Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.043

RESEARCH ARTICLE

Molecular Detection of Outer Membrane Protein 2 Gene in Isolated *Brucella* from Sheep and Goats in Sharazor/Kurdistan Region of Iraq

Nahla Muhammad Saeed[1], Sirwan Muhammad Talib[1] and Hiewa Othman Dyary[2]*

[1]Department of Microbiology; [2]Department of Basic Sciences, College of Veterinary Medicine, University of Sulaimani, New Sulaimani, Street 27, Sulaymaniyah, Kurdistan Region, Northern Iraq
*Corresponding author: dyary.othman@univsul.edu.iq

ARTICLE HISTORY (18-293)

Received: October 10, 2018
Revised: February 15, 2019
Accepted: February 28, 2019
Published online: March 22, 2019

Key words:
Brucella melitensis
Brucellosis
Outer membrane proteins
Phylogenetic analysis

A B S T R A C T

Brucellosis is an economically important zoonotic disease that is endemic in different parts of Iraq. An outbreak of abortion occurred in sheep and goats in Sharazor subdistrict, Sulaymaniyah, Iraq and *Brucella* infection was suspected to be the cause. Between November 1, 2014, and January 31, 2015, 47 tissue and vaginal swab samples were collected from 17 herds. Samples from each herd were mixed together to make 17 pooled samples. The *Brucella* genus-specific primer Bruc was used to amplify the 223 bp amplicon of bcsp31 gene. Fifteen of the 17 samples (88%) were positive for *Brucella* genus. Thereafter, five samples were randomly selected to identify the species by designing four sets of *Brucella*-species-specific primers. Sequencing of the Outer Membrane Protein 2 (omp2) gene was performed and the sequences were aligned with others in the GenBank databases. The results showed that the omp2 gene of the five isolates from Sharazor had 99-100% similarities with *Brucella melitensis* bv. 2 that was isolated from United States (accession number CP007789) and *B. melitensis* strain 20236 (accession # CP008750) isolated from human in China. The amino acid sequences of the omp2a gene in the five isolates were similar with other isolates in GenBank with the exception of *B. suis* bv. 2 (accession # CP007720). This study reported the occurrence of *B. melitensis* for the first time in Sharazor subdistrict, Sulaymaniyah, northern Iraq, using molecular techniques.

To Cite This Article: Saeed NM, Talib SM and Dyary HO, 2019. Molecular Detection of outer membrane protein 2 gene in isolated *Brucella* from sheep and goats in Sharazor/Kurdistan Region of Iraq. Pak Vet J, 39(3): 383-388. http://dx.doi.org/10.29261/pakvetj/2019.043

INTRODUCTION

Brucellosis is one of the most widespread zoonotic diseases infecting many animal species and humans. The causative agents are Gram-negative bacteria that belong to the genus *Brucella* (de Figueiredo *et al.*, 2015). The most common pathogenic species worldwide are *B. abortus* in cattle, *B. melitensis* (mainly infecting sheep and goats), and *B. suis* in pigs. These species may cause abortion and result in huge economic losses in the infected animals (Seleem *et al.*, 2010; Atluri *et al.*, 2011).

A high level of homology among the currently recognized species of *Brucella* has been revealed by DNA-DNA hybridization analyses so that *B. melitensis* was suggested to be the only representative species of this genus. The other *Brucella* species were also proposed to be biovars of *B. melitensis* (Cloeckaert *et al.*, 2002). However, the genus *Brucella* is still classified into six species, as this classification is dependent on factors such as the pathogenicity and host preference of each species (Cloeckaert *et al.*, 2001; Scholz and Vergnaud, 2013).

Besides lipopolysaccharide, the cell wall of *Brucella* is mainly composed of outer membrane proteins (omp) (Vassen *et al.*, 2019). The omp comprises two major fractions of molecular masses 36-38 and 25-27 kDa, designated group 2 and group 3, respectively (Wang *et al.*, 2010). In contrast to the lipopolysaccharide component of the outer membrane of *Brucella*, the group 2 omp seem to be of little importance as antigens and immunogens during host infection. However, group 3 omp appear to exert high levels of immunogenicity in the course of *B. ovis* infection in rams (Cloeckaert *et al.*, 2002).

The genes encoding the major omp in *Brucella* are designated as omp2a and omp2b for group 2 porin proteins, which are 36-38 kDa. The genes share 85% DNA identity, are separated by 900 bp on the

chromosome and oriented in opposite directions (Roussel *et al*., 2012).

Brucellosis is endemic in many parts of Iraq and several serological and molecular studies have revealed high incidences of the disease in small ruminants. For example, in a serological study by Al-Samarraee and Jabary (2015), blood samples were collected randomly from 160 sheep and 151 goats in Sulaymaniyah governorate, located in the north-east of Iraq. The results revealed the presence of *Brucella* antibodies in 20.0% of the sheep and 8.6% of the goat samples. In another study by Alhamada *et al.* (2017), blood samples were collected randomly from 335 sheep and 97 goats in Duhok province in the north of Iraq. It was shown that 31.7% of the sheep and 34.0% of the goats had antibodies against *Brucella*.

Sharazor is one of the 10 districts of Sulaymaniyah governorate and is located about 40 kilometers east of the latter. The district includes 49 villages and the sheep and goat population in the area is estimated to be around 84000. Different outbreaks of abortion in small ruminants have recently occurred in the area and the causative agent was reported to be *Brucella melitensis*. However, a definitive molecular characterization of the causative agent has not been conducted previously. In this study, we identified and characterized the gene encoding the omp2 and performed a phylogenetic analysis of the sequences with different isolates that were published in GenBank databases.

MATERIALS AND METHODS

Sample collection: The study was carried out in Sharazor region of Sulaymaniyah governorate/Iraq, from November 1st, 2014 to January 31st, 2015. Seventeen mixed herds of sheep and goats from nine localities were included. The samples were taken from animals which had incidences of abortion.

Forty-seven tissues and vaginal swab samples were collected from aborted fetuses and dams in 17 vaccinated and nonvaccinated herds. The animals had a history of abortion in the previous 1 to 40 days. Samples taken from each herd were pooled together and numbered from 1 to 17. Sample number 17 was subdivided into 17a (from sheep) and 17b (from goat). Of the 47 samples, 45 were vaginal swabs and two specimens were taken from aborted fetal tissues (Table 2). The swabs and tissue specimens were stored in a cooled box and transported to the laboratory in less than four hours.

Extraction of DNA: DNA extraction from the collected samples was conducted immediately after arrival at the laboratory. PrimePrep genomic DNA isolation kits (GeNet Bio, Chungnam, South Korea) were used to isolate DNA from tissue (Cat. No. K-3000) and vaginal secretions (Cat. No. K-2000). The latter was also used to extract DNA from a vaccine containing *Brucella melitensis* strain Rev. 1 (Jovac, Jordan), which was used as the positive control.

Polymerase chain reaction: Four sets of primers encoding omp2 and one set encoding bcsp31 genes, manufactured by Macrogen (Korea, Table 1), were used in the study (Baily *et al.*, 1992).

The lyophilized primers were suspended in a volume of sterile nuclease-free distilled water, to prepare a 100 pmol stock solution, and stored at -20°C. Thereafter, a tenfold dilution was made to prepare 10 pmol/µL of primers for use in the PCR.

The PCR was done in 200 µL capacity AccuPower® PCR tubes using AccuPower®PCR PreMix (Bioneer/ Korea). The PCR mix was prepared by adding 1 µL of each of the forward and reverse primers, 5 µL of the sample DNA, and 13 µL of double-distilled water. The PCR cycles included an initial denaturation for two minutes at 94°C, followed by 35 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 57°C) and extension (80 seconds at 72°C). A final extension for five minutes at 72°C was also included.

Gel electrophoresis: The PCR product (7 µL) was stained with 3 µL SYBR green dye (Bio-Rad, USA) and was run on a 1% agarose gel. The electrophoresis system (Major Science, Taiwan) was run for 40 minutes using an electrical potential of 120 volts.

DNA bands were visualized under ultraviolet light using a molecular imager (Gel Doc XR System, Bio-Rad Laboratories, USA). The size of the amplified DNA strand was estimated by comparing with a 100 base pair DNA ladder (GenDirex, USA).

Sequencing of PCR products and phylogenetic construction: The *Brucella* omp2 genes of five selected Sharazor isolates (number 2, 4, 7, 9 and 17b) were sequenced from both ends. Sequencing was conducted by Bioneer Sequencing Service (Korea). Alignment of the sequences was performed using bl2seq program. The result was published in GenBank under accession numbers KP681853, KT896745, KT896746, KT896747, and KT896748 for samples 2, 4, 7, 9 and 17b, respectively.

The sequence identity of *Brucella* omp2 gene of Sharazor isolates was confirmed by using BLAST (Basic Local Alignment Search Tool) method in the NCBI (National Center for Biotechnology Information) homepage. Expasy translate tool software was used to translate the nucleotide sequences of omp2 of Sharazor isolates to sequence amino acids.

The omp2 gene sequences of samples 2, 4, 7, 9, and 17b were comparatively analyzed and a phylogenetic tree was constructed using the Clustal W Multiple Sequence Alignment Program (MEGA6 software).

RESULTS

Detection of *Brucella* genus-specific gene: Forward and reverse Bruc primers were used to amplify a 223 bp amplicon on the Bcsp31 gene, which is specific for the genus *Brucella* and encodes a 31 kilodalton cell surface protein. The results showed that 15 of the 17 pooled samples were positive for *Brucella*, of which, 13 were vaginal swabs and the other two were tissue specimens. Two herds of sheep (number 10 and 14) were negative for *Brucella* (Table 2). Four herds (number 1, 2, 9 and 10) were previously immunized with a *B. melitensis* (strain Rev. 1) vaccine. However, three of the four samples showed positive results for *Brucella*.

Table 1: Sequences of the *Brucella* gene primers

Gene	Length	Primer	Forward primer	Reverse primer
bcsp31	223	Bruc	5'-TGGCTCGGTTGCCAATATCAA-3'	5'-CGCGCTTGCCTTTCAGGTCTG-3'
omp2b	756	Bru1	5'-GCGATCTTCCGCGACCCCTGTAG-3'	5'-CATACCTTCACCGGTTACCTCG-3'
omp2	818	Bru2	5'-CTTGCCGGTGCGGTAGGAGCCA-3'	5'-CGTTAACATCGGTTAATTCGCG-3'
omp2	873	Bru3	5'-GTGCTGCTATGAAGGGCAGT-3'	5'-CGCCCTTTACGTCGTAACGGAC-3'
omp2b	921	Bru4	5'-CGATCTGATTAGAACGAGCG-3'	5'-CGATCTGATTAGAACGAGCG-3'

Table 2: Occurrence of *Brucella* in the studied farms

Herd no.	Village name	Animal species	Type of sample	Number of samples per pool	Vaccination	PCR result
1	Greza	Sheep	Tissue	1	Yes	Positive
2	Kelakawa	Sheep	Vaginal swab	5	Yes	Positive
3	Qala	Sheep	Vaginal swab	5	No	Positive
4	Greza	Sheep	Tissue	1	No	Positive
5	Qumash	Sheep	Vaginal swab	3	No	Positive
6	Qumash	Sheep	Vaginal swab	1	No	Positive
7	Qumash	Sheep	Vaginal swab	1	No	Positive
8	Qumash	Sheep	Vaginal swab	2	No	Positive
9	Qumash	Sheep	Vaginal swab	2	Yes	Positive
10	Qumash	Sheep	Vaginal swab	1	Yes	Negative
11	Halabjay Taza	Sheep	Vaginal swab	4	No	Positive
12	Halabjay Taza	Sheep	Vaginal swab	5	No	Positive
13	Palyhero	Sheep	Vaginal swab	2	No	Positive
14	Bawakuzhyaw	Sheep	Vaginal swab	1	No	Negative
15	Bawakuzhyaw	Sheep	Vaginal swab	1	No	Positive
16	Berashka	Sheep	Vaginal swab	3	No	Positive
17a	Kanipanka	Sheep	Vaginal swab	5	No	Positive
17b	Kanipanka	Goat	Vaginal swab	4	No	Positive

***Brucella* species detection:** Five of the fifteen positive samples for *Brucella* genus (number 2, 4, 7, 9 and 17b) were randomly selected and subjected to further amplification using *Brucella* species-specific primers Bru1, Bru2, Bru3, and Bru4 (Table 1). Amplification was successful for all the primers in all the five PCR products.

Sequence identity: The nucleotide sequences of omp2 gene of samples number 2, 4, 7, 9 (sheep) and 17b (goat) were subjected to comparing with those published in the GenBank databases of the NCBI by using BLAST (Basic Logical Alignment Search Tool) program, to detect the identities and the rate of similarities between Sharazor isolates and those published in GenBank.

Comparison of the five isolates with those published in the GenBank databases showed >99% similarity when compared with *B. melitensis* strain 20236 (accession # CP008750) and *B. melitensis* bv. 2 (accession # CP007789) (Fig. 2). A lower nucleotide similarity percentage (97%) was recorded with *B. ovis* (accession # CP000708).

The CLUSTAL W Multiple Sequence Alignment results showed that there was a nucleotide substitution at A55G that differentiated the Sharazor isolates from all other aligned *B. melitensis* strains.

The vaccine strain (*B. melitensis* strain Rev1, accession # KC847057) showed an exclusive nucleotide substitution at G83A, thereby it differed from the Sharazor isolates and all other aligned *Brucella* species. Samples number 7 and 9 showed 100% nucleotide identity. Both isolates showed a unique nucleotide substitution at A162C compared with the other Sharazor and GenBank isolates.

A nucleotide substitution at C202G was present in all the five samples and the vaccine strain, which was different from the isolates from the USA with accession numbers CP007709, CP007763, CP000708, CP007705, and CP007720.

The sequences of *B. ovis* (accession # CP000708) and *B. suis* bv. 2 (accession # CP007720) showed nucleotide substitutions at G300A, T304A and C314G, so they were different from all Sharazor isolates and all other aligned *Brucella* species.

Sample number 4 showed a nucleotide substitution at G1029A, which was not present in the other samples. This substitution was also present in GenBank isolates *B. melitensis* bv. 2 (accession # CP007789), *B. melitensis* strain 20236 (accession # CP008750), *B. melitensis* M5-90 (accession # CP001851.1), and *B. melitensis* strain Rev1 (accession # KC847057). Samples number 2, isolated from sheep, and 17b, isolated from goat, showed 100% nucleotide identity.

Amino acid alignment of omp2a: The amino acid sequences of the omp2a gene result showed similar amino acid compositions in all the aligned *Brucella* isolates, with the exception of *B. suis* bv. 2 (accession # CP007720) and *B. ovis* (accession # CP000708). Both isolates exhibited amino acid substitutions at several positions such as V59I, H60S, S75T, T77S and N80K.

Amino Acid alignment of omp2b: The amino acid sequence of sample number 4 was similar to the sequence of *B. melitensis* strain 20236 (accession # CP008750), *B. melitensis* bv. 2 (accession # CP007789), *B. melitensis* M5-90, (accession # CP001851) and *B. melitensis* strain Rev1 (accession # KC847057). These isolates showed an amino acid substitution (P31S) when compared to the other aligned *Brucella* isolates.

Samples number 7 and 9 exhibited 100% amino acid sequence similarity. They differed from the other isolates by showing an exclusive amino acid substitution at W320G, which differentiated them from all the other aligned *Brucella* isolates. Furthermore, samples number 2 and 17b had 100% amino acid sequence identity.

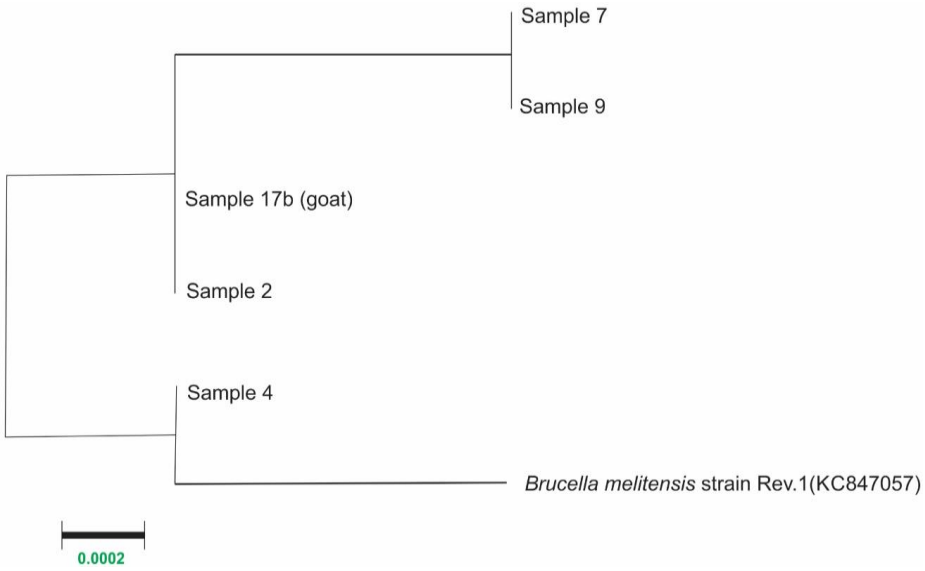

Fig. 1: Phylogenetic tree constructed based on amino acids alignment of omp2b gene of Sharazor isolates (field strains) and *Brucella melitensis* strain Rev.1 (vaccine strain). This alignment shows the formation of three groups: samples 7 and 9, sample 4 and *B. melitensis* strain Rev. 1, and samples number 2 (from sheep) and 17b (from goat).

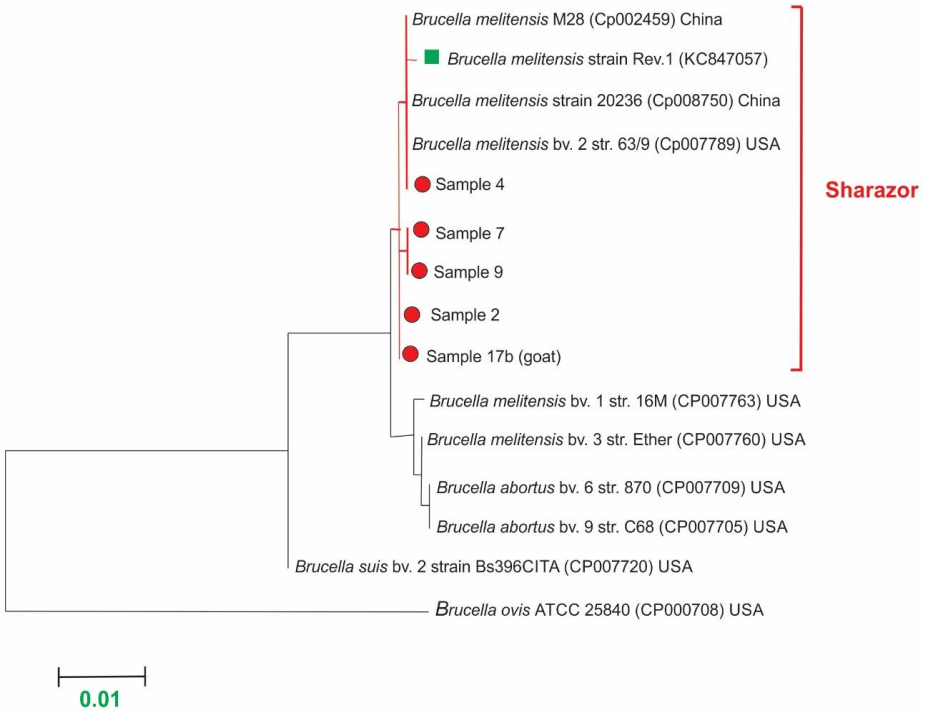

Fig. 2: Phylogenetic tree constructed based on Nucleotide sequence alignment of omp2 gene of Sharazor isolates and *Brucella* species that submitted at GenBank. All the isolates from the current study are clustered in one group.

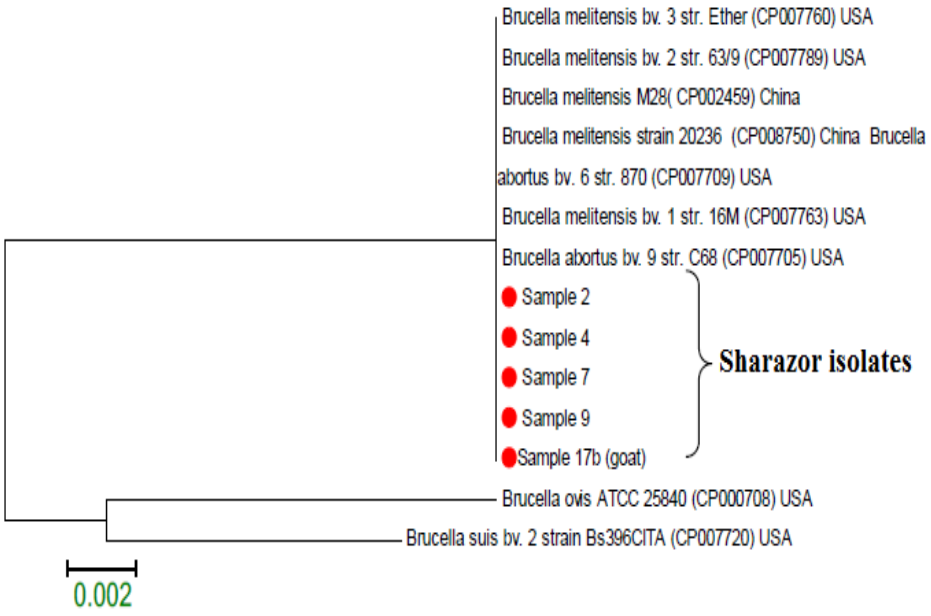

Fig. 3: Alignment of amino acids of omp2a gene from Sharazor isolates and *Brucella* species submitted at GenBank. The isolates show similar amino acid sequences with other Brucella isolates with the exception of *B. suis* bv. 2 (accession # CP007720) and *B. ovis* (accession # CP000708).

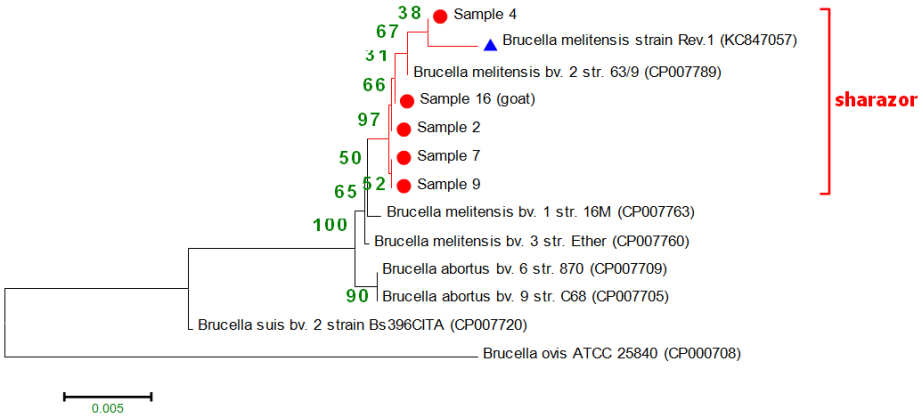

Fig. 4: Phylogenetic tree constructed based on amino acids alignment of om2b gene of Sharazor isolates and *Brucella* species that submitted at GenBank. The isolates of the current study are clustered in three groups. Samples number 7 and 9 are grouped together. Sample number 4 is clustered with *B. melitensis* strain Rev. 1 (KC847057). Samples number 2 and 17b are mostly related to *B. melitensis* strain 20236 (CP008750) from China.

Phylogenetic tree of the vaccine and field strains: A phylogenetic tree was constructed, based on amino acids alignment of omp2b, between the five samples and the vaccine strain (*B. melitensis* strain Rev. 1). The results showed the construction of three groups: samples 7 and 9, sample 4 and *B. melitensis* strain Rev. 1 vaccine, and samples 2 (sheep) and 17b (goat). So, the vaccine strain was genetically closer to sample 4 than the other Sharazor isolates (Fig. 1).

Phylogenetic tree based on nucleotide sequence alignment of the omp2 gene: The topology of the phylogenetic tree indicated that all the five isolates were clustered in one group together with *B. melitensis* bv. 2 (CP007789), *B. melitensis* strain 20236 (CP008750), *B. melitensis* M5-90 (CP001851.1), and *B. melitensis* strain Rev. 1 (KC847057). This phylogenetic tree revealed that the Sharazor isolates were more genetically related to *B. melitensis bv*. 2 (CP007789) (Fig. 2).

Phylogenetic tree based on the alignment of amino acids in the omp2a protein: The alignment of amino acid sequences of the omp2a protein showed little variation among the *Brucella* isolates as they were clustered together (Fig. 3), with the exception of *B. suis* bv. 2 (CP007720) and *B. ovis* ATCC 2840 (CP000708).

Phylogenetic tree based on the alignment of amino acids in the omp2b protein: The omp2b protein amino acid sequences showed the formation of different groups. Samples number 7 and 9 were clustered together while sample 4 was clustered with *B. melitensis* strain Rev. 1 (KC847057), *B. melitensis* strain 20236 (CP008750), *B. melitensis* bv. 2 (CP007789), and *B. melitensis* M26 (CP002459). Furthermore, samples number 2 and 17b were clustered together and they were mostly related to *B. melitensis* strain 20236 (CP008750) from China (Fig. 4).

DISCUSSION

Despite the fact that the incidence of abortion due to brucellosis has declined in the surrounding areas, *Brucella* is still a major cause of abortion in small ruminants in Sharazor. The high prevalence of brucellosis is probably due to the poor management and husbandry practices by the farmers, such as grazing of different flocks of sheep on the same pasture and sharing of rams for mating between different herds.

The genus-specific primers of *Brucella* used in the current study (Bruc), which encode the 31 kilodalton cell surface protein, revealed that 88% of the tested samples were infected by the pathogen. The high rate of positive samples indicates that brucellosis is endemic in the region and that the vaccination programs regularly conducted by the Veterinary Public Services did not reduce the prevalence rate of the disease.

The DNA-DNA hybridization assays revealed that there were more than 90% homologies among the *Brucella* isolates, which is an indication that the isolates were different biovars of *B. melitensis*. The molecular genotyping techniques used for the differentiation of *Brucella* species showed a significant DNA polymorphism. One of these loci is the omp2 gene, which encodes the 36 kDa outer membrane protein 2 of *Brucella* species (Yousefi *et al.*, 2016; Yousefi *et al.*, 2018). This gene reveals diversities among different isolates and special primers have been designed for the identification of *Brucella* species, biovars, and strains (Cloeckaert *et al.*, 2002; He, 2012; Kaden *et al.*, 2017).

The omp2 sequences of Sharazor and ten other previously mentioned *Brucella* isolates were analyzed by using Clustal Omega Multiple Sequence Alignment program. The results revealed a 99% nucleotide and amino acid sequences similarities with *B. melitensis* biovar 2 (GenBank accession number CP007789). This outcome indicates that the Sharazor isolates belong to *B. melitensis* biovar 2. This result coincides with other molecular assay outcomes which were conducted in Turkey (Büyükcangaz *et al.*, 2009; Ica *et al.*, 2012), Iran (Behroozikhah *et al.*, 2012), and southern Iraq (AL-tememy *et al.*, 2013), as the isolated species in these studies were also reported to be *B. melitensis* biovar 2.

The amino acid sequence alignment of omp2a in Sharazor isolates with other GenBank isolates showed a high degree of similarity among the aligned sequences, with the exception of *B. suis* biovar 2 (accession number CP007720) and *B. ovis* (accession number CP000708). In contrast, a higher degree of polymorphism was detected when the amino acid sequences of the omp2b gene were aligned together. The same outcome has been previously reported by other researchers (Di *et al.*, 2016).

The omp2 gene was used to differentiate the vaccine strain (*B. melitensis* strain Rev. 1, GenBank accession number KC847057) from the field strains. This locus was widely used in other molecular research as a target for the differentiation of field strain from vaccine strain of

Brucella species (Noutsios *et al.*, 2012; Saeedzadeh *et al.*, 2013). The results showed a unique nucleotide substitution at G83A. Therefore, the vaccine strain was different from the Sharazor and all other aligned *Brucella* isolates. However, the phylogenetic tree that was constructed based on the amino acid sequence of Omp2b gene showed clustering of sample 4 with *B. melitensis* strain Rev. 1 in one group and showed the same ancestor and genetic relationship. These results approved that the vaccine strain used in the immunization of sheep and goats in the Sharazor region is a suitable candidate, in particular with respect to Omp2 gene, for the control of Brucellosis in this region.

Conclusions: Despite the regular vaccination campaigns conducted by the Veterinary Public Services, brucellosis is still the main cause of abortion in small ruminants in Sharazor subdistrict of Sulaymaniyah governorate. Failure of the vaccination program is mainly due to the improper management and husbandry practices followed by the farmers. The causative agent of brucellosis in the area is *B. melitensis* and it seems that there are no strain variations among the different isolates in the region. The four sets of primers for the Omp2 gene, which were used in this study, are suitable for the identification of *Brucella* from sheep and goats. *Brucella melitensis* strain Rev. 1 is a suitable vaccine to be used in the immunization of sheep and goats in Sharazor.

Author contribution: All the authors equally contributed to the study.

REFERENCES

Al-Samarraee IA and Jabary OM, 2015. Detection of *Brucella* antibodies of sheep and goats by using two serological tests in Al-Sulaimanya governorate. Iraqi J Vet Sci 39:32-7.

AL-tememy HA, Al-jubort KH and Abdulmajeed BA, 2013. Pathological and molecular diagnosis of *Brucella melitensis* in the fetal and placental tissues of aborted ewes in Al-Najaf city. Kufa J Vet Sci 4:28-40.

Alhamada AG, Habib I, Barnes A, *et al.,* 2017. Risk factors associated with *Brucella* seropositivity in sheep and goats in Duhok Province, Iraq. Vet Sci 4:65.

Atluri VL, Xavier, MN, De Jong MF, *et al.,* 2011. Interactions of the human pathogenic *Brucella* species with their hosts. Annu Rev Microbiol 65:523-41.

Baily G, Krahn J, Drasar B, *et al.,* 1992. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. Trop Med Int Health 95:271-5.

Behroozikhah AM, Bagheri Nejad R, Amiri K, *et al.,* 2012. Identification at biovar level of *Brucella* isolates causing abortion in small ruminants of Iran. J Pathog 2012:357235.

Büyükcangaz E, Şen A and Kahya S, 2009. Isolation and biotyping of *Brucella melitensis* from aborted sheep and goat fetuses. Turk J Vet Anim Sci 33:311-6.

Cloeckaert A, Verger JM, Grayon M, *et al.,* 2001. Classification of *Brucella* spp. isolated from marine mammals by DNA polymorphism at the omp2 locus. Microbes Infect 3:729-38.

Cloeckaert A, Vizcaíno N, Paquet JY, *et al.,* 2002. Major outer membrane proteins of *Brucella* spp.: past, present and future. Vet Microbiol 90:229-47.

de Figueiredo P, Ficht TA, Rice-Ficht A, *et al.,* 2015. Pathogenesis and immunobiology of brucellosis: review of *Brucella*–host interactions. Am J Pathol 185:1505-17.

Di D, Jiang H, Tian L, *et al.* 2016. Comparative genomic analysis between newly sequenced *Brucella suis* Vaccine Strain S2 and the virulent *Brucella suis* strain 1330. BMC Genomics 17:741.

He Y, 2012. Analyses of *Brucella* pathogenesis, host immunity, and vaccine targets using systems biology and bioinformatics. Front Cell Inf Microbiol 2:2.

Ica T, Aydin F, Gümüşsoy KS, *et al.,* 2012. Conventional and molecular biotyping of *Brucella* strains isolated from cattle, sheep and human. Ankara Üniv Vet Fak Derg 59:259-64.

Kaden R, Ferrari S, Alm E, *et al.,* 2017. A novel real-time PCR assay for specific detection of *Brucella melitensis*. BMC Infect Dis 17:230.

Noutsios GT, Papi RM, Ekateriniadou LV, *et al.,* 2012. Molecular typing of *Brucella melitensis* endemic strains and differentiation from the vaccine strain Rev-1. Vet Res Commun 36:7-20.

Roussel G, Matagne A, De Bolle X, *et al.,* 2012. Purification, refolding and characterization of the trimeric Omp2a outer membrane porin from *Brucella melitensis*. Protein Expr Purif 83:198-204.

Saeedzadeh A, Sharifiyazdi H and Firouzi R, 2013. Molecular characterization of *Brucella melitensis* Rev. 1 strain in aborted sheep and goats in Iran. Comp Clin Path 22:409-12.

Scholz H and Vergnaud G, 2013. Molecular characterisation of *Brucella* species. Rev Sci Tech 32:149-62.

Seleem MN, Boyle SM and Sriranganathan N, 2010. Brucellosis: a re-emerging zoonosis. Vet Microbiol 140:392-8.

Vassen V, Valotteau C, Feuillie C, *et al.,* 2019. Localized incorporation of outer membrane components in the pathogen *Brucella abortus*. EMBO J 11:e100323.

Wang Y, Chen Z, Qiao F, *et al.,* 2010. The type IV secretion system affects the expression of Omp25/Omp31 and the outer membrane properties of *Brucella melitensis*. FEMS Microbiol Lett 303:92-100.

Yousefi S, Abbassi-Daloii T, Sekhavati MH, *et al.,* 2018. Evaluation of immune responses induced by polymeric OMP25-BLS *Brucella* antigen. Microbiol Pathog 115:50-6.

Yousefi S, Tahmoorespur M and Sekhavati MH, 2016. Cloning, expression and molecular analysis of Iranian *Brucella melitensis* Omp25 gene for designing a subunit vaccine. Res Pharm Sci 11:412-8.