



Seroprevalence and 16S rRNA gene sequence analysis of *Brucella spp.* among domestic ruminants in Northern Border, Saudi Arabia

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Article History

Received: 02 September 2019

Reviewed: 06/September/2019 to 21/October/2019

Accepted: 24 October 2019

Prepared: 27 October 2019

Published: January - February 2020

Citation

Muaz Magzob Abdellatif, Yahia Hassan Ali Osman, Hussam Hassan Arafat, Ahmed Zein Elabdeen Mahmoud. Seroprevalence and 16S rRNA gene sequence analysis of *Brucella spp.* among domestic ruminants in Northern Border, Saudi Arabia. *Medical Science*, 2020, 24(101), 165-173

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General Note



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ABSTRACT

Brucellosis is a worldwide bacterial zoonotic disease. In this study, seroprevalence of *Brucella spp* among domestic ruminants in Hail, Saudi Arabia was investigated and strains recovered from camels were identified based on 16S rRNA gene sequence analysis. The overall seropositivity was 10.6%, goats had a higher seroprevalence of 12.1% compared to 11.4% in sheep and 6.2% for camels as tested by RBPT. Nucleotide identity of 16S rRNA among Hail camel brucella, strains was 99.9%, while identity with *Brucella* strains recovered from GenBank ranged from 100 to 89.3%. To put an efficient control program in action, further research to identify the strains involved; highlights potential risks that maintain the infection in animals and/or the environment should be done.

Keywords: Seroprevalence, *Brucella*, 16S rRNA gene, Domestic Ruminants, Hail, Saudi Arabia

1. INTRODUCTION

Brucellosis is a highly contagious zoonosis worldwide caused by *Brucella spp.* and affecting domestic and wild mammals (Godfroid et al., 2011; Ducrotoy et al., 2017). Sheep and goats are the main hosts for *Brucella melitensis*, while cattle are the main host for *Brucella abortus*. Both brucella species are known to have the highest impact on domestic livestock productivity and human health (Poester et al., 2010; Pappas, 2010; De Oliveira et al., 2011; Godfroid et al., 2011). Camels are susceptible and considered to be secondary hosts for both *B. abortus* and *B. melitensis* (Abbas and Agab, 2002; Dawood, 2008; Gwida et al., 2012; Hadush et al., 2013).

Transmission of Brucellosis from animals to people is often through consumption of unpasteurized milk and dairy products (Cooper., 1992; Shimol et al., 2012), however, direct contact, with livestock abortion material, is more important (Pappas et al., 2006; Schelling et al., 2007). It is a major agricultural and public health issue in Saudi Arabia (WHO., 1999; Memish and Venkatesh., 2001; Mantur and Amarnath., 2008; Asaad and Jobran., 2012; Musallam et al., 2016; Alyousef & Aldoghaither, 2018). Rose Bengal Plate Agglutination Test (RBPT) is a simple rapid agglutination assay used as a screening test for detecting *Brucella spp.* antibodies (Gwida et al., 2011; Diaz et al., 2011). The 16S rRNA provides specific sequences useful for identification of *Brucella spp.* (Weisburg et al., 1991; Bricker and Halling., 1994; Casañas et al., 2001; Kumar et al., 2011; Singh et al., 2013).

The present study was carried out to elucidate the prevalence of *Brucella* among sheep, goats and camels reared together in Hail, Saudi Arabia and to identify the strains circulating in camels based on sequence analysis of 16S rRNA gene.

2. MATERIALS AND METHODS

Sera

Sera (n=1100) were collected randomly from clinically healthy non-vaccinated sheep, goats and camels of different ages and sex from slaughterhouse in Hail district, Saudi Arabia during January to April 2019, stored at – 20°C until tested.

RBPT

Collected sera were screened for presence of antibodies to Brucella antigen by the RBPT. Test Kits were obtained from Lillidale Diagnostics, BH21 4HU, United Kingdom. An antigen prepared from *B. abortus* (Strain 99) stained with Rose Bengal dye and suspended in acid buffer (pH 3.65) was used to detect antibodies against *B. abortus* and *B. melitensis* on test plate, equal volume (30µl) of both antigen and sera were mixed, shaken for 5 min. and read.

DNA extraction and PCR

Camel sera samples (n=6) tested positive to RBPT were selected for DNA extraction using DNeasy Blood and Tissue Kit (QIAGEN) according to manufacturer's protocol.

Amplification of 16S rRNA

Partial sequence of 16S rRNA were amplified using *Doctor Taq polymerase* according to the manufacturer's instructions (*Doctor protein INC, Korea*) using 10F (AGTTTGATCTGGCTC) and 1525R (AAGGAGGTGATCCAGCC) oligonucleotide primers as described by Bautista et al. (2009).

PCR program was performed as follows: initial denaturation at 95 °C for 2 min., followed by 35 cycles of denaturation at 95 °C for 1 min, primer annealing at 52 °C for 1 min. and extension at 72 °C for 1 min. and a final extension of 72 °C for 10 min.

Purification and Sequencing of 16S rRNA

Post-PCR reaction product (5 μ l) was mixed with 2 μ l of ExoASP-IT[®] (usb) for a combined 7 μ l reaction volume. Incubated at 37°C for 15 min, then ExoSAP-IT was inactivated by incubation at 80°C for 15 min.

The purified products were Sanger-sequenced with BigDye terminator v3.1 Cycle Sequencing Kit and a 3730xl automated sequencer (ABI PRISM 3730XL Analyzer). Nucleotide sequences were determined on both strands of amplicons at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea).

Nucleotide sequence accession number

Sequences obtained have been deposited in GenBank under accession number MN235862 and MN235870.

Sequence analysis

Obtained sequences were edited and assembled using EditSeq and SeqMan within Lasergene 7.1.0 (DNASTAR, Inc, Madison, WI, USA). Sequence alignment was performed using neighbor-joining method to determine the phylogenetic relationship with sequences retrieved from GenBank using BLASTn tool (<https://blast.ncbi.nlm.nih.gov4/Blast>).

3. RESULTS

RBPT

Out of 1100 tested sera, 117 (10.6%) were found to be positive for *Brucella spp.* antibodies, with a higher seropositivity detected in goats (12.1%) (Table 1, Fig.1).

16S rRNA sequence analysis

Nucleotide identity of 16S rRNA among Hail camel *Brucella*, strains was found to be 99.9%, while identity with *Brucella* strains recovered from GenBank ranged from 97.8-100% with *Brucella melitensis*, 97.9-100% with *Brucella abortus*, 99.1-100% with *Brucella canis* and 89.3-97.8% with *Brucella spp.* (Figure 2&3). Phylogenetic tree showed Hail camel *brucella*, strains cluster within one clade (Figure 4).

Table 1 Seroprevalence of *Brucella spp.* among sheep, goats and camels as tested by RBPT

Species		Positive	Negative	Total
Sheep	Count	58	453	511
	% within Species	11.4%	88.6%	100.0%
	% within Result	49.6%	46.1%	46.5%
	% of Total	5.3%	41.2%	46.5%
Goats	Count	46	334	380
	% within Species	12.1%	87.9%	100.0%
	% within Result	39.3%	34.0%	34.5%
	% of Total	4.2%	30.4%	34.5%
Camels	Count	13	196	209
	% within Species	6.2%	93.8%	100.0%
	% within Result	11.1%	19.9%	19.0%
	% of Total	1.2%	17.8%	19.0%
Total	Count	117	983	1100
	% within Species	10.6%	89.4%	100.0%
	% within Result	100.0%	100.0%	100.0%
	% of Total	10.6%	89.4%	100.0%

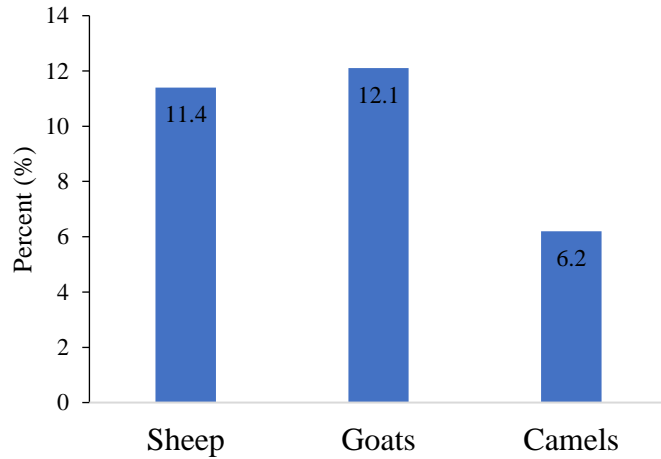


Figure 1 Percentage of *Brucella* spp. antibodies among sheep, goats and camels as detected by RBPT.



Figure 2 Ethidium bromide stained agarose gel (1%), PCR was carried on camel sera positive to RBPT. M: 100bp ladder, lane 1: control +ve, lane 2 and 3 positive test samples, lane 4,5,6,7 negative samples.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
1	100.0	99.9	99.3	99.8	99.9	98.7	99.9	99.9	99.9	99.9	99.9	99.9	98.2	99.9	98.1	99.8	90.5	91.2	99.9	99.9	99.9	98.2	89.7	95.9	93.1	97.4	99.9	93.4	99.0	1	<i>Brucella abortus</i> EF192470.	
2	0.0	99.9	99.6	99.8	99.8	98.5	99.9	99.9	99.9	99.9	99.9	99.9	98.1	99.8	98.0	99.8	90.4	91.1	99.9	99.9	99.9	98.3	88.4	95.0	93.1	97.4	99.9	93.4	99.0	2	<i>Brucella abortus</i> KF780867.	
3	0.1	0.1	99.4	99.9	99.9	98.6	100.0	100.0	100.0	99.9	99.9	99.9	98.3	99.9	98.1	99.8	90.3	91.0	100.0	99.9	99.9	98.3	88.2	94.7	93.3	97.4	100.0	93.4	99.1	3	<i>Brucella abortus</i> KF780868.1	
4	0.7	0.4	0.7	99.9	99.4	98.0	99.3	99.3	99.9	99.4	99.3	99.3	97.7	99.4	97.4	99.2	88.2	89.0	100.0	99.3	99.3	97.7	88.9	95.0	93.3	97.4	100.0	92.8	99.1	4	<i>Brucella abortus</i> KF780869.	
5	0.2	0.2	0.1	0.1	99.9	98.5	99.9	99.9	99.9	99.9	99.9	99.9	98.2	99.9	98.0	99.7	89.9	90.6	100.0	99.9	99.9	98.2	88.7	95.2	93.3	97.4	100.0	93.3	99.1	5	<i>Brucella abortus</i> KF780870.1	
6	0.1	0.2	0.1	0.7	0.1	98.6	100.0	100.0	100.0	99.9	99.9	100.0	98.3	100.0	98.2	99.8	91.0	91.7	100.0	100.0	100.0	98.4	87.5	93.3	93.3	97.4	100.0	92.7	99.1	6	<i>Brucella abortus</i> NR_042460.1	
7	1.2	1.4	1.4	2.0	1.5	1.4	98.7	98.7	98.8	98.7	98.7	98.6	97.3	98.6	97.2	98.4	88.0	88.3	99.0	98.7	98.7	97.3	86.4	92.5	92.9	96.2	98.8	91.1	97.9	7	<i>Brucella abortus</i> X13695.	
8	0.1	0.1	0.0	0.7	0.1	0.0	1.3	100.0	100.0	100.0	100.0	100.0	98.3	100.0	98.1	99.9	90.5	91.2	100.0	100.0	100.0	98.3	88.6	94.9	93.3	97.4	100.0	93.5	99.1	8	<i>Brucella canis</i> AB474584.1	
9	0.1	0.1	0.0	0.7	0.1	0.0	1.3	0.0	100.0	100.0	100.0	100.0	98.3	100.0	98.1	99.9	90.5	91.2	100.0	100.0	100.0	98.3	89.0	95.1	93.3	97.4	100.0	93.5	99.1	9	<i>Brucella canis</i> AB479093.1	
10	0.1	0.1	0.0	0.1	0.1	0.0	1.1	0.0	0.0	100.0	100.0	100.0	98.3	100.0	98.1	99.9	90.3	91.1	100.0	100.0	100.0	98.3	89.9	96.1	93.3	97.4	100.0	93.4	99.1	10	<i>Brucella canis</i> JN591318.	
11	0.1	0.2	0.1	0.7	0.1	0.1	1.3	0.0	0.0	0.0	100.0	100.0	98.4	99.9	98.2	99.8	89.9	89.9	100.0	100.0	100.0	98.4	87.5	93.3	93.3	97.4	100.0	92.0	99.1	11	<i>Brucella canis</i> NR_074286.	
12	0.1	0.1	0.1	0.7	0.1	0.1	1.3	0.0	0.0	0.0	0.0	100.0	98.4	99.9	98.2	99.8	89.9	89.9	100.0	100.0	100.0	98.4	87.5	93.3	93.3	97.4	100.0	92.0	99.1	12	<i>Brucella melitensis</i> NR_074111.	
13	0.1	0.1	0.1	0.7	0.1	0.0	1.3	0.0	0.0	0.0	0.0	0.0	98.4	100.0	98.2	99.8	91.0	91.7	100.0	100.0	100.0	98.4	87.5	93.7	93.3	97.4	100.0	93.3	99.1	13	<i>Brucella melitensis</i> AY922323.1	
14	1.8	1.9	1.7	2.3	1.8	1.7	2.7	1.7	1.7	1.7	1.6	1.6	1.7	98.3	100.0	98.1	87.1	87.3	98.3	98.4	98.4	100.0	86.6	91.8	93.5	99.8	97.8	91.5	96.9	14	<i>Brucella melitensis</i> EF187230.	
15	0.1	0.2	0.1	0.7	0.1	0.0	1.4	0.0	0.0	0.0	0.1	0.1	0.0	1.7	98.2	99.8	91.0	91.7	100.0	100.0	100.0	98.4	87.5	93.3	93.3	97.4	100.0	92.7	99.1	15	<i>Brucella melitensis</i> KX863494.	
16	2.0	2.0	2.0	2.6	2.1	1.9	2.8	1.9	1.9	1.9	1.9	1.9	0.0	1.9	98.0	87.8	88.5	98.1	98.2	98.2	100.0	89.1	93.3	94.6	99.8	97.8	92.9	96.9	16	<i>Brucella melitensis</i> KY243030.		
17	0.1	0.1	0.1	0.7	0.1	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	1.9	90.7	91.4	99.9	99.8	99.8	98.1	87.3	93.7	93.1	97.1	99.8	93.1	98.9	17	<i>Brucella melitensis</i> L26166.	
18	4.9	4.9	5.2	7.5	5.5	4.8	8.3	4.9	4.9	5.0	6.1	6.1	4.6	9.3	4.8	8.2	4.6	7.1	90.6	91.8	91.1	91.1	87.9	85.6	75.3	0.0	86.7	90.2	84.4	90.2	18	<i>Brucella melitensis</i> MF164084
19	4.9	4.9	5.1	7.4	5.5	4.7	8.5	4.9	4.9	5.0	5.9	5.9	4.6	9.1	4.7	8.2	4.6	7.1	90.6	91.8	91.8	88.6	86.1	75.5	0.0	87.4	90.6	84.4	90.6	19	<i>Brucella melitensis</i> MF164092	
20	0.1	0.1	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	1.9	0.0	5.2	5.3	100.0	100.0	98.3	88.8	96.5	93.3	97.5	100.0	93.3	99.1	20	<i>Brucella melitensis</i> MK684240.	
21	0.1	0.1	0.1	0.7	0.1	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	1.9	0.0	4.5	4.5	0.0	100.0	98.4	87.6	93.4	93.3	97.4	100.0	92.9	99.1	21	<i>Brucella melitensis</i> NR_118257.1	
22	0.1	0.1	0.1	0.7	0.1	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	1.9	0.0	4.5	4.5	0.0	0.0	98.4	87.6	93.4	93.3	97.4	100.0	92.9	99.1	22	<i>Brucella melitensis</i> NR_118257	
23	1.8	1.8	1.8	2.4	1.8	1.7	2.7	1.7	1.7	1.7	1.7	1.7	0.0	1.7	0.0	1.7	0.1	8.1	8.1	1.7	1.7	86.6	92.2	93.5	99.8	97.8	92.6	96.9	23	<i>Brucella</i> sp. EF514908		
24	11.1	12.6	12.8	12.1	12.3	13.7	15.0	12.3	11.9	10.9	13.7	13.7	13.7	14.7	13.7	11.8	13.7	10.8	11.1	12.2	13.5	13.5	14.7	84.7	93.5	86.1	90.1	85.0	89.3	24	<i>Brucella</i> sp. GU085225	
25	4.1	5.0	5.3	5.0	4.8	6.8	7.7	5.2	4.9	3.8	6.8	6.8	6.4	8.6	6.8	6.9	6.2	24.6	25.3	3.4	6.7	6.7	8.1	17.0	93.3	92.9	97.5	88.6	96.6	25	<i>Brucella</i> sp. HF542111.	
26	7.3	7.3	7.0	7.0	7.0	7.0	7.5	7.0	7.0	7.0	7.0	7.0	7.0	6.8	7.0	5.7	7.0	0.0	7.0	7.0	6.8	6.8	7.0	6.8	6.8	7.0	96.7	92.3	87.6	89.3	26	<i>Brucella</i> sp. KY453877.
27	2.7	2.7	2.7	2.7	2.7	2.7	3.8	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	0.2	2.7	9.2	9.2	2.6	2.7	2.7	0.2	15.4	7.3	3.3	97.5	92.9	97.5	27	<i>Brucella</i> sp. HM196294.	
28	0.1	0.1	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	2.2	0.0	5.2	5.2	0.0	0.0	0.0	2.2	10.6	2.3	8.2	2.6	92.8	99.9	28	<i>Brucella</i> sp. NBU1 MN235862. ←	
29	4.4	4.4	4.4	5.1	4.5	5.2	6.9	4.3	4.3	4.3	5.9	5.9	4.6	6.5	5.2	5.0	4.5	11.6	11.9	4.4	4.9	4.9	5.3	14.1	9.6	11.4	4.7	4.7	91.8	29	<i>Brucella</i> sp. U71005.	
30	1.1	1.1	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	3.2	1.0	3.2	1.0	5.2	5.2	1.0	1.0	1.0	3.2	11.6	3.3	11.7	2.6	0.1	5.8	30	<i>Brucella</i> sp. NBU2 MN235870. ←	

Figure 3 Percentage of identity and divergence among Hail camel *Brucella* strains (indicated with arrows) related to strains retrieved from GenBank.

4. DISCUSSION

Brucellosis is of wide spread all over the world, variable seroprevalence were reported in goats, 4.1% in Lao People's Democratic Republic (Burns et al., 2018) and 3.9% in China (Rahman et al., 2019). In sheep, seroprevalence of *Brucella* were, 11% in Tajikistan (Rajala et al., 2016), 13.37% in Guinea (Loureiro et al., 2017), 9% in Nigeria (Aworh et al., 2017) and 7.4% in Sudan (Abdalla et al., 2019).

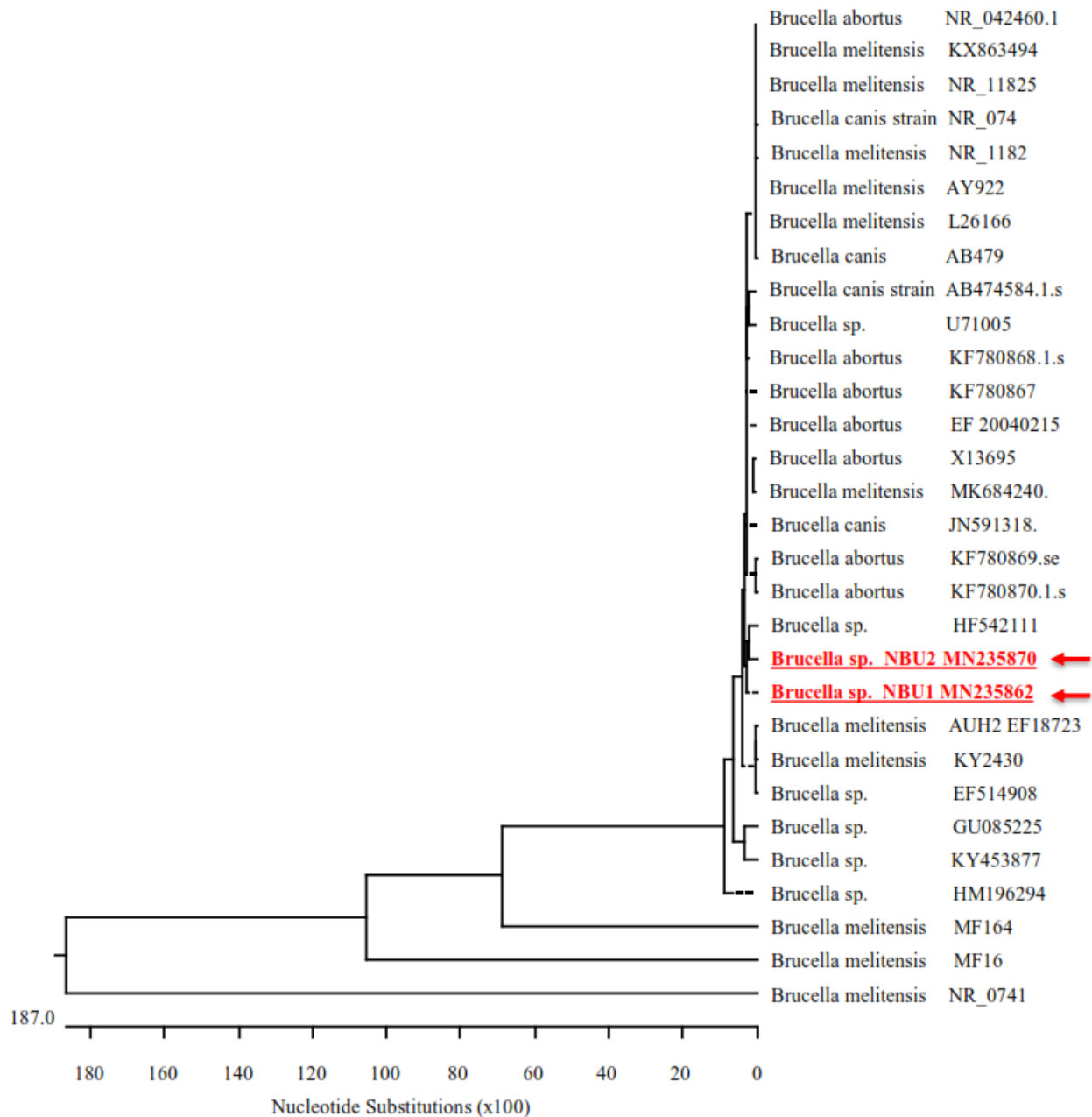


Figure 4 Phylogenetic analysis of the identified Hail camel *Brucella* strains (indicated with arrows) related to strains recovered from GenBank based on *16S rRNA* gene sequence alignment (Tree was generated using *Lasergene* 7.1).

In this study seroprevalence of *Brucella spp.* in sheep, goats and their close contact camels was measured in Hail, Saudi Arabia during January to April, 2019. The overall seropositivity was 10.6%, with higher percentage in goats (12.1%), followed by sheep (11.4%) then camels (6.2%). The results are comparable to that obtained by Abd El-rahim and Asghar (2014) who recorded an overall incidence of 9.3% of *Brucella.spp* in small ruminants in western region of the country. However, within species, the prevalence rate was higher in sheep (15.6%) and lower (3.9%) in goats. Very low incidence of *Brucella.spp* in goats (0.8%) and sheep (0.5%) in Makkah was previously reported (Radwan et al., 1983), meanwhile, higher incidence was published in Asir region, 18.2% in goats and

12.3% in sheep (Bilal et al., 1991). Threat of brucellosis in the country may be due to the importation of live animals and animal products, nevertheless it has decreased in recent years due to the vaccination, restrict importation and cooperation between veterinarians and the public health sector (Jokhdar., 2009; Ali & Alluwaimi., 2009).

Our results are in line with previous reports suggested that goats are more susceptible to *Brucella* infection than sheep (Quinn et al., 2011), it was 45% in goats, 22% in sheep in Jordan (Musallam et al., 2016); 19.6% in goats, 9% in sheep in Nigeria (Aworh et al., 2017). Also, it was found to be 33.4% in goats and 9% in sheep in Libya (Al-Griw et al., 2017), 34.0% in goats, 31% in sheep in Iraq (Alhamada et al., 2017). This could be partly due to the fact that sheep excrete the organism for shorter periods than goats. This may reduce the potential for spread of the disease within and between sheep flocks (Radostits et al., 2000). Other reports showed that sheep were more likely to be seropositive than goats (Hegazy et al., 2011; Kandeel et al., 2014; Rajala et al., 2016), which may be influenced by sampling and/or the immunity of the species investigated. Variation may be affected with the biology of the bacteria, management (animal, herd, farm), and farmer's knowledge about the disease (Burrige., 1981; Kadohira et al., 1997; Schelling et al., 2003; Coelho et al., 2008; Cowie et al., 2014).

Camels are not known to be primary hosts for any of the *Brucella* species, and the infection rate depends upon the infection rate in the primary host animals in contact with them, this may suggest the role of small ruminants in the occurrence of camel brucellosis (Agab et al., 1994). Seropositivity of *Brucella* in camels in the present work is similar to that published by Al-Ruwaili et al. (2012) in Hail, but unlike the prevalence reported in Al-Assafia (9.1%), Arar (7.7%), Domat-Aljandal (5.5%) and sakaka (2.4%). Comparable rates in camels were obtained in different countries, 4.4% in Ethiopia (Domenech et al., 1977), 5.5, 8.0% in Sudan (Omer et al., 2010); 7.3% in Egypt (El-Boshy et al., 2009), 5% in Pakistan (Fatima et al., 2016). Low seroprevalence in camels (1.8%) was reported in Riyadh region (Alshaikh et al., 2007), as well as in Ethiopia (2%) (Megersa et al., 2011; Admasu et al., 2017) 4% in Somaliland (Ghanem et al., 2009). Higher prevalence of 30.5 and 23.8% were also documented in Sudan (Musa et al., 2008), 19.4% in Jordan (Dawood, 2008), 9.3% in Nigeria (Salisu et al., 2017). Higher percentage may point to that the screened camels were at a high risk (Al-Majali et al., 2008; Omer et al., 2010).

Identification of *Brucella spp.* using commercial systems has not been consistent and misidentifications regularly encountered (Jokhdar., 2009; Ali and Alluwaimi., 2009; Dash et al., 2012). Dash et al. (2012) performed *16S rRNA* sequencing for identification of *Brucella spp.* misidentified as *Bergeyella zoohelcum*. The genetic discrimination of *Brucella spp.* remains a challenging task owing to its genetic homogeneity. Suitability of partial *16S rRNA gene* sequencing has been demonstrated for identification of pathogenic bacteria, including *Brucella* (Woo et al., 2003; Lau et al., 2006; Ruppitsch et al., 2007). In this research, *16S rRNA* gene was amplified using universal primers described previously (Bautista et al., 2009) and confirmed as camel *Brucella*. The *16S rRNA* component of 30S small subunit of prokaryotic ribosomes contains hyper-variable regions that provide specific sequences useful for bacterial identification, so it was used for confirmatory identification of *Brucella* (Weisburg et al., 1991). Sequencing Analysis of *16S rRNA* exhibits nucleotide identity of 99.9% among identified *Brucella* strains of Hail, when compared to strains retrieved from the GenBank, it showed identity homology of 97.8-100% with *Brucella melitensis*, 97.9-100% with *Brucella abortus*, 99.1-100% with *Brucella canis* and 89.3-97.8% with *Brucella spp.* The identity of the sequence is consistent with previous studies (Bricker., 2000; Halling et al., 2005), ncbi-blast search finds sequences differ from the consensus sequence which agreed with Vizcaíno et al. (2000) who declared that there was some variability in the *16S rRNA* locus, but not enough to separate the *Brucella spp.* Low variability in the *16S rRNA* locus has been noted as an impediment in using *16S rRNA gene* sequencing to discriminate at the species level (Vizcaino et al., 2000; Sacchi et al., 2002; Nilsson et al., 2003). Mustafa et al. (2017) finds 90-99% sequence identity with *B. melitensis*, variation may be due to using species specific types of primers to perform PCR, while we used selected universal primers previously designed.

5. CONCLUSION

The study showed that *Brucella spp.* is circulating in the region; further studies are needed to identify the strains and the risk factors to adopt strict prevention and prophylactic plans to eradicate the microbe.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding

The authors gratefully acknowledge the approval and the support of this research study by the grant number SAR-2018-3-9-F-7796 from the Deanship of Scientific Research at Northern Border University, Arar, Saudi Arabia.

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