RESEARCH ARTICLE　　　　　　　　　　　　　　　　**OPEN ACCESS**

Seroprevalence and Identification of *Brucella melitensis* Based on a *Glycosyltransferase* Gene among Ruminants in Rafha, Saudi Arabia

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Abstract

Brucellosis is a zoonotic disease with veterinary, public health, and economic implications. The study aimed to estimate the seroprevalence of *Brucella spp.* among ruminants in Rafha, Saudi Arabia during January to October 2022 and to identify camel strains based on *a glycosyltransferase gene* sequence. Sera (n=1012) were collected from non-vaccinated sheep, goats, camels and cattle of different sex, age and breed randomly from the abattoirs to investigate the circulating brucella antibodies using RBPT. One hundred and eighteen sera (9.7%) were reactive for *Brucella spp.* IgG immunoglobulins, with higher percentages detected in sheep (11.4%), females (13.3%), adults (10.7%), and naieme breed (13.9%). Significant correlation between *Brucella spp.* antibodies and animal species (0.095), age (0.077) was found, while strong correlation between antibodies and sex was observed. *Glycosyltransferase gene* was amplified and sequenced from camel reactive sera (n=6). Camel strains displayed multiple nucleotide substitutions and deletions, nucleotide identity among local strains is 96.2-100%. Phylogenetic analysis showed that *Brucella spp.* strains clustered in two groups, Rafha strains clustered in one group together with other strains. Further investigation is needed to determine the prevalence of the bacteria among farm animals and to identify the strains involved to improve the preventive measures and strategies adopted for control.

Keywords: *Brucella spp.*, Seroprevalence, *Glycosyltransferase Gene,* Ruminants, Rafha, Saudi Arabia

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INTRODUCTION

Brucellosis is a zoonotic disease causing serious consequences for animal production and human health.[1-3] *Brucella spp.* is the causal agent of the disease with *B. abortus* and *B. melitensis* being mainly important in ruminant and human infections. It infects almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and human.[1] Human illness spread via contaminated dairy products and intimate contact with infected animals.[4,5] Families bred camel, along with goats and sheep for a variety of purposes. In addition to providing physical labor, a camel's wool can be woven into cloth, milk can be drunk, and for meat and leather. The disease was reported in different parts of the Kingdom of Saudi Arabia.[6-8] It is regarded as a main public health and agricultural problem in the country.[9-15] The RBT is an affordable, quick, simple and efficient screening and a diagnostic test for individual animals and herds.[7,16] To adopt effective control measures, large-scale epidemiological studies are required. The *glycosyltransferase gene* coding for O-antigen production is a crucial virulence protein is conserved among *Brucella* species.[17-20] Studies have shown that it is conserved in all *Brucella* species. Furthermore, characterization of the gene can clarify the relationship between genotype and their use in differential diagnosis. Analysis of this gene can identify the circulating strains and elucidate the similarity between genetic constitution of *Brucella spp.*[16,20]

No published research on the prevalence of brucellosis in ruminants or on genotyping of *Brucella* species based on the *glycosyltransferase gene* in Rafha, Saudi Arabia. The objective of the current study aimed to assess the prevalence of brucellosis among ruminants in Rafha, Saudi Arabia and to identify camel *Brucella* strains based on a *glycosyltransferase gene* sequence.

MATERIALS AND METHODS

Ethical Approval

Animals were treated in accordance with ethical considerations and the research was authorized by the Local Committee of Bioethics (HAP-09-A-043) at Northern Border University, KSA, issued the decision no. (3/44/H/2022).

Sample Collection

Sera (n = 1212) were collected randomly from the slaughterhouse in Rafha, Saudi Arabia during January to October 2022 from sheep, goats, camels, and cattle of various sex, age, and breed (Table 1).

Rose Bengal Plate Test (RBPT)

The presence of anti-*Brucella* IgG antibodies were examined in serum samples. The antigen was obtained from Lillidale Diagnostics, BH21 4HU, United Kingdom. Test sera and RBPT antigen were mixed in an equal volume (30µl), shaken for 5 minutes, and then read.

Statistical analysis

The correlation between seropositivity for IgG and species, sex, age, and animal breed were assessed utilizing Spearman's rank correlation coefficient (P-value = 0.01). Analysis was done using SPSS25 (Statistical Package for Social Sciences 25) (Table 2).

DNA extraction

Following the manufacturer's protocol, DNA were extracted from camel sera (n=10) that were reactive by RBPT using the DNeasy Blood Kit (QIAGEN).

Amplification of a *glycosyltransferase gene*

The *glycosyltransferase gene* was amplified using the procedure and the primer sequences (F:5'-GAGTAGACACGGGAAATC-3' and R:5'-GATAAACACGCCGAGCTT-3') published by Etemadi et al. (2008). Conventional PCR was carried out using *QIAGEN kits* as follows; denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 30s, 55°C for 30s, 72°C for 90s, and a final extension at 72°C for 8 min.

Purification of amplicons

Amplicons (5µl) were combined with 25µl of ExoASP-IT® (usb) for a total reaction volume of 75µl. ExoSAP-IT was incubated at 37°C for 15 minutes before being deactivated by heating at 80°C for 15 minutes. Purified PCR products were Sanger-sequenced using a 3730xl automated sequencer and the BigDye terminator v3.1 Cycle Sequencing Kit (ABI PRISM 3730XL Analyzer).

Table 1. Seroprevalence of brucellosis as detected by RBPT among species, sex, age and breed in Rafha, Saudi Arabia during January to October 2022

Species		Sheep	Goat	Camel	Cattle	Total
Positive	N	102	6	10	0	118
	% within Species	11.4	4.7	5.6	0.0	9.7
Negative	N	789	123	170	12	1094
	% within Species	88.6	95.3	94.4	100.0	90.3%
Total	N	891	129	180	12	1212

Sex		Male	Female	Total
Positive	N	39	79	118
	% within Sex	6.3	13.3	9.7
Negative	N	579	515	1094
	% within Sex	52.9	47.1	100.0
Total	N	618	594	1212

Age		Young	Adult	Total
Positive	N	9	109	118
	% within Age	4.5	10.7	9.7
Negative	N	189	905	1094
	% within Age	95.5	89.3	90.3
Total	N	198	1014	1212

Breed		Mgater (Camels)	Baladi (Goats)	Naime (Sheep)	Brbre (Sheep)	Friesian (Cattle)	Hendi (Cattle)	Syrian (Goats)	Mjahim (Camels)	Swakni (Sheep)	Total
Positive	N	6	6	99	3	0	0	0	4	0	118
	% within Breed	3.8	5.6	13.9	2.0	0.0	0.0	0.0	19.0	0.0	9.7
Negative	N	153	102	615	144	6	6	21	17	30	1094
	% within Breed	96.2	94.4	86.1	98.0	100.0	100.0	100.0	81.0	100.0	90.3
Total	N	159	108	714	147	6	6	21	21	30	1212

The Macrogen sequencing facility sequenced both strands (Macrogen Inc., Seoul, Korea).

Sequence analysis

Lasergene 7.1.0 was used to edit and assemble nucleotides using *EditSeq and SeqMan* (DNASTAR, Inc, Madison, WI, USA) (DNASTAR, Inc, Madison, WI, USA). The BLASTn program (https://blast.ncbi.nlm.nih.gov4/Blast) was used to align local sequences with those from GenBank (Table 3), and a phylogenetic tree was constructed using the neighbor-joining approach.

RESULTS

RBPT

Out of the tested sera one hundred and eighteen sera (9.7%) were reactive for *Brucella spp.* IgG immunoglobulins, 11.4% among sheep with higher incidence among naieme breed (13.9%), 13.3% among females and 10.7% among adults across all species (Figure 1).

Statistical analysis

Seropositivity significantly correlated with animal species (0.095), age (-0.077) and sex (-0.118) (Table 2).

Glycosyltransferase gene sequencing

Glycosyltransferase gene was amplified and sequenced from camel sera (n=6). Sequences were deposited in the *GenBank* (Accession numbers MN934944, MN934945, MN934946, MN934947, MN934948 and MN934949).

Sequence analysis

Local strains exhibited multiple nucleotide substitutions and deletions (Figure 2), identity among sequences was 96.2-100% (Figure 3), while identity with strains retrieved from GenBank was 42.1-99.9% (Figure 2). Phylogenetic analysis displayed *Brucella spp*. in two branches, Rafha strains clustered in one group together with other strains (Figure 4).

DISCUSSION

Brucellosis is a zoonotic bacterial disease caused by various *Brucella* species, which mainly infect cattle, swine, goats, sheep and dogs.[24] In the current study, prevalence of brucellosis in Rafha. Saudi Arabia in sheep, goats and camels was determined by RBPT, as well as, camel strains were identified based on a *glycosyltransferase gene* analysis. Sero-positivity was assumed to be attributable to infection of brucellosis since

Table 2. Spearman correlation of seroprevalence of brucellosis as detected by RBPT with species sex, age and animal breed in Rafha, Saudi Arabia during January to October 2022

		Seropositivity	Species	Sex	Age	Breed
Seropositivity	Correlation Coefficient	1.000	.095**	-.118**	-.077**	.001
	Sig. (2-tailed)	.	.001	.000	.007	.968
	N	1212	1212	1212	1212	1212
Species	Correlation Coefficient	.095**	1.000	-.327**	-.655**	-.576**
	Sig. (2-tailed)	.001	.	.000	.000	.000
	N	1212	1212	1212	1212	1212
Sex	Correlation Coefficient	-.118**	-.327**	1.000	.366**	.014
	Sig. (2-tailed)	.000	.000	.	.000	.620
	N	1212	1212	1212	1212	1212
Age	Correlation Coefficient	-.077**	-.655**	.366**	1.000	.450**
	Sig. (2-tailed)	.007	.000	.000	.	.000
	N	1212	1212	1212	1212	1212
Breed	Correlation Coefficient	.001	-.576**	.014	.450**	1.000
	Sig. (2-tailed)	.968	.000	.620	.000	.
	N	1212	1212	1212	1212	1212

**. Correlation is significant at the 0.01 level (2-tailed)

immunization has never been practiced in the area.

The findings showed that the disease spread among ruminants and it occurred at a higher rate in sheep. The detected prevalence of brucellosis in sheep (11.4%), is similar to that published in Saudi Arabia[14] as well as in India.[25] It was slightly lower than that reported in other parts of the country, including western region (15.6%) and Makkah (12.3-14.2%).[7,8,26] Much higher seroprevalence (31.7%) was reported at Duhok in northern Iraq.[27] However, it was slightly higher than the reported one (7.3%) in Aljouf region, Saudi Arabia,[15] and in Aseer and Jazan (5.1%) in southern Saudi Arabia,[28] also that found (8.3%) in India.[29] Nevertheless, none of tested sheep sera in Farasan Islands in the Red Sea in southwestern Saudi Arabia were found to be
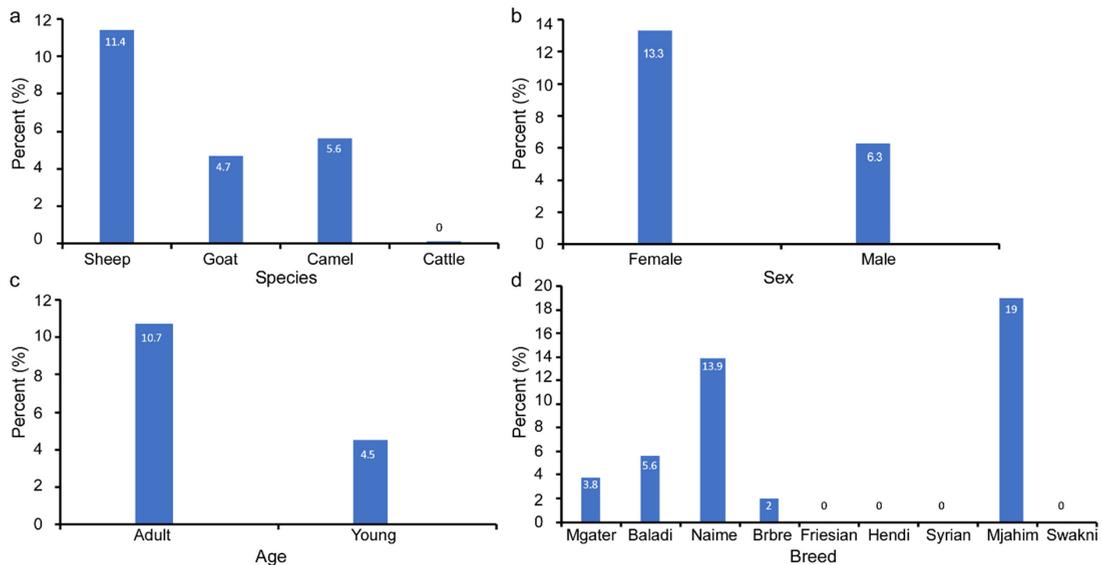

Figure 1. Seroprevalence rate of *Brucella spp.* antibodies as tested by RBPT according to species (a), sex (b), age (c) and breed (d) in Rafha, Saudi Arabia during January to October 2022
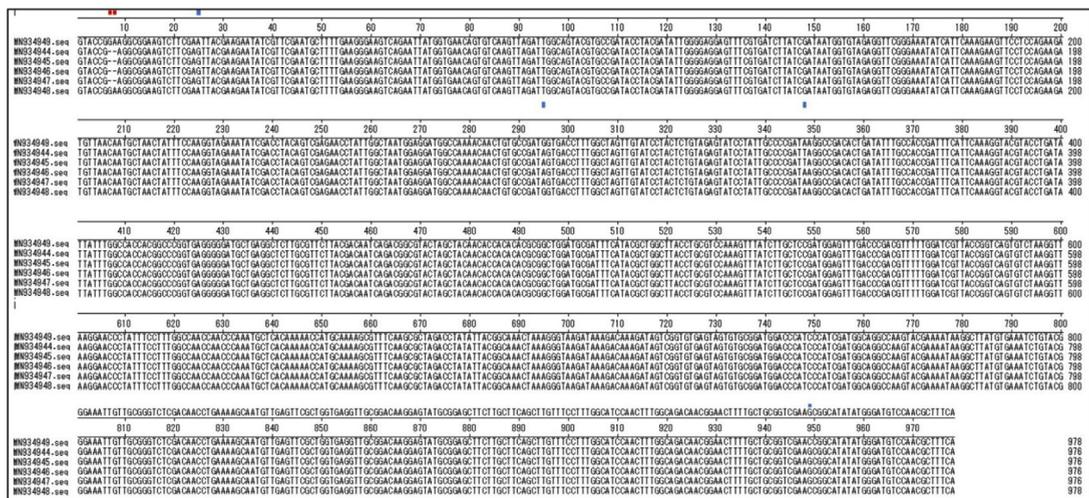

Figure 2. Sequence alignment of a *glycosyltransferase gene* identified from *brucella spp.* strains from camel in Rafha, Saudi Arabia during January to October 2022

positive.[30] Discrepancy may be due to the breed involved, herd size, management, and seasonality of the disease.

The detected percent of caprine brucellosis (4.7%) is alike to previous records in Bangladesh.[31,32] However, Abdellatif et al.[14] detected much higher seropositivity (12.1%) in Hail, Saudi Arabia. Meanwhile it was 8.8%, in Medina.[33] A very low seroprevalence (0.6%) was detected in goats in Farasan Islands in Saudi Arabia[30] which is expected due to the isolated nature of the island. Brucellosis is endemic in many

Percent Identity

	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		
1	■	100.0	100.0	100.0	99.9	99.9	42.2	42.1	42.1	100.0	42.3	99.9	99.9	42.1	99.9	99.9	42.2	42.2	42.2	99.9	99.9	100.0	100.0	99.7	99.7	1	AY065979
2	0.0	■	100.0	100.0	99.9	99.9	42.2	42.1	42.1	100.0	42.3	99.9	99.9	42.1	99.9	99.9	42.2	42.2	42.2	99.9	99.9	100.0	100.0	99.7	99.7	2	CP001578
3	0.0	0.0	■	100.0	99.9	99.9	42.2	42.1	42.1	100.0	42.3	99.9	99.9	42.1	99.9	99.9	42.2	42.2	42.2	99.9	99.9	100.0	100.0	99.7	99.7	3	CP007717
4	0.0	0.0	0.0	■	99.9	99.9	42.2	42.1	42.1	100.0	42.3	99.9	99.9	42.1	99.9	99.9	42.2	42.2	42.2	99.9	99.9	100.0	100.0	99.7	99.7	4	CP018506
5	0.1	0.1	0.1	0.1	■	100.0	42.2	42.1	42.1	99.9	42.3	99.8	99.8	42.1	99.8	99.8	42.2	42.2	42.2	99.8	99.8	99.9	99.9	99.8	99.8	5	CP018532
6	0.1	0.1	0.1	0.1	0.0	■	42.2	42.1	42.1	99.9	42.3	99.8	99.8	42.1	99.8	99.8	42.2	42.2	42.2	99.8	99.8	99.9	99.9	99.8	99.8	6	CP018554
7	115.7	115.7	115.7	115.7	115.7	115.7	■	99.8	99.8	42.2	99.8	42.1	42.1	99.8	42.1	42.1	100.0	99.9	100.0	42.3	42.3	42.2	42.2	42.2	42.2	7	CP022875
8	116.3	116.3	116.3	116.3	116.3	116.3	0.2	■	100.0	42.1	99.8	42.0	42.2	99.8	42.0	42.0	99.8	99.9	99.8	42.2	42.2	42.1	42.1	42.1	42.1	8	CP023223
9	116.3	116.3	116.3	116.3	116.3	116.3	0.2	0.0	■	42.1	99.8	42.0	42.2	99.8	42.0	42.0	99.8	99.9	99.8	42.2	42.2	42.1	42.1	42.1	42.1	9	CP023308
10	0.0	0.0	0.0	0.0	0.1	0.1	115.7	116.3	116.3	■	42.3	99.9	99.9	42.1	99.9	99.9	42.2	42.2	42.2	99.9	99.9	100.0	100.0	99.7	99.7	10	CP025821
11	115.2	115.2	115.2	115.2	115.2	115.2	0.2	0.2	0.2	115.2	■	42.2	42.4	99.8	42.2	42.2	99.8	99.9	99.8	42.4	42.4	42.3	42.3	42.3	42.3	11	CP027643
12	0.1	0.1	0.1	0.1	0.2	0.2	116.3	117.0	117.0	0.1	115.8	■	99.8	42.0	100.0	100.0	42.1	42.1	42.1	99.8	99.8	99.9	99.9	99.6	99.6	12	CP033079
13	0.1	0.1	0.1	0.1	0.2	0.2	115.2	115.8	115.8	0.1	114.7	0.2	■	42.2	99.8	99.8	42.3	42.3	42.3	99.8	99.8	99.9	99.9	99.6	99.6	13	CP054955
14	116.1	116.1	116.1	116.1	116.1	116.1	0.2	0.2	0.2	116.1	0.2	116.8	115.7	■	42.0	42.0	99.8	99.9	99.8	42.2	42.2	42.1	42.1	42.1	42.1	14	CP061816
15	0.1	0.1	0.1	0.1	0.2	0.2	116.3	117.0	117.0	0.1	115.8	0.0	0.2	116.8	■	100.0	42.1	42.1	42.1	99.8	99.8	99.9	99.9	99.6	99.6	15	CP066175
16	0.1	0.1	0.1	0.1	0.2	0.2	116.3	117.0	117.0	0.1	115.8	0.0	0.2	116.8	0.0	■	42.1	42.1	42.1	99.8	99.8	99.9	99.9	99.6	99.6	16	LT671512
17	115.7	115.7	115.7	115.7	115.7	115.7	0.0	0.2	0.2	115.7	0.2	116.3	115.2	0.2	116.3	116.3	■	99.9	100.0	42.3	42.3	42.2	42.2	42.2	42.2	17	LT962916
18	115.7	115.7	115.7	115.7	115.7	115.7	0.1	0.1	0.1	115.7	0.1	116.3	115.2	0.1	116.3	116.3	0.1	■	99.9	42.3	42.3	42.2	42.2	42.2	42.2	18	LT962945
19	115.7	115.7	115.7	115.7	115.7	115.7	0.0	0.2	0.2	115.7	0.2	116.3	115.2	0.2	116.3	116.3	0.0	0.1	■	42.3	42.3	42.2	42.2	42.2	42.2	19	LT963350.
20	0.1	0.1	0.1	0.1	0.2	0.2	115.2	115.8	115.8	0.1	114.7	0.2	0.2	115.7	0.2	0.2	115.2	115.2	115.2	■	100.0	99.9	99.9	99.6	99.6	20	MN934944
21	0.1	0.1	0.1	0.1	0.2	0.2	115.2	115.8	115.8	0.1	114.7	0.2	0.2	115.7	0.2	0.2	115.2	115.2	115.2	0.0	■	99.9	99.9	99.6	99.6	21	MN934945
22	0.0	0.0	0.0	0.0	0.1	0.1	115.7	116.3	116.3	0.0	115.2	0.1	0.1	116.1	0.1	0.1	115.7	115.7	115.7	0.1	0.1	■	100.0	99.7	99.7	22	MN934946
23	0.0	0.0	0.0	0.0	0.1	0.1	115.7	116.3	116.3	0.0	115.2	0.1	0.1	116.1	0.1	0.1	115.7	115.7	115.7	0.1	0.1	0.0	■	99.7	99.7	23	MN934947
24	0.3	0.3	0.3	0.3	0.2	0.2	115.7	116.3	116.3	0.3	115.2	0.4	0.4	116.1	0.4	0.4	115.7	115.7	115.7	0.4	0.4	0.3	0.3	■	100.0	24	MN934948
25	0.3	0.3	0.3	0.3	0.2	0.2	115.7	116.3	116.3	0.3	115.2	0.4	0.4	116.1	0.4	0.4	115.7	115.7	115.7	0.4	0.4	0.3	0.3	0.0	■	25	MN934949
	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		

Figure 3. Percentage of identity of a *glycosyltransferase gene* sequences from *Brucella spp.* strains identified from camel in Rafha, Saudi Arabia and sequences retrieved from GenBank

Table 3. Annotation of sequences retrieved from GenBank using BLASTn tool for phylogenetic analysis

No.	GenBank	Host	Species	strain	Country	Reference
1.	CP025821	Homo sapiens	*Brucella melitensis*	CIT31	China	Unpublished
2.	LT962945	Homo sapiens	*Brucella melitensis*	1	Norway	Unpublished
3.	CP018506	Homo sapiens	*Brucella melitensis*	BwIM_SOM_36a	Somalia	21
4.	CP007717	Sus scrofa	*Brucella suis*	513UK	United Kingdom	22
5.	CP001578	Vole	*Brucella microti*	CCM 4915	Czech Republic	23
6.	AY065979	Unknown	*Brucella melitensis*	16M	USA	Unpublished
7.	CP033079	Elk	*Brucella abortus*	BJ1	China	Unpublished
8.	CP027643	Dog	*Brucella canis*	GB1	China	Unpublished
9.	LT671512	Bos taurus	*Brucella abortus*	Wisconsin	USA	Unpublished
10.	LT963350	Homo sapiens	*Brucella melitensis*	1	Norway	Unpublished
11.	LT962916	Homo sapiens	*Brucella melitensis*	1	Norway	Unpublished
12.	CP023308	Bubalus bubalis	*Brucella abortus*	9510	Italy	Unpublished
13.	CP023223	Bubalus bubalis	*Brucella abortus*	67761	Italy	Unpublished
14.	CP022875	Bos taurus	*Brucella melitensis*	BL	China	Unpublished
15.	CP018554	Homo sapiens	*Brucella melitensis*	BwIM_TUR_39	Turkey	21
16.	CP018532.1	Homo sapiens	*Brucella melitensis*	BwIM_SYR_41	Syria	21
17.	CP066175	Sheep	*Brucella abortus*	68	Ukraine	Unpublished
18.	CP061816	Cystophora cristata	*Brucella pinnipedialis*	23a-1	Svalbard	Unpublished
19.	CP054955	Sus scrofa	*Brucella suis*	CVI_72	Slovenia	Unpublished

countries, variable seroprevalence of the disease had been reported. A very high prevalence (34%) in goats have been detected in Iraq [27] and (27.7%) in Jordan.[34] In Dhofar Province at Southern Oman, goat sera showed 13% seropositivity.[35] In India, 5.8% seropositivity was determined.[29] It was found to be 14.8% in North West Libya,[36] which was higher than our present study. In contrast, Rahman & Ahasan[37] declared that the rate of brucellosis was 1.98% in Bangladesh. Despite contradictions in the literature, reports showed that sheep was more likely to be reactive than goats,[38-40] which may be influenced by sampling, circulating strain, immunity of the species, management (animal, herd, farm), and/or owner's awareness about the disease.[41-43] The occurrence of camel brucellosis (5.6%) was slightly comparable to the obtained results in Hail (6.2%)[14] and Alzulfi, Saudi Arabia (6.5%) Salih et al, higher than that reported (3.5%.) at Aseer and Jazan in southern Saudi Arabia[28] and disagree with the previous data (1.9%)

reported in Riyadh by Alshaikh et al. Variable seroprevalence were found in other Gulf countries, in Dhofar, Southern Oman, 3.4% of camel sera tested positive.[35] A far higher seroprevalence of brucellosis in camel sera (20.6%) was reported in Qatar.[35] The rate also contrast with that reported in Ethiopia (2.2%),[44] 4.1%,[45] and 3.37%.[46] Higher rate was recorded in Sudan ((37.5%) [47] and Somalia (7%).[48] The exposure may be increased as a consequence of the intense animal rearing.[49] Furthermore, the infections can also spread due to the absence of control measures, mixed grazing of camel herds with other herds and animals in the pasture, and drinking points. All cattle sera in the current study were seronegative, it may be related to the number of animals tested because cattle were not bred in the region. Previous research reported bovine brucellosis as 18.1% in Jordan,[12] 1.9% in China,[50] 6.3% in Pakistan.[51] Seroprevalence was found to be variable depending on sampling size, species, sex and size of herd.[3]
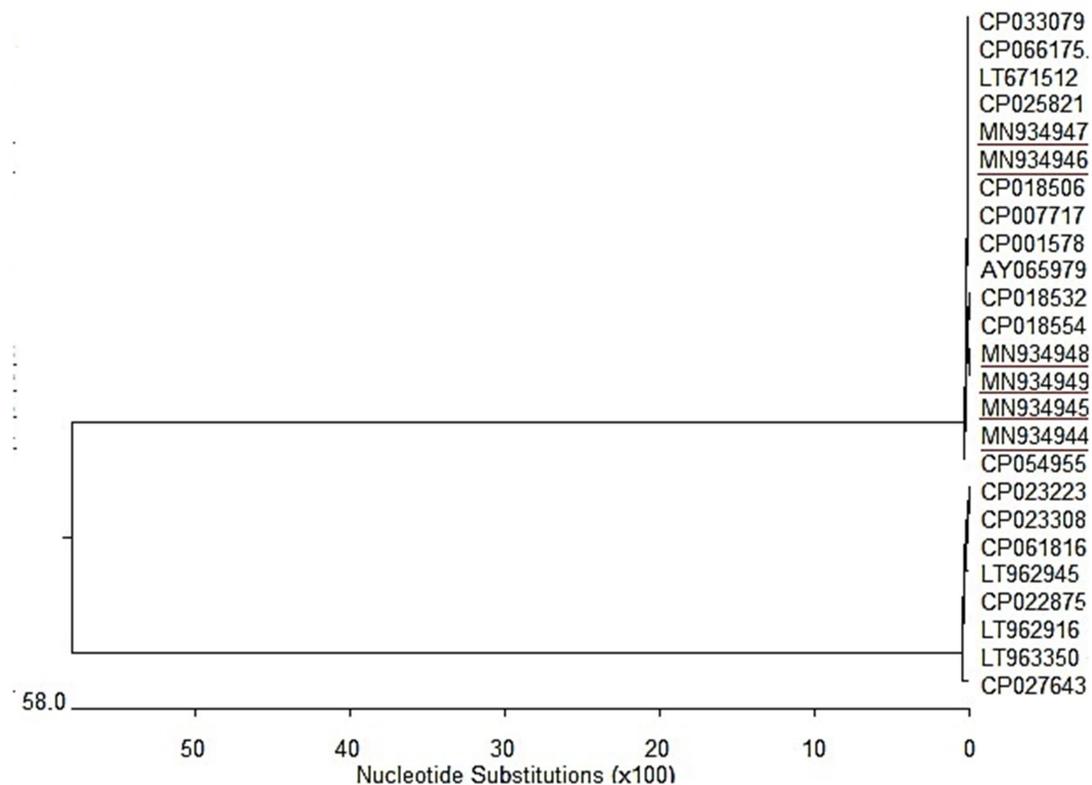

Figure 4. Phylogenetic analysis of a *glycosyltransferase* gene sequences from *Brucella* spp. strains identified from camel in Rafha, Saudi Arabia and sequences retrieved form GenBank

Sex wise, seroprevalence was comparable to the previous reports.[15,52] But it differs with other literature.[34,53,54] Difference may be due to the immune response or the interaction of other risk factors. Age-level showed that was higher in adult animals, it's supported by previous research.[15,40] It may be owing to sex hormones, which may stimulate the growth of the bacteria, and tend to increase in concentration with age and sexual maturity. This might be true since older animals keep on in the flock for a long time, and they had a longer duration of contact. The higher percentages in naimee breed may be due the number of animals tested.

Analysis of data revealed significant correlation between seroprevalence of *Brucella spp.* and species, sex and age. In contrary, there was no association between the prevalence and animal breed. Results were in accordance to that observed by Rahman et al. However, it opposed with Akhter et al.[55] who found that none of these factors was linked with brucellosis. Inconsistency may be attributed to variance in the risk factors involved at both animal and herd level.[56]

Traditional identification of brucellosis depends on the isolation of the bacteria.[57] Owing to various restrictions in the isolation of the bacteria including requirement for biosafety facilities, workers expertise, and hazard of contamination, numerous molecular procedures to identify and discriminate *Brucella* species have been developed.[58] In the present investigation, camel strains were identified as *B. melitensis* based on *glycosyltransferase gene* amplification and sequencing. Comparative analysis of the nucleotide sequence among camel strains exhibited 96.2-100% similarity with multiple nucleotide substitutions and deletions. Phylogenetic analysis based on *Glycosyltransferase gene* sequence to clarify the genetic relation between local strains and other sequences deposited in GenBank. Unfortunately, there were no sequences of the *Glycosyltransferase gene* of the *Brucella* from Saudi Arabia found in GenBank to be added in the tree. Analysis display *Brucella* strains in two branches, Rafha strains clustered in one group together with other strains. The results agreed with Etemady et al., who report considerable genetic diversity among *B. melitensis* and conservation of *B. abortus* strains.

CONCLUSION

The prevalence of brucellosis was 11.4% in sheep, 4.7% in goats and 5.6% in camels. The seropositivity was higher in females (13.3%), adults (10.7%), and naieme breed (13.9%). Circulating strains were identified from camel sera as *B. melitensis* based on a *glycosyltransferase* gene. Analysis revealed multiple nucleotide substitutions and deletions, displaying variable identities. Further investigation to identify the circulating strains and to understand factors implicated in the epidemiology is needed to improve the preventative measures and control policy adopted.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Local Committee of Bioethics (HAP-09-A-043) at Northern Border University, Arar, Saudi Arabia, wide letter number 3/44/H/2022.

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