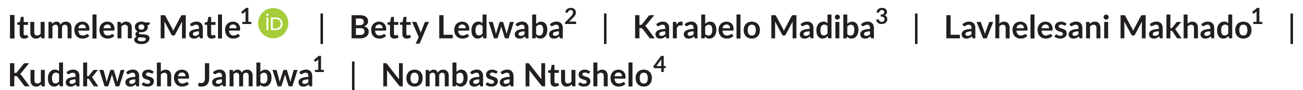
DOI: 10.1002/vms3.483

ORIGINAL ARTICLE

WILEY

Characterisation of *Brucella* species and biovars in South Africa between 2008 and 2018 using laboratory diagnostic data



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Abstract

Background: Brucellosis is an infectious zoonotic bacterial disease of humans and other animals. In the Republic of South Africa (RSA), animal brucellosis is widespread and the current available data on the prevalence of this disease rely solely on serological testing. The primary limitation of brucellosis serology is the lack of discriminatory powers to differentiate between *Brucella* species and biovars as well as the cross-reactivity observed with other Gram-negative bacteria.

Aim: The aim of this study was to conduct a retrospective laboratory-based survey on *Brucella* species and biovars isolated from various animal species in SA between 2008 and 2018.

Material and Methods: The isolation of *Brucella* species and biovar typing was performed using conventional microbiological techniques.

Results and Discussion: A total of 963 strains of Brucella species were included in this study with a frequency of detection for *B. abortus* (n = 883; 91.6%) followed by *B. melitensis* (n = 42; 4.4%), *B. ovis* (n = 29; 3.0%) and *B. canis* (n = 9; 0.9%). Of the 883 strains of *B. abortus*, 90.1% were typed as *B. abortus* biovar-1 while 5.7% as *B. abortus* biovar-2, and 3.3% and 0.5% were *B. abortus* S19 and B. *abortus* RB51 vaccine strains, respectively. Among the 42 *B. melitensis* strains, 71.4% were reported as *B. melitensis* biovar-1 and 26.2% as *B. melitensis* biovar-3 while 2.4% was *B. melitensis* biovar-2.

Conclusion: A retrospective study, such as this one, provides useful information that can be critical in formulating policies and strategies for the control and eradication of brucellosis in animal populations in RSA.

KEYWORDS
Brucella species, biovar, retrospective study, South Africa

1 | 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

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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* (Scholz 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) in terms of the bovine brucellosis scheme (R.2483 of 9 Dec 1988) which was established 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 whist 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% CO_2 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 CO_2 requirement), production of hydrogen sulphide (H_2S), 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

	***Brucella* species (%)**				
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

TABLE 2 *Brucella abortus* biovar distribution per animal species between 2008 and 2018

Brucella biovars	Animal species (%)				Total
	Antelope	Cattle	Goats	Unspecified wildlife animal	Total (n = 883)
B. abortus biovar-1	11 (1.4)	772 (96.5)	0 (0)	17 (2.1)	800
B. abortus biovar-2	0 (0)	50 (100)	0 (0)	0 (0)	50
B. abortus S19	0 (0)	29 (100)	0 (0)	0 (0)	29
B. abortus RB51	0 (0)	4 (100)	0 (0)	0 (0)	4

TABLE 3 *Brucella melitensis* biovar distribution per animal species between 2008 and 2018

Brucella biovars	Animal species (%)				Total
	Antelope	Cattle	Goats	Unspecified wildlife	Total (n = 42)
B. melitensis biovar-1	6 (20)	1 (3.3)	22 (73.3)	1 (3.3)	30
B. melitensis biovar-2	0 (0)	0 (0)	1 (100)	0 (0)	1
B. melitensis biovar-3	6 (54.6)	2 (18.2)	3 (27.3)	0 (0)	11

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 antelope 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 (0.91%) 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 0.1%) 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.

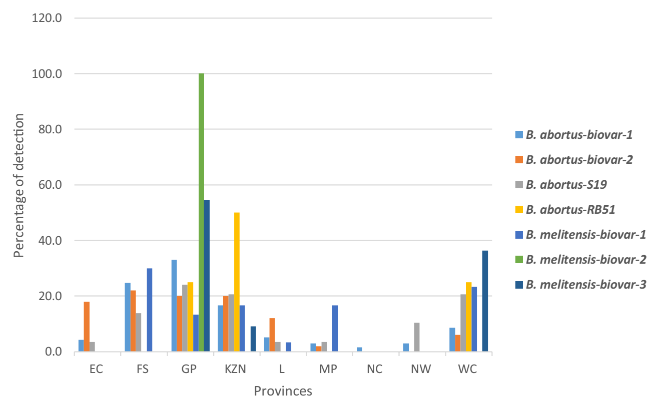

FIGURE 1 Distribution of *Brucella* species biovars in different provinces of South Africa EC, Eastern Cape; FS, Free State; GP, Gauteng; KZN, KwaZulu Natal; L, Limpopo; MP, Mpumalanga; NC, Northern Cape; NW, North West; WC, Western Cape

4 | 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,

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	3			
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)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (20.7)	6 (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

protecting human health (Behroozikhah et al., 2012). It also provides useful information on the changing patterns of animal brucellosis, 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 *Brucell*a 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

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 ($5–8 \times 10^{10}$) 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/Food%20Import%20&%20Export%20Standard/docs/brucella%20Melitensis.pdf). The seroprevelance 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/Food%20Import%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; Zhu 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 el., 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.

ACKNOWLEDGEMENTS

The authors thank the ARC for providing all research facilities and funding for this work. The authors also thank Mr Kekana from ARC: OVR for technical support.

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://publons.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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How to cite this article: Matle I, Ledwaba B, Madiba K, Makhado L, Jambwa K, Ntushelo N. Characterisation of *Brucella* species and biovars in South Africa between 2008 and 2018 using laboratory diagnostic data. *Vet Med Sci*. 2021;7:1245–1253. https://doi.org/10.1002/vms3.483