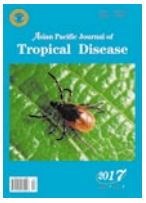
Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com



Original article https://doi.org/10.12980/apjtd.7.2017D6-431

Molecular characterization of *Brucella* species in cattle, sheep and goats obtained from selected municipalities in the Eastern Cape, South Africa

Lesley-Anne Caine[1,2*], Uchechukwu Uchechukwu Nwodo[1,2], Anthony Ifeanyin Okoh[1,2], Ezekiel Green[1,2,3]

[1]*SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice 5700, South Africa*

[2]*Department of Biochemistry and Microbiology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa*

[3]*Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P. O. Box 17011, Doornfontein Campus, 2028 Gauteng, South Africa*

ARTICLE INFO

Article history:
Received 30 Nov 2016
Received in revised form 19 Dec, 2nd revised form 21 Dec 2016
Accepted 2 Apr 2017
Available online 13 May 2017

Keywords:
Brucella
Cattle
Sheep
Goats

ABSTRACT

Objective: To detect *Brucella* spp. isolated from raw milk, blood and lymph nodes samples of cattle, sheep and goats.
Methods: A total of 1 955 samples comprising raw milk, blood and lymph nodes were collected from 880 cattle, 555 sheep and 520 goats from four municipalities in the Eastern Cape Province, South Africa. *Brucella* isolates were recovered from theraw milk, lymph nodes and blood samples using *Brucella* selective media.
Results: Detection of the *Bru* gene was observed in 130 isolates ranging from 81 (62.3%) in cattle to 16 (12.3%) in sheep. In cattle, the highest number of isolates was observed in the lymph node samples (24.6%), while the lowest was inraw milk (13.8%). In sheep, the highest number was observed in the blood samples (7.7%) and the lowest (0.8%) in the sheep's lymph nodes (0.8%), while in goats we detected the highest number of isolates in the blood samples (20%) and the lowest inraw milk (2.3%). *Brucella abortus* (*B. abortus*) (56.9%), *Brucella melitensis* (37.7%) and *B. abortus* vaccine strain S19 (5.4%) were confirmed from the isolates.
Conclusions: The present study adds to the data available regarding *B. abortus* and *Brucella melitensis* infections in cattle, goats and sheep populations and highlights the effectiveness and advantages of AMOS-PCR over culture. The results also indicate the need for effective control measures to be implemented.

1. Introduction

Brucellosis is a highly contagious zoonotic disease affecting livestock and humans[1]. *Brucella melitensis* (*B. melitensis*) and *Brucella abortus* (*B. abortus*) tend to infect small ruminants and cattle respectively, causing abortions and infertility[2], resulting in huge economic losses. These animals are the most common source of human brucellosis, a debilitating chronic condition which when left untreated can lead to permanent sequelae[3]. The disease has been reported in most countries with the exception of a few countries where it has been eradicated. Although brucellosis is a notifiable disease in many countries, official figures do not fully reflect the number of cases reported annually. The number is probably underestimated as a result of underreporting and misdiagnosis[4]. Information is scanty for many areas where brucellosis is endemic, and this is the case in South Africa, and in particular Eastern Cape Province, where livestock is a form of subsistence farming. Diagnosis of the causative agents of brucellosis is the cornerstone of any control program and is usually based on immunological, bacteriological and molecular findings.

Serological tests have proved to be either too sensitive, giving false-positive results, or too specific, giving false-negative results[5-7]. The presence of antibodies does not always mean an active case of brucellosis, as vaccinated animals tend to yield persistent post-vaccinal immune responses and other Gram-negative bacteria such as *Yersinia enterocolitica* cross-react with *Brucella* spp.[8,9]. Polymerase chain reaction (PCR) such as *Brucella* AMOS-PCR, is a recent advance for rapid and accurate diagnosis of brucellosis that has been shown to overcome the

*Corresponding author: Miss Lesley-Anne Caine, SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice 5700, South Africa.
Tel: +27-717-579-386
Fax: +27-866-219-191
E-mail: 200906584@ufh.ac.za

Foundation Project: Supported by the National Research Foundation (NRF) and the South Africa Medical Research Council (SAMRC) (Grant No. SAMRC/UFH/P790).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

limitations of conventional methodology. Vaccination with live, attenuated *B. abortus* strains (S19 and RB51) and Riv1 vaccine for small ruminants has been effective in preventing *B. abortus* and *B. melitensis* infections and abortions in cattle, sheep and goats. The *Brucella* AMOS-PCR identifies and differentiates between *B. abortus*, *B. melitensis*, *Brucella ovis* and *Brucella suis* bacteria. It was developed to differentiate the vaccine strains from the field strains[10]; with almost 100% sensitivity[11], it is advantageous when serological testing often fails[12]. It also produces results in just 24 h which provides useful and early information to regulatory officials. AMOS-PCR assay uses a five primer cocktail. One primer anneals to the IS711 element. By design, a positive PCR product of 498-bp is specific for *B. abortus*, a positive PCR product of 731-bp is specific for *B. melitensis*, a positive PCR product of 976-bp is specific for *Brucella ovis* and a positive PCR product of 285-bp is specific for *Brucella suis*. In addition, a positive PCR product of 364-bp is specific for the RB51 vaccine strain and a positive PCR product of 178-bp is specific for S19 which is a short sequence[13] of the *eri* gene (essential for erythritol catabolism), present in all *Brucella* strains except for *B. abortus* S19[14]. This PCR procedure has high potential as a rapid screening test for differentiating the two *Brucella* vaccines from the virulent field strains of *Brucella*[15].

Reports on brucellosis in Southern Africa are mainly restricted to cattle with the exclusion of small ruminants (sheep and goats) and are widely based on serological surveys[16,17]. Consequently, this present study aimed at detecting and identifying *Brucella* spp. from cattle, sheep and goats from four different municipalities in the Eastern Cape Province, South Africa.

2. Materials and methods

2.1. Ethical considerations

All experimental procedures involving animals were conducted in accordance to the OIE[18] and approved by the University of Fort Hare Ethics committee.

2.2. Sample collection

A total of 1955 samples comprising milk, blood and lymph nodes were collected from 880 cattle, 555 sheep and 520 goats (Table 1) from the livestock production sector of the Amathole District Municipality, Buffalo City Metropolitan Municipality, OR Tambo District Municipality as well as random samples collected from cattle slaughtered in the Queenstown and East London abattoirs (Figure 1). Blood samples from cattle were collected from the caudal tail vein while those from sheep and goats were collected from the jugular vein. All samples were collected with individual needles and stored in sterile EDTA vacutainer tubes[18]. The tubes containing blood were tilted to avoid blood clotting and immediately stored

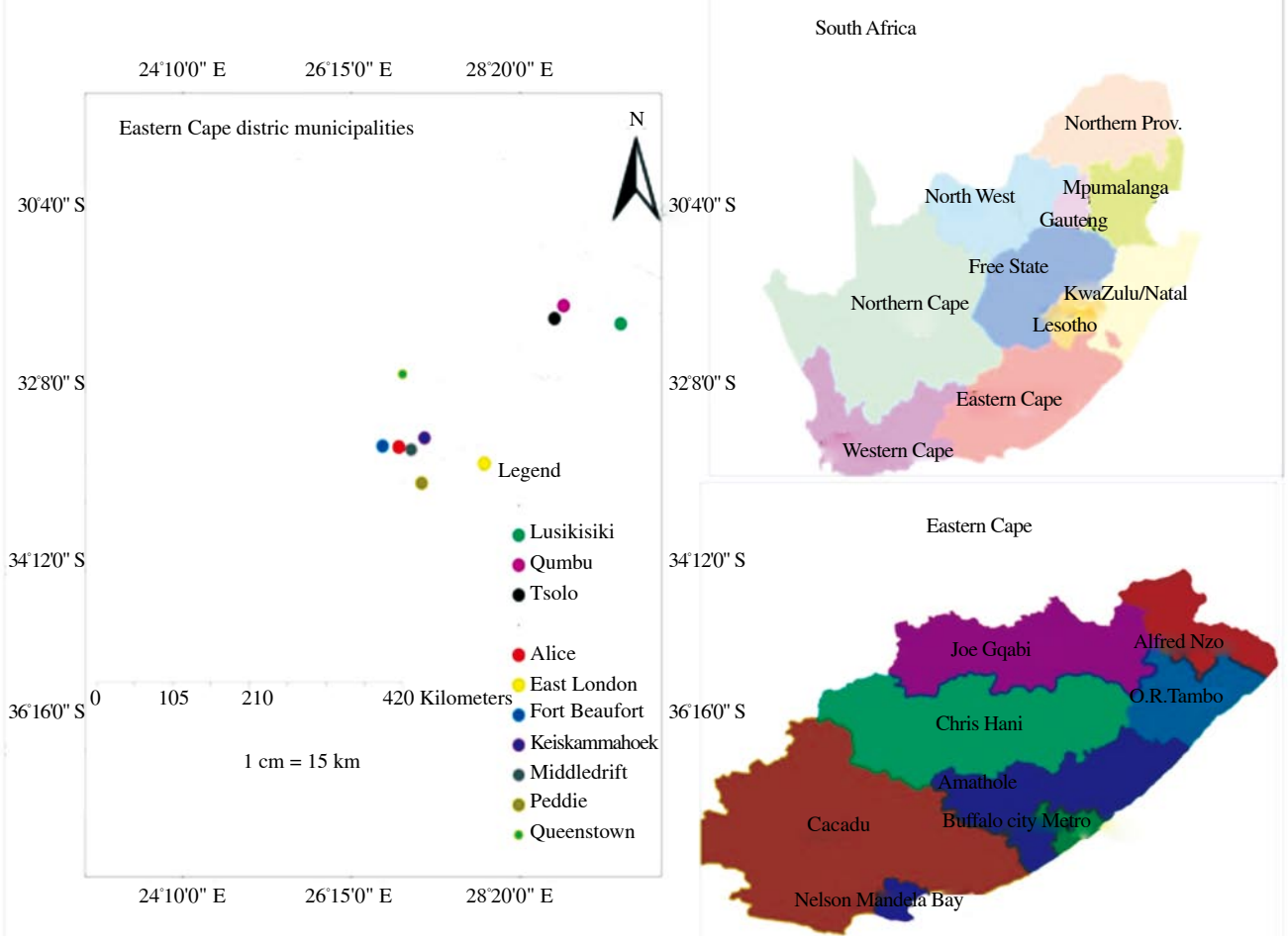


Figure 1. Map showing the municipalities where samples were collected from cattle, sheep and goats.

on ice until further analysis could be conducted. Raw milk samples were collected in individual sterile bottles from each quarter of dairy cows, sheep and goats based on the method of Alton *et al.*[19] and kept at 4 ˚C for further analysis. Tissue samples of the mammary lymph nodes from cattle slaughtered in the Queenstown and East London abattoirs were collected and processed for bacterial isolation in accordance with the description of Alton *et al.*[19].

Milk samples were centrifuged at 8 753 r/min for 15 min. The cream and deposit obtained after the skim milk had been discarded was mixed and spread with a swab-stick on *Brucella* Agar (Merck, Johannesburg, South Africa) with *Brucella* supplement (Liofilchem, Roseto D.A., Italy). The plates were incubated at 37 ˚C with 5%–10% CO_2. The presence of *Brucella* colonies were inspected for after 2, 4 and 7 days. Blood from cattle, sheep and goats were inoculated into a Castaneda biphasic medium which consisted of both a solid and liquid *Brucella* medium (Merck, Johannesburg, South Africa) with *Brucella* supplement (Liofilchem, Roseto D.A., Italy). The Castaneda bottles were incubated for 21 days, supplemented with 5% CO_2, with periodic tipping[18,20]. Lymph nodes were immersed in alcohol and flamed before being cut into small pieces and spread on the surface of *Brucella* Agar[19].

Table 1
The number of samples collected in each municipality.

Municipality	No. of samples collected	Cattle	Sheep	Goats
Amathole (A)	755	405	135	215
Blood		270	100	165
Raw milk		135	35	50
Lymph nodes		0	0	0
OR Tambo (B)	700	275	315	110
Blood		175	265	100
Raw milk		100	50	10
Lymph nodes		0	0	0
Buffalo City (C)	255	100	55	100
Blood		40	35	85
Raw milk		10	0	15
Lymph nodes		50	20	0
Lukanji local (D)	245	100	50	95
Blood		50	25	25
Raw milk		8	5	15
Lymph nodes		42	20	55
Total	1 955	880	555	520

2.3. DNA extraction and molecular characterization of *Brucella* species

DNA was extracted from presumptive isolates using the Zymo Research bacterial or fungal mini-prep kit following the manufacturer's instructions. Genus-specific primers (*Bru*-F, *Bru*-R) for identification of *Brucella* sequences and species-specific primers shown in (Table 2) were used as recorded by Khamesipour *et al.*[21] and Bricker and Halling[13,14]. The thermal conditions for the PCR were as follows: initial denaturation at 95 ˚C for 5 min, followed by 35 cycles of denaturation at 94 ˚C for 45 s, annealing at 64.9 ˚C for 1 min and 72 ˚C extension for 1 min. A final elongation step at 72 ˚C for 5 min for the *Bru* gene and initial denaturation at 94 ˚C for 2 min, followed by 35 cycles of denaturation at 95 ˚C for 20 s, annealing at 60 ˚C for 20 s and 72 ˚C extension for 30 s. A final elongation step at 72 ˚C for 7 min for all *Brucella* spp.

2.4. Gel electrophoresis

Agarose gel (1.5%) was prepared using 1× TBE buffer [10× TBE buffer: 1 mol/L Tris, 1 mol/L Boric acid, 50 mmol/L EDTA (pH 8.3)]. The gel was stained with 5 μL ethidium bromide[10,15]. A KAPA universal DNA molecular weight marker and a 100 bp ladder (Fermentas) were used as size standards. Gel electrophoresis was performed at 100 V for 45 min and amplicons were visualized under UV light and photographed using an Alliance 4.7 XD-79 System (Uvitec, Cambridge, UK).

3. Results

3.1. Confirmation of bacterial isolates

From the 1 955 samples collected, the *Bru* gene was successfully amplified from 130 (14.8%) isolates (Tables 3–5). The targeted gene was amplified and showed a base pair size of 245 on an agarose gel. We detected the highest number of isolates, 81 (62.3%), in cattle, while the lowest number, 16 (12.3%), was observed in the samples from sheep. The study detected the highest number of isolates in the blood and lymph node samples of cattle.

Table 2
Primer sets and expected amplicon sizes specific for the different *Brucella* species and vaccine stains.

Strain	Primer set	Primer sequence (5'-3')	Amplicon size (bp)	References
Brucella species	*Bru*	CTATTATCCGATTGGTGGTCTG	245	[21]
	Bru	GGTAAAGCGTCGCCAGAAGG		
B. abortus	*IS711*	TGCCGATCACTTTCAAGGGCCTTCAT	498	[15]
	AB	GACGAACGGAATTTTTCCAATCCC		
B. melitensis	*IS711*	TGCCGATCACTTTCAAGGGCCTTCAT	731	[15]
	BM	AAATCGCGTCCTTGCTGGTCTGA		
B. abortus vaccine strain S19	*ERI1*	GCGCCGCGAAGAACTTATCAA	178	[15]
	ERI2	CGCCATGTTAGCGGCGGTGA		
B. abortus vaccine strain RB51	*IS711*	TGCCGATCACTTTCAAGGGCCTTCAT	364	[15]
	RB51	CCCCGGAAGATATGCTTCGATCC		
Rev-1 vaccine	*P1*	TGGAGGTCAGAAATGAAC	282	[22]
	P2	GAGTGCGAAACGAGCGC		

Table 3
Identification of *Brucella* spp. from blood, raw milk and lymph nodes of cattle by molecular characterization.

Municipality	No. of samples tested	*Bru* gene	*B. abortus*	*B. melitensis*	*B. abortus* vaccine strain
Amathole	405				
Blood		9	9	0	1
Raw mikl		7	7	0	1
Lymph node		0	0	0	0
OR Tambo	275				
Blood		15	13	0	0
Raw mikl		7	6	0	1
Lymph node		0	0	0	0
Buffalo City	100				
Blood		4	4	0	1
Raw mikl		2	2	0	0
Lymph node		15	13	0	2
Lukanji local	100				
Blood		3	3	0	0
Raw mikl		2	2	0	0
Lymph node		17	15	0	1
Total	880	81	74	0	7

Table 4
Identification of *Brucella* spp. from blood, raw milk and lymph nodes of sheep by molecular characterization.

Municipality	No. of samples tested	*Bru* gene	*B. abortus*	*B. melitensis*	*Rev-1* vaccine strain
Amathole	135				
Blood		8	0	8	0
Raw mikl		5	0	5	0
Lymph node		0	0	0	0
OR Tambo	315				
Blood		0	0	0	0
Raw mikl		0	0	0	0
Lymph node		0	0	0	0
Buffalo City	55				
Blood		1	0	1	0
Raw mikl		0	0	0	0
Lymph node		1	0	1	0
Lukanji local	50				
Blood		1	0	1	0
Raw mikl		0	0	0	0
Lymph node		0	0	0	0
Total	555	16	0	16	0

Table 5
Identification of *Brucella* spp. from blood, raw milk and lymph nodes of goats by molecular characterization.

Municipality	No. of samples tested	*Bru* gene	*B. abortus*	*B. melitensis*	*Rev-1* vaccine strain
Amathole	215				
Blood		9	0	9	0
Raw mikl		1	0	1	0
Lymph node		0	0	0	0
OR Tambo	110				
Blood		15	0	15	0
Raw mikl		2	0	2	0
Lymph node		0	0	0	0
Buffalo City	100				
Blood		1	0	1	0
Raw mikl		0	0	0	0
Lymph node		2	0	2	0
Lukanji local	95				
Blood		1	0	1	0
Raw mikl		0	0	0	0
Lymph node		2	0	2	0
Total	520	33	0	33	0

3.2. Brucella species characterization

The 130 isolates were further confirmed by PCR using primers (Table 2) which targeted *B. abortus*, *B. melitensis*, *B. abortus* vaccine strain S19, *B. melitensis* Rev-1 vaccine strain and *B. abortus* vaccine strain RB51. The PCR products were then subjected to 1.5% agarose gel for electrophoresis which was observed at 498, 731 and 178 base pairs. Only *B. abortus* (56.9%), *B. melitensis* (37.7%) and *B. abortus* vaccine strain S19 (5.4%) were confirmed from the isolates (Tables 3–5). *B. melitensis* Rev-1 vaccine strain and *B. abortus* vaccine strain RB51 were not amplified from any of our isolates.

4. Discussion

Demand for meat and milk in South Africa has risen in the last 10–20 years[23]. This is an indication that the livestock industry is expected to yield more products for human consumption to combat hunger. However this industry is negatively affected by microorganisms causing infectious diseases such as brucellosis, caused by the *Brucella* species[24,25]. Brucellosis remains a major public health concern in sub-Saharan Africa, where similar livestock systems, environmental conditions and cultural aspects occur[26,27]. Cattle and small ruminants are assumed to be the main source of human infection and a number of control options exist, with vaccination and/or test-and-slaughter of positive animals being the corner-stones of most control programmes. At the time of this study, this is the first report on detection of *Brucella* spp. in blood, raw milk, and lymph nodes of cattle, sheep and goats from the Eastern Cape Province, South Africa.

A total of 130 of the isolates we recorded were *Brucella* of which 62.3% were from cattle, 25.4% from goats and 12.3% from sheep. It has often been reported that sensitivity of culture medium depends on the disease stage, *Brucella* spp., culture medium, quality of circulating bacteria and culture techniques used[28,29]. Our results indicate that, a lower number of the *Bru* gene was detected in raw milk samples compared to lymph nodes. The lower incidence in milk samples, however, may not be an indication that these animals are healthy or not infected with *Brucella* spp. as pathogens can be located in the lymph nodes, also could be due to the fact that most of the milk samples from our study were collected from commercial dairy cattle where farmers usually follow a vaccination and control strategy, whereas other livestock animals in small holding farms are tested at most every 5 years and consequently the brucellosis is less controlled in these farming areas[16]. It should also be noted that culture was performed before DNA could be detected in the present study.

The detection of *Brucella* in the lymph nodes samples differed from cattle (24.6%), sheep (0.8%) and goats (3.1%) in our study. This results suggest that the organism might be readily detectable in lymph tissues, however, Corbel and Brinley-Morgan[30] found it difficult to detect *Brucella* from the tissue samples. In this regard, stages of *Brucella* infection play an important role when samples are collected for detection and culture of *Brucella* spp. It was proposed that after penetration, the organisms are phagocytosed by neutrophils and macrophages which carry them to the regional lymph nodes where they multiply and induce a lymphadenitis. Hence the presence of *Brucella* in lymph nodes depends on the time it took the organism to be carried to the lymph nodes. On the other hand, a high rate

of isolation of the *Brucella* organism from the lymphoid tissue was reported by Khamesipour *et al*.[21] an outcome contrary to our study. It is important to note that lymph nodes samples were collected post-mortem. This has been indicated as being far from an ideal situation for a diagnostic test, however, a suggestion was put forward that biopsy samples from mammary lymph tissues would be worthy of investigation[31].

The *Bru* gene was detected in a higher percentage in our study from blood samples than lymph node samples, a result varying with the results of Khamesipour *et al.*[32] who discovered a higher percentage of this gene in lymph node samples compared to the blood samples. In our study, *Brucella* was observed in 23.9% of cattle, 7.7% in sheep and 20% in goat's blood samples. Contrary to the study done by O'Leary *et al*.[31], whole blood in our study attested to be a good sample for *Brucella* detection. O'Leary *et al.*[31] suggested that since bacteria are taken up by macrophages and non-professional phagocytes, white blood cells templates might be worthy templates for use in PCR detection. In addition, similar results where DNA was extracted from blood and used in screening animals for brucellosis were documented by Madboly *et al*.[33] in buffalo, Keid *et al*.[34] in dogs and Khamesipour *et al*.[21] in cattle and sheep. The identification of *Brucella* spp. is important in contributing to better understanding of geographical transmission patterns of *Brucella* in different animal species in Eastern Cape Province, South Africa. It also plays a role in specific control measures to be implemented.

All the *Brucella* spp. were identified as *B. abortus*, *B. melitensis* and *B. abortus* vaccine strain S19 in our study. These results, agree to the report of Bricker and Halling[14] that a positive PCR product of 498-bp is specific for *B. abortus*, a positive PCR product of 731-bp is specific for *B. melitensis*, and a positive PCR product of 178-bp is specific for S19 a short sequence of the *eri* gene (essential for erythritol catabolism).

Considering all the isolates obtained in our study, a higher percentage was confirmed as *B. abortus* (56.9%) compared to *B. melitensis* (37.7%) a result contrary to Mohamed *et al*.[10] who detected a lower percentage (16.4%) of *B. melitensis* from infected cattle and sheep in Egypt. This could potentially cause abortions and infertility resulting in huge economic losses. The isolation of *B. abortus* and *B. melitensis* from lymph nodes, blood and milk of infected animal confirms the presence of disease[26]. In our study, *B. abortus* (56.9%) was isolated from cattle while *B. melitensis* (37.7%) was isolated from goats and sheep. This further strengthens the observations of Corbel[35], who mentioned that *B. melitensis* and *B. abortus* preferentially infect small ruminants and cattle, respectively. *Brucella* spp. are mostly transmitted to humans by direct contact with infectious animal tissue, inhalation of aerosolized droplets or the consumption of raw milk and its products. Many people in the rural areas of the Eastern Cape Province of South Africa consume raw milk and believe that raw milk and its products have advantages or value over the pasteurized milk[36-38]. In South Africa, the prevalence of *B. abortus* in cattle is relatively high and outbreaks are reported from all provinces; in contrast, outbreaks of *B. melitensis* in sheep and/or goats have been rare but could be on the rise[39]. Cattle, sheep and goats in the rural areas of the Eastern Cape are generally grazed on communal pastures that usually move over distances of several kilometres which could lead to contamination of large areas as calving is not restricted to a specific place, such as a pen. Consequently, this could function as a source of infection for other groups of livestock within that community utilizing the same pasture, a factor that has been found to be important in the risk of infection[16].

The confirmation of the *B. abortus* vaccine strain S19 (5.4%) implies that AMOS-PCR can differentiate between vaccinated animals with any type of vaccine (S19, RB51 and Rev-1) from infected animals. This technique could therefore stop the slaughtering of vaccinated animals in the Eastern Cape. *Brucella* vaccines used in South Africa for livestock are the *B. melitensis* Rev 1, the live *B. abortus* strain 19, and *B. abortus* strain RB51[17]. One of the reasons S19 was detected in our study is due to the fact that it is the vaccine of choice in the Eastern Cape for the prevention of brucellosis in cattle because it has been reported to be superior[40]. The *B. abortus* vaccine strain RB51 and *B. melitensis* Rev 1 were not detected in any of the isolates in this study, which is an indication that the detected *B. abortus* and *B. melitensis* species are a true reflection of the presence of bovine, caprine and ovine brucellosis in the four municipalities of the Eastern Cape Province of South Africa.

The present study adds to the data available regarding *B. abortus*, and *B. melitensis* infections in cattle, goats and sheep populations and highlights the effectiveness and advantages of AMOS-PCR over culture. The results indicate the need for effective control measures to be implemented such, more regular testing of blood, as well as test and slaughter approach, and vaccination and control of all livestock entering the Eastern Cape are highly recommended as strict preventive measures to assist in the suppression of brucellosis in the herd.

Conflict of interest statement

None declared.

Acknowledgments

This study was funded by the National Research Foundation (NRF) and the South Africa Medical Research Council (SAMRC) (Grant No. SAMRC/UFH/P790).

References

[1] Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos E. The new global map of human brucellosis. *Lancet Infect Dis* 2006; **6**: 91-9.

[2] Miller R, Nakavuma JL, Ssajjakambwe P, Vudriko P, Musisi N, Kaneene JB. The prevalence of brucellosis in cattle , goats and humans in rural Uganda : a comparative study. *Transbound Emerg Dis* 2016; **63**(6): e197-210.

[3] Zinsstag J, Schelling E, Solera J, Blasco JM, Moriyón I. Brucellosis. In: Palmer SR, Soulsby L, Torgeson PR, Brown DG, editors. *Handbook of zoonoses.* Oxford, New York: Oxford University Press; 2011, p. 54-62.

[4] Musallam II, Abo-Shehada M, Omar M, Guitian J. Cross-sectional study of brucellosis in Jordan: prevalence, risk factors and spatial distribution in small ruminants and cattle. *Prev Vet Med* 2015; **118**(4): 387-96.

[5] Gupta VK, Shivasharanappa N, Kumar V, Kumar A. Diagnostic evaluation of serological assays and different gene based PCR for detection of *Brucella melitensis* in goat. *Small Rumin Res* 2014; **117**(1):

94-102.

[6] Priyadarshini A, Sarangi LN, Palai HK, Mishra R, Behera PC. Brucellosis in cattle and occupationally exposed human beings: a serosurvey in Odisha, India. *Pure Appl Microbiol* 2013; **7**: 3255-60.

[7] Moussa IM, Omnia ME, Amin AS, Ashagan MH, Selin SA. Evaluation of the currently used polymerase chain reaction assays for molecular detection of *Brucella* species. *Afr J Microbiol Res* 2011; **5**: 1511-20.

[8] See W, Edwards WH, Dauwalter S, Almendra C, Kardos MD, Lowell JL, et al. *Yersinia enterolitica* an unlikely cause of positive brucellosis tests in greater yellowstone ecosystem bison (*Bison bison*). *J Wild Dis* 2012; **48**(3): 537-41.

[9] Corbel MJ. Recent advance in the study of *Brucella* antigen and their serological cross reaction. *Vet Bull* 1985; **55**: 927.

[10] Mohamed AG, Ramadan KM, Monem HA, Toukhy Essam EL, Khairy EA. Amos PCR as a rapid screening method for differentiation of infected and vaccinated cattle and sheep with brucellosis. *Glob Vet* 2013; **11**(1): 748-56.

[11] Mitka SC, Anetakis E, Souliou ED, Kansouzidou A. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. *J Clin Microbiol* 2007; **45**: 1211-8.

[12] Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. *Croat Med J* 2010; **51**: 296-305.

[13] Bricker BJ, Halling SM. Differentiation of *Brucella abortus* bv. 1, 2 and 4, *Brucella melitensis*, *Brucella ovis* and *Brucella suis* bv. 1 by PCR. *J Clin Microbiol* 1994; **32**: 2660-6.

[14] Bricker BJ, Halling SM. Enhancement of the *Brucella* AMOS-PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. *J Clin Microbiol* 1995; **33**: 1640-2.

[15] Ewalt DR, Bricker BJ. Validation of the abbreviated *Brucella* AMOS PCR as a rapid screening method for differentiation of *Brucella abortus* field strain isolates and the vaccine strains, 19 and RB51. *J Clin Microbiol* 2000; **38**: 3085-6.

[16] Hesterberg UW, Bagnall R, Perrett K, Bosch B, Horner R, Gummow B. A serological prevalence survey of *Brucella abortus* in cattle of rural communitiesin the province of KwaZulu-Natal, South Africa. *J S Afr Vet Assoc* 2008; **79**: 15-8.

[17] Njiro SM, Kidanemariam AG, Tsotetsi AM, Katsande TC, Mnisi M, Lubisi BA, et al. A study of some infectious causes of reproductive disorders in cattle owned by resource-poor farmers in Gauteng Province, South Africa. *J S Afr Vet Assoc* 2011; **82**(4): 213-8.

[18] Office International des Épizooties (OIE). Manual of diagnostic tests and vaccines (mammals , birds and bees). 6th ed. Vol. 2. Paris: Office International des Épizooties; 2008, p. 66.

[19] Alton GG, Angus RD, Verger JM. Diagnosis of bovine brucellosis: principles, practice and problems. *Surveillance* 1989; **16**(2): 3-6.

[20] Carmichael LE. Vaccination. In: Nielsen K, Duncan JR, editors. *Animal brucellosis.* Boca Raton: CRC; 1990, p. 335-50.

[21] Khamesipour F, Doosti A, Taheri H. Molecular detection of *Brucella* spp. in the semen, testis and blood samples of cattle and sheep. *J Pure Appl Microbiol* 2013; **7**: 495-500.

[22] Mullis KB, Faloona FA. Specific synthesis of DNA *in vitro* via a polymerase catalysed chain reaction. *Methods Enzym* 1987; **155**: 335-50.

[23] Godfroid J, Al DS, Pappas G, Roth F, Matope G, Muma J, et al. A "One Health" surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comp Immunol Microbiol Infect Dis* 2013; **36**: 241-8.

[24] Cloeckaert A, Vizcaino N. DNA polymorphism and taxonomy of *Brucella* species. In: Lopez-Goni I, Moriyon I, editors. *Brucella molecular and cellular biology.* Wymondham: Horizon Bioscience; 2004, p. 1-24.

[25] Araj GF. Update on laboratory diagnosis of human brucellosis. *Int J Antimicrob Agents* 2010; **36S**: S12-7.

[26] Refai M. Incidence and control of brucellosis in the Near East Region. *Vet Microbiol* 2002; **90**: 81-110.

[27] Gwida M, Al Dahouk S, Melzer F, Rosler U, Neubauer H, Tomaso H. Brucellosis – regionally emerging zoonotic disease? *Croat Med J* 2010; **51**: 289-95.

[28] Karthik K, Rathore R, Thomas P, Elamurugan A, Arun TR, Dhama K. Serological and molecular detection of *Brucella abortus* from cattle by RBPT, STAT and PCR and sample suitability of whole blood for PCR. *Asian J Anim Vet Adv* 2014; **9**(4): 262-9.

[29] Pathak AD, Dubal ZB, Karunakaran M, Doijad SP, Raorane AV, Dhuri RB, et al. Apparent seroprevalence, isolation and identification of risk factors for brucellosis among dairy cattle in Goa, India. *Comp Immunol Microbiol Infect Dis* 2016; **47**: 1-6.

[30] Corbel MJ, Brinley-Morgan WJ. Genus *Brucella* Meyer and Shaw 1920. In: Krieg NR, Holt JC, editors. *Bergey's manual of systematic bacteriology.* Baltimore, Md: Williams and Wilkins Co.; 1984, p. 377-88.

[31] O'Leary S, Sheahan M, Sweeney T. *Brucella abortus* detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Res Vet Sci* 2006; **81**: 170-6.

[32] Khamesipour F, Rahimi E, Shakerian A, Doosti A, Momtaz H. Molecular study of the prevalence of *Brucella abortus* and *Brucella melitensis* in the blood and lymph node samples of slaughtered camels by polymerase chain reaction (PCR) in Iran. *Acta Vet* 2014; **64**(2): 245-56.

[33] Madboly AA, Ghazi YA, Sokkar SM, Desouky HM, Ahmed YF. Serological, bacteriological, molecular and immunohistochemical techniques for diagnosis of brucellosis in buffaloes. *J Middle East Appl Sci* 2014; **4**(3): 762-70.

[34] Keid LB, Soares RM, Vasconellos SA, Salgado VR, Megid J, Richtzenhain J. Comparison of a PCR assay in whole blood and serum specimens for canine brucellosis diagnosis. *Vet Rec* 2010; **167**: 96-9.

[35] Corbel MJ. *Brucellosis in humans and animals.* Geneva: World Health Organization; 2016, p. 1-102.

[36] Guh A, Phan Q, Nelson R, Purviance K, Milardo E, Kinney S, et al. Outbreak of *Escherichia coli* O157 associated with raw milk, Connecticut, 2008. *Clin Infect Dis* 2010; **51**: 1411-7.

[37] Oliver SP, Jayarao BM, Almedia RA. Food borne pathogens in milk and the dairy environment food safety and public health implications. *Foodborne Pathog Dis* 2005; **2**: 1115-29.

[38] Caine L, Nwodo U, Okoh A, Ndip R, Green E. Occurrence of virulence genes associated with diarrheagenic *Escherichia coli* isolated from raw cow's milk from two commercial dairy farms in the Eastern Cape Province, South Africa. *Int J Environ Res Public Health* 2014; **11**(11): 11950-63.

[39] Mbizeni S. Brucellosis in South Africa: progress and challenges. South Africa: Department of Agriculture, Forestry, Fisheries; 2015. [Online] Available from: http://repository.up.ac.za/bitstream/handle/2263/49187/mbizeni_brucellosis_sa2015.pdf?sequence=1&isAllowed=y [Accessed on 28th October, 2016]

[40] Food and Agriculture Organization. Brucellosis in humans and animals. Rome: Food and Agriculture Organization; 2006.