	GPS EAST			
O.6.9	GPS SOUTH			

(O.7) CONTACT PERSON DETAILS (From CAS/laboratory sample submission form)

O.7.1	FIRST NAME	
O.7.2	SURNAME	
O.7.3	CELL NUMBER	
O.7.4	LANDLINE	
O.7.5	EMAIL ADDRESS	

(O.8) INTERVIEWER DETAILS (Please mark with an X who will be conducting the interview. Please fill in the cell number of the person selected)

O.8.1	AHT NAME		
O.8.2	STATE VET		
O.8.3	CELL NUMBER		

(O.9) RECRUITED? (Y/N)

(O.9) INTERVIEW DATE

TO BE COMPLETED BY AHT/STATE VET CONDUCTING THE FIELD INVESTIGATION

(O.10.1) Is the information captured above (O.2 – O.7) complete AND correct? Y/N

(O.10.2) If No, please complete AND correct

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(A) INTERVIEWEE DETAILS

(A.1) PERSON INTERVIEWED (Must be in control/manager of cattle and present during period of outbreak for a case farm)

(A.1.1)FIRST NAME	(A.1.2) SURNAME	(A.1.3) CELL NUMBER	(A.1.4) EMAIL

(A.2) STATUS (Please mark with an X)

A.2.1	OWNER	
A.2.2	MANAGER	
A.2.3	EMPLOYEE	
A.2.4	FAMILY MEMBER	
A.2.5	OTHER, Please specify beneath:	

(B) HERD DETAILS

(B.1) HOLDINGS (Please complete the table below: each row is a holding on which the owner's cattle are kept)

(B.1.1) HOLDING CURRENTLY ON

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)
1				

(B.1.2.1) IS THIS THE MAIN HOLDING? (Y=Yes, N=No, U=Unknown) ☐

(B.1.2.2) IF NO, PLEASE STATE THE FARM NAME, FARM NUMBER AND PLOT NUMBER OF MAIN HOLDING: FARM NAME:_____FARM NO._____PLOT NO._____

(B.1.3) OTHER HOLDINGS WHERE YOU KEEP YOUR CATTLE

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark with an X. Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)	Km from B.1.1 (main holding)
2					
3					
4					
5					

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(A) INTERVIEWEE DETAILS

(A.1) PERSON INTERVIEWED (Must be in control/manager of cattle and present during period of outbreak for a case farm)

(A.1.1)FIRST NAME	(A.1.2) SURNAME	(A.1.3) CELL NUMBER	(A.1.4) EMAIL

(A.2) STATUS (Please mark with an X)

A.2.1	OWNER	
A.2.2	MANAGER	
A.2.3	EMPLOYEE	
A.2.4	FAMILY MEMBER	
A.2.5	OTHER, Please specify beneath:	

(B) HERD DETAILS

(B.1) HOLDINGS (Please complete the table below: each row is a holding on which the owner's cattle are kept)

(B.1.1) HOLDING CURRENTLY ON

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)
1				

(B.1.2.1) IS THIS THE MAIN HOLDING? (Y=Yes, N=No, U=Unknown) ☐

(B.1.2.2) IF NO, PLEASE STATE THE FARM NAME, FARM NUMBER AND PLOT NUMBER OF MAIN HOLDING: FARM NAME:_____FARM NO._____PLOT NO._____

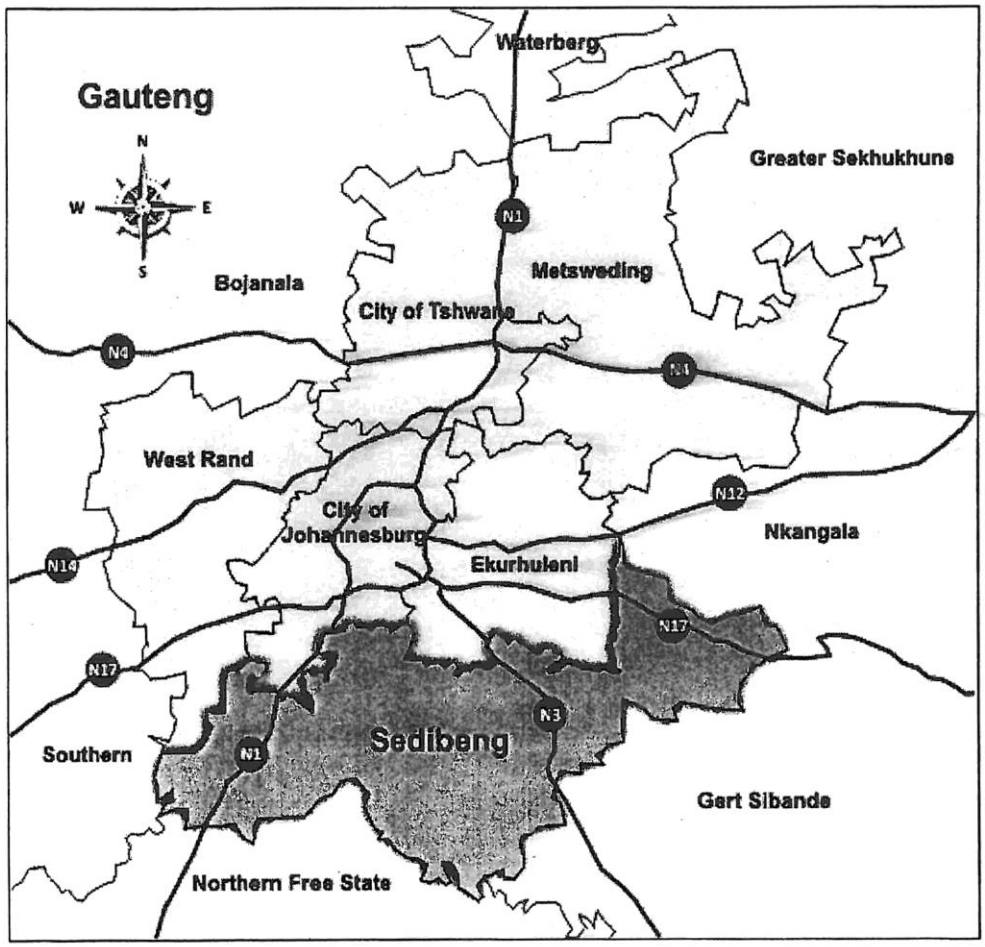
(B.1.3) OTHER HOLDINGS WHERE YOU KEEP YOUR CATTLE

No.	Farm/Plot Name	Total No. of Cattle	Was Brucellosis diagnosed on this farm within the last 5 years? (Please mark with an X. Y=Yes, N=No OR U=Unknown)	Is this a government sponsored farm/project? (Please mark Y=Yes, N=No OR U=Unknown)	Km from B.1.1 (main holding)
2					
3					
4					
5					

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

6					

(B.1.4) Please mark the above farms/plots on the map provided (indicate the approximate spatial position using the numbers of the farm from B.1.1 and B.1.2. You can use arrows and mark each arrow with the correct farm/plot number. If it is OUTSIDE the province, please mark the holding # (B.1) OUTSIDE the map border



BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(B.2) TYPE OF HERD

B.2	TYPE OF HERD (Multiple herds may be selected. Please mark the appropriate herds with an X)		ON WHICH FARMS/PLOTS MENTIONED ABOVE (B.1.1 & B.1.2) ARE THE SELECTED HERDS FARMED?					
			1	2	3	4	5	6
B.2.1	STUD HERD							
B.2.1.1	DAIRY							
B.2.1.2	BEEF							
B.2.2	MIXED HERD							
B.2.3	COMMERCIAL HERD							
B.2.4	COMMUNAL GRAZING							
B.2.5	SPECULATOR HERD							
B.2.6	OTHER, PLEASE SPECIFY BENEATH							

(B.3) CATTLE BREED (Please mark with an X)

B.3.1	FRESIAN	
B.3.2	JERSEY	
B.3.3	BONSMARA	
B.3.4	BRAHMAN	
B.3.5	NGUNI	
B.3.6	OTHER, PLEASE SPECIFY BENEATH	

(B.4.1) HERD STRUCTURE ON REFERENCE DATE (see O.3) (Numbers 1 – 6 below refer to farms/plots from B.1.1 and B.1.2)

B.4.1	CATTLE	NO. OF CATTLE ON REFERENCE DATE ON FARMS/PLOTS					
		1	2	3	4	5	6
B.4.1.1	CALVES						
B.4.1.2	HEIFERS						
B.4.1.3	COWS (2-5YEARS)						
B.4.1.4	COWS (5-10YEARS)						
B.4.1.6	COWS (>10YEARS)						
B.4.1.7	BULLS						

(B.4.2) HERD STRUCTURE AT PRESENT (Numbers 1 – 6 below refer to farms/plots from B.1.1 and B.1.2)

B.4.2	CATTLE	NO. OF CATTLE AT PRESENT ON FARMS/PLOTS					
		1	2	3	4	5	6
B.4.2.1	CALVES						
B.4.2.2	HEIFERS						
B.4.2.3	COWS (2-5YEARS)						
B.4.2.4	COWS (5-10YEARS)						
B.4.2.6	COWS (>10YEARS)						
B.4.2.7	BULLS						

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(B.4.3) Total Number of OTHER animals on the farm at present:

Sheep	Goats	Pigs	Wildlife-Buffalo	Wildlife-Antelope

(C) MOVEMENT

(C.1) Total No. of cattle introduced in the 12 months before the reference date (see O.3) ☐

(C.2) No. of introductions into herd (WHERE INTERVIEW IS TAKING PLACE) in the 12 months before the reference date (see O.3) (i.e. Number of events when cattle were introduced into the herd) ☐

(C.3) MOVEMENT IN

	SOURCE	No. of Cattle	District	Province
C.3.1.1	Farm – Neighbour			
C.3.1.2	Farm - <20km away			
C.3.1.3	Farm - >20km away			
C.3.2	Auction			
C.3.3	Speculators			
C.3.4.1	Communal grazing – common herd (shared grazing)			
C.3.4.2	Communal herd - <30km away (your cattle do NOT share grazing with this herd)			
C.3.4.3	Communal grazing - >30km away			

(C.4) Were the cattle tested for brucellosis after their arrival? (Y=Yes, N=No, U=Unknown) ☐

(C.5) Were the cattle vaccinated before arrival onto your farm? (Y=Yes, N=No, U=Unknown) ☐

(C.5.1) If Y, what vaccine was used?

S19 ☐	RB51 ☐	S19 & RB51 ☐	UNKNOWN ☐

(C.6.1) Were the cattle isolated after arrival? (Y=Yes, N=No, U=Unknown) ☐

(C.6.2) If Y, for how long ____days

(C.7) Do you check the brucellosis status of the herds you purchase your cattle from before purchase? (Y=Yes, N=No, U=Unknown) ☐

(C.8) Distance to the nearest infected herd? _____km | unknown ☐

(C.9) Were any of your neighbouring farms positive for brucellosis within the last 3 years? (Y=Yes, N=No, U=Unknown) ☐

(C.10) Do any of your neighbours keep wildlife on their farm? (Y=Yes, N=No, U=Unknown) ☐

(C. 11) Do any of your workers own their own cattle? (Y=Yes, N=No, U=Unknown) ☐

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(C.11.1)If Yes, please fill the following table:

NAME OF EMPLOYEE	NO. OF CATTLE	GRAZES WITH YOUR HERD? (Y=Yes, N=No, U=Unknown)

(D) MANAGEMENT

(D.1) BREEDING

D.1 Do you...(Y=Yes, N=No, U=Unknown)

D.1.1	Use bull from another herd?	
D.1.2	Use AI and bull?	
D.1.3	Use AI only?	
D.1.4	Use bull from your own herd?	

(D.2) WHICH OF THE FOLLOWING DID YOU EXPERIENCE IN THE YEAR BEFORE THE REFERENCE DATE? (Please mark with an X)

D.2.1	ABORTIONS IN HERD	
D.2.2	WEAK CALVES	
D.2.3	RETAINED PLACENTAS	
D.2.4	REDUCTION IN NUMBER OF CALVES	
D.2.5	REDUCTION IN MILK YEILD	
D.2.6	REDUCTION IN CONCEPTION RATE	
D.2.7	HYGROMAS IN CATTLE	
D.2.8	REPORTS OF BRUCELLOSIS ON NEIGHBORING FARMS	
D.2.9	MORE THEN 3 MONTHLY CONSECUTIVE POSITIVE MRTS	

(D.3.1) WHICH OF THE FOLLOWING SYMPTOMS WERE EXPERIENCED BY ANY PERSON ON THE FARM/S WITHIN THE LAST YEAR? (Please mark with an X)

D.3.1.1	FEVER	
D.3.1.2	ANOREXIA(no appetite & not eating)	
D.3.1.3	FATIGUE(tiredness & sleepiness)	
D.3.1.4	HEADACHE	
D.3.1.5	DEPRESSION	
D.3.1.6	ANTHRALGIA(i.e. joint pain)	
D.3.1.7	MYALGIA(i.e. muscle pain)	
D.3.1.8	BACK PAIN	
D.3.1.9	EPISODES OF PROFUSE SWEATING	

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

(D.3.2) If Y, to any of the above symptoms (D.3) how would you classify this person/these persons? (Select most appropriate option by marking with an X. If more than one person per category, state the number of persons showing any of the above symptoms)

Family Members	Employees	Family members of Employees

(D.3.2.1) What sort of animal contact do the people indicated in D.3.2 have with cattle?
(Please specify Y/N/U for each option. Y=Yes, N=No, U=Unknown, NA=Not Applicable)

D.3.2.1.1	CONSUME UNPASTEURISED DAIRY PRODUCTS FROM CATTLE ON FARM	
D.3.2.1.2	VACCINATE COWS WITH BRUCELLOSIS VACCINE	
D.3.2.1.3	HANDLE COWS DURING BIRTH PROCESS	
D.3.2.1.4	OTHER, PLEASE SPECIFY BENEATH	

(D.4) CALVING PRACTICES

D.4 Please answer each of the following (Please specify Y/N/U for each option. Y=Yes, N=No, U=Unknown, NA=Not Applicable)

D.4.1	Do you segregate cows at calving?	
D.4.2	Do dry cows calve together?	
D.4.2.1	If Y, do they calve indoors?	
D.4.2.1.1	If Y, do they calve in individual pens?	
D.4.3	Do you observe each calving?	
D.4.4	Calving practice (management of cows during calving) other than those mentioned above? If Y, please specify beneath	

(D.5) BIOSECURITY

D.5.1 Are your cattle fenced in on your holdings? Y/N/U

D.5.2 How would you describe your cattle handling facilities? (Please mark the most appropriate option with an X)

D.5.2.1	Non-existent	
D.5.2.2	Fair	
D.5.2.3	Good	
D.5.2.4	Excellent	

D.5.3 How do you identify your cattle?

D.5.3.1	Markings only	
D.5.3.2	Ear Tags	
D.5.3.3	Branding	
D.5.3.4	Other	

[redacted]

(E.1) Has your herd been vaccinated within the last 3 years? (Y=Yes, N=No, U=Unknown) ☐

(E.1.1) If Y, please select the vaccine used:

BRUCELLOSIS IN CATTLE: CASE CONTROL STUDY, 2014-2015
QUESTIONNAIRE

S19 ☐	RB51 ☐	S19 & RB51 ☐	UNKNOWN ☐

(E.2) Have your positive cattle ever been C-branded? (Y=Yes, N=No, U=Unknown)
(E.3) Have you received training in Brucellosis control and management? (Y=Yes, N=No) ☐☐

(E.4) Have your workers received training in Brucellosis control and management within the last 3 years? (Y=Yes, N=No, U=Unknown) ☐

(E.4.1) If Y, who conducted the training?

THANK YOU FOR YOUR TIME AND INPUT. YOUR CONTRIBUTION IS VERY IMPORTANT IN UNDERSTANDING BRUCELLOSIS IN ORDER TO CONTROL IT.

(F.1) WOULD YOU LIKE FURTHER INFORMATION ON HOW TO CONTROL BRUCELLOSIS IN YOUR HERD? (Y=Yes, N=No) ☐

INTERVIEWER NAME & SURNAME _____

INTERVIEWER SIGNATURE _____

DATE OF INTERVIEW _____

APPENDIX 2: Zoonotic study knowledge and risk factors questionnaire

INFORMED CONSENT

Dear Participant

I am a PhD student from the School of Veterinary Tropical Diseases, University of Pretoria. You are invited to volunteer to participate in this survey:

BRUCELLOSIS IN GAUTENG, 2014-2016

This letter gives information to help you to decide if you want to take part in this study. Before you agree you should fully understand what is involved. If you do not understand the information or have any other questions, do not hesitate to ask us. You should not agree to take part unless you are completely happy about what we expect of you.

The purpose of this survey is to determine:

(1) The risk of your herd getting or maintaining Brucellosis
(2) The risk of you/your co-workers/your employees getting or having Brucellosis
(3) Your current knowledge and understanding of Brucellosis

Your participation will help us to:

(1) Identify the most important risk factors to control in order to reduce Brucellosis in your herd, and/or prevent your herd being infected in the future.
(2) Determine the extent of Brucellosis in people.
(3) Target education/awareness campaigns to capacitate you/your co-workers your family and other farmers/farmworkers to detect, respond to, and most IMPORATANTLY to PREVENT Brucellosis in your animals and the people you know.

I am going to ask you some questions in order to complete a questionnaire as well as request a blood sample from you. We are going to take 5 ml of your blood. All unused blood will be discarded according to strict biosecurity measures at the National Institute for Communicable Diseases Laboratory. We will let you know the result of your brucella blood test and if it is positive, you will receive a referral letter from the medical practitioner of the research team for further medical management. The questionnaire will take approximately 15 minutes to complete.

The questionnaire will be kept in a safe place to ensure confidentiality. Your identity and the name of your farm will not be used in any analysis or reports. I will be available to help you with the questionnaire or to fill it in on your behalf.

In order to determine if Brucellosis is infecting people, we do a test on the blood of a person to see if his/her body has detected and responded to the brucellosis bacteria by looking for the presence of antibodies to the brucellosis bacteria. The person drawing your blood is a qualified medical doctor. There are two tests that will be done on you blood: a serological test for Brucellosis and Leptospirosis. Leptospirosis is another bacterium that you can get from handling animals, and we want to make sure that if you did feel sick, it was not due to these two organisms.

Your participation in this study is voluntary. Compensation will not be given for participation and there are no foreseeable risks and no direct personal benefit for the study to the participants. You can refuse to participate or stop at any time without giving any reason. The information you give us remains anonymous. Once you have given the questionnaire back to us, you cannot recall your consent. You will not be identified as a participant in any publication that comes from this study.

Note: The implication of completing the questionnaire is that informed consent has been obtained from you. Thus any information derived from your form (which will be totally anonymous) may be used for e.g. publication, by the researchers. We sincerely appreciate your help.

The Research Ethics committee of the University of Pretoria granted ethics approval for this study.

Yours truly,

Dr. K. Govindasamy (BVSc, MPH)

Page 1 of 2

INFORMED CONSENT

I hereby confirm that I have been informed by the investigator, Dr Govindasamy, about the nature, conduct, benefits and risks of the survey. I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding the investigation.

I am aware that the results of the survey, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a survey report.

I may, at any stage, without prejudice, withdraw my consent and participation in the survey. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the survey.

Patient's name _____
(Please print)

Patient's signature _____ Date _____

I, Dr _____ herewith confirm that the above participant has been informed fully about the nature, conduct and risks of the above survey.

Investigator's name _____
(Please print)

Investigator's signature _____ Date _____

Witness's name* _____ Witness's signature _____ Date _____
(Please print)

*Consent procedure should be witnessed whenever possible.

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Zoonotic Study Interview Unique Number:
Date:__/__/____
Case Farm Unique Number:_____
Aht Official :_____ Ehp Official_____

1. Demographics of farm worker

(1.1) PERSONEL DETAILS

NAME			
SURNAME			
LANDLINE			
CELL PHONE			
ADDRESS			
DATE OF BIRTH			
SEX	MALE	FEMALE	
EDUCATION	PRIMARY		
	SECONDARY		
	TERTIARY		
	OTHER TRAINING		

(1.2) OCCUPATION (Please select the most appropriate)

FARM OWNER	
FARM MANAGER	
FARM WORKER	
FAMILY MEMBER OF FARMER	
OTHER	

(1.3.1) If Other, please specify

(1.4) Do you live on this farm? Y/N
(1.4)1. If Y, how many live within your house? ☐
(1.4)2. How many of these are children less than 18 years? ☐

(1.5) Do you own cattle? Y/N
(1.5)1. If Y, how many of your cattle graze on this farm? ☐
(1.5)2. How many of your cattle graze on other farms? ☐

(1.6) Do you work with cattle off this farm? Y/N ☐

2. CLINICAL SYMPTOMS & HEALTH SEEKING BEHAVIOUR

(2.1.1) ARE YOU CURRENTLY ON MEDICATION? YES/NO

(2.1.2) IF YES, PLEASE SPECIFY _____

_____

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(2.2) HAVE YOU EXPERIENCED ANY OF THE FOLLOWING SYMPTOMS WITHIN THE LAST 6 MONTHS?

FEVER	
SWEATING	
NIGHT SWEATING	
FATIGUE	
ANOREXIA	
WEIGHT LOSS	
HEADACHE	
PAIN IN YOUR JOINTS	
GENERALIZED ACHING	

(2.3) HOW FREQUENTLY DO YOU EXPERIENCE THE ABOVE SYMPTOMS?

(2.4) WHAT DO YOU DO WHEN/IF YOU EXPERIENCE ANY OF THE ABOVE SYMPTOMS?

VISIT LOCAL CLINIC	
VISIT GP	
GO TO HOSPITAL	
VISIT TRADITIONAL HEALER	
PRAY/VISIT CHURCH/PASTOR	
NOTHING, I JUST IGNORE IT	
SELF MEDICATE	

(2.5) IF SELF MEDICATE, PLEASE SPECIFY_____

(2.6.1) WHEN/IF YOU VISIT A HEALTH PROFESSIONAL, DOES S/HE ASK YOU IF YOU HAVE BEEN IN CONTACT WITH ANIMALS? YES/NO

(2.6.2) WHEN/IF YOU VISIT A HEALTH PROFESSIONAL, DO YOU INFORM HIM/HER THAT YOU SUSPECT IT COULD BE BRUCELLOSIS? YES/NO

(2.7) HOW FAR IS YOUR CLOSEST CLINIC/HEALTH FACILITY? _____KM

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(2.8) HOW DO YOU GET TO YOUR HEALTH FACILITY?

DRIVE	
WALK	
TAXI	
LIFT	

3. EXPOSURE RISK FACTORS

(3.1). **THIS QUESTION APPLIES TO YOU HANDLING CATTLE ON THIS FARM.** PLEASE ANSWER THE FOLLOWING QUESTIONS (Y=YES, N=NO, NA=NOT APPLICABLE)

DO YOU DRINK RAW (UNPASTEURIZED) MILK	
DO YOU EAT UNPASTEURIZED CREAM/CHEESE	
DO YOU HANDLE COWS AT CALVING	
DO YOU HANDLE AFTERBIRTH/PLACENTA	
DO YOU HANDLE NEWBORN CALVES	
DO YOU VACCINATE CATTLE FOR BRUCELLOSIS	
DO YOU MILK COWS	
DO YOU TRANSPORT MILK	
DO YOU TRANSPORT LIVE ANIMALS	
DO YOU EAT DURING WORKING HOURS	
DO YOU WASH YOUR HAND AFTER ANIMAL HANDLING?	
DO YOU SLAUGHTER CATTLE	

(3.2) HOW LONG HAVE YOU WORKED ON THIS FARM? _____(MTHS/YRS)

(3.3) ANSWER THIS QUESTION ONLY IF YOU OWN YOUR OWN CATTLE. **THIS QUESTION APPLIES TO YOU HANDLING CATTLE THAT YOU OWN.** PLEASE ANSWER THE FOLLOWING QUESTIONS (Y=YES, N=NO, NA=NOT APPLICABLE)

DO YOU DRINK RAW (UNPASTEURIZED) MILK	
DO YOU EAT UNPASTEURIZED CREAM/CHEESE	
DO YOU HANDLE COWS AT CALVING	

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DO YOU HANDLE AFTERBIRTH/PLACENTA	
DO YOU HANDLE NEWBORN CALVES	
DO YOU VACCINATE CATTLE FOR BRUCELLOSIS	
DO YOU MILK COWS	
DO YOU TRANSPORT MILK	
DO YOU TRANSPORT LIVE ANIMALS	
DO YOU EAT DURING WORKING HOURS	
DO YOU WASH YOUR HAND AFTER ANIMAL HANDLING?	
DO YOU SLAUGHTER CATTLE	

4. KNOWLEDGE PERCEPTIONS ATTITUDES

(5.1) RATE THE FOLLOWING IN ORDER OF IMPORTANCE TO YOU (5=MOST IMPORTANT)

MAKING A PROFIT	
MY HEALTH	
PROVIDING FOR MY FAMILY	
THE HEALTH OF MY FAMILY MEMBERS	
THE HEALTH OF MY CO-WORKERS/EMPLOYEES	

(5.2) WHAT IS YOUR OPINION ON THE FOLLOWING STATEMENTS (Y=YES, N=NO, DK=DON'T KNOW)

DRINKING RAW MILK IS SAFE AND HEALTHY	
BRUCELLOSIS CAUSES ABORTIONS IN CATTLE	
BRUCELLOSIS CAUSES CALVES TO BE BORN WEAK	
BRUCELLOSIS CAN BE IN THE HERD AND NOT CAUSE ABORTIONS	
BRUCELLOSIS CAN CAUSE DISEASE IN PEOPLE	

(5.3) PLEASE ANSWER Y/N TO THE FOLLOWING QUESTIONS

DO YOU KNOW THE SYMPTOMS OF BRUCELLOSIS IN CATTLE?	
DO YOU KNOW THE SYMPTOMS OF BRUCELLOSIS IN PEOPLE?	
DO YOU UNDERSTAND HOW BRUCELLOSIS IN A HERD RESULTS IN A DROP IN PROFIT?	
DO YOU UNDERSTAND HOW YOUR ABILITY TO WORK IS REDUCED IF YOU HAVE BRUCELLOSIS?	

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DO YOU UNDERSTAND HOW THE ABILITY OF YOUR CO-WORKERS ARE REDUCED IF THEY ARE INFECTED WITH BRUCELLOSIS?	

(5.4) WHAT DO YOU DO WHEN YOU SEE THAT THERE HAS BEEN AN ABORTION IN YOUR HERD (MARK ONLY THOSE OPTIONS WHICH APPLY TO YOU)

THROW AWAY FETUS AND DO NOTHING MORE		
REPORT IT TO FARMER/MANAGER		

WE HAVE REACHED THE END OF OUR QUESTIONNAIRE. THANK YOU FOR YOUR TIME AND INPUT. YOUR CONTRIBUTION IS VERY IMPORTANT IN UNDERSTANDING BRUCELLOSIS IN ORDER TO CONTROL IT.

(F.1) WOULD YOU LIKE FURTHER INFORMATION ON HOW TO CONTROL BRUCELLOSIS IN YOUR HERD? (Y=Yes, N=No) ☐

INTERVIEWER NAME & SURNAME

INTERVIEWER SIGNATURE

DATE OF INTERVIEW

APPENDIX 3: Animal ethics approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Epidemiology of bovine brucellosis, Gauteng, 2014-2016**
PROJECT NUMBER	**V011-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. K Govidasamy**

STUDENT NUMBER (where applicable)	**UP_99250374**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**n/a**	
NUMBER OF ANIMALS	**n/a**	
Approval period to use animals for research/testing purposes		**February 2016-February 2017**
SUPERVISOR	**Prof D Abernethy**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date **9 March 2016**
CHAIRMAN: UP Animal Ethics Committee	Signature

APPENDIX 4a: Human ethics approval certificate

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

31/03/2015

Approval Certificate
New Application

Ethics Reference No.: 74/2015

Title: The prevalence and impact of Brucella abortus in personnel on cattle farms infected with brucellosis: Pilot study

Dear Dr Bernice Harris

The **New Application** as supported by documents specified in your cover letter dated 24/02/2015 for your research received on the 23/03/2015, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 25/03/2015.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**74/2015**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

☎ 012 354 1677 🖷 0866516047 deepeka.behari@up.ac.za http://www.healthethics-up.co.za
✉ Private Bag X323, Arcadia, 0007 - 31 Bophelo Road, HW Snyman South Building, Level 2, Room 2.33, Gezina, Pretoria

APPENDIX 4b: Human ethics extension approval

APPLICATION FOR APPROVAL OF AMENDMENT

Date: 13 July 2016

Prof CW van Staden
Chair: Faculty of Health Sciences Research Ethics Committee
University of Pretoria
Steve Biko Academic Hospital
Tel: 012 356 3084/5
E Mail: manda.smith@up.ac.za / fhsethics@up.ac.za

Tswelopele Building, Level 4, Rooms 4-59 and 4-60
Dr Savage Road, Gezina, Pretoria
Private Bag X323, ARCADIA,0007

Dear Prof CW van Staden

PROTOCOL NO. (UP NUMBER): 74/2015

PROTOCOL NAME: THE PREVALENCE AND IMPACT OF BRUCELLA ABORTUS IN PERSONNEL ON CATTLE FARMS INFECTED WITH BRUCELLOSIS

Version No. 2 dated 13 July 2016
(The above Version No. and date applicable to your changes must be reflected as a "footer" on your attached Amendment.)

Please note:
- The Principal Investigator must indicate the significance of the Amendment;
- That it is the duty of the Principal Investigator to clearly indicate on a separate letter how the new amended document has been changed from the original and the reasons must be supplied that necessitated these changes; and
- Changed text must be highlighted in different colour in your attached document to indicate the changes made.

1. NATURE OF AMENDMENT:

(1) Extension of sample frame and sample size: Sample frame will now include a sample of 150 abattoir workers and 29 State Veterinary Personnel who are in contact with brucellosis infected cattle on a frequent basis.
(2) Extension of study time frame and of Ethics approval: To 31/03/2017

2. RATIONALE:

(1) Reasons for extending sample:
  (a) The state regulatory officers (AHTs and SVs) felt that they should be prioritized since this is a GDARD sponsored project. They felt that their brucellosis status should be known as well.
  (b) Internal collaboration with the Veterinary Public Health (VPH) component of Veterinary Services of GDARD resulted in overlapping project objectives, viz. to determine the seroprevalence of Brucellosis in abattoir workers in Gauteng. In light of limited resources and delivering an efficient, effective, timely service, it was proposed that this sample group be included in the current study.
(2) Reasons for extension of study frame and of Ethics approval:
  (a) An extended period of unanticipated convalescence of the principal investigator from April 2015 to December 2015.
  (b) The availability of the farmers and farm workers
  (c) Loss at recruitment of the anticipated sample
  (d) The availability of the medical doctor

3. CHANGES:

(1) TITLE: THE PREVALENCE AND IMPACT OF BRUCELLA ABORTUS IN HIGH RISK PERSONNELL EXPOSED TO CATTLE INFECTED WITH BRUCELLOSIS
(2) OBJECTIVES 1, 2 and 3 (to include extended sample frame and sample size) (pg 2)
(3) RESEARCH METHODOLOGY:
  a. Rationale for selecting abattoir workers' sample (pg 2)

APPENDIX 5a: Brucellosis awareness poster

Brucellosis

Undulant Fever, Mediterranean Fever or Malta Fever

Brucellosis is a highly infectious bacterial disease of cattle, sheep, goats and pigs which can infect humans. This is gotten from direct contact with animals secretion, excretions and animal products such as meat and milk.

Causes

Brucella abortus (from cows)
Brucella mellitensis (from sheep/goat)
Brucella suis (from pigs)

Transmission

Brucella spp. can be transmitted through direct contact with the infected animal secretions, excretion and animal by raw products like meat and milk through the eyes, nose and broken skin.

People at risk

Veterinarians
Animal health assistants
Abattoir workers
Livestock handlers
Consumers of raw or undercooked meat and unpasteurized milk

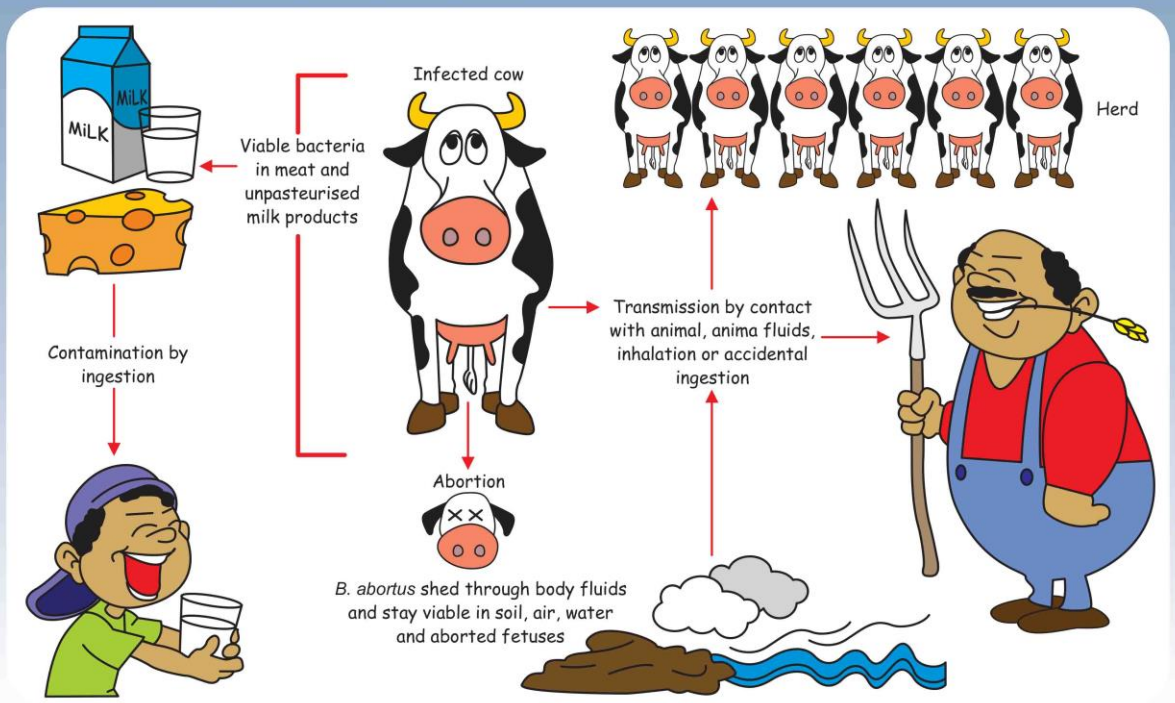
Clinical signs in humans

Clinical signs may occurs from week, months to years

- Septiceamia
- Loss of appetite
- Headaches
- Chills
- Night sweating
- Weight loss
- Join pain
- Muscle pain
- Back pain
- Orchitis

Preventative measures

- Always put on your protective cloths / gears during work
- Do not eat raw or improperly cooked meat
- Do not drink unpasteurized milk
- Wash and disinfect hands after working with animal by products.



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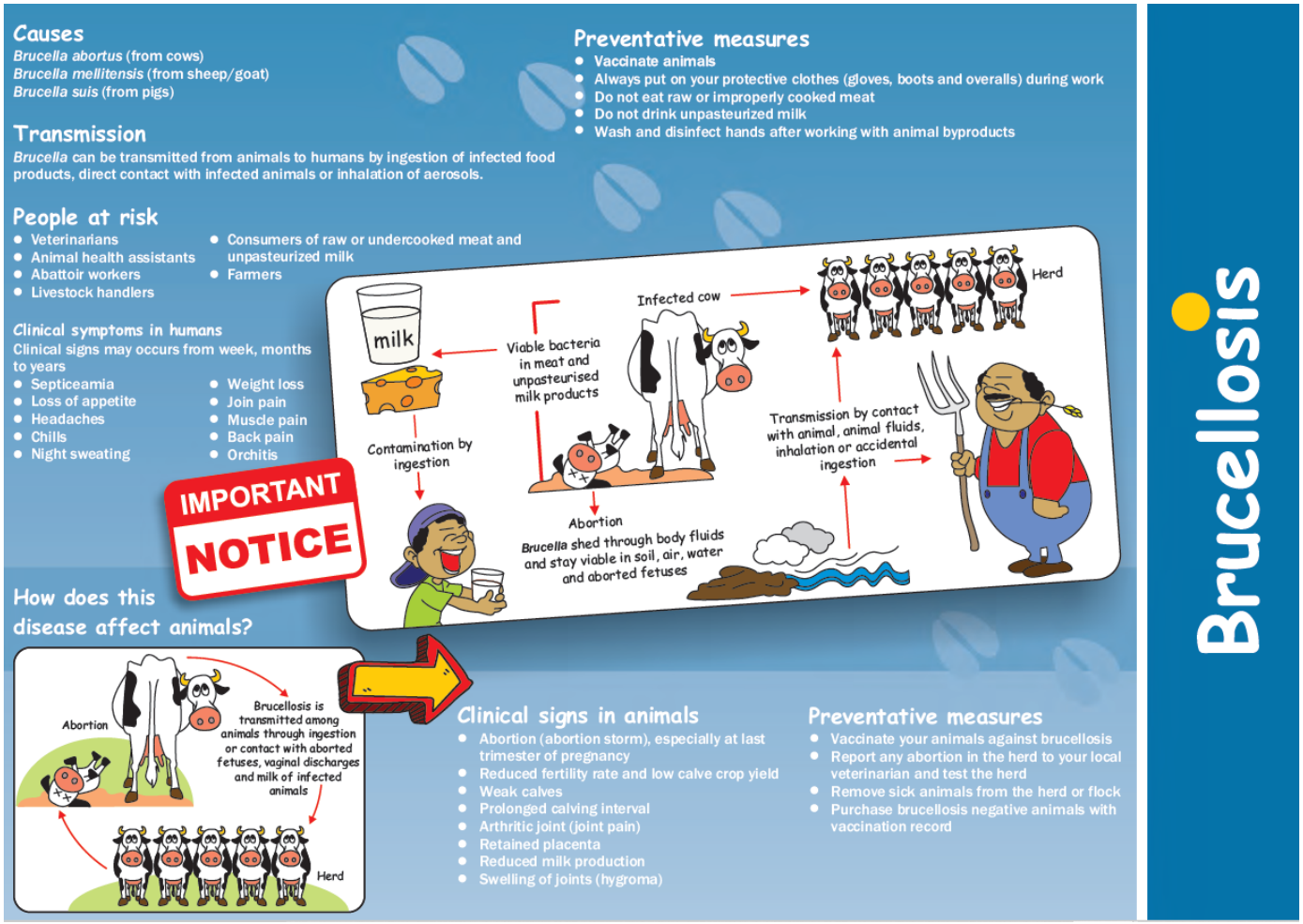
APPENDIX 5b: Brucellosis awareness brochure



APPENDIX 5c: Brucellosis awareness mug design

Coffee Mug Design