Research Article

BASRAH JOURNAL OF VETERINARY RESEARCH, 2021, 20(02):68-79
Journal homepage: www.basjvet.org

Molecular Detection of *Brucella ovis* in Aborted Ewes in Sulaimani Governorate

Eman D. Arif
Department of Microbiology, College of Veterinary Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq
Corresponding author: Eman D. Arif
Email: eman.aref@univsul.edu.iq
ORCID ID:

Received: Nov.29, 2021; Accepted: Dec. 28, 2021; Available Online Dec. 2021

Abstract

Abortion causes substantial economic losses in sheep flocks. In addition to financial compensation, abortion is a sharp aspect of public health. *Brucella*, *Campylobacter*, *Salmonella* and *Chlamydia* are the most examples of diseases associated with the abortion of ewes. Brucellosis is a zoonotic bacterial infection produced by a variety of species of *Brucella*. It has a significant economic impact on domestic animals, primarily ewes. The current study investigated *Brucella ovis* from aborted fetuses and vaginal swab samples collected from sheep flocks in the Sulaimani governorate by the polymerase chain reaction. Thirty-eight aborted fetuses and 70 vaginal swabs were collected from sheep flocks in three districts of Sulaimani governorate (Kalar, Chamchamal and Said Sadiq) from March 2018 to June 2019. The pathogen was identified in clinical specimens using conventional PCR. *Brucella ovis* was isolated from 26 of 38 aborted fetuses (68.4%) and 21 of 70 vaginal swabs (30 %) of aborted ewes. The *Brucella ovis* gene *ompA* was sequenced, and phylogenetic analysis of the *ompA* gene sequences revealed that *Brucella ovis* isolated established a distinct branch and a lower relationship of *Brucella ovis* to the *Brucella melitensis* species. It has been concluded that *Brucella ovis* is the most pathogenic *Brucella* species and a major cause of ewe's abortion in the Sulaimani governorate.

Keywords: Abortion, *Brucella ovis*, polymerase chain reaction, sheep

Introduction

Zoonotic disease Brucellosis, a global health and economic relevance, causes significant loss in domestic animals, particularly cows, sheep, and goats. Cattle are reared near with goats and sheep in several regions, especially in South of Europe and West of Asia (1, 2). Syria, Saudi Arabia, Iran, and Iraq have very high rates of Brucellosis (3). Infections caused by *Brucella* bacteria are known as bacterial brucellosis. It is a major zoonotic disease that is also a leading factor of reproductive injuries in animals. The most common pathogens that cause Brucellosis are *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs, and *B. canis* in dogs (4,5,6). The most visible clinical manifestation in animals is spontaneous abortion, whilst weak or stillborn lambs and low milk production are other clinical observations. Sheep is abortion induced mostly by *Brucella melitensis* or *B. ovis* (7, 8). *Brucella* can live on a pasture for up to 15-25 days, and transfer to the ewe can occur by mucous membrane contact with diseased rams or contaminated substances (vaginal, preputial and conjunctival) (9).Abortion secretions in polluted soil is a concern to animal and human health. Although ewes are normally asymptomatic, they can abort in the third trimester, give birth to a weak lamb , or have a stillbirth. Following an abortion, ewes eliminate the infection in a few weeks (10, 11).

In many countries, serological brucellosis diagnosis is implemented as a feature to consider for disease control and eradication. Serological responses in sheep, on the other hand, can be inconclusive and unspecific because not all infected animals produce detectable levels of antibodies, and cross-reactions with antigens other than those found in *Brucella* can lead to inaccurate results (12). As a result, bacterial culture or PCR should be used to confirm a *Brucella* diagnosis (13, 14). Culture procedures are not always successful, and handling microorganisms is dangerous as well as time-consuming (15).In seasoned laboratories, the rate of isolation is relatively low (16). Molecular approaches have been widely employed to improve detection performance in terms of speed and to reduce dangers with *Brucella* exposure (17). The use of molecular procedures such as nucleotide sequencing has resulted in reliable *Brucella* spp. typing at the genus, species, and biovar levels (18).

The present study aimed to investigate *Brucella ovis*, one of the critical abortion agents, from aborted fetuses and vaginal swab samples collected from sheep flocks in the Sulaimani governorate by PCR.

MATERIALS AND METHODS

Study area and collection of samples

Between March 2018 and June 2019, 108 samples were collected from various flocks in three districts of Sulaimani governorate with a history of abortion. We collected 38 samples of aborted fetuses and 70 vaginal swab samples have been taken from the vaginas of aborted ewes in each of Kalar, Said Sadiq, and Chamchamal. In these districts, the sheep flock's management method is traditional; the animal flocks are owned by different people. Indoors, before being allowed to graze on pasture, grain, hay, and silage are fed to the sheep. Various animal species may share a pasture, or flocks may exchange rams to improve fertility. Tissue was taken from previously aborted fetuses (liver) and their dams (vaginal swabs) with abortions within the previous 2–4 days using disposable blades and scissors. Collected samples were kept in plastic containers, labeled, and sent to the Research Center / College of Veterinary Medicine / University of Sulaimani in a refrigerated package, where they were identified as *Brucella ovis* the same day.

Extraction of DNA

A DNA extraction kit was used on the samples to extract the DNA (GeNet Bio, South Korea). The manufacturer's instructions were followed during the process. DNA quality was measured spectrophotometrically, and low concentration samples (lower than 100 ng/µL) were eliminated from further analyzes.

Oligonucleotides and PCR amplification

Saleh *et al*. (19) presented the primers used for amplifying a 200 bp segment of the *ompA* gene, with forwarding (5´- GACGCCATCCAGGAACAG -3') and reverse (5´- GTATACGATCTGGTCCTGC -3') primers. Macrogen® (South Korea) was progressed the primers for our research. The total DNA was amplified using PCR Add Start Taq Master (PCR Add Start Taq Master) (Korea, Add bio). For the experiment, 0.2 mL PCR tubes were used. In the PCR tube, l0 µL of master mix, 5 µL of DNA, and 1 µL (10 pmol) of each forward and reverse primer was placed. By adding 3 µL of DEPC-treated water, the ultimate volume of 20 µL was obtained. The first step in the thermal cycler process was denaturation at 95 °C for 5 min. There were 30 cycles of denaturation (95°C for 1 min) and annealing (57 °C for 1 min) followed by an extension at 72°C for 5 min. Finally, the sample was subjected to an additional 5 min of extension at 72°C. After loading 7 µL of PCR products on to 1% agarose gel in 1 Tris/Borate/EDTA (TBE) Buffer, the PCR products were analyzed. A 5 µL safe dye was used to stain the gel. Using the Safe-Blue

Illuminator/Electrophoresis System, electrophoresis was performed for 50 min at 120 volts. By comparing PCR result amplicons to a 100 bp DNA ladder, migration patterns were studied.

Sequencing of the ompA gene and phylogenetic analysis

South Korea's Macrogen Sequencing Facility sequenced the PCR results of the *ompA* gene. The external protein membrane gene analysis (*ompA*) was used to characterize and classify Gram-negative coccobacillus. A pathogen that induced extreme epididymis inflammation in rams and abortion in ewes, known as *Brucella ovis*, was isolated from northing Iraq in 2018. The partial *ompA* gene sequence (200 bp) of the isolate was extracted from the liver and the two strands were sequenced. The sequence was compared to those in the available databases using BLAST and matched with their nearest neighbors using MegaX.

Results

Samples

Genomic DNA was successfully extracted from fetal samples and vaginal swabs of aborted ewes using the DNA extraction kit. In the current investigation, 108 samples were collected from Kalar, Said Sadiq and Chamchamal, where abortions had been observed. Twenty-six samples (68.4%) from aborted fetuses and 21 samples (30%) from vaginal swabs of PCR were used to identify *Brucella ovis* in aborted ewes (Table 1).

Identification of *Brucella ovis*

In the present study, *Brucella ovis* was positive for the *ompA* gene according to agarose gel electrophoresis, which indicated a 200 bp amplicon (Figure1).The sequencing of the PCR product was determined to corroborate the results.

Table 1: PCR results for *Brucella ovis* detection in aborted fetuses and vaginal swabs of aborted ewes.

District name	Fetal samples		Vaginal swabs	
	Samples tested	Samples positive (No. %)	Samples tested	Samples positive (No. %)
Kalar	18	15 (83.3)	38	10 (26.3)
Said Sadiq	13	7 (53.8)	12	6 (50.0)
Chamchamal	7	4 (57.1)	20	5 (25.0)
Total	38	26 (68.4)	70	21(30)

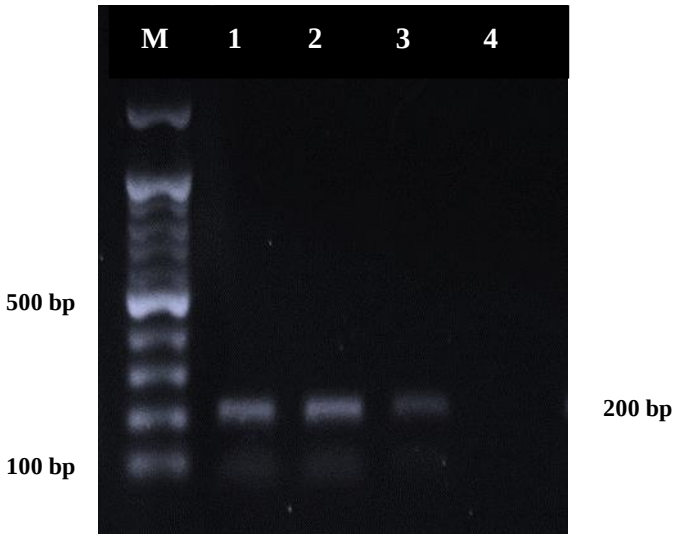

Figure1: Specific amplification of target DNA from *Brucella ovis* by PCR using specific primers. Lane M: Show 100 bp DNA size marker, lane 4: Negative control (no DNA in the PCR reaction mix) and lane 1, 2, 3: An aborted ewe's samples (200bp).

Phylogenetic analysis

(Figure 2) showed the DNA sequencing of *Brucella ovis* and sides of mutation. The taxonomic position of *Brucella ovis* showed neighbour-joining analysis of the *ompA* gene sequence alignment. All accessions were grouped into three main clades. Phylogenetic analysis of the *ompA* gene sequences revealed that *Brucella ovis* isolated established a distinct branch and a lower relationship of *Brucella ovis* to the *Brucella melitensis* species was seen (Figure 3).

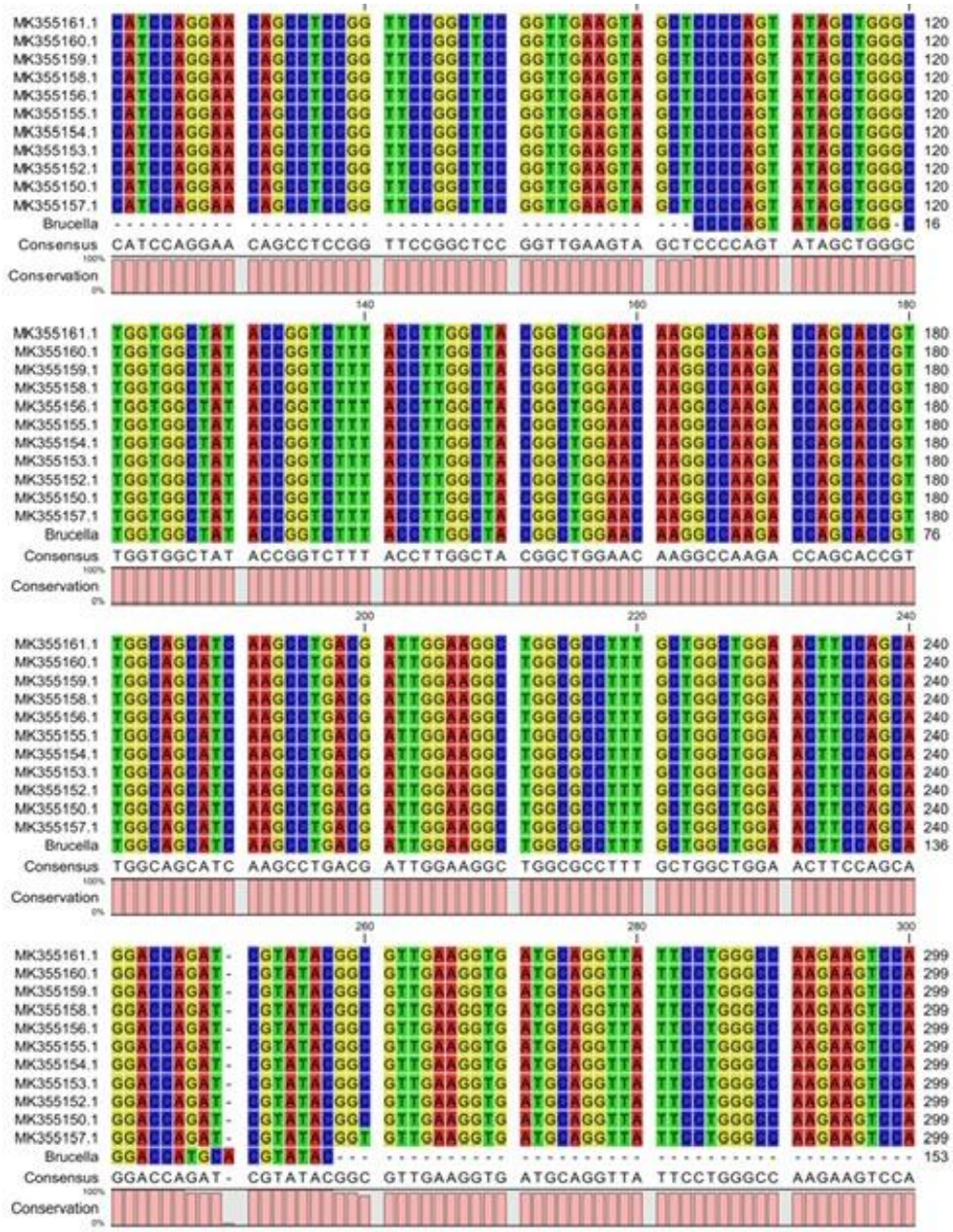

Figure 2: DNA sequencing of *Brucella ovis* and sides of mutation.

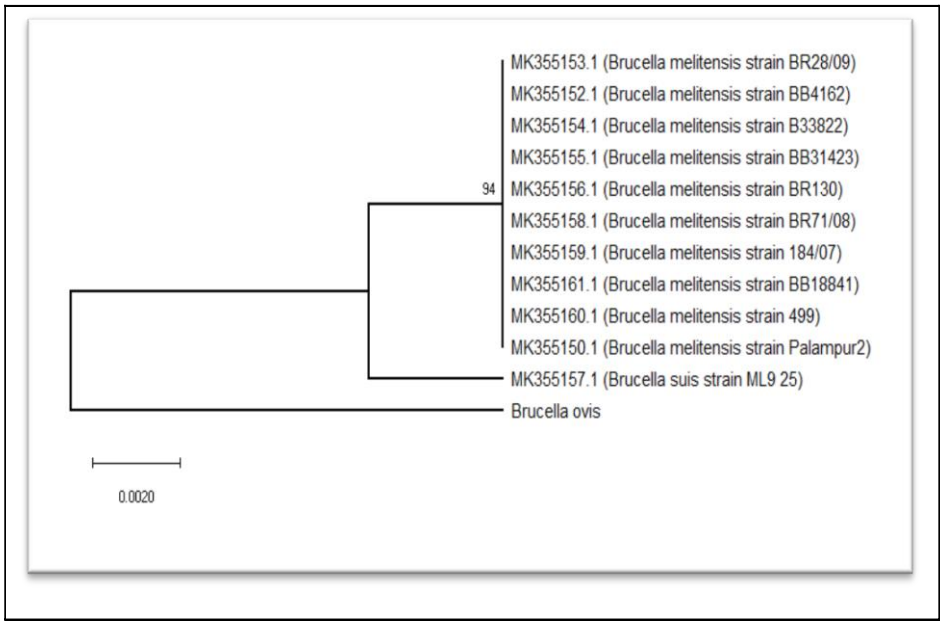

Figure 3: A maximum likelihood dendrogram based on *ompA* gene sequences showing the phylogenetic relationships between *Brucella ovis* and some associated taxa. At branch nodes, bootstrap values based on 100 replicates were displayed. Per nucleotide location, there were 0.002 substitutions.

Disussion

The most important source of income for sheep farmers comes from selling the lambs, while abortion which occurred mostly in the last months of gestation caused a reduction in the total number of lambs per farm (19). One of the most common serious causes of parturient disturbance in small ruminants is brucellosis (20).

In the present study, aborted fetuses and vaginal swabs from aborted ewes were investigated by PCR to detect *Brucella*. Many studies previously used this method (1, 21, 22). According to our data, in ewes, brucellosis causes abortion. *Brucella ovis* is the one of the common causes of ewe abortion in Sulaimani governorate. *B. ovis* was isolated in 68.4% of aborted fetuses and 30% of vaginal swabs from aborted ewes using PCR. A bacteriological or molecular diagnosis should be used to supplement the correct *Brucella* diagnostic window (13, 23, 24). Because *Brucella* culture is dangerous, no *Brucella* organisms were separated in this experiment. The benefits of the PCR method include its efficiency, safety, and resistance to contamination by other microorganisms present in the tissue samples used for isolation, which explains why the PCR test is preferable for diagnosing brucellosis in infected sheep tissues (25, 26).

Numerous cases of brucellosis are found in sheep in Sulaimani governorate each year. In the Sharazor subdistrict of Sulaimani governorate, in small ruminants, *Brucella* is

indeed a significant cause of abortion. PCR indicated that 15 of the 17 samples (88 %) were positive for the *Brucella* genus in sheep (21). Farmers' poor management and husbandry techniques, such as grazing multiple flocks of sheep on the same pasture and sharing rams for mating across multiple herds likely to be to blame for the high brucellosis prevalence. In another study conducted in Sulaimani governorate, Abortion in sheep is caused mainly by *B. melitensis* with prevalence rates of (32.7 %) (22) .This contradicted the current study's conclusion that *Brucella ovis* is the most pathogenic species for sheep abortion in Sulaimani governorate. This difference may be due to some environmental factors and diagnostic techniques.

According to other researchers, *B. melitensis* is one of the most common causes of abortion in ewes. A research that was carried out in Sistan region, south-eastern Iran showed that 15 fetuses out of 78 aborted ewe's fetuses (19.2%) (27). Mohammadi study in the cities of Kalaleh and Gonbad-e Qabus showed that 10 fetuses out of 57 aborted ewe's fetuses (17, 5%) (28). On the other hand, in another study, on aborted fetuses in XUAR, northwest of China, 34 (28%) specimens out of 120 samples were infected with *B. melitensis* (1). Differences between studies may be attributed to geographical region, diagnostic techniques, the animal's breed, collection, and timing of materials.

According to the present study, phylogenetic analysis of the *ompA* gene sequences revealed that *Brucella ovis* isolated established a distinct branch and a lower relationship of *Brucella ovis* to the *Brucella melitensis* species (Figure 3). All strains of the first clade (*Brucella melitensis*) and the second clade (*Brucella suis*) showed similarity of 96.51%. All isolates were submitted by researchers at the Indian Veterinary Research Institute, Department of Veterinary Public Health. As shown in the phylogenetic tree *Brucella ovis* of the current study is unique to Iraq.

Conclusion: In conclusion, Brucellosis is a major cause of ewe's abortion, accounting for the majority of sheep abortions in the area. The PCR assay is an efficient and sensitive technique for detecting *Brucella*. *Brucella ovis* is the most pathogenic *Brucella* species in the governorate of Sulaimani.

Acknowledgments

The author would like to thank College of Veterinary Medicine at University of Sulaimani, Sulaimani governorate, Iraq.

Conflict of Interest

The author declares that he has no conflict of interest.

References

1. Zhang, H., Shengnan, S. O., Benben, W. A., Jiang, Y.,Wenxing, W. U., Fei, G. U. O., Chuangfu, C. H. (2018). *Brucella melitensis* isolated from aborted cow and sheep fetuses in northwest of China. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *24*(2), 307-310.

2. Selim, A., Attia, K., Ramadan, E., Hafez, Y. M., Salman, A. (2019). Seroprevalence and molecular characterization of *Brucella* species in naturally infected cattle and sheep. *Preventive veterinary medicine*, *171*(1), 1-7.

3. Al Dahouk, S., Nöckler, K. (2011). Implications of laboratory diagnosis on brucellosis therapy. Expert review of anti-infective therapy, 9(7), 833-845.

4. Reisberg, K., Selim, A. M., Gaede, W. (2013). Simultaneous detection of *Chlamydia* spp., *Coxiella burnetii*, and *Neospora caninum* in abortion material of ruminants by multiplex real-time polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation*, *25*(5), 614-619.

5. Pirot, R. Q. (2020). Seroprevalence of Human, Sheep and Goat brucellosis in Raparin District. Journal of University of Raparin, 7(1), 58-79.

6. Júnior, G. N., Megid, J., Mathias, L. A., Paulin, L., Vicente, A. F., Cortez, A., Ribeiro, M. G. (2017). Performance of microbiological, serological, molecular, and modified seminal plasma methods in the diagnosis of *Brucella abortus* in semen and serum of bovine bulls. *Biologicals*, *48*(1), 6-9.

7. Norman, F. F., Monge-Maillo, B., Chamorro-Tojeiro, S., Pérez-Molina, J. A., López-Vélez, R. (2016). Imported brucellosis: a case series and literature review. *Travel medicine and infectious disease*, *14*(3), 182-199.

8. Poester, F. P., Samartino, L. E., Santos, R. L. (2013). Pathogenesis and pathobiology of brucellosis in livestock. *Rev Sci Tech*, *32*(1), 105-15.

9. Wareth, G., Melzer, F., Tomaso, H., Roesler, U., Neubauer, H. (2015). Detection of *Brucella abortus* DNA in aborted goats and sheep in Egypt by real-time PCR. *BMC Research Notes*, *8*(1), 1-5.

10. Shirazi, Z., Khalili, M., Sadeghi, B., Sharifi, H., Ashrafganjooyi, S. (2017). Detection of *Brucella* spp. in the sheep and goats milks from southeastern Iran using culture and PCR. *Journal of Medical Microbiology and Infectious Diseases*, *5*(3), 40-42.

11. Andriopoulos, P., Tsironi, M. (2016). Molecular diagnosis of brucellosis: a brief report. *Adv Mol Diagn*, *1*(2), 108-112.

12. Ilhan, Z., Solmaz, H., Aksakal, A., Gulhan, T., Ekin, I. H., Boynukara, B. (2008). Detection of *Brucella melitensis* DNA in the milk of sheep after abortion

by PCR assay. *Archivos de Medicina Veterinaria, 40*(2), 141-146.

13. Marianelli, C., Martucciello, A., Tarantino, M., Vecchio, R., Iovane, G., Galiero, G. (2008). Evaluation of molecular methods for the detection of *Brucella* species in water buffalo milk. *Journal of Dairy Science, 91*(10), 3779-3786.

14. Scholz, H. C., Vergnaud, G. (2013).Molecular characterisation of *Brucella* species. *Revue scientifique et technique (International Office of Epizootics), 32*(1), 149-162.

15. Refai, M. (2003). Application of biotechnology in the diagnosis and control of brucellosis in the Near East Region. *World Journal of Microbiology and Biotechnology, 19*(5), 443-449.

16. Wareth, G., Hikal, A., Refai, M., Melzer, F., Roesler, U., Neubauer, H. (2014). Animal brucellosis in Egypt. *Journal of Infection in Developing Countries, 8*(11), 1365-1373.

17. Probert, W. S., Schrader, K. N., Khuong, N. Y., Bystrom, S. L., Graves, M. H. (2004). Real-time multiplex PCR assay for detection of *Brucella* spp., *B. abortus*, and *B. melitensis. Journal of clinical microbiology, 42*(3), 1290-1293.

18. De Massis, F., Ancora, M., Atzeni, M., Rolesu, S., Bandino, E., Danzetta, M. L., Scacchia, M. (2015). MLVA as an epidemiological tool to trace back Brucella melitensis biovar 1 re-emergence in Italy. *Transboundary and emerging diseases, 62*(5), 463-469.

19. Saleh, M., Harkinezhad, M. T., Marefat, A., Salmani, V. (2013). An outbreak of abortion in Afshari sheep with probable involvement of *Campylobacter fetus*. *Iranian Journal of Veterinary Medicine, 7*(1), 51-56.

20. Zong-Lin, H. E., Xiakla, B., Kang, Q., Ayinuer, Y. R. M., Zhu, T. T., Wan, X. J., Wu-Chang, G. U. (2014). Epidemiological survey of livestock brucellosis in Aksu area. *Grass-Feeding Livest, 5*, 60-66.

21. Saeed, N. M., Talib, S. M., Dyary, H. O. (2019). Molecular Detection of Outer Membrane Protein 2 Gene in Isolated *Brucella* from Sheep and Goats in Sharazor/Kurdistan Region of Iraq. *Pakistan Veterinary Journal, 39*(3), 383-388.

22. Nassrullah, O.J., Mohammed, O.M., Rachid,S.K. (2020).Isolation and molecular identification of *Brucella melttiensis* in aborted fetuses of sheep and goats in Sulaimani Province. *J S M C, 10* (3), 257-256.

23. Marianelli, C., Petrucca, A., Pasquali, P., Ciuchini, F., Papadopoulou, S., Cipriani, P. (2008). Use of MLVA-16 typing to trace the source of a laboratory-acquired *Brucella* infection. *Journal of Hospital Infection, 68*(3), 274-276.

24. Shakerian, A., Deo, P., Rahimi, E., Shahjavan, A. R., Khamesipour, F.

(2016). Molecular detection of *Brucella melitensis* in sheep and goat milk in Iran. *Tropical Journal of Pharmaceutical Research*, *15*(5), 913-918.

25. Hussein, A., Mohamed, R.H., Abdel-Ra'ouf, A.M., Abu-Elnaga, E.M., Mohamed, R.S., Hussein, A.A., Wehrend, X. (2019).Diagnosis of Brucellosis in recently aborted ewes using serological tests and polymerase chain reaction. *J Applied Sci*, *19*(2), 77-81.

26. Rahman, A. A., Saegerman, C., Berkvens, D., Melzer, F., Neubauer, H., Fretin, D.,Ward, M. P. (2017). *Brucella abortus* is prevalent in both humans and animals in Bangladesh. *Zoonoses and public health*, *64*(5), 394-399.

27. Mahdavi Roshan, H., Saadati, D., Najimi, M. (2018). Molecular detection of Brucella melitensis, Coxiella burnetii and Salmonella abortus ovis in aborted fetuses of Baluchi sheep in Sistan region, south-eastern Iran. *Iranian Journal of Veterinary Research*, *19*(2), 128-132.

28. Mohammadi, N. (2016). A survey of the prevalence of Brucella melitensis, Campylobacter fetus and Chlamydophila abortus induced abortion in sheep flocks in Kalaleh and Gonbad Kavus by PCR (Doctoral dissertation, University of Zabol).

الكشف الجزيئي عن جراثيم *Brucella ovis* في النعاج المجهضة في محافظة السليمانيه

*ايمان ظاهر عارف

*فرع الاحياء المجهرية ، كلية الطب البيطري ،جامعة السليمانية ،السليمانية ، اقليم كوردستان ،العراق

الخلاصة

يتسبب الإجهاض في خسائر اقتصادية كبيرة في قطعان الأغنام. بالإضافة إلى التعويض المالي ، يعد الإجهاض جانبًا حادًا من جوانب الصحة العامة. تعد البروسيلا ، كامبيلوبكتر ، السالمونيلا والكلاميديا من أكثر الأمثلة على الأمراض المرتبطة بإجهاض النعاج. الحمى المالطية هي عدوى جرثومية حيوانية المصدر تنتج عن أنواع مختلفة من جراثيم البروسيلا والتي تأثير اقتصادي كبير. تحرت الدراسة الحالية عن *Brucella ovis* في الاجنة المجهضة وعينات المسحات المهبلية التي تم جمعها من قطعان الاغنام في محافظة السليمانية عن طريق تفاعل البلمرة المتسلسل. تم جمع 38 جنينًا مجهضًا و 70 مسحة مهبلية من قطعان الأغنام في ثلاث مناطق من محافظة السليمانية (كلار وجمجمال وسيد صادق) من اذار 2018 إلى حزيران 2019. تم التعرف على العامل الممرض في العينات السريرية باستخدام PCR التقليدي. تم عزل *Brucella ovis* من 26 من 38 جنين مجهض (68.4٪) و 21 من 70 مسحة مهبلية (30٪) من النعاج المجهضة. تم إجراء تسلسل لجين *Brucella ovis ompA* ، وكشف تحليل النشوء والتطور لتسلسل الجين *ompA* أن *Brucella ovis* المعزولة أسست فرعًا متميزًا وعلاقة أقل ل *Brucella ovis* بأنواع *Brucella melitensis*. وقد تم التوصل إلى ان *Brucella ovis* هي أكثر أنواع البروسيلا المسببة للأمراض والسبب الرئيسي لإجهاض النعاج في محافظة السليمانية.