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Detection of *ure*, *emerD* and *znuA* Virulence Genes in *Brucella melitensis* Isolated from Aborted Fetuses of Sheep in Thi-Qar Governorate

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Abstract: Brucellosis is a major and widespread bacterial zoonosis. it is common throughout the world This has serious economic and public health consequences. Sheep, goats, and cows are examples of domestic animals.; suffer significant losses due to Brucellosis. The aim of study was to detect the ure, emerD and znuA virulence genes in *Br. melitensis*, samples were collected from eight fields of sheep in Thi-Qar Governorate south of Iraq during the period November 2022 - to June 2023, from animals that had abortions or certain disease symptoms, , 32 out of 67 mother sheep prove positive results for indirect Elisa ,32samples of fetal fluids fetus of sheep in Thi-Qar Governorate appeared 32 a positive for the Conventional PCR technique so 32 isolates of *Brucella melitensis* were determined, Generally. the result of fetal fluids was found in most prevalent gene was *ure*, which was found twenty isolates. (68. 75%): while the znuA gene had the lowest proportion of all analyzed genes due to its presence five isolates (15.62%). It was concluded gene were found in certain isolates, such as the *emrD* gene which found in 14 isolates. (43.01%).

Keywords: RBPT, Elisa, *Br.melitensis*, Sheep.

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Introduction

Brucellosis is a major and widespread bacterial zoonosis. it is common throughout the world This has serious economic and public health consequences. Sheep, goats, and cows are examples of domestic animals.; suffer significant losses due to Brucellosis. It is a significant of zoonotic disease that be lead to Animal reproduction issues (1,2). Brucellosis is a significant and widespread bacterial illness that affects both humans and animals (3). it affects cattle, camels, sheep and goats, producing economic losses owing to a reduction in milk output, sterility, and serial abortion instances in field animals, Aside from the economic costs, it is a dangerous medical condition disseminated mostly by contaminated milk or the consumption of dairy products made from infected unpasteurized milk., Its symptoms in infected humans include edoema, fever, and headache (4). *Brucella* is a genus of seven species of Gram-negative facultative anaerobic coccobacilli, non-spore producing or motile bacteria, all of these species are

capable of invading humans, After entering Brucella is conveyed to the lymph nodes through the digestive system, where it begins to reproduce before being carried outside of the lymphatic system, where it enters the circulation and subsequently to other organs such as the liver and spleen, Brucella can also damage immune cells by generating guanine monophosphate and adenine monophosphate, both of which suppress the immune system (5).

Iraq is one of the nations where brucellosis is present is a big problem(6). Particularly in poor nations Enterobacteriaceae(such prostatitis as *Brucella melitensis*)is associated with epididymitis, orchitis and, suggesting that they may have a role in infertility in man (7, 8).

Brucella is an intracellular bacterium that reduces the effects of antibiotics. of while also increasing treatment length; several studies have alluded to taking many antibiotics at the same time over an extended period of time in order to obtain a cure (9).is also regarded as one of the most economically significant livestock reproductive disorders, resulting in abortion, sterility, and decreased output (10).

Materials and methods

Sample collection

Thirty-tow of fetal fluid contents samples as well as blood samples of its mother sheep were gathered from eight fields in the research region, including Al-Nasirya city and Al-Shatra fields (45 Km north of thiqar) as well AL-Refaai field and Al-Nasirya fields (70 Km north of Thiqar) addition to AL- Aslah, Bathaa, AL-Garaf and Said dakheel. Distrisct

Samples collected during the period November 2022 - to June 2023, from cases of animals suffered from abortion or had specific illness signs.

Thirty-two (fetal fluid) samples of fetuses following to 32 mother sheep that were collected among sixty seven sample of whole mother sheep , in fact that thirty two were taken from fetus animal which shows direct abortion evidence of brucellosis (according to history of its mother abortion and positive results of ELISA test).

Three ml of fetal fluid sample volume were withdrawn from each animal fetus were identified using the standard methods.

Bacterial DNA extraction

The QIAamp DNA blood kit and QIAamp DNA Mini Kit Handbook was used to extract the genomic DNA of bacterial isolates (11).

Detection of Virulence genes

This work employed specific primers for PCR analysis of the *ure, emrD,* and *znuA* genes, as described in (Table1), which were synthesised by the Macrogen Company in South Korea. As recommended by the procedure, the lyophilized forward and reverse primers were suspended in TB buffer (0.45 gm agarose in 30 ml buffer).

The PCR reaction was carried out in 25 ml reaction mixture that contained 7 ml of bacterial DNA, 2 ml of (1.5mM) Mgcl2(Q solution): (12.5) 1 ml of master mix, 1 ml of each primer (forward and reverse): and (1.5) 1 ml of nuclease free water. these ingredients were carefully combined and centrifuged before being placed in a heat cycler, the PCR include, initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 minutes.

Analysis of PCR product

The gel was made by adding 0.45 gm of agarose to 30 ml of TBE

buffer, boiling it, and then plating it in the tray. The gel was allowed until it reached 45° before adding 3l of ethidium bromide solution (l/ml).

In the wells, 10l of PCR products were deposited, followed by 8l of 1000 bp ladder mixed with 2l of loading dye and placed in the first well. The tray was placed in a tank containing 1X TBE buffer, and a voltage of 90 V/cm2 was applied for 30 minutes, until the trapping dye nearly reached the edge of the gel, before being studied under a UV light source (13).

PCR assay

DNA amplification system provided by Promega-company, USA and genus specific *ure*, *emrD* and *znuA* primer pair designed by Macrogen to amplify a 2100bp, 203bp and 204 bp respectively as shown in (Table 1).

Table (1): Oligonucleotide primers used in the PCR assay

Genes	Primer Sequences (5' - 3')	Annealing Temperature	Amplicon Sizes (bp)	References
Ure	F: GCTTGCCCTTGAATTCCTTTGT	62 ºC	2100bp	Hamdy and Zaki (14)
	R: ATCTGCGAATTTGCCGGACT			
emrD	F: CTCGTCGGAATGTCCATTTT	60 ºC	204bp	AboKsour, (15)
	R: CTGACGAGAATCCCCATGTT			
znuA	F: TTAAACCCGTTGGGTTCATC -	57 ºC	203bp	
	R: TCGCTCCTGGTAATTTGCTT			

Results and discussion

The current study shed light on the most common bacterial cause of reproductive failure in sheep in numerous Thi-Qar governorate locations. the study established the prevalence of *B. melitensis* as a cause of reproductive failure and the importance of putative risk factors in this disease (14). The present study showed that most *B. melitensis* isolates from aborted fetuses of sheep have virulence factors genes include *ure*, *emerD* and *znuA* in their genome, most *B. melitensis* isolates had *ure* genes that has been hypothesized to play a role in the pathogenesis of disease.

The results showed high frequent of *ure* gene 20/32(62.5%): of *B. melitensis* comparing with the isolation percentages of the other genes, *emerD* which has14 isolates and percentage (43.01%), The *znuA* that has 5 isolates (15.62%) as shown in Figure (1). (15): (16) reported that the *ure*, *emerD* and *znuA* primer pair was found to be more sensitive for identification of Brucella organisms.

Using conventional PCR, Chi square analysis was utilised to detect distinct risk variables related with Brucella infection in small ruminants. in general, this study discovered a considerable variation in Brucella-positive samples between the Thi-Qar regions. PCR is a potential method for diagnosing brucellosis. It is a potentially beneficial technology that has been employed alone or in combination with other methods to detect Brucella spp. from isolated bacteria or heavily contaminated aborted foetuses (17).

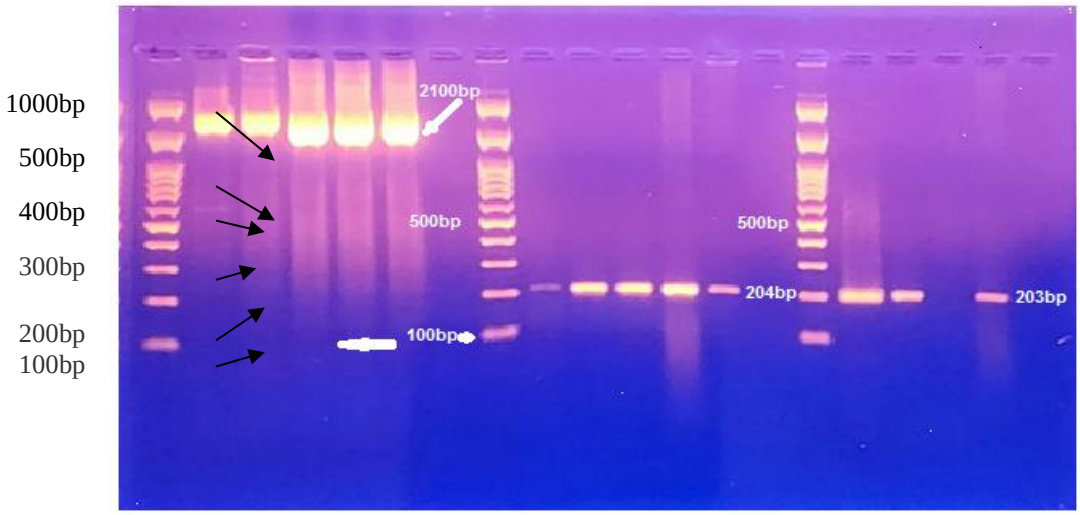

Figure (1): Agarose gel electrophoresis of PCR product of three *brucella* virulence genes. Lane M, 1000 bp ladder, lanes 1- 5: PCR product of *ure* gene: 16-PCR product of *emerD* gene: 14, lanes 8: 12-product of *ZnuA* gene 5, lane13 -15 N, Negative control, lanes15

Because of the geographical situation, it is not surprising that the highest prevalence rate of PCR-positive samples was recorded in this district, especially since there is no proper risk assessment and risk analysis conducted on the imported animals, which may contribute to the increased prevalence of various diseases, including brucellosis. Furthermore, the greater frequency of brucellosis in sheep in this location may be due to inadequate management as well as environmental variables (18). According to the current study, the abortion frequency in sheep that had one abortion was greater than in sheep that had two or more abortions, this is a marker of the prior abortion's immune response owing to acquired or specific immunity generated after exposure to the same antigens (19).

Furthermore, the current study's findings demonstrated that flock size is a risk factor, with more positive sheep detected in large and medium flocks than in small groups (20). especially in low-income countries, due to loss of work or income as a consequence of illness and reduced profitability in the livestock sector (21, 22, 23) and may be supported by the fact that an increase in flock size is typically associated with an increase in flock density, which is one of the contributing factors to Brucella infection, particularly after abortion (24). The herding of several animal species as a single big and dense flock, namely maintaining sheep with cattle, has been recognised as a significant risk factor for Brucella seropositivity (25). This conclusion highlights the significance of animal and animal product imports without adequate risk assessment in both cases. Border crossings, both legal and informal. Another possible reason for this observation is the sharing of male animals in this region during the mating season (26, 27).

Conclusion

Brucellosis is quite common in this area, mostly at the herd level, and it is both an economic and a public health problem. Thereby this study offers a clear insight into the high virulence and pathogenic characteristics of *B. melitensis*, and may be helpful to veterinary officials and public health authorities to set national campaigns for the control and eradication of this hazard.

Addition to the samples that are requested from sheep that want isolation, due to the difficulty in obtaining samples from the lymph nodes, Bone, chronic injury, causes synovial development, as well as the development of the spinal cord, which are sites of osteoarthritis bacteria in it (28). This study used PCR to identify the key risk variables for brucellosis positive in sheep from several Thi-Qar governorate regions. These findings revealed that the district, history of abortion, previously treated animals, and frequency of abortion were all strongly linked to brucellosis in sheep in this region. This suggests that animals from outside the herd are a significant source of Brucella infection. As a result, a strong brucellosis control method should be developed in Iraq, as well as effective precautions for animal transportation, particularly unlawful imports.another side Sheep live under the effect of seasonal climate variations, which are frequently linked to changes in latitude and altitude of a location with regard to total insolation received (29). These findings can be used to launch a large-scale research to determine the optimal disease management strategy and treatment in this region. However, further research on the expression of virulence genes and molecular typing methods covering wider geographical areas of Iraq is needed to determine the predominant strains of Brucella with gene expression properties with a view to developing an effective vaccine.

Statistical analysis

This study designed by that used in the analysis of variance for data of virulence genes values independent t-test, Dunnett's test at a 5% level of significance. Moreover, all frequency data was analyzed by Pearson's chi-squared test and Fisher's exact test. Data were processed and analyzed by using statistical program social science (SPSS-v 26) 2019 and the results (30).

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