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The status of bovine brucellosis in Gauteng Province, South Africa, 2020

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ABSTRACT

Introduction: A population-based survey was carried out to estimate the prevalence of bovine brucellosis in an endemic area (Gauteng Province, South Africa) and to study possible risk factors. **Methods:** In 2019-2020, 254 herds (9111 cows) were randomly selected, tested serologically and classified as positive/negative using a two-test system. **Results:** The herd-level (13.6%), individual-level (1.7%) and within-herd (8.9%) prevalences were estimated. Several risk factors are identified in this population that could help to target disease control – large herds, shared communal grazing land, herds being unvaccinated, and testing with isolation of new cattle before introduction to the herd. **Conclusion:** Overall, the bovine brucellosis prevalence had not changed in the past four years but it was less than the prevalence within surrounding countries in the region. The reported vaccination coverage had improved since the previous survey but remained inadequate with many owners not knowing the vaccination status of their cattle. We discuss if elimination is possible in this province considering the prevalence level, impending control policy changes and communal farming practices. We describe lessons learned in the field by Provincial Veterinary Services and suggest what is needed for successful control and eventual eradication in this setting.

Introduction

Brucellosis is an important bacterial disease affecting domestic animals, wildlife and people [1]. Despite being a priority zoonotic disease [2], it remains relatively neglected by veterinary and medical sectors in sub-Saharan Africa [3]. The disease in all animals is significant, but control programs mainly exist for brucellosis in cattle [3]. Bovine brucellosis is most often caused by *Brucella abortus* biovars but can be caused by other *Brucella* species where cattle are raised with other animals [1,4]. Cattle owners experience substantial economic losses from late-term abortions, decreased milk production and herd infertility due to bovine brucellosis [1,4]. It spreads between herds by the introduction of infected individuals and between cattle by ingestion of the bacteria within infected birth products or discharges [4,5]. It is also a public health problem, causing febrile illness in people and possible complications affecting other organs systems [6]. Occupational exposure is common among farm personnel, animal healthcare workers, abattoir workers and laboratory staff who come into contact with the bacteria by ingestion, inhalation, mucous membrane contact or injection [1,4,5]. The most effective way to prevent the disease in humans is to control it in animals [2,3,7].

Countries like Australia, New Zealand, Japan, Canada and some members of the European Union have eliminated brucellosis [8]. Yet it remains a major problem for low-income countries [9], where public animal health services have declined due to competing health problems demanding attention, decreased government resources for operational costs of disease control and lack of epidemiological data to justify disease control programs [3,10]. Surveillance and active control of brucellosis in sub-Saharan Africa is mainly implemented in southern Africa and only recently [3]. In South Africa (SA) brucellosis has been a controlled animal disease since 1984 [11-12]. To eradicate brucellosis in SA, the Bovine Brucellosis Scheme was launched in 1979, and comprised of vaccination of susceptible heifers/cows; permanent identification, branding ('C brand') and voluntary slaughter of positive cattle; a herd accreditation program; declaration of eradication areas for mandatory identification, testing and slaughter of positive cattle [13]. In the 1980´s, brucellosis control was prioritised by national veterinary services and there was good progress [4] but the disease later got out of control when responsibility of continued vaccination and testing shifted from government to livestock owners and state veterinary services became decentralised in 1994 [7]. Currently, the Bovine Brucellosis Scheme is voluntary, and nine Provincial State Veterinary Services independently manage the programs within the Scheme. There are programs for monitoring brucellosis-negative herds, surveillance to detect newly infected herds, and managing infected herds. Infected herds fall under compulsory statutory control [5]. Unfortunately, compliance by cattle owners and enforcement of legislation are lacking countrywide. Thus, the Bovine Brucellosis Control Policy was reviewed in 2020 to encompass more compulsory actions, stricter controls, and possible incentives. These changes must still be changed in the legislation [7].

Vaccination against brucellosis reduces the disease incidence in the most economical way in endemic settings [7,8,14]. It is a valuable tool used in elimination programs to reduce prevalence low enough that test-and-slaughter becomes a feasible method for stamping it out [10]. In sub-Saharan Africa, vaccination programs have mainly been utilised in southern Africa, with SA probably having the most sustained program. Outside of southern Africa, livestock vaccination for brucellosis is rarely done or on ad hoc basis [3,15]. Two vaccines are registered for use in bovines in SA, namely *B. abortus* strain 19 (S19) and *B. abortus* strain RB51 (RB51) [5]. It is compulsory for owners to vaccinate heifers aged 4-8 months with either strain. Booster vaccinations with RB51 are recommended for more complete protection of cows in endemic areas or infected herds [5]. Vaccination of adult cows with S19 is prohibited without written permission from the Provincial Director [11]. In Gauteng Province, Gauteng Veterinary Services (GVS) administer S19 vaccinations to heifers aged 4-8 months and RB51 booster vaccinations for older cows at no cost in the public sector.

Bovine brucellosis is widespread across sub-Saharan Africa, but prevalence is poorly estimated. This is due to lack of national surveys, different diagnostic methods, biased (non-random) sampling strategies and different definitions of sample populations [3,10,16]. Generally, estimates for southern African countries seem lower, e.g., 6-10% in Zimbabwe [16,17], 10% in Mozambique [16], 14% in SA [9] and 6-21% in Zambia [16,18-20]. Whereas reported estimates were higher in other countries, e.g., 1-15% in Ethiopia [16,21], 1.7-19% in Rwanda [22,23], 0.2-22% in Kenya [22,24], 29-32% in South Sudan [22,25], 0.2-48% in Tanzania [22,26], 1.2-44% in Uganda [22,27]; and 4-37% in Nigeria [15,28]. The true prevalence in SA is unknown with no national surveys performed. It is accepted to be endemic and under-reported since herd testing is optional and cattle movements are uncontrolled [4]. Infected herds reported in 2015-2019 were concentrated on the Highveld plateau (Free State, North West, Gauteng and Mpumalanga Provinces) [7]. A GVS survey in 2015 reported serological prevalence estimates of 14% (n=176) in herds and 1.3% (n=2365) in cattle in Gauteng Province [29]. Another survey in 2001-2003 reported 1.5% serological prevalence in cattle from rural communal grazing areas in KwaZulu-Natal Province [30].

Several risk factors for bovine brucellosis infection are recognised. These include increasing cow age [3,19-21,23,24,26]; pastoral production systems [3,21,22,24]; increasing herd size [3,10,21,23,24,27]; cattle raised with multiple livestock species [21]; female cattle [19,26,28]; certain breeds [23,28]; a history of increased abortion events [3,17,20,23,27]; and geographic area [10,19]. Factors that are apparently protective include zero-grazing small-holdings; decreasing herd size; decreasing landholding size [3] and increasing vaccination coverage [20].

Any changes in the disease status in Gauteng Province since the 2015 GVS survey are unknown. Regular cross-sectional surveys are recommended to monitor the progress of control programs [10]. So GVS repeated a survey of brucellosis in Gauteng cattle in 2019. Our objectives were to describe cattle herds sampled, estimate prevalence, and explore possible predictors of bovine brucellosis.

Methods

Setting & study design

Gauteng Province is the smallest but most populated province in SA. The estimated cattle population is 500 000 cattle (4% of the national herd), excluding feedlots [31]. Cattle are raised on privately owned holdings or communal areas, which are open state-owned land managed by traditional leaders and used by their communities to live on and for agriculture (e.g., grazing for livestock). This was a cross-sectional survey that targeted all cattle herds in Gauteng Province. It was carried out by the Provincial Veterinary Services, called Gauteng Veterinary Services (GVS) from June 2019 to November 2020. GVS is subdivided into the Germiston, Pretoria and Randfontein state veterinarian areas.

Sample size calculation

With the Epi-Z calculator tool (Cristóbal Zepeda, USDA-APHIS-VS, cristobal.zepeda@aphis.usda.gov) we calculated a sample size of 296 herds for between-herd prevalence assuming 30% expected prevalence, 95% precision and an estimated population of 3500 herds. For within-herd prevalence, all qualifying cows per herd were included.

Sample population & sampling strategy

To increase the precision of the prevalence estimates, the planned sample size was rounded up to 320 herds (24 more than the required by the calculation) which was about 10% of the known number of herds. We randomly selected the 320 herds to be sampled from the list of known cattle herds recorded in the 2016 GVS animal census (n=3200). This was done using the random function in Microsoft Excel (Office 365) which naturally resulted in the selected herds being distributed across the state vet areas based on the known population density (number of herds). The proportions of herd by state vet area were checked after making the selection to confirm this. A herd was eligible if there was ≥1 cow that qualified for testing. Cattle that qualified for testing were defined as cows (females), that had already mated with a bull or any cow ≥18 months in herds where the bull is kept in the herd year-round. All qualifying cows were sampled in every selected herd. Trained animal health technicians (AHT) went to the selected herds and either recruited them (if it still existed and qualified for testing) or the nearest qualifying herd. The herd owner was contacted for the necessary permissions. Often a second visit was needed to collect the samples and complete the survey. If it was impossible to recruit a herd at the allocated location, due to the absence of cattle or facilities to enable safe sample collection or owner refusal, then a new location in that municipality was randomly selected. Feedlots were excluded.

Data collection

For each recruited herd, the AHT responsible for the area collected specimens and epidemiological data. Clotted serum specimens collected were labelled, refrigerated and transported to the Agricultural Research Council´s Onderstepoort Veterinary Research Institute´s (ARC-OVR) laboratory. Following the national policy, all sera were screened for Brucella antibodies using the Rose Bengal test (RBT) and then any positive sera were confirmed using the complement fixation test (CFT) [5,7]. The CFT is specific and uses standardised units [1]. Since adult vaccination with S19 is not permitted by GVS, the cut-off for individual seropositivity of a CFT titre ≥ 30 IU/ml was used [1,5]. The mobile electronic application called EpiCollect5 (EpiCollect5, Imperial College, London, UK, 2019 https://five.epicollect.net/) was used to capture answers from the herd owners/stockmen to a structured questionnaire about the herd and its management.

Data management

The laboratory test results were captured in Microsoft Excel (Office 365) and the questionnaire data were exported from EpiCollect5. These data were merged using the unique herd identifier in Stata version 15 (StataCorp, College Station, TX, USA) to create a single dataset. A categorical variable for herd size was created, whereby herds with more than 20 cows ≥ 18 months were classified as large and those with fewer were classified as small. This classification comes from GVS field observations where it was seen that, generally, herds with > 20 reproductive cows could be profitable on a commercial scale and anything smaller is commonly used for subsistence farming. A binary outcome variable to indicate herd positivity was created. A herd was regarded as positive if at least one serum sample from the herd was seropositive.

Data analysis

The sampled herds were summarised using simple descriptive analysis. The herd prevalence was calculated as a proportion of the number of positive herds out of all herds tested. The cattle prevalence was calculated as a proportion of the number of seropositive individual cows out of all cows tested. The within-herd prevalence was the cattle prevalence among infected herds only. Prevalence estimates and 95% confidence intervals were calculated in Stata version 15.

The inferential analysis explored associations between selected exposure factors and herd positivity or individual cow seropositivity. We used Pearson´s chi-square test and simple logistic regression for the univariable analyses and considered all factors with p value <0.1 for inclusion in the multivariable models. Those factors in the final herd-level model were also considered for the cow-level model. Backward selection of variables, with a p value threshold of 0.1, was used to remove insignificant exposure factors in the selection of each multivariable model. In the cow-level analysis the clustered sandwich estimator was used for cluster-robust standard errors, to adjust for the grouping of cows in herds [32]. In the final models, only variables with a p value of <0.05 were considered statistically significant. The Hosmer-Lemeshaw goodness of fit test was used to assess the fit of both models, with groups of ten quantiles for the cow-level model.

Ethical considerations

Herd owners participated on a voluntary basis and gave written consent for brucellosis testing. The data analysed and reported were anonymised. As per Act No. 35 of 1984 GVS are mandated to control brucellosis and conduct surveillance. Thus, a waiver was obtained from the national department of Agriculture, Land Reform and Rural Development to exempt this work from a research permit requirement as in section 20 of Act. Furthermore, research ethics approval was obtained for this analysis from the Animal Ethics Committee at the Faculty of Veterinary Sciences, University of Pretoria (REC042-22).

Results

Participating herds

The sampled herds are summarised in **Table 1**. In total, 254 herds and 9111 cattle were sampled. The total herd size ranged 2-700 with a median of 38 and interquartile range of 13-81. Slightly less herds were sampled in 2019 (n=109, 43%) than 2020 (n=145, 57%). From March-June 2020 little sampling was done because of movement restrictions imposed by the government due to the COVID-19 pandemic. The survey was intended to be completed within 2019 but was extended into 2020 since the sample size reached was inadequate. With 11% (n=28) of the herds were in the Randfontein state veterinarian area, the remainder were almost equally split between the Germiston (n=115, 45%) and Pretoria (n=111, 44%) state veterinarian areas. Slightly more than half (n=146, 57%) were large herds. Most were beef only herds (n=176, 69%) followed by mixed (n=38, 15%) and then dairy herds (n=26, 10%). Considering how new introductions to herds were managed, almost a third each reported having a closed herd, i.e., no new cattle brought into the herd (n=70, 28%), testing and isolating new cattle before adding them to the herd (n=95, 37%), or adding new cattle to the herd without knowing their brucellosis status (n=77, 30%).

The RB51 vaccine was reportedly used most often, in 57% (n=146) of herds to vaccinate heifers and 44% (n=113) of herds to vaccinate adult cows. In contrast, the S19 vaccine was reportedly used much less, in 26% (n=66) of herds to vaccinate heifers and 4% (n=11) of herds to vaccinate adult cows. A quarter of owners (n=61, 24%) stated that their herds were not vaccinated with either vaccine and 17% (n=43) did not know their herd´s vaccination status. Most herds were sampled in winter and spring time (July to November). The herd locations were geographically scattered across Gauteng Province (**Figure 1**), except the herds in the Randfontein state vet area were underrepresented and clustered in two areas.

Spatial distribution of cattle herds sampled for bovine brucellosis survey in Gauteng Province, South Africa, 2019-2020 **Figure 1**. The map was made for this paper using Esri ArcGIS 10.2, with the 2016 provincial boundary shapefile from South African Municipal Demarcation Board (https://dataportal-mdb-sa.opendata.arcgis.com/).

Prevalence estimates

Valid results were received for 251 herds (9094 cattle). With 34 positive herds (157 positive cattle), an overall herd prevalence of 13.6% (95% CI: 9.8 - 18.4%) and cattle prevalence of 1.7% (95% CI: 1.5 - 2.0%) were estimated. The cattle prevalence within infected herds was 8.9% (95% CI: 7.6 - 10.3%) (**Table 2**).

Predictors of brucellosis positivity

The inferential analysis findings at herd-level and cow-level data are shown in **Table 3**. Herds were more likely to be positive for brucellosis if they were large (OR: 4.8 (1.8-12.8)), used shared grazing land (OR: 3.9 (1.7-8.7)), or were not vaccinated with either vaccine (OR: 2.9 (1.2-6.8)). Individual cow seropositivity was also associated with herds using shared grazing land (OR: 4.2 (1.8-9.9)) and herds not being vaccinated (OR: 1.9 (1.0-3.8)). An additional predictor for individual seropositivity was herds in which new cattle were isolated and tested for brucellosis before being added to the herd compared to uncontrolled introductions (OR: 0.1 (0.0-0.3)). The area, type of production and specific use of the different vaccines were analysed, but there were no associations. The post-regression goodness-of-fit tests showed favourable outcomes for both models.

Discussion

This study reports recent bovine brucellosis prevalence estimates and associated risk factors using robust methodology.

Bovine brucellosis control and eradication calls for >80% vaccination coverage in breeding herds [7,8]. We found a high proportion of unvaccinated herds (24%), and the reported vaccination coverage was low for heifers with either vaccine (62%) and adult cows with RB51 vaccine (44%). This vaccination uptake is inadequate but has improved since the 2015 GVS survey, which described an overall coverage of 36% [30]. A correspondingly low level of vaccine sales was noted, which estimated that <15% of heifers are vaccinated annually in SA [7]. About 17% of owners/stockmen did not know the vaccination status of their herds, similar to other recent South African surveys. About 25% of smallholder cattle owners in Gauteng Province said that they vaccinated their herd for brucellosis but did not know with which vaccine [33]. Another survey of 227 communal cattle owners in Eastern Cape Province also found 25% of owners did not know if their herds were vaccinated for brucellosis [34]. Whether inadequate vaccination is a result of ignorance, resistance by the farmer, or an over-reliance on state veterinary services for vaccinations can only be speculated. Further investigation into knowledge, attitudes and practices around brucellosis vaccinations is needed to improve understanding of vaccination coverage.

The overall cattle prevalence estimated here, 1.7%, is much lower than what is described for other endemic African countries with upper limits of ≥10%. This shows that even an imperfect but sustained vaccination control program is beneficial in a middle-income country scenario [3,16]. This estimate was similar to the 1.5% in a large survey of cattle in communal grazing areas of KwaZulu-Natal Province [30], 1.3% in the previous 2015 GVS provincial survey [30], and 1.4% from a study of GVS laboratory records [10]. In contrast, it was less than the 3% in a study of North West Province laboratory records [16] and 4% reported by another analysis of laboratory data for Gauteng Province [35]. Brucellosis prevalence figures arising from laboratory data analysis in SA are biased since the majority of samples are submitted for the national control scheme and should not be confused with a classical passive laboratory surveillance system.

We reported a herd prevalence of 14%. This figure is not consistently reported in other countries. The herd prevalence in North West Province (33%) was much higher and might be explained by the low vaccination coverage (7%) described [16]. This herd prevalence was much lower than the 22% reported by a study of GVS laboratory records [10] but the 2015 GVS survey reported the same estimate as 14% [30]. Therefore, this difference is judged to be due to the mentioned challenges with analysing laboratory data. Interestingly, the within-herd prevalence estimated from GVS laboratory records, 7% [10], was lower than the 9% in our survey. So herds participating in the Scheme had lower within-herd prevalence than the positive herds newly detected in our survey.

The bovine brucellosis situation in Gauteng Province did not change in the past 4 years. Provincial Veterinary Services have limited resources and competing priorities to manage so vaccination coverage and monitoring of informal herds may be insufficient [7]. GVS experienced challenges with implementing vaccinations and resistance to slaughter positive cattle. Barriers to brucellosis vaccinations observed by officials included frequent vaccine shortages from the local manufacturer (both strains); lack of safe handling facilities at livestock holdings preventing vaccination and testing; owners not implementing a calving season so that ages of replacement heifers vary throughout the year necessitating repeated visits to vaccinate the same herd; poor herd management; owner reluctance due to fear of vaccines causing abortions in cows of unknown pregnancy status; and general vaccine hesitancy or refusal by owners. Cattle owners could be uncooperative for many reasons sometimes hiding/moving the cattle when disease control actions should take place. Abattoirs with state approval to do so were reluctant to slaughter brucellosis-positive cattle, despite safety precautions being in place (observations by provincial field officials). Generally, brucellosis control has stagnated and is especially undermined at communal farming enterprises. For elimination, it was recommended that vaccination be used to reduce the herd (cattle) prevalence to <5% (<2%). Then test-and-slaughter methods are an effective final step [10,36,37]. We need better vaccination coverage particularly while cooperation with slaughter of positive cattle is low.

Herd and cow positivity were associated with herds using shared grazing land and lack of herd immunisation. Situations where cattle herds mix and share pastures have similarly been documented as high-risk for bovine brucellosis [3,21,22,26]. Higher prevalence was also observed in communal herds in North West province of SA [16]. Close contact between cattle from other herds/owners is inevitable in communal herds which are grouped together for grazing in the day and corralled separately at night. They share water points and grazing spaces, plus calving/abortion occurs any time further contaminating their environment [33]. Large herds were more likely to be positive for brucellosis in this sample. Increased herd size is a known risk factor since increased animal population density means increased contact between infected and susceptible animals [3,10,14,21,23,24,27]. In this survey, cows from herds in which new cattle were isolated and tested for brucellosis before being introduced were much less likely to be seropositive than those in herds with uncontrolled introductions. This shows the benefit of good biosecurity practice that protects cattle. But it can be difficult to change people´s practices even if the correct practices are known as illustrated by the Eastern Cape study that found a lack of health investigations of newly purchased cattle despite having the knowledge that it should be done [35]. It was good that the prevalence in the dairy sector was much lower compared to others but it is known that hand-milking of cows in smallholder Gauteng herds for personal consumption is common [33]. Thus the risk of human infection by ingesting contaminated milk remains important in our setting.

Strengths and limitations

The sample population is believed to be representative of the target population in terms of production type (beef versus dairy) [38] and land use (private land versus communal) [39]. Sometimes, the randomness of the herd selection may have been lost due to practical considerations, e.g., inability to sample due to absent safe handling facilities or needing telephone confirmation prior to visiting because of transport budget constraints. Prevalence may have been over-estimated by using a conservative CFT cut-off value. In vaccinated herds, antibody titres up to 60 IU/ml could be false positives depending on cow age, timing and vaccine strain used [5]. However, only one of the 34 positive herds was a borderline case with antibody titres between 30-60 IU/ml and no other higher titre reactors so this problem was considered small. Buffered *Brucella* antigen tests are highly sensitive and suitable as screening tests at the local level, but positive reactors should be retested with suitable confirmatory serological test methods [1]. Thus a two-test system is used in SA [5] and this survey. Some seropositive cows could be missed due to delayed seroconversion. Our reliance on serology without confirmation by bacterial isolation meant that the *Brucella* species involved in each case was unknown [39]. Although *B. abortus* is the most common causal organism in cattle [3], *B. melitensis* has been identified in South African cattle [40].

Conclusion

Eradication of brucellosis has been achieved elsewhere using the same vaccines, laboratory methods and field approaches being used in SA [8, 10]. Such campaigns are expensive, difficult and time-consuming [3,5,8,16] but can be successful with close cooperation between the producer, veterinary services and laboratory. The cattle prevalence of brucellosis in Gauteng Province is lower than in other endemic sub-Saharan African countries, possibly due to the presence of a control program for several decades. The progress in controlling the disease has stagnated, because of poor compliance with and enforcement of control measures. Possibly, the new changes to the policy will help to improve the situation. Gauteng Province is a promising area to pilot elimination but would need more resources, increased farmer awareness, cross-sector commitment, and incentives. We recommend training and engagement with communal farmers to improve understanding of the benefits and methods of brucellosis vaccinations, basic biosecurity measures to protect their cattle from infection and incentives for rapid slaughter of positive cows. This should be targeted at larger herds using communal grazing land and accompanied with cross-sectional surveys every 5 years to monitor the progress.

What is known about this topic

- Bovine brucellosis is a neglected zoonotic disease that is endemic to Gauteng Province in South Africa where an official control program is in place
- The disease can be eliminated by rigorous control programs that broadly include vaccination, controlled cattle movement and test-and-slaughter practices
- Publicly available data are lacking on the prevalence and current situation of the disease in sub-Saharan African countries

What this study adds

- Provides recent prevalence data and risk factors of bovine brucellosis at a time when the national brucellosis control strategy is being revised
- Gives robust evidence which highlights areas of progress and areas needing renewed focus for disease control in this context
- We share lessons learned by Provincial Veterinary Services in the field of bovine brucellosis control

Competing interests

The authors declare no competing interests.

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Authors' contributions

Conceptualization, L.D.B. and P.G.; methodology, L.D.B. and P.G.; software, L.D.B.; formal analysis, L.D.B.; data collection, A.G., D.M. and J.M.; writing - original draft preparation, L.D.B.; writing - review and editing, L.D.B., A.G., D.M., J.M. and P.G.; visualisation, L.D.B. All authors have read and agreed to the published version of the manuscript.

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Tables and figures

Table 1: Descriptive characteristics of 254 cattle herds sampled and exposure factors analysed in the Gauteng bovine brucellosis survey, South Africa, 2019-2020

Table 2: Prevalence estimates for bovine brucellosis in Gauteng, South Africa, 2019-2020

Table 3: Factors associated with positivity in the inferential analyses, Gauteng bovine brucellosis survey, 2019-2020

Figure 1:Spatial distribution of cattle herds sampled for bovine brucellosis survey in Gauteng Province, South Africa, 2019-2020

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Table 1: Descriptive characteristics of 254 cattle herds sampled and exposure factors analysed in the Gauteng bovine brucellosis sur vey, South Africa, 2019-2020		
Herd characteristic	**Number (n)**	**Proportion (%)**
Year		
2019	109	43
2020	145	57
State Veterinarian Area		
Randfontein	28	11
Pretoria	111	44
Germiston	115	45
Municipality		
City of Tshwane	111	44
Ekurhuleni	41	16
Lesedi	29	11
Midvaal	26	10
Rand West	13	5
Emfuleni	13	5
Mogale City	9	4
City of Johannesburg	6	2
Merafong City	6	2
Herd size		
Small (≤20 adult cows)	97	38
Large (>20 adult cows)	146	57
Production system type		
Dairy	26	10
Mixed	38	15
Beef	176	69
Management of new introductions		
Test & isolate	95	37
Uncontrolled	77	30
Closed herd	70	28
Use shared grazing land	95	
Yes	146	37
No		57
Not vaccinated with either vaccine		
Yes	61	24
No	181	71
Vaccinate heifers with S19		
Yes	66	26
No	174	69
Vaccinate heifers with RB51		
Yes	146	57
No	96	38
Vaccinate adults with S19		
Yes	11	4
No	232	91
Vaccinate adults with RB51		
Yes	113	44
No	129	51

Table 2: Prevalence estimates for bovine brucellosis in Gauteng, South Africa, 2019-2020

Group	**Proportion (n/N)**	**Prevalence (%)**	**95% Confidence Interval**
Herd prevalence	34/251	13.55	9.8 – 18.4
Within-herd prevalence	157/1775	8.85	7.6 – 10.3
Individual prevalence	157/9094	1.73	1.5 – 2.0
Laboratory results were missing for 3 herds (17 cattle).			

Table 3: Factors associated with positivity in the inferential analyses, Gauteng bovine brucellosis survey, 2019-2020

Exposure factor	Herd-level model (n=251)						Individual Cow-level model (n=9094)					
	Number Tested	% Positive	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value	Number Tested	% Sero-positive	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Year												
2020	143	14.0	1.09 (0.5 – 2.3)	0.815	Eliminated		5130	1.2	0.48 (0.1 – 1.7)	0.268	Eliminated	
2019	108	13.0	Reference				3964	2.4	Reference			
State Veterinarian Area												
Randfontein	28	28.6	4.44 (1.5 – 12.9)	0.006	Eliminated		970	7.4	13.90 (2.8 – 67.9)	0.001	Eliminated	
Germiston	114	14.9	1.95 (0.8 – 4.6)	0.127			4113	1.5	2.7 (0.9 – 7.9)	0.078		
Pretoria	109	8.3	Reference				4011	0.6	Reference			
Herd size												
Large	144	18.1	3.34 (1.3 – 8.5)	0.011	4.80 (1.8 – 12.8)	0.002	8329	1.8	1.22 (0.4 – 3.8)	0.729	Eliminated	
Small	97	6.2	Reference		Reference		765	1.4	Reference			
Production system type												
Mixed	38	18.4	2.71 (0.5 – 14.2)	0.239	Eliminated		1827	1.7	4.38 (0.7 – 27.7)	0.116	Eliminated	
Beef	174	12.6	1.74 (0.4 – 7.9)	0.474			5646	1.1	2.82 (0.6 – 13.7)	0.198		
Dairy	26	7.7	Reference				1275	0.4	Reference			
Management of new introductions												
Closed herd	69	14.5	0.60 (0.3 – 1.4)	0.241	Eliminated		2107	1.4	0.35 (0.2 – 0.8)	0.018	0.64 (0.3 – 1.6)	0.329
Test & isolate	94	5.3	0.20 (0.1 – 0.6)	0.003			4905	0.1	0.04 (0.0 – 0.1)	<0.001	0.09 (0.0 – 0.3)	<0.001
Uncontrolled	77	22.1	Reference				1730	3.8	Reference		Reference	
Use shared grazing land												
Yes	94	21.3	3.00 (1.4 – 6.5)	0.005	3.88 (1.7 – 8.7)	0.001	2354	3.4	9.59 (4.3 – 21.4)	<0.001	4.16 (1.8 – 9.9)	0.001
No	145	8.3	Reference		Reference		6373	0.4	Reference		Reference	
Not vaccinated with either vaccine												
Yes	61	19.7	1.95 (0.9 – 4.3)	0.096	2.89 (1.2 – 6.8)	0.016	1949	1.6	1.60 (0.6 – 4.2)	0.331	1.94 (1.0 – 3.8)	0.049
No	179	11.2	Reference		Reference		6793	1.0	Reference		Reference	
Vaccinate heifers with S19												
Yes	66	13.6	1.02 (0.5 – 2.3)	0.957	Eliminated		3713	1.1	0.94 (0.3 – 2.6)	0.907	Eliminated	
No	172	13.4	Reference				4990	1.2	Reference			
Vaccinate heifers with RB51												
Yes	144	13.2	0.97 (0.5 – 2.1)	0.938	Eliminated		2645	1.3	0.83 (0.3 – 2.1)	0.692	Eliminated	
No	96	13.5	Reference				6097	1.1	Reference			
Vaccinate adults with S19												
Yes	11	9.1	0.64 (0.1 – 5.2)	0.678	Eliminated		294	1.0	0.88 (0.1 – 7.6)	0.904	Eliminated	
No	230	13.5	Reference				8508	1.2	Reference			
Vaccinate adults with RB51												
Yes	111	13.5	1.10 (0.5 – 2.4)	0.798	Eliminated		4945	1.4	1.54 (0.6 – 3.7)	0.334	Eliminated	
No	129	12.4	Reference				3842	0.9	Reference			

OR: odds ratio, CI: confidence interval, n: number. N: total number. For the herd-level model, the number of observations was 239. Model fit was assessed using Hosmer-Lemeshaw goodness-of-fit test (p=0.76). For individual cow-level model, the number of observations was 8727. Standard error was adjusted for 239 clusters in unique herd number. Model fit was assessed using Hosmer-Lemeshaw goodness-of-fit test (p=0.34)

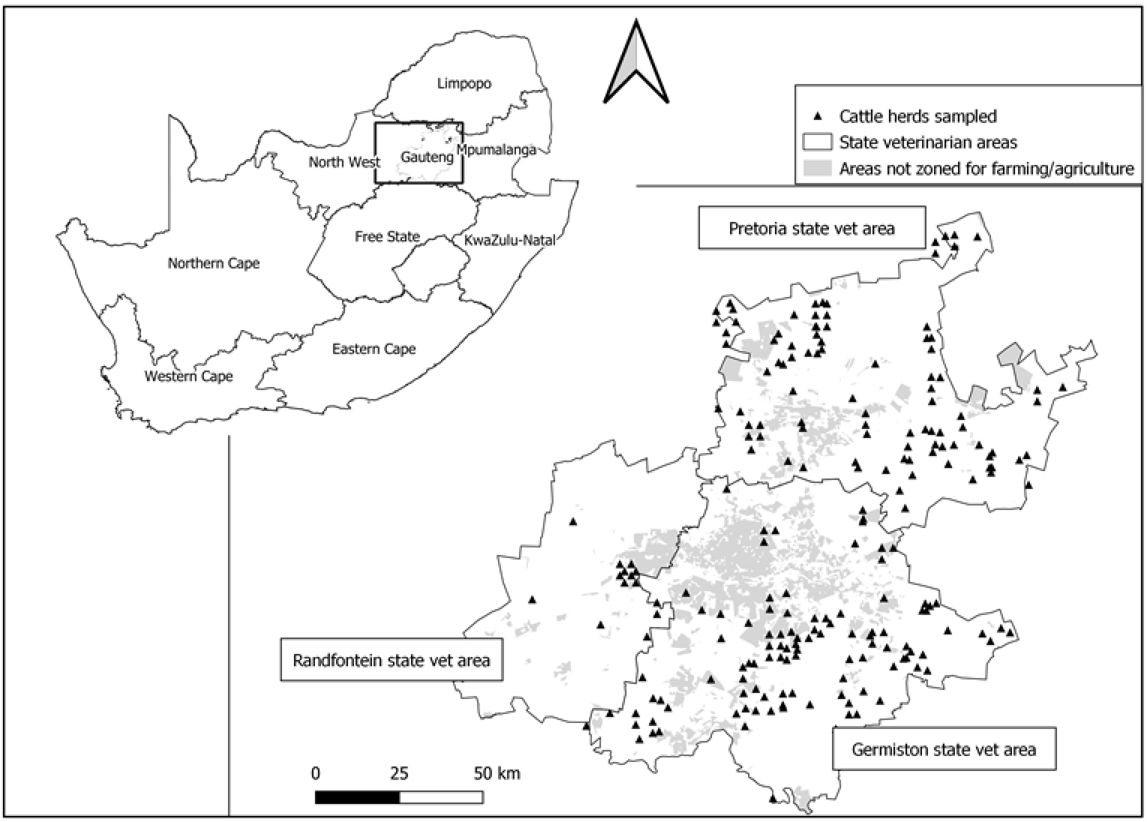

Figure 1: Spatial distribution of cattle herds sampled for bovine brucellosis survey in Gauteng Province, South Africa, 2019-2020