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RESEARCH ARTICLE

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Seroprevalence, and molecular detection of *Brucella abortus* in cattle tissues from an abattoir in Namibia

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ABSTRACT

Brucellosis is a worldwide zoonosis that is endemic in Namibia. This study estimated seroprevalence of brucellosis, and determined the presence of *Brucella* infection in slaughtered cattle using the genus-specific 16-23S rRNA interspacer PCR (ITS-PCR), and the species-specific AMOS-PCR. Between December 2018 and May 2019, sera (n = 304), pooled lymph nodes (n = 304), and individual spleen (n = 304) were collected from slaughtered cattle from 52 farms. Sera were tested for anti-Brucella antibodies using the Rose Bengal test (RBT), and the complement fixation test (CFT). Seroprevalence was 2.3% (7/304) (RBT) and 1.6% (5/304) (CFT). Prevalence of positive herds was 9.6% (5/52). Lymph node (n = 200) and spleen (n = 200) samples from seronegative cattle tested negative for *Brucella* spp. DNA on ITS-PCR, but *Brucella* spp. DNA was detected in lymph nodes (85.7%, 6/7) and spleen (85.7%, 6/7) from RBT positive cattle. ITS-PCR confirmed isolates from lymph node (51.4%, 4/7) and spleen (85.7%, 6/7) as *Brucella* spp.; while AMOS-PCR and *Brucella abortus* species specific (BaSS) PCR confirmed the isolates as *Brucella abortus*, and field strains, respectively. Provision of adequate protective gear, and the promotion of brucellosis awareness among abattoir workers is recommended to prevent zoonotic infection.

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Introduction

Brucellosis is a worldwide zoonosis caused by *Brucella* bacteria. It is a neglected [1] and thus a re-emerging disease among animal and human populations worldwide [2], with an estimated 5000 000 to 12,500,000 human cases reported annually [3–5]. The disease has been eradicated in several countries including Australia, New Zealand, Japan, Canada, Northern and Central Europe [6], but the infection remains endemic in many developing countries in Africa [7].

Brucella abortus, B. melitensis and *B. suis* cause the greatest impact on animal production [8], with huge financial losses reported in several countries [9,10]. Around the world, *Brucella abortus* is the most common cause of bovine brucellosis [9,10], but rare cases of bovine brucellosis caused by *B. melitensis* [11–15] and *B. suis* biovars [13,16,17] have been reported.

In cattle, *Brucella* infection is transmitted through the ingestion of, contact with or inhalation of contaminated or infected aborted fetal material, vaginal discharges, milk, feed, or water [18,19]. Bovine brucellosis is associated with late-term abortions, retained placenta, stillborn or weak calves, epididymitis, orchitis, and infertility [20]. In humans, *Brucellae* are a food safety and occupational hazard, with unpasteurized milk and dairy products, and contact with infected animal tissues posing the greatest risk for infection [21,22] in abattoir workers, cattle herders, veterinarians, dairy workers, livestock farmers, and laboratory workers [23]. Human brucellosis causes a severe debilitating febrile illness that manifests non-specific clinical symptoms, such as an undulating fever, headache, myalgia, back pain, weight loss, chronic fatigue, or polyarthritis [22,24].

Serological studies in Namibia have shown that bovine brucellosis is endemic at a low individual animal prevalence of 0.01–0.49% [25,26]. Control of bovine brucellosis in Namibia is primarily based on mandatory vaccination of heifers of 3–8 months with *Brucella* abortus S19 vaccine, and the added option of using *B. abortus* RB51 in cattle older than 8 months of age; importation of brucellosis-free cattle; identification and culling of seropositive animals [27].

The handling of bovine carcasses, organs, and fluids at abattoirs can expose abattoir workers to *Brucella* infection [28–30], since cattle of unknown brucellosis status are slaughtered. In 2018, 122 679 cattle were slaughtered at six cattle abattoirs in Namibia, which represents about a third of cattle

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marketed in the country [31]. Therefore, in addition to herd screening on farms, surveillance at abattoirs can be an invaluable complementary tool for detecting and acting against *Brucella* infected cattle herds.

Since previous studies on brucellosis in Namibia have been based on serological assays [25,32–35], the species causing infection in cattle have not been identified, but presumed to be *B. abortus* in sero-positive cattle. Moreover, the zoonotic risk at slaughter has not been explored. Therefore, the aim of this study was to estimate seroprevalence of brucellosis, determine the presence of *Brucella* infection, and perform molecular characterisation of isolates from cattle tissues at an abattoir in Namibia.

Materials and Methods

Study area

Namibia is located in the South-Western part of Africa at −22°58'1.42"S and 18°29'34.80"E. It is divided into 14 administrative regions, and three World Organisation for Animal Health (WOAH) recognised Foot-and-Mouth disease (FMD) zones, that is, the infected, protection and FMD-free zone without vaccination (Figure 1). The cattle population is estimated at 2.7 million.

Study abattoir

The abattoir was located in the Khomas region (Figure 1) and slaughtered cattle of different breeds originating from both commercial and communal farming systems, as well as from auctions and a feedlot. Ante-mortem and post-mortem inspections, and other routine abattoir hygiene procedures were carried out under the supervision of an official veterinarian supported by a team of meat inspectors.

Study design

A cross-sectional study design, using systematic random sampling to select slaughtered cattle for sampling, was used to estimate the prevalence of bovine brucellosis at the abattoir from December 2018 to May 2019. Over the study period, sampling was

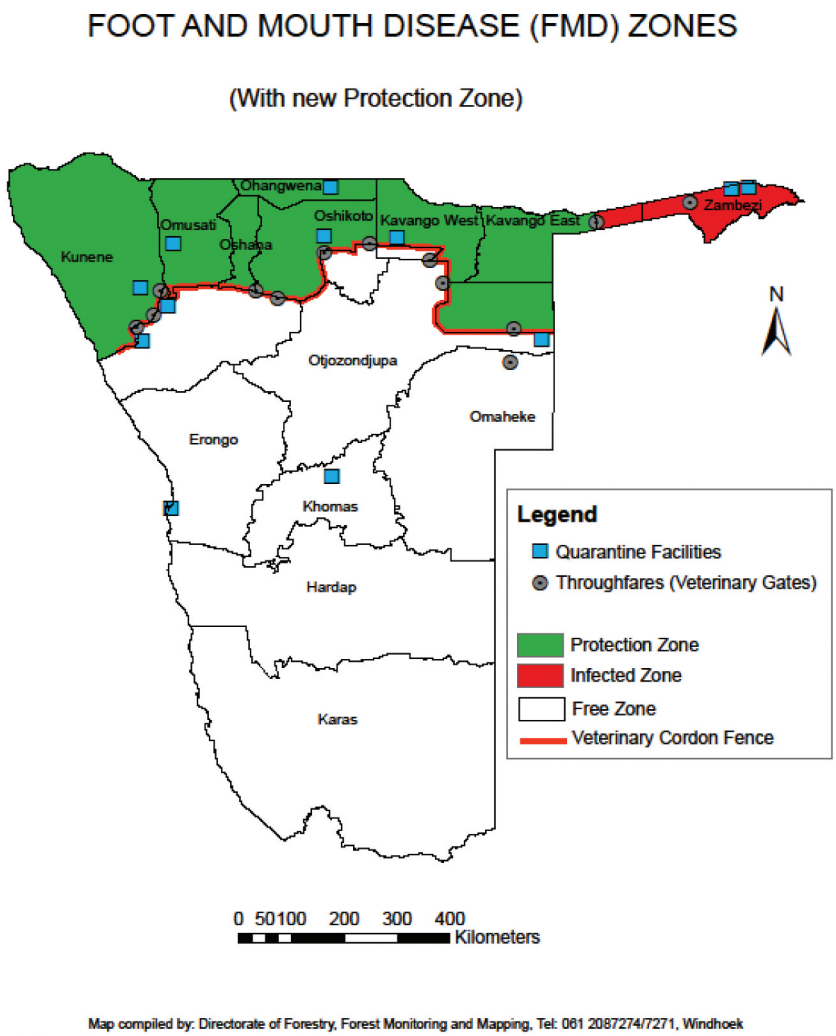

Figure 1. Map of Namibia showing the location of the Khomas region in the central part of the country.

carried out once a week on a different day of the week. Cattle of different ages brought for slaughter at the abattoir were eligible for the study. Age, sex, farm of origin and movement history of each sampled animal were retrieved from slaughter records and from the Namibia Livestock Identification and Traceability System (NamLITS) database through the state veterinarian supervising the establishment.

Sample size

To estimate seroprevalence, a sample size of 304 cattle was determined using the formula $n = 4PQ/L^2$ [36], assuming a 5% brucellosis prevalence in the cattle population, a precision of 0.025 (2.5%) and based on a 95% level of confidence.

Sample collection

Blood sampling

Blood samples ($n = 304$) were collected aseptically at the abattoir from severed jugular veins of selected cattle of both sexes. The slaughter procedure was performed following OIE guidelines on animal welfare [37]. For each animal selected for sampling, 5 ml of blood was collected in a sterile plain vacutainer blood tube for serum recovery. The collected samples were identified, securely packed and transported to the Central Veterinary Laboratory (Windhoek), where serum was recovered from clotted blood by centrifugation at 3000 rpm for 5 min. Sera were frozen at −20°C until testing.

Tissue sampling

Pieces of lymph nodes [retropharyngeal, parotid, mandibular, superficial inguinal (in males) and supra-mammary (in females)] and spleen were taken aseptically from the same cattle from which blood was collected and placed in sterile dilution bags. Lymph node samples from one animal were pooled, while spleen samples were stored separately. Samples were stored at −20°C.

Testing of sera

Serological assays were performed at the Central Veterinary Laboratory (Windhoek, Namibia). Sera were screened for anti-*Brucella* antibodies using the Rose Bengal test (RBT). On the RBT, any visible agglutination or clumps were considered as indicative of a positive result [8]. Samples testing positive on RBT were confirmed using the complement fixation test (CFT). The RBT and CFT were performed as described by the OIE [8] using standardized antigens (*B. abortus* Weybridge strain 99) for the detection of smooth anti-*Brucella* antibodies. CFT results were read after the plates were left to stand for one hour to allow unlysed cells to settle. Test results of 30 ICFTU/ml) and above were considered as positive based on the absence of haemolysis. In all cases, positive and negative controls were run with each batch of tests for the purposes of test validation.

Molecular identification of *Brucella* spp. in cattle tissues

Spleen ($n = 207$) and lymph nodes ($n = 207$) from a total of 207 cattle, that comprised seven cattle that tested positive on RBT, and 200 randomly selected seronegative cattle (on both RBT and CFT), were subjected to the *Brucella* genus-specific 16S-23S rRNA interspacer region (ITS) conventional PCR (ProFlex™ PCR System, Applied Biosystems™). Genomic DNA extraction and purification from tissues was performed following the protocol described in the PureLink® Genomic DNA kit (Life Technologies™). The concentration of extracted DNA was quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). Genus-specific 16S-23S rRNA interspacer region (ITS) primers were used in a conventional PCR to amplify a 214 bp fragment using the primers ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC. The PCR assay was performed as described by Keid et al. [38]. Primers were used at a final concentration of 0.2 µM with 1× DreamTaq Green PCR Master Mix (ThermoFisher Scientific, South Africa) and 2 µl DNA in a 15 µl PCR reaction mixture. The initial PCR assay denaturation was done at 95°C for 3 min followed by 35 cycles at 95°C for 1 min, 60°C for 2 min, 72°C for 2 min and finally at 72°C for 5 min. *Brucella melitensis* Rev 1 (Onderstepoort Biological Products, South Africa) was used as a positive control and nuclease-free water as a negative control for the PCR assay. Gel electrophoresis of amplicons was performed on a 2% agarose gel stained with ethidium bromide (1.0 g/ml) and the readings were made under ultraviolet light (UV) light.

Isolation and identification of *Brucella* spp. from cultures

Bacteriological isolation was performed at the Faculty of Veterinary Science (University of Pretoria, South Africa) in a biosafety level 2+ laboratory on tissues (spleen and lymph nodes, $n = 14$) obtained from seropositive cattle ($n = 7$). Homogenates (200 µl) were prepared from each tissue (spleen and lymph nodes) and inoculated onto Farrell's [8] and CITA [39] media. The culture plates were incubated at 37°C in the presence of 5% carbon dioxide and observed daily for 14 days for any growth of *Brucella*-like colonies (pinpoint, smooth, translucent, shiny, convex).

Colonies were presumptively identified by microscopic examination for morphology, size, and staining properties after modified Ziehl-Neelsen staining [8].

Characterization of *Brucella* spp. from cultures using AMOS-PCR

The multiplex AMOS-PCR assay was used to identify and differentiate *Brucella* spp. on cultures. The assay was performed as previously described [40,41] using DNA extracted from cultures. Four forward primers that are specific to each of the four *Brucella* species under investigation (Table 1) were used at a final concentration of 0.1 µM, to which was added 0.2 µM of the reverse primer *IS711*, 1× MyTaq™ Red PCR Mix (Bioline South Africa) and 2 µl of template DNA in a 25 µl PCR reaction mixture. The PCR assay was performed in the following cycles and conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 55.5°C for 2 min, 72°C for 2 min and a final extension step at 72°C for 10 min. Amplicons were analysed by electrophoresis using a 2% agarose gel stained with ethidium bromide that was viewed under UV light. *Brucella abortus* RB51 (Colorado Serum Company, Denver) was used as a positive control, while nuclease free water served as a negative control.

Identification and differentiation of field isolates and S19 strains using BaSS PCR

The *B. abortus* isolates identified in the AMOS-PCR assay were further characterised using the *B. abortus* species-specific (BaSS) PCR assay [42] (excluding the RB51 primers) to identify and differentiate between field isolates (wild-type biovars 1, 2, and 4) and S19 vaccine strains. The assay targets a 702bp deletion within the eryCD locus for *B. abortus* S19. The BaSS PCR was performed on DNA that was extracted and purified from the seven lymph nodes and seven spleens that originated from RBT seropositive cattle. The *B. abortus* IS711-specific element interrupting the *alk*B locus-specific primers, 16S universal primer and ery primers targeting common *ery*CD locus (absent in S19 strain) were used at a final concentration of 0.2 µM each, 1× MyTaq™ Red PCR Mix (Bioline South Africa) and 2 µl of template DNA in a 25 µl PCR reaction mixture. The PCR assay was performed in the following cycles and conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 15 sec, 52°C for 30 sec, 72°C for 2 min and a final extension step at 72°C for 10 min. Amplicons were analysed by electrophoresis using a 2% agarose gel stained with ethidium bromide that was viewed under UV light. *Brucella abortus* S19 and *B. abortus* bv 1 strains were used as a positive control, while nuclease free water served as a negative control.

Table 1. Oligonucleotide sequences of primers that were used in the AMOS-PCR assay to detect the Brucella species and the expected sizes of amplicons.

Name of primer	Sequence (5′-3′)	Size of amplicon (bp)
B. abortus	GAC GAA CGG AAT TTT TCC AAT CCC	498
B. melitensis	AAA TCG CGT CCT TGC TGG TCT GA	731
B. ovis	CGG GTT CTG GCA CCA TCG TCG GG	976
B. suis	GCG CGG TTT TCT GAA GGT GGT TCA	285
IS711	TGC CGA TCA CTT AAG GGC CTT CAT	

Data analyses

Test results were stored in Microsoft Excel® spreadsheet version 2007 (Microsoft Corporation, Redmond, WA). Abattoir seroprevalence, and prevalence of *Brucella* positive farms were determined as a percentage of cattle or farms tested that were positive on both RBT and CFT positive, respectively. The 95% confidence intervals (CI) were estimated considering CFT sensitivity and specificity of 81% and 98%, respectively. Proportions of reactors were compared between groups using the z-test calculator (https://epitools.ausvet.com.au/ztesttwo). In all cases, $p < 0.05$ was considered significant.

Results

A total of 304 cattle of varying breeds from 52 farms were sampled at the abattoir. Majority of the cattle (57.9%, $n = 176$) were female and ≥5 years old (45.7%, $n = 139$), but cattle aged 4 years ($n = 40$), 3 years ($n = 58$), 2–2.5 years ($n = 40$) and <2 years ($n = 27$) were also part of the study.

Seroprevalence

Of the 304 sera tested, 7 were positive on RBT (and 5 on both RBT and CFT), giving an apparent animal prevalence of brucellosis at the abattoir of 2.3% (7/304; 95% CI: 1.1–4.7%). However, after confirmation with the CFT assay, animal brucellosis prevalence was 1.6% (5/304; 95% CI: 0.7–3.8%). Of the five animals that tested positive on both RBT and CFT, four were males and one was a cow, and two had a history of movement between farms, and through an auction before they were slaughtered at the abattoir (Table 2). Two sera tested positive on RBT, but negative on CFT (<30 ICFTU) (Table 2). Overall, five cattle farms tested positive for anti-*Brucella* antibodies on

the CFT (Table 2), giving a prevalence of 9.6% (5/52; 95% CI: 4.2–20.6%) for positive farms.

Prevalence of anti-*Brucella* antibodies between females (0.6%, 1/176) and males (3.1%, 4/128) was not different ($z = 1.7$, $p = 0.08$). The positive titres were higher in older than in younger animals. Within age categories, prevalence was 5.0% (2/40, 2.0–2.5 years); 2.5% (1/40, 4 years); 2.9% (4/139, ≥5 years); 0.0% (0/27, <2 years) and 0.0% (0/58, 3 years).

ITS-PCR on tissues

Brucella DNA was detected in both spleen and lymph nodes of 6/7 (85.7%) cattle that were seropositive on RBT, CFT or both (Table 2) (Figure 2), but not in one animal that was seropositive on both RBT and CFT (Table 2) (Figure 2). Additionally, spleen and lymph nodes ($n = 200$ each) that were tested from randomly selected seronegative cattle, also tested negative for *Brucella* DNA. Therefore, the overall detection rate of *Brucella* DNA in tissues was 2.9% (6/207) on ITS-PCR.

Bacterial isolation

Repeated cultures of tissue homogenates (spleen, $n = 7$; lymph nodes, $n = 7$) from seropositive cattle yielded mixed growth of *Brucella* and other bacteria. From these cultures (lymph node, $n = 7$), spleen, $n = 7$), 10 *Brucella* spp. isolates were confirmed by ITS-PCR from lymph nodes (57.1%, 4/7) and spleens (85.7%, 6/7), respectively.

AMOS-PCR results

The 10 *Brucella* spp. isolates from lymph node ($n = 4$) and spleen ($n = 6$) homogenate cultures were identified as *B. abortus* by AMOS-PCR (Figure 3). *Brucella abortus* was detected more in spleens (85.7%, 6/7) than in lymph nodes (57.1%, 4/7). Results of the RBT, CFT, ITS-PCR, culture, and AMOS-PCR agreed in 57.1% (4/7) of the cattle tested (Table 1). In one case, ITS-PCR did not detect *Brucella* DNA in spleen or lymph nodes, but AMOS-PCR identified *B. abortus* in cultures.

Identification and differentiation between *Brucella abortus* field isolates and S19 using BaSS PCR

Brucella abortus isolates were confirmed as *B. abortus* field strains by BaSS-PCR. The assay amplified the 500 bp *B. abortus* IS711-specific element product in the *alk*B locus (*B. abortus* specific), and a 180 bp *ery*CD locus product that is common to all *Brucella* except *B. abortus* S19 strains (Figure 4).

Performance of test assays

The proportion of agreement between different assays was assessed using data in Table 2, and the results are indicated in Table 3. There was a high agreement between most assays (71.4–85.7%) except for the CFT and ITS-PCR assay on tissues (57.1%). Results of all tests used (RBT, CFT, ITS PCR (tissues and cultures) and AMOS-PCR (cultures)) were in agreement on samples originating from 57.1% (4/7) of the cattle tested.

This study determined a low brucellosis seroprevalence (1.6%) at a beef abattoir in Namibia, which serves as confirmation of low infection rates on the farms of origin. Seroprevalence was higher than the prevalence of up to 0.5% reported by earlier studies in Namibia [26,43], but within the range of 0.0–2.9% reported at South African [15,44] and Brazilian abattoirs [45]. The slaughter of predominantly mature cattle may have overestimated the seroprevalence of brucellosis in the current study. Higher abattoir prevalence rates than in the current study have been reported in Nigeria (3.9%) [46], Tanzania (4.7%) [47] and several African countries [48–54] where the vaccination of cattle against brucellosis is

Table 2. Characteristics of the seropositive cattle ($n = 7$), and results of serological, ITS-PCR (on tissues and cultures) and AMOS-PCR (cultures) screening (Identity numbers (ID No. correspond with ID No. in Figure 2)).

					ITS-PCR on tissues (±)		ITS-PCR on cultures (±)		AMOS-PCR on cultures (±)	
Age (years)	Sex (M/F)	History of movement	RBT (±)	CFT titre (ICFTU/ml)	Lymph nodes (ID No.)	Spleen (ID No.)	Lymph nodes (ID No.)	Spleen (ID No.)	Lymph nodes (ID No.)	Spleen (ID No.)
≥5	F	Stayed on one farm throughout	+	120 (+)	- (1)	- (8)	- (1)	+ (8)	- (1)	+ (8)
2–2.5	M	Stayed on one farm throughout	+	30 (+)	+ (2)	+ (9)	+ (2)	+ (9)	+ (2)	+ (9)
2–2.5	M	Stayed on two farms and moved through one auction	+	30 (+)	+ (3)	+ (10)	+ (3)	+ (10)	+ (3)	+ (10)
4	M	Stayed on one farm throughout	+	60 (+)	+ (4)	+ (11)	+ (4)	+ (11)	+ (4)	+ (11)
≥5	M	Moved through two farms and two auctions	+	120 (+)	+ (5)	+ (12)	+ (5)	+ (12)	+ (5)	+ (12)
≥5	M	Moved through two farms and two auctions	+	18 (-)	+ (6)	+ (13)	- (6)	+ (13)	- (6)	+ (13)
≥5	F	Stayed on one farm throughout	+	18 (-)	+ (7)	+ (14)	- (7)	- (14)	- (7)	- (14)

NOTE: ID No.: identification number of the tissue examined.

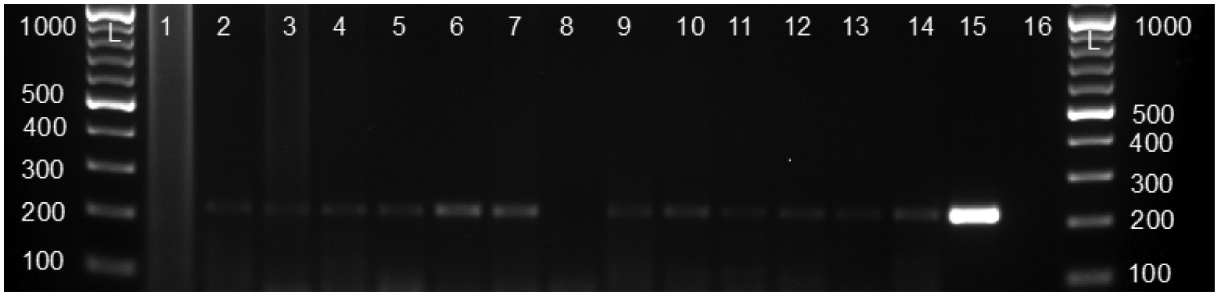

Figure 2. Genus-specific 16S-23S rRNA interspacer region (ITS) PCR amplification gel electrophoresis results of tissue samples (lymph nodes and spleen) from seropositive cattle. Lane L (100 bp marker), Lane 1–7 (lymph nodes), Lane 8–14 (spleen), Lane 15 (positive control: B. melitensis Rev 1), Lane 16 (negative control: nuclease-free water).

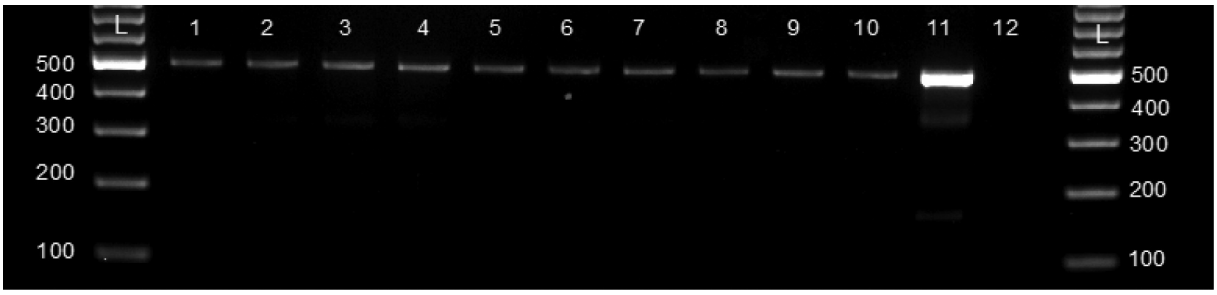

Figure 3. AMOS-PCR products from the amplification of the IS711 gene using Brucella species-specific primers. Lane L (100 bp marker), Lane 1–6 (spleen), Lane 7–10 (lymph nodes), Lane 11 (B. abortus RB51- positive control), Lane 12 (negative control: nuclease free water).

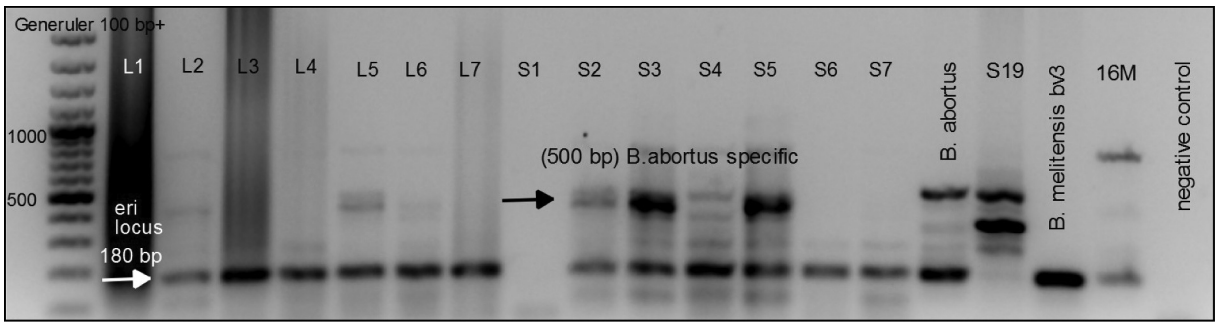

Figure 4. BaSS-PCR products showing the amplification of a 500 bp B. abortus IS711 specific element product in the alkB locus, and a 180 bp eryCD locus product that is common to all Brucella except B. abortus S19 strains. Lane L1-L7 - lymph nodes, Lane S1-S7 - spleen, thereafter, positive, and negative controls.

Table 3. A comparison of assay agreement levels on various samples obtained from seropositive cattle ($n = 7$) (extracted from Table 2).

Assays	Number of test results in agreement	Agreement (%)
RBT and CFT	5	71.4
RBT and ITS-PCR (tissues)	6	85.7
RBT and ITS-PCR (cultures)	6	85.7
RBT and AMOS-PCR (cultures)	6	85.7
CFT and ITS-PCR (tissues)	4	57.1
CFT and ITS-PCR (cultures)	6	85.7
CFT and AMOS-PCR (cultures)	6	85.7
ITS-PCR (tissues) and ITS-PCR (cultures)	6	85.7
ITS-PCR (tissues) and AMOS-PCR (cultures)	6	85.7

Discussion.

limited to absent. The proportion of brucellosis infected cattle farms identified at the abattoir (9.6%) was low, and similar to the prevalence of infected farms (9.3%) reported previously in Namibia [26], which is an endorsement of the effectiveness of brucellosis control measures implemented in the country.

The positive titre and the number of seropositive cases recorded at the abattoir were higher in older than younger cattle, in agreement with studies elsewhere [55,56], and serve to confirm that brucellosis is a disease of sexually mature cattle [57,58]. The higher number of seropositive cases in older than younger animals can be ascribed to a longer exposure time to infection in the herd [56], the absence of

seroconversion or higher resistance to *Brucella* infection in younger animals [59]. In contrast to previous studies that found more *Brucella* infected female than male cattle [52,60,61], the current study found no differences in prevalence between sexes as has also been reported by Shafee et al. [62]. In this study, there was no apparent association between animal movement history and seropositivity, as some seropositive cattle had stayed on one farm throughout their lifetime, while others had been traded between farms and moved through auctions.

Although the vaccination status of cattle in this study was unknown, the contribution of vaccine antibodies to the observed seroprevalence was excluded because all *B. abortus* isolates amplified an 180bp product (*ery*CD locus), which is present in field strains, but absent in the S19 strain due to a 702bp deletion within the eryCD locus for *B. abortus* S19 [63]. Moreover, the detection of four CFT seropositive male cattle points to natural *Brucella* infection (past or present) rather than vaccine strains as the source of the detected antibodies, since male cattle are not vaccinated against brucellosis. In Namibia, vaccination of heifers of 3–8 months of age using S19 is mandatory. The option for vaccinating female cattle older than 8 months of age with RB51 is provided in the Animal Health regulations [27]. The brucellosis vaccination requirement is strictly enforced in the commercial cattle rearing sector, but not in the communal cattle rearing sector [26], both of which supplied slaughter cattle to the study abattoir.

Although the number of samples that were used to compare test agreements were small and included only samples from cattle that were positive on RBT, a higher proportion of agreement was observed between ITS-PCR (on tissues) and RBT than CFT. Thus, despite its drawbacks, the RBT is a useful screening test for identifying *Brucella* positive cattle and any negative results on the confirmatory CFT may need to be investigated further using other tests. The identification of *Brucella* DNA in tissues (lymph nodes and spleen) from two RBT positive and CFT negative cattle reinforces the need to use several tests including ITS-PCR to complement the diagnosis of brucellosis [64] in cattle. Under normal circumstances, sera that test positive on RBT and negative on CFT, are considered negative. The discrepancy between the results of the CFT and ITS-PCR may be explained, in part, by the stage of *Brucella* infection at the time of testing. Serological testing of cattle in the early stages of infection using CFT is associated with negative results due to low IgG titres [65]. The absence of *Brucella* DNA in tissues from one RBT and CFT-positive cow, may be due to low bacterial levels in the early stage of infection [66] or infection that was cleared [67]. ITS-PCR was used in this study to detect *Brucella* spp. in cattle tissues and in cultures. The assay has been reported to detect extremely low levels of *Brucella* DNA in tissues [38], including tissues from seronegative animals [15]. In this study, the high sensitivity of ITS-PCR was demonstrated by the detection of *Brucella* DNA in mixed cultures, in which the concentration of bacteria was low. The ITS-PCR *Brucella* spp. detection rate of 2.9% in tissues determined by this study was lower than the detection rate of 12.5% reported at South African abattoirs [15], which may reflect the differences in risk factors and prevalence of bovine brucellosis between the two countries. Results of this study show that ITS-PCR is a useful tool for brucellosis surveillance and confirmation, which needs to be validated for use by laboratories.

Considering that ITS-PCR can only identify *Brucella* to the genus level, the species-specific AMOS-PCR was used to confirm the isolation of *B. abortus* in Namibia from ten spleen and lymph node homogenate cultures. Results of ITS-PCR and AMOS-PCR assays on cultures were in agreement, confirming the widely reported sensitivity of molecular techniques [68]. The AMOS-PCR assay that was used in this study cannot differentiate between field and vaccine strains of *B. abortus*. We therefore used the BaSS PCR to confirm the identified *B. abortus* as field strains based on the presence of an *ery*CD locus. Discriminating field infections from vaccine strains is very important because serious control measures such as isolation of the farm, and slaughter of affected animals are imposed whenever a positive case is reported. To the best of our knowledge, this is the first report on the molecular identification of *B. abortus* in cattle tissues in Namibia. The detection of *B. abortus* is not surprising since it is the most common cause of the disease in bovines worldwide [9,10]. Due to the low bacterial concentration in the tissues from apparently healthy cattle, and subsequent low bacterial growth on culture plates, which was overwhelmed by other bacteria, pure cultures could not be obtained for submission to a reference laboratory for biotyping. However, since the AMOS-PCR only detects *B. abortus* biovar 1, 2 and 4 [40], isolates in the current study can only be one of these biovars. The BaSS-PCR was used as recommended by Bricker and Halling [69] and Bricker et al. [42] to confirm that none of the isolated strains were *B. abortus* S19, as the *ery* locus that is common to all *Brucella* except S19, was amplified to produce a 180 bp product.

Despite the low seroprevalence and isolation rate of *Brucella* spp., it is of concern that a zoonotic pathogen was isolated from cattle tissues at an abattoir. Therefore, it is recommended that abattoir workers follow prescribed biosafety procedures including the wearing of adequate personal protective

gear to prevent possible infection [47,70], because brucellosis does not present typical signs or lesions to permit the exclusion of affected cattle or meat at ante- or post-mortem inspection. It was encouraging that the study abattoir provided employees with adequate protective clothing for their specific responsibilities. Health education training is also recommended among abattoir workers and meat handlers in general, to create awareness of and prevent brucellosis and other potential zoonoses.

There were limitations associated with the study. The low concentration of brucellae in the tissues and cultures precluded the growth of pure cultures for biotyping. Therefore, it is imperative that future studies should confirm the biovars of *B. abortus* isolated in Namibia using Bruce-ladder PCR [71].

Conclusion

In conclusion, the study isolated *Brucella abortus* from cattle tissues and determined a low seroprevalence of bovine brucellosis at a major abattoir in Namibia. However, the risk of exposure to *Brucella* infection among abattoir workers exists, especially where biosafety procedures are neglected by the workers or not enforced by the authority.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Author contributions

Conceptualisation: OM and HvH; Sample Collection and Laboratory Analysis: OM, GA, FK, FBK, JNK, GT-Z, SK; Writing-Original Draft preparation: OM, HvH, FBK, AM-S, FK, JNK, GT-Z, SK, GA; Writing – Review and Editing, OM, HvH, FBK, AM-S, FK, JNK, GT-Z, SK, GA.

Data availability statement

The datasets used and/or analysed during the current study are included in this manuscript.

Ethics approval and animal welfare

The study protocol was approved by the Chief Veterinary Officer, Ministry of Agriculture, Water and Land Reform (Namibia); Director of Animal Health (South Africa) according to Act 35 of 1984 (REF 12/11/1/1/6 (905); Research Ethics Committee (REC 056–20) and Animal Ethics Committee (V055–18) of the University of Pretoria. Samples were collected from cattle that were slaughtered humanely at the abattoir.

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