INTRODUCTION

The genus Brucella consists of pathogenic members that have zoonotic potential and are of veterinary and economic importance (Moreno, 2014). The pathogenic members of the genus Brucella cause a disease known as brucellosis in a wide variety of domestic animals (Corbel, 1997), wild animals (Godfroid, 2002), marine animals (Ewalt et al., 1994; Ross et al., 1996) and humans (Wojno et al., 2014).
et al., 2016). In animals, brucellosis is characterized mostly by abortions as well as reduced fertility, poor weight gain and decline in milk production (Dematawewa & Berger, 1998; Lucy, 2001). In humans, the disease causes non-specific symptoms such as anorexia, malaise, back pain, headaches, abdominal pain and chills, which mostly lead to misdiagnosis (Pappas et al., 2006). Spontaneous abortion may occur in pregnant women mostly in the first trimesters of pregnancy (Khan et al., 2001). Farmers, animal health professionals, veterinarians, abattoir workers and other people handling animals are in a high-risk group of getting brucellosis (Pappas et al., 2006; Young, 1995). However, the general public is also at risk through the consumption of contaminated, unpasteurised raw milk and dairy products of infected animals (Young, 1995).

Twelve species are recognized within the genus Brucella which includes the six terrestrial species B. abortus, B. melitensis, B. canis, B, ovis, B. suis, B. neotomae (Corbel & Brinley-Morgan, 1984) as well as the atypical species which includes B. microti (Schoelz et al., 2008), B. inopinata (Scholz et al., 2010), B. pappi (Whatmore et al., 2014), B. vulpis (Scholz et al., 2016) B. ceti and B. pinnipedialis (Foster et al., 2007). The zoonotic species of Brucella worldwide are B. abortus, B. melitensis, B. suis and B. canis (Corbel, 1997; Galinska & Zagórski, 2013; Pappas et al., 2006; Wojno et al., 2016).

Brucella abortus consists of eight different biovars (1–7 and 9) while B. melitensis has three biovars (1–3) and B. suis has five biovars (1–4 and 5). Other Brucella species have not been differentiated into biovars. Members of the genus Brucella prefer certain animal species as their specific hosts (Verger et al., 1985). However, this host preference is not limited since almost all species in the genus with the exception of B. ovis, can infect other hosts beside their preferred hosts, even though the infection of these other hosts is mostly mild and restrained (El-Sayed & Awad, 2018). Moreover cross infection can occur among different hosts due to mixed husbandry systems (Madu et al., 2016).

Brucellosis is listed as a notifiable disease by the World Organisation for Animal Health. It is also a controlled disease in animals in Republic of South Africa (RSA) under Section 10 of Animal 41 Diseases Act 35 of 1984. This scheme involves serological testing and surveillance of high-risk farms, especially dairy and stud herds that are suspected or has confirmed brucellosis cases, as well as the compulsory vaccination of 4–8 months old heifers with 5 × 10^10 organisms of B. abortus S19 vaccine (Bosman, 1980). The scheme is under review since its current status of ‘voluntary participation’ is limiting the efficient control of brucellosis (DAFF, 2017). In a recent Bovine Brucellosis Control Policy document (https://www.dalrrd.gov.za/vetweb/pamphlets&Information/Policy/Bovine%20Brucellosis%20Policy.pdf), it is indicated that the disease is currently not under control in the country; however, it is prioritised and identified as a model for disease control in the RSA Veterinary Strategy 2016–2026. General, bovine brucellosis is endemic in the RSA, particularly in areas with intensive production systems (DAFF, 2015, 2017; Hesterberg et al., 2007; Kolo et al., 2019). Previous studies have shown that brucellosis has serious economic implications for the dairy and meat industries globally, especially in low-income countries including South Africa (McDermott et al., 2013). Moreover DAFF (2017) indicated that approximately 10% of dairy herds in RSA are infected, causing monetary losses amounting to R20 million ($1 million) annually. However, the baseline data on the prevalence of animal brucellosis in RSA are mostly based on testing. An important limitation of brucellosis serology is the inability to differentiate Brucella species and biovars which induced antibodies in the host and the zoonotic potential associated with those species as well as the cross-reactivity observed with other Gram-negative bacteria.

The bacteriology laboratory of the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) is the only national reference centre for isolation and typing of Brucella species in RSA. The laboratory receives diagnostic samples collected from different hosts in all the nine provinces in the country. The aim of this study was to analyse phenotypic typing results of Brucella species and biovars collected over an 11-year period (2008–2018) from all the nine provinces of RSA.

## 2 MATERIALS AND METHODS

### 2.1 Study area

The RSA is located at the southernmost tip of the African continent. It has a total land area of 1,220,813 km^2 which is bounded by 2,798 km of coastline spreading along the South Atlantic and the Indian Oceans. It shares borders with Namibia, Botswana, Zimbabwe, Lesotho, Mozambique and Eswatini. The country, divided into nine provinces, has an animal population of approximately 13.6 million beef cattle, 1.4 million dairy cattle, 24.6 78 million sheep, 7 million goats and 3 million game species (farmed; DAFF, 2017). For the control of brucellosis, the country uses live attenuated B. abortus S19 and RB51 vaccines in cattle and B. melitensis Rev1 in sheep and goat.

### 2.2 Data source

A retrospective study was conducted from the data of animal samples submitted to ARC-OVR between 2008 and 2018 for routine screening, which includes isolation and phenotypic typing of Brucella isolates. The samples were collected from different animal species including cattle, sheep, goats, dogs and game (wildlife) across the nine provinces of RSA. The sample types included organs, aborted foetuses, foetal tissues, lymph nodes, placenta, milk, semen, vaginal swabs and abomasal fluids as well as bacterial cultures. Bacterial culture samples were received from provincial veterinary laboratories and had been identified as Brucella species. Samples were transported to the laboratory according to the prescribed World Health Organisation (WHO) safety guideline (WHO, 1997) and the IATA—Infectious Substances Shipping Guidelines (IATA, 2006). The isolation of Brucella species and biovar typing was performed using conventional microbiological.
2.3 | Conventional microbiological techniques

2.3.1 | Isolation

The samples (field and reference) were processed based on their matrices (tissues, milk and abomasal fluids). All the tissue samples were subjected to homogenisation for 120 s in 5 ml sterile saline using a tissue homogenizer (Bead Ruptor 24 Elite; Omni International) and 0.5–2 ml of each homogenate was inoculated on 5% Sheep Blood Agar and Farrell's media (Onderstepoort Biological Products). The milk samples were centrifuged at 8,000 g at 4°C for 15 min. The supernatant was discarded whilst the cream and sediment were cultured separately on the media as mentioned above. Swabs and abomasal fluid samples were directly inoculated on the same media. The inoculated plates were incubated at 37°C in 5% CO₂ and checked every other day for presumptive Brucella colonies from day 3 up to 10 days.

2.3.2 | Confirmation and typing

The presumptive Brucella colonies were identified by staining with modified Ziehl–Neelsen (Stamp’s), the reaction to oxidase and catalase, as well as hydrolysis of urea and confirmed as Brucella species. Biovar and vaccine strains determination was performed using phenotypical tests which includes growth in different atmospheric conditions (aerobic and CO₂ requirement), production of hydrogen sulphide (H₂S), growth in the presence of different concentration of thionin acetate (20 mg/ml) and basic fuchsin (20 mg/ml) and agglutination with monospecific antisera A, M and R (Animal and Plant Health Agency). Furthermore, the isolates were tested for resistance or sensitivity to Brucella phages (Tb, Wb and Iz) at routine testing dilutions. The sugar and antibiotic sensitivity to erythritol (1,000 μg), penicillin G (10 units), streptomycin (10 μg) and rifampicin (30 μg) were also investigated as described. Based on the above reactions to the above phenotypical, biochemical, antibiotic and dyes tests the isolates were allocated into biovars and vaccine strains (S19 and RB51) using the OIE terrestrial manual (2009; 2016; 2018).

2.3.3 | Reference strains

The following bacterial strains were used as controls in this study, B. abortus biovar-1 ATCC23448, B. melitensis 16M/NCTC 10,094 as well as the lab-isolated references B. melitensis Rev1, and B. abortus S19.

2.3.4 | Data management and analysis

The type of data collected during this study was qualitative. A descriptive statistical analysis was used to summarize, display, explore and examine the data as described previously by Ott and Longnecker (2001). The analysis includes a frequency distribution of data using Bar graphs or Tables. The frequency distribution of data was done using the FREQ procedure of SAS software (Version 9.4; SAS Institute Inc.) and bar graphs were done using Microsoft Excel (2016).

3 | RESULTS

Table 1 summarizes the Brucella data that were collected during the period between 2008 and 2018. During the period under review a total of 963 strains of Brucella species were isolated and typed at the ARC-OVR Brucella reference laboratory. Of the 963 isolated strains of Brucella, the largest proportion was recovered from samples collected in cattle (n = 859; 89.2%), followed by sheep (n = 29; 3.0%), goats (n = 26; 2.7%), antelope (n = 13; 1.3%) and dogs (n = 10; 1.0%). A proportion of the samples (n = 18; 1.9%) was from unspecified wildlife animals.

The 963 isolated strains in this study represent four species of Brucella with 91.7% (n = 883) identified as B. abortus, 4.4% (n = 42) as B. melitensis, 3.0% (n = 29) as B. ovis and 0.9% (n = 9) as B. canis. Table 1 also gives a comparison of four isolated Brucella species over 11-year period which shows that B. abortus was reported throughout the duration of the study however, the frequency of detection differed depending on the year. The highest frequency for B. abortus was reported in the year 2012 while the lowest was in 2018. The other three species of Brucella were detected at a low frequency throughout the study period. However, a slightly elevated frequency of B. melitensis was observed in 2009 and 2015.

Analysis of the results according to the biovars revealed that of 883 B. abortus strains, 90.1% (n = 800) were identified as B. abortus biovar-1, and 5.7% (n = 50) as B. abortus biovar-2. It was also revealed that 3.3% (n = 29) and 0.5% (n = 4) of B. abortus strains were reported as S19 and RB51 vaccine strains, respectively (Table 2). Brucella abortus biovar-1 was predominantly reported from cattle samples (96.5%) followed by unspecified wildlife (2.1%) and antelope (1.4%). Brucella abortus biovar-2, S19 and RB51 strains were reported only in cattle samples (Table 3).

### TABLE 1 Summary of laboratory diagnostic data for characterisation of Brucella species in the Republic of South Africa

| Year | B. abortus | B. canis | B. melitensis | B. ovis | Total |
|------|------------|----------|---------------|---------|-------|
| 2008 | 66         | 0        | 7             | 6       | 79    |
| 2009 | 78         | 0        | 12            | 4       | 94    |
| 2010 | 107        | 1        | 3             | 0       | 111   |
| 2011 | 97         | 1        | 1             | 1       | 100   |
| 2012 | 121        | 0        | 2             | 0       | 123   |
| 2013 | 68         | 1        | 0             | 2       | 71    |
| 2014 | 90         | 1        | 4             | 0       | 95    |
| 2015 | 87         | 3        | 10            | 3       | 103   |
| 2016 | 68         | 0        | 1             | 0       | 69    |
| 2017 | 51         | 2        | 0             | 4       | 57    |
| 2018 | 50         | 0        | 2             | 9       | 61    |
| Total | 883 (91.7) | 9 (0.94) | 42 (4.4)      | 29 (3.0) | 963   |
Differentiation of *B. melitensis* strains into biovars showed that 71.4% \((n = 30/42)\) were reported as *B. melitensis* biovar-1 and 26.2% \((n = 11/42)\) as *B. melitensis* biovar-3 while 2.4% \((n = 1/42)\) were *B. melitensis* biovar-2 (Table 3). *Brucella melitensis* biovar-1 was most numerous in goat samples at 73.3% \((n = 22/30)\) followed by antelope samples at 20% \((n = 6/30)\). However, *B. melitensis* biovar-3 was reported at a higher frequency of 54.6% \((n = 6/11)\) in antelopes than the frequency of 27.3% \((n = 3/11)\) in goats. Interestingly, 18.2% \((n = 2/11)\) and 3.3% \((n = 1/11)\) of samples from cattle were positive for *B. melitensis* biovar-3 and *B. melitensis* biovar-1, respectively (Table 3). *Brucella canis* and *B. ovis* were only reported in dog and sheep samples, respectively.

The distribution of *Brucella* biovars varied greatly based on geographical location. Gauteng and Western Cape were shown (Figure 1) to be the only provinces that reported all biovars of *B. abortus* and *B. melitensis* with the exception of biovar-2 of *B. melitensis* in Western Cape. Further analysis revealed a high frequency of *B. abortus* biovars in the Free State \((22.3\%, n = 213/954)\) and KwaZulu-Natal \((15.8\%, n = 151/954)\) provinces and a low frequency in the Northern Cape \((1.6\%, n = 13/954)\). Moreover *B. melitensis* biovar-1 was reported in six provinces, namely, Free State \((26.7\%)\), Western Cape \((23.3\%)\), KwaZulu-Natal \((16.7\%)\), Mpumalanga \((16.7\%)\), Gauteng \((13.3\%)\) and Limpopo \((3.3\%)\). *Brucella melitensis* biovar-3 was reported most frequently from Gauteng \((54.5\%)\) followed by Western Cape \((36.4\%)\) and KwaZulu-Natal \((3.9\%)\) provinces (Figure 1). *Brucella abortus* S19 vaccine strain was reported in varied frequencies across all provinces with absences in North West province while *B. abortus* RB51 was detected in Gauteng \((25\%)\), Western Cape \((25\%)\) and KwaZulu-Natal \((50\%)\).

Of the 963 isolated strains of *Brucella* species in this study, the frequencies of detection were high in organ samples \((n = 617; 64.0\%)\) and lymph node samples \((n = 134; 13.9\%); Table 4\). The detection rates of different *Brucella* biovars varied greatly in organ and lymph node samples. A low frequency of occurrence was reported from milk \((n = 26; 2.6\%)\), abomasal fluids \((n = 14; 0.6\%)\), semen \((n = 6; 0.1\%)\), placenta and cotyledon \((n = 5; 0.5\%)\) (Table 4). Interestingly, *B. abortus* S19 was isolated from an intestinal sample.

### DISCUSSION

Animal brucellosis is endemic in most regions of RSA, based on serological testing, however, the diversity of the biovars causing this disease throughout the country is unknown. A retrospective laboratory-based study, such as this one is considered as a critical component of epidemiological surveys to evaluate the success of animal brucellosis control measures and preventive strategies, thus,
which is important in determining the animal species and geographical areas that are prone to contamination by Brucella species and biovars (Sayan & Gürbilek, 2014). In addition, a retrospective seroprevalence study conducted previously (Kolo et al., 2020) showed overall seropositivity of 5.85% (44,687/764,276) for brucellosis in cattle, sheep, pigs and goats, with individual animal species seropositivity at 6.31%, 2.09%, 0.63% and 0.13%, respectively, from 2007 to 2015. The above-mentioned study also showed that the provincial seropositivity distribution among tested animals varies widely with a range of 1.84%–17.65%. Although the study (Kolo et al., 2020) only accounts for samples tested at ARC-OVR laboratory, it is important as the results of study indicate the likelihood of infections within the country.

A review of laboratory-generated data on Brucella species and biovars in animals is an uncomplicated method to assess the occurrence of the disease in the country. However, it should be emphasised that isolation and typing of Brucella species and biovars performed in this study is not a systematic review of all brucellosis cases and outbreaks in RSA. Therefore, the data presented might be biased. Considering the duration covered (11 years) by this study and the sample size analysed, the data generated provide current and important overview information on Brucella species and biovars in RSA. The main focus of the present study was to show the distribution of Brucella species and biovars in different animal hosts and geographical areas throughout the nine provinces of RSA. Isolation of the pathogen is regarded as the gold standard in the diagnosis of brucellosis (OIE, 2016). A total of 963 Brucella strains were isolated between 2008 and 2018 at the Brucella reference laboratory (ARC-OVR). The reason for a low number of Brucella strains isolated and reported in this study compared to 44,687 seropositive animals tested between 2007 and 2015 in RSA (Kolo et al., 2020) could be that bacteriological diagnosis using biotyping is less sensitive, expensive, time-consuming and involves hazardous culture hence is only recommended as a confirmatory test (Poester et al., 2010). It is also subjective meaning that requires expertise and it is likely prone to inconsistency between laboratories (DAFF, 2017; Lucero et al., 2005; Whatmore et al., 2014). Furthermore, this method is likely to become less relevant as new Brucella species that are atypical and diverge from classical criteria emerge continuously. (Whatmore et al., 2014). The high biosafety and security risks associated with bacteriological diagnosis of Brucella species are also important factors to consider (OIE, 2016).

The number of Brucella strains recovered from different animal species varied greatly with 89.2%, 3.0%, 2.7%, 1.1% and 1.0% for cattle, sheep, goats, antelope and dogs, respectively. As a result of the brucellosis scheme in SA, which is aimed at testing cattle (DAFF, 2017), it was no surprise that the majority of samples in the present study were from cattle. The scheme is in some way biased toward the cattle population while other livestock have not received much attention; thus, resulting in higher numbers of Brucella species isolated from cattle as compared to sheep, goats and dogs.

Only four Brucella species were identified during this retrospective study with a frequency of detection of 3.0%, 0.9%, 4.4% and 91.6% for B. ovis, B. canis, B. melitensis and B. abortus, respectively. Previous studies regarding the occurrence of Brucella species in different animal species reported the presence of B. abortus (53.4%), B. melitensis (43.2%), B. ovis (0.2%), B. suis (3.1%), and B. ceti (0.1%) in Italy (De Massis et al., 2019; Di Giannatale et al., 2008). In 2007, Lucero and co-workers also reported the occurrence of B. abortus (38.5%), B. melitensis (42.5%), B. suis (16.3%), B. ovis (1.6%) and B. canis (1.1%) among livestock in Latin American countries. Cao et al. (2018) reported the following occurrence of Brucella species; B. melitensis (86.4%), B. abortus (10.1%) and B. suis (3.0%) in livestock from four provinces in China. Although Karagul et al. (2018) suggested that the distribution of Brucella species seem to differ based on geographical locations, substantial evidence that indicates the

### TABLE 4 Distribution of Brucella species and biovars in different sample types

| Sample types          | B. abortus (%) | B. melitensis (%) | B. canis | B. ovis | Total |
|-----------------------|----------------|-------------------|----------|---------|-------|
|                       | 1   | 2   | S19 | RB51 | 1   | 2   | S19 | RB51 | 1   | 2   | S19 | RB51 | 1   | 2   | S19 | RB51 | 1   | 2   | S19 | RB51 |
| Previously isolated organisms | 69 (8.6) | 13 (26.0) | 16 (55.2) | 3 (75.0) | 17 (56.7) | 0 (0) | 10 (90.9) | 9 (100) | 13 (44.8) | 150 (15.6) |
| Lymph nodes           | 122 (15.3) | 1 (2.0) | 5 (17.2) | 0 (0) | 5 (16.7) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 134 (13.9) |
| Organs                | 577 (72.1) | 22 (44.0) | 2 (6.9) | 0 (0) | 5 (16.7) | 0 (0) | 1 (9.1) | 0 (0) | 10 (34.5) | 617 (64.1) |
| Milk                  | 26 (3.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 26 (2.7) |
| Cotyledon             | 0 (0) | 2 (4.0) | 3 (10.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5 (0.5) |
| Placenta              | 0 (0) | 5 (10.0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5 (0.5) |
| Semen                 | 0 (0) | 0 (0) | 1 (3.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.1) |
| Intestine             | 0 (0) | 0 (0) | 1 (3.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.1) |
| Abomasal fluids       | 1 (0.13) | 7 (14.0) | 2 (6.9) | 1 (25.0) | 3 (10.0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 14 (0.6) |
| Vaginal swabs         | 4 (0.5) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 4 (0.4) |
| Total                 | 800 | 50 | 29 | 4 | 30 | 1 | 11 | 9 | 29 | 963 |
cause of this variation in the occurrence of Brucella species is currently poorly understood.

The detection frequency for B. abortus was high from 2010 to 2012 and low between 2017 and 2018; however, the reason for this variation could be due to the severe drought with the country experienced in 2015–2018 period as it may have affected livestock (AgriSA, 2019; Klipple & Costello, 1960). The high detection frequency for B. abortus reported in this study coincide with findings reported by Kolo et al. (2020) which found cattle seropositive samples were the highest during the same period (2010–2014) as our study. The differentiation of B. abortus into biovars showed the isolation of B. abortus biovar-1 and B. abortus biovar-2 in cattle, antelope and unspecified wildlife in RSA. Furthermore, the high isolation frequency of B. abortus biovar-1 reported in the majority of the samples from cattle, antelope and unspecified wildlife corresponds with previous RSA reports that indicated that B. abortus biovar-1 has been predominantly isolated from bovine in all provinces countrywide (Bishop et al., 1994; Chisi et al., 2017; Gradwell, 1977). Moreover studies from the neighboring Zimbabwe also showed a high frequency of B. abortus biovar-1 isolation from a wide range of animals (Matope et al., 2010; Mohan et al., 1996). The results obtained with our study as well as the previous studies carried out in RSA suggest that B. abortus biovars 3–7 and 9 possibly do not occur in the country since they have not been reported and can currently be considered exotic.

The isolation of B. abortus biovar-2 was reported only in cattle in this study but to a lesser extent as compared to B. abortus biovar-1. This appears to be a standard in RSA and Zimbabwe given that the results are in agreement with previous findings in the two countries (Bishop et al., 1994; Chisi et al., 2017; Matope et al., 2010; Mohan et al., 1996). Aparicio (2013) also indicated that this biovar has been isolated from cattle around the world. Brucella abortus biovar-1 has also been reported as prevalent in livestock from northwest provinces of China (Cao et al., 2018). The frequency of isolation for B. abortus biovar-2 in the current study was low than the 10% previously reported in 1981/2 in KwaZulu Natal and could be considered epidemiologically important in the emergence of the disease in some part of the country (Coetzer & Tustin, 2004). However, B. abortus biovar-2 was reported as the most predominant biovar in Israel (Crawford et al., 1990).

Brucella abortus vaccine strains 19 and RB51 were also isolated from cattle in the present study. Both vaccine strains are predominantly used for vaccination of cattle against brucellosis in RSA (Simposon et al., 2018). The vaccination of all heifers between the ages of 3–8 months with B. abortus S19 is mandatory as part of the brucellosis control scheme in RSA. Moreover the availability of B. abortus S19 vaccine has been problematic for some time (DAFF, 2017; Hesterberg et al., 2007) and B. abortus RB51 has been used as an alternative, hence its isolation from the cattle. To a greater extent, isolation of B. abortus vaccine strain 19 from cattle suggests that cattle older than the recommended age of 3–8 months (pregnant adult cattle) might have been vaccinated previously. After vaccination of cattle with one, two or three doses prior to breeding age, McDiarmid (1957) recovered S19 from 10% of milk samples and 1.5% of samples from cases of abortion. Furthermore, the vaccination of infected animals with S19 does not cure nor alter the normal course of the disease. In another study, Diaz et al. (1968) has shown that the serological screening of most animals vaccinated post the recommended age resulted in persistent reactions against the antigenic O-chain of the smooth lipopolysaccharide from Brucella strains. Moreover other studies also indicated that although B. abortus S19 live vaccine provide protection to 70% of the cattle vaccinated against the virulent wild-type strains, the full dose (5 × 10^10) of this attenuated vaccine can also induce abortions if given to pregnant animals (Nicoletti, 1990; Schurig et al., 2002).

Isolation of B. melitensis remained low throughout the 11-year period of this study with a slight increase observed in 2009 and 2015. The increase can be attributed to two outbreaks of B. melitensis that occurred in Gauteng (Kungwini local municipality) and Western Cape (Beaufort West local municipality) respectively (Wojno et al., 2016; https://www.dalrrd.gov.za/doaDev/sideMenu/Import%20&%20Export%20Standard/docs/brucella%20Melitensis.pdf). The seroprevalence of brucellosis was also high in 2009–2010 in goats, which coincide with outbreak in Gauteng in 2010, however, not the outbreak in 2015 as serological tests might have been done by the Western Cape provincial laboratory (https://www.dalrrd.gov.za/doaDev/sideMenu/Import%20&%20Export%20Standard/docs/brucella%20Melitensis.pdf). All three biovars (1–3) of B. melitensis were reported in the current study with B. melitensis biovar-1 representing 90% of isolates from goats and antelope across nine provinces of RSA over the past 11 years. In the past, B. melitensis biovar-1 has been isolated from goats in Sub Saharan Africa and Asia (Ducrotoy et al., 2017; Zhou et al., 2020). Glover et al. (2020) reported the isolation of B. melitensis biovar-1 and biovar-3 in antelopes in RSA. B. melitensis biovar-1 and B. melitensis biovar-3 were also reported in cattle in the current study. Although B. melitensis biovar-1 and 3 infections in cattle are rare, their isolation has been reported in different countries including RSA (Corbel, 1988; Karagul et al., 2018; Kolo et al., 2019). Even if the preferred hosts for B. melitensis includes goats, sheep and antelope, spill over to other animals can occur particularly in enzootic areas (Nyirenda et al., 2016). For instance, Verger et al. (1989) reported B. melitensis in cattle in France, while Zhang et al. (2018) isolated B. melitensis aborted cow and sheep fetuses in Northwest of China. Most importantly, isolation of B. melitensis biovars in cattle, goats and antelopes in the current study present a huge challenge in veterinary and public health as this species is highly pathogenic to humans. Subsequently, it poses a risk of spillover to other farms and other species around those geographical areas (Godfroid et al., 2014). This also has serious implications for control of brucellosis in the country since the current brucellosis testing scheme targets cattle only.

A low frequency of B. canis and B. ovis isolation was reported in the present study and it can be attributed to the fact that they have never been actively surveyed as they are not considered a priority in RSA as it is not zoonotic pathogen. However, there is a lot of
serological testing performed for *B. ovis* in rams in country although not a survey outbreaks in sheep have been identified. Previous studies showed that few localised cases of *B. canis* in Hermanus, Bedford, Knysna and Somerset West in the Western Cape Province, of RSA (Gous et al., 2005; Van Helden, 2012).

Globally, various *Brucella* species occur in different countries or even areas within the country. Previous studies indicated that 90% of brucellosis infections in RSA are due to *B. abortus* biovar 1 and 10% are due to *B. abortus* biovar 2 (Bishop et al., 1994). This trend was also observed in this study with a substantial number of *B. abortus* infections (90.6%) throughout all the nine provinces of the country being due to *B. abortus* biovar-1 and to a lesser extent from *B. abortus* biovar-2 (5.67%), *B. abortus* vaccine S19 (3.28%) and *B. abortus* vaccine strain RB51 (0.45%). The lower frequency of infection due to biovars of *B. melitensis* observed in this study is presumably due to the fact that goats and sheep, which are preferred hosts of *B. melitensis* are infrequently tested as compared to cattle. However, the occurrence of *B. melitensis* in Gauteng, Kwa-Zulu Natal and Western Cape Provinces of RSA was possible since it has been reported previously (Ribeiro et al., 1990; Wojno et al., 2016).

The sample types required for the isolation of *Brucella* are mostly chosen based on the observed clinical signs; however, isolation in the present study was performed using sample types submitted to the laboratory. Samples sent for routine screening/diagnosis should be stored properly considering the sample type and the distance between the collection site and the laboratory, so as to avoid contamination or sample spoilage (Alton et al., 1988). Thus, the extent of isolation cannot be correctly determined based on this factor since high isolation rates will likely be recorded from the most submitted sample types, which was the case with organs and lymph nodes. However, it is worth mentioning that the isolation of *B. abortus* S19 vaccine strain from intestines was unanticipated. Nevertheless, suitable samples types for isolation of *Brucella* from animals usually include stomach contents of the aborted foetuses, foetal membranes, vaginal secretions, milk, hygromas fluids, tissue samples from the udder, mammary and genital lymph nodes, etc. whereas in humans it can be isolated from blood, urine and cerebrospinal fluid (Alton et al., 1988; Bishop et al., 1994).

## 5 | CONCLUSION

In conclusion, a retrospective analysis and review of 11-year data on *Brucella* species and biovars from the General Bacteriology Reference Laboratory at ARC-OVR has indicated that animal brucellosis is widespread in RSA. The current study has provided baseline data on the distribution of *Brucella* species and biovars in the country and it forms a basis for prospective epidemiological studies. The data generated in this study gives an insight into provinces that possibly need interventions for better control measures for brucellosis. It will also be critical in the improvement of policy to control animal brucellosis in the country. Future studies should involve molecular typing such as Multi Locus VNTR Analysis and whole genome sequence of *Brucella* isolates from this study (OVR depositary) in order to obtain have data that can be used for source tracking and comparison with strains that are associated with brucellosis is in human beings, which is significant from a ‘one health’ standpoint.

## 6 | STUDY LIMITATION

The main limitation of this study was bias in samples submitted for testing, as not all suspect cases of brucellosis in the country were included. Also, sample submission forms were not fully completed so we could not capture all the necessary information to facilitate drawing more concise interpretations.

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## AUTHOR CONTRIBUTION

Itumeleng Matle: Conceptualization; Funding acquisition; Supervision; Writing-original draft. Betty Ledwaba: Writing-review & editing. Kudakwashe Jambwa: Methodology; Writing-review & editing. Lavhelesani Makhado: Data curation; Methodology. Karabelo Madiba: Methodology; Writing-review & editing. Nombasa Ntushelo: Formal analysis; Methodology; Writing-review & editing.

## PEER REVIEW

The peer review history for this article is available at https://pubns.com/publon/10.1002/vms3.483.

## DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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## REFERENCES

AgricSA. (2019). https://www.dailymaverick.co.za/article/2019-11-13-south-africa-unprepared-for-drought-disaster-agrisa-warns-government/. Accessed on 17 February 2021

Alton, G., Jones, L., Angus, R., & Verger, J. (1988). Techniques for the Brucellosis laboratory. Institute National de la Recherche Agronomique.

Aparicio, E. D. (2013). Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis*, *Brucella suis* and *Brucella abortus*. *Revue Scientifique et Technique de l'OIE*, 32(1), 53–60. https://doi.org/10.20506/rst.32.1.2187

Behrozikhah, A. M., Bagheri Nejad, R., Amiri, K., & Bahonar, A. R. (2012). Identification at biovar level of *Brucella* isolates causing abortion
three abattoirs of Northern Nigeria. *Journal of Veterinary Medicine and Animal Health, 8*(3), 15–20.

Matope, G., Makaya, P. V., Dhihiwayo, S., Gadha, S., Madekurozwa, R. L., & Pfukenyi, D. M. (2010). A retrospective study of Brucellosis sero-prevalence in commercial and smallholder cattle farms of Zimbabwe. *Bulletin of Animal Health and Production in Africa, 58*(4), 340–348.

McDermott, J., Grace, D., & Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique, 32*(1), 249–261. https://doi.org/10.1050/rest.32.2.2197

McDiarmid, A. (1957). The degree and duration of immunity in cattle resulting from vaccination with S. 19 Br. abortus vaccine and its implication in the future control and eventual eradication of brucellosis. *Veterinary Record, 69*, 877–879.

Mohan, M., Makaya, P. V., Muvavarirwa, P., Matope, G., Mahembe, E., & Pawandwa, A. (1996). Brucellosis surveillance and control in Zimbabwe: Bacteriological and serological investigation in dairy herds. *Onderstepoort Journal of Veterinary Research, 63*, 47–51.

Moreno, E. (2014). Retrospective and prospective perspectives on zoonotic brucellosis. *Frontiers in Microbiology, 5*, 213. https://doi.org/10.3389/fmicb.2014.00213

Nicoletti, P. (1990). Vaccination against Brucella. *Advances in Biotechnological Processes, 13*, 147–168.

Nyirenda, M., Letlojane, L., & Syakalima, M. (2016). Prevalence of Brucella abortus in buffaloes of Mafikeng game reserve, North West province, South Africa: A retrospective study. *Indian Journal of Animal Research, 50*(2), 281–283.

OIE. (2016). Brucellosis (infections with B. abortus and B. suis). Chapter 2.1.4. In *Manual of diagnostic tests and vaccines for terrestrial animals*. World Health Organization. http://www.oie.int. Accessed on 28 March 2020

Ott, R. L., & Longnecker, M. (2001). *An introduction to statistical methods and data analysis: Brooks*. Cole, Cengage Learning, 1273 pp.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsiansos, E. V. (2006). The new global map of human brucellosis. *The Lancet Infectious Diseases, 6*(2), 91–99. https://doi.org/10.1016/S1473-3099(06)70382-6

Poester, F., Nielsen, K., Ernesto Samaritno, L., & Ling Yu, W. (2010). Diagnosis of brucellosis. *The Open Veterinary Science Journal, 4*(1).

Ribeiro, L. M. M., Herr, S., Chaparro, F., & Van der Vyver, F. H. (1990). The isolation and serology of Brucella melitensis in a herd of goats in central RSA. *Onderstepoort Journal of Veterinary Research*, 57, 143–145.

Ross, H. M., Jahans, K. L., MacMillan, A. P., Reid, R. J., Thompson, P. M., & Foster, G. (1996). *Brucella* species infection in North Sea seal and cetacean populations. *The Veterinary Record, 138*(26), 647–648.

Sayan, M., & Gürbilek, S. E. (2014). Single nucleotide polymorphism analysis of the rpoB gene region for genotyping of Brucella melitensis strains isolated from field in Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23*(3), 411–415.

Scholz, H. C., Hubalek, Z., Sedláček, I., Vergnaud, G., Tomaszo, H., Al Dahouk, S., Melzer, F., Kämpfer, P., Neubauer, H., Cloeckaert, A., Maqurt, M., Zygmunt, M. S., Whatmore, A. M., Falsen, E., Bahn, P., Göllner, C., Pfiffer, M., Huber, B., Busse, H. J., & Nöckler, K. (2008). *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. *International Journal of Systematic and Evolutionary Microbiology, 58*, 375–382. https://doi.org/10.1099/ijs.0.05356-0

Scholz, H. C., Nöckler, K., Göllner, C., Bahn, P., Vergnaud, G., Tomaszo, H., Al Dahouk, S., Kämpfer, P., Cloeckaert, A., Maqurt, M., Zygmunt, M. S., Whatmore, A. M., Pfiffer, M., Huber, B., Busse, H. J., & De B. K. (2010). *Brucella inopinata* sp. nov., isolated from a breast implant infection. *International Journal of Systematic and Evolutionary Microbiology, 60*, 801–808. https://doi.org/10.1099/ijs.0.011148-0

Scholz, H. C., Revilla-Fernandez, S., Al Dahouk, S., Hammerl, J. A., Zygmunt, M. S., Cloeckaert, A., Koylass, M., Whatmore, A. M., Blom, J., Vergnaud, G., Witte, A., Aistleitner, K., & Hofer, E. (2016). *Brucella vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). *International Journal of Systematic and Evolutionary Microbiology, 66*, 2090–2098. https://doi.org/10.1099/ijsem.0.005998

Schurig, G. G., Srinanganathan, N., & Corbel, M. J. (2002). Brucellosis vaccines: Past, present and future. *Veterinary Microbiology, 90*(1–4), 479–496. https://doi.org/10.1016/S0378-1135(02)00255-9

Simpson, G. J., Marriottt, T., Rourke, E., Chiulando, A., Letteson, J. S., & Godfroid, J. (2018). Immunological response to Brucella abortus strain 19 vaccination of cattle in a communal area in South Africa. *Journal of the South African Veterinary Association, 89*(1), 1–7.

Van Helden, L. (2012). *Information sheet: Brucella canis*. stellenbosch: Western Cape Government Department of Agriculture.

Verger, J. M., Garin-Bastuji, B., Grayon, M., & Mahé, A. M. (1989). La brucellose bovine à Brucella melitensis en France [Bovine brucellosis caused by Brucella melitensis in France]. *Annales de Veterinary Research, 20*(1), 93–102. French. PMID: 2930137.

Verger, J., Grimont, P. F., Grimont, P., & Grayon, M. (1985). *Brucella*, a mono-specific genus as shown by deoxyribonucleic acid hybridization. *International Journal of Systematic Bacteriology, 35*, 292–295. https://doi.org/10.1099/00207713-35-3-292

Whatmore, A. M., Davison, N., Cloeckaert, A., Al Dahouk, S., Zygmunt, M. S., Breve, S. D., Perrett, L. L., Koylass, M. S., Vergnaud, G., Quance, C., Scholz, H. C., Dick, E. J., Hubbard, G., & Schlabritz-Loutsevitch, N. E. (2014). *Brucella papionis* sp. nov., isolated from baboons (*Papio spp*.). *International Journal of Systematic and Evolutionary Microbiology, 64*(Pt 12), 4120–4128. https://doi.org/10.1099/0.665482-0

Wojno, J. M., Moodley, C., Pienaar, J., Beylis, N., Jacobsz, L., Nicol, M. P., Rossouw, J., & Bamford, C. (2016). Human brucellosis in South Africa: Public health and diagnostic pitfalls. *South African Medical Journal, 106*(9), 883–885. https://doi.org/10.7196/SAMJ.2016.v106i9.11020

World Health Organization (WHO). (1997). *Guidelines for the safe transport of infectious substances and diagnostic specimens*. WHO/EMC/97.3. WHO, 15 pp. https://www.who.int/csr/resources/publications/biosafety/WHO_EMC_97_3_EN/en/

Young, E. J. (1995). An overview of human brucellosis. *Clinical Infectious Diseases, 21*(2), 283–289. https://doi.org/10.1093/clinids/21.2.283

Zhang, H., Song, S., Wang, B., Jiang, Y., Wu, W., Guo, F., Liu, Y., Wang, Q., Zhang, J., Zhang, H., & Sheng, J. (2018). *Brucella melitensis* isolated from aborted cow and sheep fetuses in northwest of China. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 24*(2), 307–310.

Zhu, X., Zhao, Z., Ma, S., Guo, Z., Wang, M., Li, Z., & Liu, Z. (2020). *Brucella melitensis*, a latent “travel bacterium”, continual spread and expansion from Northern to Southern China and its relationship to worldwide lineages. *Emerging Microbes & Infections, 9*(1), 1618–1627. https://doi.org/10.1002/emom.2020.1788995

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