AN EPIDEMIOLOGICAL ANALYSIS OF THE OCCURRENCE OF BRUCELLOSIS IN CATTLE AND ADOPTED CONTROL MEASURES IN SOUTH AFRICA DURING THE PERIOD 2014 TO 2019

BY

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DECLARATION

I, SIMANGO EMILY, declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) has not been submitted by me for a degree at this or any other university, this is my own work in design and execution, and that all materials contained herein has been duly acknowledged.

Signature: Ms Simango E   Date: November 2023

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DEDICATION

This study is dedicated to my beloved family (Simango Kedibone, Simango Emily, Simango Mathule and Simango Mpho).

ABSTRACT

Bovine brucellosis is among the most neglected zoonotic diseases in developing countries, where it is endemic and a growing challenge to public health. The development of cost-effective control measures of the disease can only be affirmed by knowledge of the disease epidemiology and the ability to define its risk profiles. The study aimed to document the trend of bovine brucellosis and the control measures adopted following reported cases during the period 2014 to 2019 in South Africa. Data on confirmed cases of bovine brucellosis was retrieved from the website of the World Organisation of Animal Health (WOAH). Data was analysed using the Statistical Package for Social Sciences (IBM SPSS, 2022) version 29.0. Descriptive analysis (frequencies and percentages) and the Analysis of variance (ANOVA) were utilized for statistical significance ($p<0.05$). The data retrieved revealed an overall average bovine brucellosis prevalence rate of 8.48%. There were statistically significance differences in bovine brucellosis prevalence across the provinces except for the year 2016 ($p<0.05$), with Eastern Cape province having the highest prevalence rate in 2016. Vaccination, kill and disposed were the documented control measures in place for the control of bovine brucellosis in the current study, with vaccination being the most commonly adopted strategy. The study identified gaps, such as the lack of invaluable information on the adoption of comprehensive control measures, testing of only suspect cases, and export livestock, that may contribute to brucellosis underreporting in South Africa. More research on the epidemiology alongside the adoption of comprehensive control measures can help to reduce the outbreaks of bovine brucellosis and economic impact in the South African livestock sector.

Keywords: Risk factors, Abortion, Bovine, Prevalence, Endemic.

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LIST OF ABBREVIATIONS

DAFF	Department of Agriculture, Forestry and Fisheries
KZN	KwaZulu Natal
GDARD	Gauteng Department of Agriculture and Rural Development
WOAH	World Organization of Animal Health
ANOVA	Analysis of Variance
iELISA	indirect Enzyme-linked Immunosorbent Assay
RBTP	Rose Bengal Agglutination Plate
CFT	Complement Fixation Test
S19	Strain19
NLS	National Livestock Statistics
RB51	Strain RB51

CHAPTER 1
INTRODUCTION

1.1. Background

Livestock production and the supply of animal products play a key role in contributing to food security in South Africa (Frean *et al*., 2018). Animal products play an important role in the food industry and are important contributors to safe and affordable high-quality protein for a growing population (Mpofu, 2018, Schelling *et al*., 2021). Bovine brucellosis is a chronic disease that negatively impacts cattle reproduction and production by causing abortions, still births, weak calves, retained placentas, decreased milk yield, and reduced fertility in breeding stock (Fürst *et al*., 2017, Kolo *et al*., 2019, Modisane, 2019). In developed countries, bovine brucellosis is well-controlled or completely eradicated while the disease is endemic to most African countries (Govindasamy *et al*., 2021). Prevalences of bovine brucellosis were documented in previous studies in South Africa. Bishop (1984) reported a prevalence of 1.50 % for cattle sampled at the Cato Ridge abattoir in Kwazulu-Natal province in 1984. Hesterberg *et al*. (2008) reported 1.45 % prevalence in cattle sampled in communities in the KwaZulu-Natal province from 2001 to 2003 while Kolo *et al*. (2019) reported prevalence rates from 0.75 % to 2 % in communal cattle in the North-West Province and average prevalence of 5.5% (seropositivity) in cattle slaughtered in the abattoirs located in the Gauteng province, South Africa.

The risk factors associated with the transmission of brucellosis are host, agent, management, and environmental (Sandengu, 2018, Oosthuizen *et al*., 2019). Brucella organisms are most frequently acquired through ingestion, conjunctival inoculation, and skin contamination while udder inoculation from infected milking cups is another possibility. Gloveret *et al*. (2020), stated that the diagnosis of bovine brucellosis is confirmed by isolating and identifying the causing agent of the disease. However, to be able to screen a large number of cattle, the diagnostic tests should be inexpensive, easy to perform, rapid, highly sensitive, and fairly specific (Frean *et al*., 2018, Ledwaba *et al*., 2021, Schelling *et al*., 2021). Treatment is often unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs since Brucella species are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system (Tempia *et al*., 2019). Nyarku, (2020) and Ledwaba *et al*. (2021) added that treatment failures may be due to the inability of the drug to penetrate the cell membrane barrier.

1.2. Problem statement

Livestock production is the mainstay of South African agriculture. Frean *et al*. (2018) estimated that 60% of the South African population relies on agriculture with cattle production playing a key role. Brucellosis in cattle is usually caused by biovars of *Brucella abortus* (OIE, 2008). Brucellosis is endemic worldwide with a substantial impact on both human and cattle health as well as the economy (Al Dahouk *et al*., 2013, Suarez-Eaquivel *et al*., 2020). Brucellosis causes reproductive loss through infertility, stillbirths, irregular estrus cycles, and reduced meat and milk production in cattle (Scott *et al.*, 2007). In addition, brucellosis poses a barrier to the importation and exportation of cattle thereby constraining livestock trade (Mahomed *et al*., 2015). According to Moreno (2021), the global occurrence of brucellosis is often high due to factors such as unrestricted movement of cattle and lack of vaccination of susceptible animals. Pearce and Merletti (2006), Peters (2014) and Berezowski *et al*. (2019) defined bovine brucellosis as a neglected tropical disease whose epidemiology is not well understood. Globally, most studies focused primarily on implementing national brucellosis control programs (OIE, 2008, Dorneles *et al*., 2015, Moreno, 2021). In South Africa, farmers are responsible for vaccinating their herds and government veterinary services assist farmers where resources allow (DAFF, 2017). Therefore, brucellosis prevalence among herds tends to spike, as there is generally poor compliance with brucellosis vaccination requirements, and testing is not compulsory (DAFF, 2017). Additionally, lack of proper perimeter fencing around farms, mixing of animal groups and communal grazing practices create a potential risk for disease spread (Frean *et al*., 2018, Govindasamy, 2020).

1.3. Rationale

Reports on brucellosis prevalence based on studies published between 2003 and 2015 on sub-Saharan African countries reported an occurrence rate ranging from 1.0 % to 36.6 % in livestock raised under various production systems (Maichomo *et al*., 2009, Megersa *et al*., 2011, Mpofu, 2018). Hull and Schumaker (2018) and Manafe *et al*. (2022) reported an increase in the prevalence of bovine brucellosis in South Africa during the period 2018 to 2019. Kiro *et al*. (2016) reported that the epidemiology of brucellosis is not well understood, and the provided information is often inadequate, missing, or biased, particularly in sub-Saharan Africa. Several studies reported practices and knowledge of farmers regarding control measures adopted following bovine brucellosis cases in Africa (Olsen and Tatum, 2010, Oladele *et al*., 2013, Wolff *et al*., 2019, Nyarku, 2020). Despite the economic and social importance of the disease, there is a progressive reduction in public funds availed for bovine brucellosis control programs in most developing countries (Khurana *et al*., 2021, Moriyón *et al*., 2023). Most of the studies that analysed brucellosis control measures implemented by farmers were conducted in developed countries including Australia, New Zealand, Japan, and Canada (Renault *et al*., 2018; Guntheretal, 2019). Mdlulwa *et al*. (2021) reported that in developing countries, the control measures for brucellosis are often limited to vaccinations and restrictions on animal movements.

1.4. Aim

The study sought to document the trend of bovine brucellosis and the control measures adopted following cases during the period 2014 to 2019 in South Africa.

1.5. Objectives

The objectives of the study were to:

i. Determine the prevalence of bovine brucellosis and identify the most affected provinces in South Africa during the period 2014 to 2021.

ii. Evaluate the control measures adopted following reported cases of bovine brucellosis in South Africa during the period 2014 to 2021.

1.6. Research questions

The study sought to provide answers to the following questions:

i. What was the prevalence of bovine brucellosis and which provinces were most affected in South Africa during the period 2014 to 2021?

ii. Which control measures were adopted following reported cases of bovine brucellosis in South Africa during the period 2014 to 2021?

CHAPTER 2
LITERATURE REVIEW

2.1. Introduction

Several studies emphasised the importance of livestock in the social lives of people either as a source of food, status, and acceptance within a community (Abdelbaset *et al*., 2018; Dadar *et al*., 2021; El-Diasty *et al*., 2022). This importance of livestock in the lives of rural communities is supported by the fact that some cultures encourage keeping more animals of a particular kind, for instance in some cultures, goats may be preferred to other animals because of their anti-predatory behaviour (Tempia *et al*., 2019, Oduniyi *et al*., 2020). Although sheep and goats fulfil the same uses as cattle, they do not elicit the same emotional impact in people as cattle do. Brucellosis is a highly contagious zoonotic disease and causes significant reproductive losses in livestock. It is caused by gram-negative facultative intracellular bacteria of the genus *Brucella* (Akakpo *et al*., 2010; WHO, 2017). Whatmore *et al*. (2014), reported *Brucella abortus* to be the second most common zoonotic *Brucella* spp. after *B. melitensis.* The epidemiology of bovine brucellosis is complex (Pearce and Merletti, 2006; Peters, 2014; Berezowski *et al*., 2019). Important factors that contribute to its high prevalence and spread in cattle include farming systems and practices, low levels of farm sanitation, lack of enforcement of legislation around livestock movement practices, mixing and trading of animals, and sharing of grazing pastures. Bovine brucellosis has been eradicated in many developed countries but remains endemic in the developing world because of, among other factors, a lack of resources and control programmes (Frean *et al*., 2018; Kolo *et al*., 2018). The purpose of this chapter is to give a complete overview of literature from various sources on the epidemiological analysis of the occurrence of bovine brucellosis and the control measures that have been implemented. Furthermore, this literature review outlines the current understanding of bovine brucellosis so that a framework is set to explore emerging disease problems and research objectives. The cattle farming systems are explained and an overview of the concepts of bovine brucellosis (prevalence, risk factors, socioeconomic effects, control measures, and transmission mechanisms) are provided. The effect of season on the occurrence of bovine brucellosis is also discussed.

2.2. Cattle farming systems in South Africa

The cattle farming systems in South Africa can be broadly divided into commercial and communal. In South Africa, the contribution of cattle production to agricultural output ranges between 25-30 % per year, excluding the values of draught power, manure, and other products (Musemwa *et al*., 2008). The country is known for its rich and immensely diverse cattle breeds that are distributed across different agro-ecologies and socio-cultural settings, where they are kept as a source of food, family income, providing draught power, foreign exchange earnings and social and cultural functions (Scarpa *et al*., 2003; Biradar *et al*., 2013, Madisane, 2019), thus contributing to household livelihoods, food security, and poverty alleviation (Meissner *et al*., 2013, Mpofu, 2018). South Africa has an estimated population of 12.8 million cattle of which the majority is owned by approximately 4 million black farmers who reside and farm on agricultural land in the former homeland areas of South Africa (NLS, 2018). Compared to other provinces, the Eastern Cape Province has more than 29% of the cattle population in South Africa (DAFF, 2018).

According to Statistics South Africa (2016), there are approximately 34% of households own between 1 to 10 herds of cattle, and 22.8% which own between 11 and 100 herds of cattle in the Eastern Cape communal farming areas. Nqeno (2008), Karimuribo *et al*., (2011), Mthi *et al*., (2016), and Yawa (2017) indicated that lack of breeding plans, feed shortage, poor infrastructure, diseases, and poor management practices are the major constraints contributing to low productivity in communal farming systems. Extensive cattle production contributes to a high prevalence of bovine brucellosis mainly through large herd sizes and movement of herds. According to Omer *et al*. (2010) and Oosthuizen *et al*. (2019), large herd sizes enhance the exposure potential through increased contact within the herd and with other infected herds, common feeding and watering points and relatively poor management, thus promoting the transmission of the disease. The unregulated movement of cattle from infected herds or areas to bovine brucellosis-free herds or areas is the major cause of failure in bovine brucellosis eradication programs (Fürst *et al*., 2017, Govindasamy *et al*., 2021). The low incidence of brucellosis in small herds may be related to herd management. Thus, small herds often graze nearby pastures, allowing interactions with other herds to be controlled, or using communal methods (Sandengu, 2018, Frean *et al*., 2018)

2.3. Prevalence of bovine brucellosis in South Africa

Bovine brucellosis is endemic to all provinces of South Africa. However, it is mostly concentrated in the central and highveld regions. The uncontrolled movement of cattle and the shortage of vaccinations for susceptible animals have contributed to the increased incidence rate across the country (Ducrotoy *et al*., 2015). South Africa is among the African countries where knowledge of animal diseases such as brucellosis is still not widely disseminated. Additionally, brucellosis has been known to be present from 1996 to 2004 with outbreaks ranging from 219 and 457 cases reported annually to the World Organisation of Animal Health. The prevalence of bovine brucellosis in communal grazing areas of Kwa-Zulu Natal from 2001 to 2003 was found to be 1.45 % (0.84-2.21 %) and varied from 0 to 15.6% between magisterial districts (Hesterberg *et al*., 2008). Over the same period, the individual prevalence was estimated at 0.75% – 2% in communal cattle in North-West Province. Manafe *et al*. (2022) reported an increase in the occurrence of bovine brucellosis during the period 2018 to 2019, with 139 and 423 reported cases respectfully.

According to the KZN Department of Agriculture and Rural Development, 70% of the cases reported in 2020 arose consequently from the communal dip tanks in the area where there is generally poor compliance with bovine brucellosis control program. The Gauteng Department of Agriculture and Rural Development (GDARD) reported a 1.27% prevalence in the cattle population in Gauteng province from 2015 to 2016 using the RBT and CFT tests (GDARD, 2016). Brucellosis was reported to be more prevalent in the north-eastern area of the province due to few private veterinarians, lack of resources to contain the disease, and lack of compliance from livestock owners among other factors.

2.4. Risk factors associated with bovine brucellosis in South Africa

The epidemiology of cattle brucellosis is influenced by several factors including those associated with disease transmission between herds as well as those influencing the maintenance and spread of infection within herds (Moreno, 2021). Understanding the epidemiology of brucellosis is, therefore, vital for strategising evidence-based disease control measures. However, information regarding the epidemiology of bovine brucellosis in South Africa is inadequate (Pearce and Merletti, 2006, Peters, 2014, Berezowski *et al*., 2019). Consequently, the adoption of appropriate preventive measures is often not undertaken. The risk of cattle exposure and infection is considerable and influenced by many factors, which include lack of movement controls, sharing of farmland, lack of vaccination programs, and larger herd sizes (AlMajali *et al*., 2009; Bedaso *et al*., 2018). Lack of knowledge of the disease among farmers and farm workers has also been proved to be a significant risk factor (Tesfaye *et al*., 2011; Wossene *et al*., 2020). The organism's survival in the environment may play a role in disease epidemiology. The long incubation period of brucellosis, as well as the presence of latently infected animals, offer a significant danger of infection since extended periods may pass without any overt signs of infection within a herd (Ogugua *et al*., 2018, Khan *et al*., 2021). The incubation period varies between 50 and 225 days depending on the age of the animal and the stage of gestation at which infection occurs (Nicoletti, 2010, Deka *et al*., 2018, Bifo *et al*., 2020, Etefa *et al*., 2022). Up to their first abortion, heifers born to seropositive cows may be serologically negative and latently infected (Sagamiko *et al*., 2018)

Addis (2015) placed the risk factors for brucellosis into four categories which are the environment and reservoirs, host factors, and management. Infected animals contaminate the environment, creating favourable conditions for the brucella bacteria. The ability of brucella to persist outside the mammalian hosts is relatively high under favourable conditions (Dorneles *et al*., 2015, Kaden *et al*., 2018). Brucella pathogens may retain infectivity for several months in water, aborted foetuses and foetal membranes, faeces and liquid manure, wool, hay, and fomites (Corbel, 2006, Abubakar *et al*., 2012, Rahman *et al*., 2014). Brucella is able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil. Survival is prolonged at low temperatures, especially below 0 C (Falenski *et al*., 2011, Méndez-González *et al*., 2011, Jansen *et al*., 2019).

Host factors include the age, sex, and breed of cattle, and herd sizes while management factors include issues of disease prevention and cattle movement control (Yilma *et al*., 2016; Bendaso *et al*., 2018). Age has been expressed as the intrinsic factor related to brucella infection (Abubakar *et al*., 2010). A higher prevalence of brucella organisms has been determined in adult cattle than in young cattle (Ashagrie *et al*., 2011, Borba *et al*., 2013). However, the higher prevalence of brucella in adult cattle has also been related to longer interaction with infected herds. Sexually mature and pregnant cattle are more susceptible than sexually immature cattle (Matope *et al*., 2011, Sandengu, 2018, Oosthuizen *et al*., 2019). This is because the Brucella organisms cause a response in the reproductive system due to the concentration of erythritol sugar, which is produced in cattle foetal tissues and stimulates the growth of Brucella organisms. It has been reported that bovine brucellosis is more prevalent in crossbred cattle than indigenous cattle (Muma *et al*., 2007, Nahar and Ahmed, 2009, Tsegaye *et al*., 2016).

Another risk factor for Brucella infection is herd sizes, with large herds having the highest risk. The spread of the disease across herds in different areas has been linked to the movement of infected animals (de Alencar Mota *et al*., 2016). In terms of management factors, according to Patel *et al*. (2014), several factors relate to the provision of facilities such as calving camps, and quarantine camps for the animals especially when breed and type of animal is a significant risk factor for the development of brucellosis in animals. Muma *et al*. (2012) stated that the indiscriminate buying and selling of animals and bringing them to the herd has been proved to act as a risk factor. It is therefore important that when farmers purchase cattle even from other farmers, they must give enough attention to the disease status of the animals they are bringing in (Eyob *et al*., 2019).

2.5. Socio-economic effects of bovine brucellosis

Bovine brucellosis negatively affects livestock productivity (de Alencar Mota *et al.* 2016). The dairy production sector is of primary public health concern because of high reported brucellosis cases (Poester *et al*., 2013, El-Diasty *et al*., 2021). The economic losses due to the disease are considered both in terms of animal production loss and public health. In addition to production loss, the disease is an impediment to free animal movement and is a barrier to import and export livestock trade. Brucellosis causes direct socio-economic losses in communities that rely on cattle production for their livelihoods (Meissner *et al*., 2013, Madisane, 2019). Losses in animals are attributed to direct effects on their offspring due to abortion, stillbirth, and infertility whereas indirect losses are due to reduction in milk yields and human brucellosis infections (Lakew *et al*., 2019). However, infections in cattle do not always lead to abortion but can persist in a herd without any clinical symptoms, other than the birth of weak or nonviable calves and a reduction in milk yield (Rossetti *et al*., 2017, Bosilkovski *et al*., 2021). Although data on bovine brucellosis is lacking, the economic effects of brucellosis on livestock species can still be estimated, because prices can be estimated for direct losses due to morbidity and mortality and indirect losses due to treatment costs. The economic effects of animal diseases vary by country as influenced by the country's livestock dynamics and grazing patterns (McDermott *et al*., 2013). In South Africa, 290-460 bovine brucellosis outbreaks were reported annually to the OIE between 1996 and 2004 (OIE, 2006) while the herd prevalence in the intensively farmed areas was 14.7% in 1990, resulting in approximately R300 million in losses annually (Hesterberg *et al*., 2008, DAFF, 2018).

2.6. Control measures for bovine brucellosis in South Africa

Brucellosis is among the infectious diseases that are controlled by national and local animal health and disease control services (Frean *et al*., 2018). In South Africa, brucellosis is a controlled disease in terms of the Animal Disease Act, 1984 (Act No. 35 of 1984) and the Animal Diseases Regulations. The compatible relationships of brucella species with the hosts including variable incubation periods, long survival time in both extracellular and intracellular environments, asymptomatic carrier stages, and resistance to treatment are the major challenges when controlling bovine brucellosis (Falenski *et al*., 2011, Méndez-González *et al*., 2011, Jansen *et al*., 2019). Strategies implemented in South Africa for controlling brucellosis include surveillance, vaccination, movement control, biosecurity, and a testing, isolation, and slaughter policy (Durrani *et al*., 2020).

2.6.1. Surveillance

Comprehensive national disease surveillance programs are necessary to recognize infected herds and to allow any subsequent corrective and preventive measures to be taken (Asanishvili *et al*., 2016). Marageni *et al*. (2017) stated that there are a variety of serological tests that can be used in the field to detect the presence of brucellosis in a cattle herd and the most often used fast screening test is the Rose Bengal plate test (RBPT). Most of the existing rapid screening tests that can be employed in the field (for example, the RBPT and the Brucella milk ring tests) have limited sensitivity and specificity, making effective surveillance of bovine brucellosis at the individual level difficult (Chisi *et al*., 2017, Kolo *et al*., 2019). Serological surveillance should be combined with bacteriological examinations using tissue samples or secretions from questionable or diseased animals (Fasina *et al*., 2015, Chaters *et al*., 2019). In addition to the challenges associated with individual animal testing, its effectiveness is dependent on rigorous epidemiological investigations and a good animal identification and movement control system, especially given the disease's long incubation period, which allows for the disease's gradual spread (Calba *et al*., 2016). Animal surveillance by serological tests to determine infected animals, control of brucellosis transmission to non-infected animal herds, and elimination of animal carriers of the bacteria such as dogs, cats, and mice from the proximity of the cattle herds to eradicate the sources of infection are all required for effective brucellosis control in animals (Alton *et al*., 2015, Kiros *et al*., 2016). From September 2015 to August 2016, an estimated 3,476,000 cattle were slaughtered at South African abattoirs (DAFF, 2016). These abattoir facilities can also be used to monitor disease control policies, detect newly introduced disease agents, and evaluate intervention programs such as brucellosis vaccination (DAFF, 2017). Most importantly, abattoir surveys can facilitate early intervention to reduce epidemic animal loss. As a result, brucellosis abattoir surveillance research can generate baseline data on the disease's occurrence among the cattle population, especially when the animals come from various farms to be processed into wholesome meat products for human consumption (Alto *et al*., 2015, Fasina *et al*., 2015, DAFF, 2017). However, abattoir data may not produce reliable prevalence estimates because the population of slaughtered cattle tends not to correctly represent the target population which may affect the validity of results from such facilities.

2.6.2. Vaccination

Vaccination as the sole means of brucellosis control has been proven to be effective (Getahun *et al*., 2021). Reduction in the number of positive animals in a herd is directly related to the percentage of vaccinated animals. Aparicio (2013) and Khan and Zahoor, (2018) stated that proceeding from a control to an eradication program, through a test and slaughter program is necessary. Matle *et al*. (2021) isolated *Brucella abortus* vaccine strains 19 and RB51 from cattle and both vaccine strains are predominantly used for vaccination of cattle against brucellosis in South Africa. These vaccines reduce the risk of abortion and transmission but do not provide sterile immunity. S19 has proven to be highly effective and beneficial in decreasing and eradicating bovine brucellosis globally throughout the years (Sancho *et al*., 2015, Matle *et al*., 2021). Its effectiveness, however, is not without disadvantages. It is highly immunogenic and has been linked to post-vaccination miscarriages as well as interference with serological tests (Miranda *et al*., 2016, Pascual *et al*., 2017).

Dorneles *et al*. (2015) stated that due to RB51 lacking the LPS-O antigen seen in S19, it does not produce post-vaccination antibodies and therefore, it is preferred for booster vaccinations or vaccinations in adult females but is also associated with post-vaccination abortions and premature births (Dougherty *et al*., 2013, Alves *et al*., 2015). The effectiveness of RB51 and S19 are similar in their prevention of both abortion and foetal transmission. However, Pascual *et al*. (2017) suggested a variation in the effectiveness of S19 that is age-related and that RB51 seems more effective when the prevalence is low. Moreover, the availability of *B. abortus* S19 vaccine has been problematic for some time and *B. abortus* RB51 has been used as an alternative, hence its isolation from the cattle. In South Africa, vaccination is restricted to heifers between the ages of four and eight months. Repeat or booster vaccinations with Strain 19 is illegal and vaccinations should be done only with RB51 (DAFF, 2013, Kiros *et al*., 2016).

2.6.3. Biosecurity protocols

Biosecurity in terms of bovine brucellosis refers to the hygienic practices that minimise exposure of susceptible animals to *Brucella abortus* pathogens to reduce the prevalence (Harris *et al*., 2020). This can be done at the national, regional, or farm level. In an outbreak situation, isolation of infected animals, proper disposal of placentae and aborted foetuses, and disinfection of premises all reduce the risk of transmission (Zamri-Saad and Kamarudin, 2016). Personal hygiene among agricultural personnel contributes to the prevention of the spread of *Brucella abortus* pathogen between farms via fomites (Zhou, 2018). The movement of infected animals is a major contributor to the spread of brucellosis. Contaminated farms must be restricted from moving their animals, and animals within contaminated herds should be identified individually to provide for traceability (Simpson *et al*., 2018, Tempia *et al*., 2019). To avoid reinfection, replacement stock must be recruited from brucellosis-free herds while newly introduced animals should be quarantined or retained (Kiros *et al*., 2016, Frean *et al*., 2018). Farmers are also encouraged to test their animals before moving them. Biosecurity is critical, but it is not always possible, particularly in locations where trans humance and nomadism are practiced (Sancho *et al*., 2015)

2.6.4. Test and slaughter

Test and slaughter is a useful technique in eradication, but it is difficult to implement partly due to cultural and religious views that may limit testing and slaughter policies. Culling cattle, for example, is a taboo in India and has been prohibited (Dadar *et al*., 2021, PA *et al*., 2023). Close collaboration between veterinary services and farmer compliance is critical to eradication success. Farmers may be encouraged to comply by offering competitive compensation which makes the practice to be an expensive tool. Nyanhongo *et al*. (2017) and Govindasamy *et al*. (2021) stated that test and slaughter practices may not be successful unless combined with additional control measures such as mass vaccination. Caetano *et al*. (2016) and Kolo *et al.* (2021) stated that mass vaccination helps to reduce the disease prevalence. Therefore, vaccination of young replacement stock can be combined with testing and slaughter of adult animals (Docrotoy *et al*., 2018, Singh *et al*., 2018).

2.7. Bovine brucellosis transmission mechanisms

Species of the genus Brucella can be transmitted via horizontal or vertical routes (Meltzer *et al*., 2010). Brucella organisms are found in higher concentrations in the uterine environment of pregnant animals. The aborted foetuses, placental membranes, and uterine discharges act as the main sources of infection. In utero infection or milk and colostrum can also be sources of disease transmission to the new-born calf. Few infected cattle become chronic carriers. Brucella can survive for longer periods in conditions of high humidity, low temperatures, no sunlight, and in soil; and can remain viable for several months in water, aborted foetuses, and manure under appropriate conditions (Méndez-González *et al*., 2011, OIE, 2018, Jansen *et al*., 2019). A publication by the South African Department of Agriculture, Forestry and Fisheries stated that there is a great concern for brucellosis transmission given the intensity and widespread distribution of outbreaks in cattle throughout the country

(DAFF, 2017). Animals contact the infection through ingestion of contaminated feeds and water or by contacting aborted foetuses, foetal membranes, and discharges from uterine tissues. Inhalation can also be a mode of transmission while infected bulls may spread infection between herds by natural service or artificial insemination (Acha and Szyfers, 2001). Tukana and Gummow (2017) indicated that susceptible cattle sharing common water sources with brucella-positive animals are among other reasons for the spread of brucellosis. *Brucella abortus* can also be spread through fomites such as buildings, equipment, and clothes. Reservoirs of infection have been reported in a wide range of domestic animals, birds, and carnivores such as dogs. The transmission of brucellosis by ticks, fleas, or mosquitoes from an infected herd to a non-infected herd has not been proven (OIE, 2009).

2.8. Seasonal effect of bovine brucellosis in South Africa

The incidence and prevalence of most infectious diseases are directly linked to seasonal weather variations. Seasonal weather variations influence the dynamics of infectious diseases by affecting the host-pathogen interactions which alters the components of reproduction (Dorneles *et al*., 2015, Kaden *et al*., 2018, Frean *et al*., 2018, Govindasamy, 2020). Cold or wet seasons are associated with high disease incidences due to the abundance, survival, and virulence of pathogens. On the other hand, warm or dry seasons are associated with decreased disease incidences due to increased outdoor activities and exposure of pathogens to ultraviolet light (OIE, 2008, Dorneles *et al*., 2015, Moreno, 2021). In addition, the survival of pathogens outside their hosts depends on other environmental factors such as humidity, salinity, temperature, and soil pH (Abubakar *et al*., 2012, Rahman *et al*., 2014, Dinknesh *et al*., 2019; Gelma *et al*., 2019).

2.9. Conclusion

Brucellosis is one of the most common zoonotic diseases worldwide except in developed countries where bovine brucellosis has been eradicated. In developing countries, brucellosis appears to be more endemic, especially in sub-Saharan countries, and its prevalence is influenced by management factors, socio-economic and inadequate information. A robust surveillance mechanism to identify infected animals, prevention of spread from infected animals and herds to non-infected herds, removal Brucella infection reservoirs, and preventive measures to prevent the reintroduction of the disease in a herd are all necessary components of an efficient system for controlling animal brucellosis.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study area

The secondary data used in this study was collected in South Africa. South Africa has a total area of 1,221 million km^2 and lies at 30.5595° S and 22.9375° E. Average annual rainfall in South Africa is about 464 mm, with Western Cape receiving majority of the rainfall in winter and the rest if the country receiving summer while the average temperature ranges from 15ºC to 36ºC in summer and -2ºC to 36ºC in winter.

3.2. Data collection procedures

The study used secondary data from the World Organization of Animal Health (WOAH) which is available on the public domain. WOAH is an intergovernmental organization that supports, coordinates, and promotes animal disease control. It is responsible for providing all available information about exceptional disease events that occur in the member countries, as well as follow-up reports and six-monthly reports on WOAH listed disease situations in each country. The information portal allows the data to be viewed, analysed, and extracted in different formats. The data focused on the outbreaks and reported cases of brucellosis in cattle in each province during the period 2014 to 2019. Additionally, the data included control measures adopted which include vaccination, test, slaughter, killed and disposed.

3.3. Data analysis

The data was analyzed using Statistical Package for Social Sciences (IBM SPSS, 2022) version 29.0. The Shapiro-Wilk test was used to assess the normality of the data before the analysis. Descriptive statistics (frequencies and percentages) were used to achieve the three objectives. The one-way analysis of Variance (ANOVA) was used to evaluate the statistically significant differences in the prevalence of brucellosis between the years and between the provinces. The level of significance was observed at $p<0.05$.

CHAPTER 4

RESULTS

The results of the number of cases of bovine brucellosis against the susceptible cattle population per annum in each of the provinces in the study area are presented in Table 4.1. There were no records on the prevalence of bovine brucellosis in Limpopo, Gauteng, and KwaZulu-Natal in 2014, and Mpumalanga in 2015 and other provinces.

Table 4.1: Prevalence of bovine brucellosis in South Africa during the period 2014 to 2019.

Year	**Prevalence of bovine brucellosis (%)**								
	L	NW	GP	MP	NC	FS	KZN	WC	EC
2014	-	7.94	-	22.96	17.15	19.84	-	9.86	0.13
2015	0.83	55.52	33.85	-	12.86	22.88	2.89	26.35	8.06
2016	7.05	5.57	-	7.11	-	11.86	11.28	4.00	4.83
2017	8.01	14.16	-	5.76	11.11	4.85	-	-	6.16
2018	-	13.35	-	4.80	-	8.20	13.86	-	-
2019	21.58	15.56	-	3.04	-	6.31	19.94	8.45	-

–: no information available, L: Limpopo, NW: North West, GP: Gauteng, MP: Mpumalanga, NC: Northern Cape, FS: Free State, KZN: KwaZulu-Natal, WC: Western Cape, EC: Eastern Cape.

Table 4.2 illustrates the overall mean of bovine brucellosis prevalence across the years (2014 to 2019). It can be seen in Table 4.2 below that the year 2015 had the highest prevalence rate (p<0,05) whereas 2018 had the lowest.

Table 4.2: Overall mean of bovine brucellosis prevalence across the study period.

Year	**Overall mean prevalence (%)**
2014	8.65
2015	18.14
2016	5.74
2017	5,56
2018	4.47
2019	8.32

Results in Table 4.3 show control and prevention measures that were implemented against bovine brucellosis during the study period. The implemented methods were killing and disposal, and vaccination. Killing and disposal was the most common practice in 2015. It can be seen from Table 4.3 that a higher number of cattle were vaccinated in 2014, while 2019 had the lowest number of vaccinated animals.

Table 4.3: Control measures adopted following the reported cases of brucellosis in cattle in the study area from 2014 to 2019.

Year	**Control Measures**	
	Killing and Disposal N (avg)	Vaccination N (avg)
2014	109 (7)	59 498 (7 437)
2015	828 (52)	41 420 (2 589)
2016	638 (46)	7 396 (528)
2017	118 (8)	6 309 (451)
2018	286 (29)	7 913 (791)
2019	295 (21)	5 155 (368)

Table 4.4 compares the prevalence of bovine brucellosis across all the provinces from 2014 to 2019. There were statistically significance differences in bovine brucellosis prevalence across the provinces except for the year 2016 (p>0.05) with the Eastern Cape province having the highest prevalence.

Table 4.4 Prevalence of bovine brucellosis in South Africa during the period 2014 to 2019.

Year	**Prevalence according to provinces (Mean±SE)**									**p-value**
	L	NW	GP	MP	NC	FS	KZN	WC	EC	
2014	-	7.93±0.45	-	22.95±15.92	17.15±0.06	19.84±2.79	-	9.85±8.43	0.13±0.00	0.02
2015	10.84±0.00	20.51±19.68	2.89±0.00	9.92±0.00	22.88±0.00	20.96±12.89	5.69±0.00	27.82±14.95	9.83±0.00	0.01
2016	0.43±0.00	10.56±0.66	-	7.11±0.06	-	11.85±7.52	11.28±0.00	4.00±0.00	42.82±35.01	0.17
2017	8.01±6.34	14.16±0.97	-	5.76±0.84	11.11±0	4.84±0.41	3.05±0.00	-	6.16±0.00	0.04
2018	-	13.34±2.92	-	4.80±2.09	-	8.19±1.81	13.86±0.00	-	-	0.03
2019	21.57±19.69	15.56±4.20	-	3.04±1.26	-	6.31±1.34	19.94±15.96	8.45±0.00	-	0.05

–: no information available L: Limpopo, NW: North West, GP: Gauteng, MP: Mpumalanga, NC: Northern Cape, FS: Free State, KZN: KwaZulu-Natal, WC: Western Cape, EC: Eastern Cape.

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

According to the findings of this study, the overall prevalence of bovine brucellosis was 8.48% in South Africa. Ayoola *et al*. (2017) and Mfune *et al*. (2021) reported a prevalence of 7. 81% in Nigeria and 7.53% in Zambia. The higher prevalence rate reported in our study may be attributable to the uncontrolled movement of cattle herds in search of feed, which results in interherd interactions, and the consequent spread of bovine brucellosis (Kiros *et al*., 2016, Docrotoy *et al*., 2017). Asgedom *et al*. (2016), Awah-Ndukum *et al*. (2018), and Chaka *et al*. (2018) reported bovine brucellosis prevalence of 2.4%, 5.4% and 9.7% respectively. The differences between the findings of these studies in comparison to the present study might have been brought about by the different diagnostic tests used. Indirect ELISA was reported to provide the best sensitivity and specificity as compared to both RBPT and CFT (Getachew *et al*., 2016). The findings of this study were in close agreement with the findings of Tebug *et al*. (2014) with 7.7% in Malawi, Sagamiko *et al*. (2018) with 9.3% in Tanzania, and Ogugua *et al*. (2018) with 10.1% in Nigeria. On the other hand, there were reports with relatively lower prevalence of bovine brucellosis in other sub-Saharan countries such as Ethiopia with an overall of 0.6% (Getahun *et al*., 2022) and Uganda with 1.2% (Nguna *et al*., 2019). This variation in the prevalence of brucellosis in cattle might be due to management-related factors such as region, locality, herd size, knowledge of brucellosis, and risk factors such as sex and age. The majority of the previous studies indication a higher prevalence, were carried out in intensive production systems from numerous owners gathered or at abattoirs. The mean annual bovine brucellosis prevalence was reported at 18.14% in 2015, then gradually decreased over the years. This is a wide variation in the calculated prevalences over the years, suggesting that progress has been made in controlling the disease. When compared with the prevalence for the sub-Saharan Africa, estimated to be 36.6% found by Ducrotoy *et al*. (2015), our study findings fell below the range.

In the current study, the control measures implemented for the control of bovine brucellosis were vaccination, kill and disposed. The high prevalence recorded in the present study regardless of the implemented control measures may be due to unrestricted movement of cattle herds. Muma *et al*. (2013) and Musallam *et al*. (2015) reported cattle movement to be an important and consistent risk factor in the transmission of bovine brucellosis and often leading to outbreaks. There is no effective control of the movement of livestock in the study area due to cattle moving freely between grazing areas, into reserves, sale of animals, and possible use of the cattle for traditional practices such as *lobola* in the communal farms (Bruckner 2014, Govindasamy *et al*., 2021, Kgasi and Michel 2023). It was also reported in a study by Sichewo *et al.* (2020) that the free movement of cattle in communal farming, sharing of pastures, and watering points in the same area were significant risk factors for bovine brucellosis in cattle herds. The findings in our study demonstrate that traditional disease control strategies of killing and disposing are difficult to apply due to resource (financial), logistical, and political constraints. Culling and compensation are indeed a very concerning and emotive issue within both stakeholder groups of farmers and veterinary officials (Ducrotoy *et al*., 2017). Kgasi and Michel (2023) results are in parallel with the current study that killing and disposal is a difficult control measure to adopt, they discovered that farmers and veterinary officials acknowledge that killing and disposal can help reduce or eradicate the disease; however, any animal removal should be accompanied by compensation. Research in Spain found that veterinarians were strongly in favour of the killing and disposal approach (Cowie *et al*., 2015). Furthermore, Adesoka *et al.* (2013), Kiros *et al.* (2016) and Makwavarara (2018) also reported that farmers are unwilling to have their animals killed if no compensation is provided, and they also do not trust the government to reimburse them.

Recent studies identified gaps and inadequacies in knowledge transfer from veterinary officials to farmers, which has been identified as important in order to successfully manage the spread of bovine brucellosis in rural communities (Ndengu *et al*., 2017; Khan and Zahoor 2018). Mfune *et al*. (2021) reported that farmers and veterinary officials both believe that current strategies for controlling bovine brucellosis are ineffective because diseases have been present in their area for some time and are yet to be eradicated. Reasons for the government strategy's ineffectiveness included difficulty isolating animals in communal lands, a lack of compensation for farmers, a lack of disease control operational structure, a lack of knowledge transfer, inadequate vaccination, and a lack of resources and under-staffing (Zhang *et al*., 2018, Dhand *et al*., 2021, Dadar *et al*., 2021). Non-compliance with the prescribed control measures has over the years led to the increase and spread of this disease (Ndazigaruye *et al*., 2018), Tesfaye *et al*. (2011) and Patel *et al*. (2014) reported that

33% of farmers had no knowledge about brucellosis let alone that it is a zoonotic disease. Furthermore, Modisane (2019) reported that at least 50% of the respondents in rural communities of North-West province did not vaccinate their herds for brucellosis resulting in the spread of the disease. Frean *et al*. (2018) reported that due to inadequate surveillance and vaccination programs, South African farms are at high risk of contracting bovine brucellosis in their herds. Financial constraints in South Africa as a developing country make control difficult and eradication rather elusive.

The findings of this study show that there was no statistical relationship between South African provinces regarding the prevalence of bovine brucellosis during the study period except in the year 2016 with Eastern Cape Province having the highest prevalence rate. Caine *et al*. (2017) results on the prevalence of bovine brucellosis in the Eastern Cape are in parallel with the current study. The high prevalence recorded in the current study may be due to lack of knowledge regarding bovine brucellosis. A study by Manafe *et al*. (2022) reported a low level of education attained by relevant respondents because of limited opportunities for higher-level education in the rural settings where most smallholder farmers operate. This finding is in close agreement with a study by Yawa *et al*. (2020) report of low levels of education among cattle farmers in communal areas in the Eastern Cape Province of South Africa. Commercial farming in the Eastern Cape was found to be a higher risk compared to communal farming, although the finding was not statistically significant (Segwagwe *et al*., 2018, Kolo *et al*., 2019). Semi-intensive breeding is widely applied by commercial farms whereas extensive management systems are common amongst rural and smallholder farms. Previous studies have found that practising intensive farming in commercial farms tends to promote the transmission and persistence of Brucella spp. infection especially following abortions (Matope *et al*., 2010, Tempia *et al*., 2019). Thus, the potential for spread to contact animals is extremely high, especially if cattle are overcrowded, as in most dairy herds.

Caine *et al*. (2017) and Sandengu (2018) reported that temperatures were gradually decreasing while rainfall was increasing in the Eastern Cape Province in line with changes in the climatic factors reported across Western Cape and Kwazulu-Natal Province. Furthermore, Brucella organisms can survive in an aborted foetus in the shade and also in liquid manure stored in tanks for up to eight months, three to four months in faeces, 2-3 months in wet soil and 1-2 months in dry soil (Godfroid *et al*., 2004, Botopele, 2022). Thus, although bacteria will dessicate quickly under high temperatures, they may persist in damp, moist conditions for prolonged periods unless proper disinfection methods are used. The results of bovine brucellosis by Kolo *et al*. (2022), Marageni *et al*. (2017), Modisane (2019) and Seanego *et al.* (2022) fell below the reported range. Such observations may imply the existence of similar risk factors that favour the persistence of the disease.

5.2. Conclusion

The current study revealed 8.48 % overall prevalence of bovine brucellosis in South Africa. This study offered baseline data that will help to enhance informed disease control policies in the country. Although certain critical epidemiological information was lacking, the findings of our study can be used to drive, monitor, adjust, or establish policies to reduce the issues posed by brucellosis in the country's cattle sector. Furthermore, the knowledge of risk factors in disease transmission provides guidelines on how and where to implement control measures. A disease control programme must be able to identify these risks, prioritise them and provide effective risk mitigation methods.

5.3. Recommendations

The recommendations of the current study are:

- Continuous research on bovine brucellosis should continue, notably in the areas of vaccine development, diagnostic procedures, and economic effects.
- The South African policy should adopt a more appropriate and cost-effective mechanism for guaranteeing that affected cows that test positive for the disease are slaughtered and owners are compensated appropriately.
- When drafting new policies, it is important to ensure the participation of relevant stakeholders such as the cattle industry and rural communities.
- To decrease the future shedding of the Brucella bacteria by cows, the government could gather and create vaccination programs to encourage the vaccination of heifers. According to the Brucellosis control program, heifers should be vaccinated between the ages of 4 and 8 months. Other approaches, such as vaccination with a decreased dose sub-conjunctival, could be used by state veterinarians where suitable.

- Finally, brucellosis is a reportable disease in South Africa, and our study identified gaps, such as the lack of invaluable information on the livestock tested and the testing of only suspect cases and export livestock, that may contribute to brucellosis under-reporting in the country. It is therefore critical to overcome these constraints in order to generate accurate data for the creation of an annual report and summary for program evaluation purposes.

CHAPTER 6

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