Research Article

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Real Time- Polymerase Chain Reaction (RT-PCR) Test to Diagnose Brucellosis in Sheep in ANBAR Province

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Abstract

The aims of the study were to determine incidence ratio of brucellosis in aborted ewes in Anbar governorate using real time PCR technique and bacterial culture and evaluating the efficiency of bacterial culture. 50 samples of stomach contents of an aborted fetus and vaginal swabs from aborted ewes were collected. The incidence ratio of brucellosis in ewes in Anbar governorate were 72% in PCR test However in bacterial culture were 46%. The positive prediction value and Negative prediction value and Sensitivity and Specificity of bacterial culture were (100%, 51.8 %,63%,100%) respectively when use Real time PCR as an assessment test. Conclusions: Anbar province is endemic with brucellosis, especially in sheep. The Real Time PCR is a fast, easy and accurate test in detecting even small numbers of bacteria in a sample, Significant superiority of polymerase chain reaction technology over bacterial culture.

Key words: Brucellosis in Anbar, ewes, Real time PCR, Bacterial culture.

Introduction

Brucellosis is one of zoonotic and infectious disease that spread all over the world especially in the Middle East and cause by facultative intracellular bacteria called Brucella (1). This bacterium is a coccobacillary in shape and Gram-negative, non-motile, non-spore forming, non-capsulated, aerobic This genus contains ten species incuding (*Brucella.melitensis*, *Brucella abortus*,

Brucella suis, *Brucella canis*, *Brucella ovis*, *Brucella neotomae*, *Brucella microti*, *Brucella ceti*, *Brucella pinnipedialis*) (2) French agency for food, environmental and occupational health and safety (3). The disease can infect wide range of living organisms including human and animal (small ruminant, cattle, camels, dog, cat, equine, swine, aquatic creatures, reindeer, weeds) Especially those who are sexually mature (4, 5). Huge economic losses cause by abortion of pregnant animals, low milk production, permanent and temporary sterility, Losses in calves and lambs breeding stations, as well as the cost of treatment and control and slaughter programs (6).

The most important signs of Brucellosis in adult female are abortion in the third trimester of pregnancy, especially in small ruminants, in addition to endometritis and placentitis and in male causing epididymitis or orchitis and Hygromas especially in ram and wither fistula in horse (7, 6). The disease is transmitted from animal to human through direct contact with infected animals, or by eating food contaminated with bacteria, most cases occur by ingesting unpasteurized milk or contaminated products (8). The diagnosis of Brucellosis depends on two types of tests: first, direct tests: which reveal the presence of the pathogen and indirect tests: which detect the presence of immune responses (9,10). Indirect serological tests have many disadvantages, including obtaining false positive results (11). Therefore, the polymerase chain reaction and bacterial culture were used for the purpose of accurate isolation. Bacterial isolation is the most confirmatory of the diagnosis, because it reveals the presence of the pathogen in the very early stages of the disease, so it is the gold standard for diagnosis (12). Brucella colonies appear on Brucella media after 4 to 7 days of incubation at a temperature of 37 degrees and are in the form of white to pale round pearly colonies (13). One of the disadvantages of this method is that it takes a long time (14). Polymerase Chain reaction (PCR)It is an easy method to replicate or create copies of the DNA sequence using enzymes or primers outside the body (15). The main purpose of using this technique is to make rapid copies of millions to billions copies of a specific and specific part of the DNA, this allows scientists to take a very small sample of DNA and amplify it into a large amount enough to study (16).

Material and Methods

Sample collection: Abomasa contents of aborted fetuses as well as vaginal swabs collected from 50 aborted ewes in in Anbar province.

Bacterial culture: Brucella Agar base (HIMEDIA- Indian) and Selective Supplement were used and add 5% sterile serum for bovine or horses also to media. Use The culture loop for cultured stomach contents, while the vaginal swabs were cultured by Planning the swab on the surface of the petri dish. Each sample was cultured on two petri dish of Brucella

culture Agar, the first dish was incubated aerobically (17). While the second dish was incubated anaerobically in the presence of 5% of CO_2 (Carbon dioxide) using aerobic conditions (jar candle) and the dishes were Incubate For a period of less than 7 days (17).

Real Time- Polymerase Chain Reaction (RT-PCR): The Kit Extraction DNA™ gSYNC used genomic DNA extraction Manufactured by Biotech Geneaid.(use by the manufacturer's instructions)**,** used a v1 Detection Kit Brucella from the Bosphorus company Turkish bosphore and contain: Mix Master PCR(DNA Poymerase ,PCR Buufer,Mix of dNTP) ,Interna Control ,Postive control,DH_2O.and use by manufacturer's instructions (The stage of preparing the reaction mixture: making sure that all components of the kit are melted before use and the mixture was prepared using the materials shown in the following table (1):

Figure 1: Aborted ewe fetus

Table (1): material that use polymerase chain reaction and his quantities

Substances used in preparing the reaction mixture	Quantities in microns
PCR Master Mix	14.9
Internal Control	0.1
Negative / positive control or DNA samples	10
Total	25

Table (2): Primer used

Target	Primer-Prob	Target gene	Sequence
Brucella genus	Forward	bcsp31	5'-GCTCGGTTGCCAATATCAATGC-3'
	Reverse		5'-GGGTAAAGCGTCGCCAGAAG-3'
	Probe		FAM-AAATCTTCCACCTTGCCCTTGCCATCA –BHQ1'

Table (3): Programming the Real time PCR instrument

Initial denaturation	**95°C**	**14:30 min**
Denaturation	97°C	00:30 min
Annealing and Synthesis	54°C	01:30 min
(Data Collection)		
Hold	22°C	05:00 min

Result and Discussion

Bacterial Culture

The results of bacterial culture of samples from aborted fetuses and vaginal swabs from 50 aborted ewes showed that Brucella bacteria were isolated from 23 samples with an isolation rate of 46%. When the polymerase chain reaction test was performed on 50 different samples of stomach contents of an aborted fetus or vaginal swabs of aborted ewes using Real Time PCR device, positive results were obtained by 72% (36 sample from 50). All isolates, when culture on Brucella agar after 5-7 days of aerobic and anaerobic incubation at 37 °C, gave colonies with smooth appearance, convex, pale to pearly white color colonies. The result reached by this study Close to the result obtained by *Al-abdali* , Were was the result 40% in sheep using bacterial isolation (18). Also, the percentage obtained from this study was close to the percentage reached by (19), which is 42% in sheep and goats using bacterial isolation in Iraq. While the percentage obtained increased from the percentage recorded by (20) Which reach 31% when bacterial isolation from the

contents of aborted sheep embryos was used. And also, about the percentage recorded by **(**21**)**, which is 33%, where bacterial isolation was used from the contents of aborted fetuses of sheep in Iraq (Mosul). Real time Polymerase Chain reaction (RT-PCR), Since there was no previous study in Anbar province, Real Time PCR was adopted to diagnose Brucellosis, so the results of this study were compared with other regions, where some research gave results that reached 100% when using this test to detect Brucella in aborted sheep and goats (22). (23) indicated that the detection rate of Brucella bacteria using the polymerase chain reaction was 57.1%. The percentage of isolation of Brucella by using the polymerase chain reaction was 66.6% in sheep (24, 25) found that the rate of Brucellosis isolate was 48.1% when using the polymerase chain reaction technique to diagnose brucellosis among sheep and goat abortions in Jordan. The difference in the percentage obtained in the study from the percentages recorded in other studies is due to the geographical distribution, the different places, the methods of control and public awareness in the places where the studies were carried out, as well as the fact that the samples were taken at different times, which is not a condition in conjunction with the occurrence of abortion.

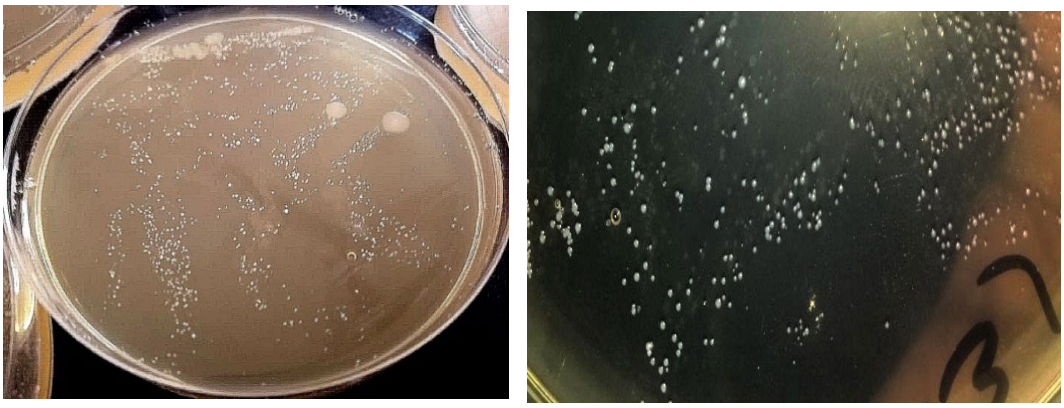
Finding of present study showed that 72% of the samples gave positive results for the detection of Brucellosis by using the Real time PCR Technique in Anbar province, and it is normal to obtain such results because when the samples were taken, it was found that there was a great complaint by the owners about repeated abortions, especially for the herds owners in large numbers. It was also noticed that the breeders were ignorant of the way to get rid of aborted fetuses, as they do not follow healthy methods to get rid of aborted fetuses, as well as the absence of health awareness in the animal trade, especially rams, where most owners export rams to other areas without conducting tests on them. It also shows the absence of control programs, as it was found from the history of the case that most of the herds are not vaccinated against Brucella. All these reasons would increase the risk of disease spreading among the herd easily.

Compare between bacterial culture and RT-PCR. When comparing the results of bacterial with the results of the PCR, it was found that 36 positive samples for the PCR test were 23 positive samples for bacterial isolation and 13 negative samples for bacterial isolation. While the 14 negative cases of PCR, none of them were positive for bacterial isolation, and all of them were Negative for bacterial isolation. This difference may be due when using the polymerase chain reaction as an assessment tool is that these bacteria are opportunistic and may also be intracellular. Also, the polymerase device detects very small quantities of bacteria and also dead that the bacterial culture fails to detect.

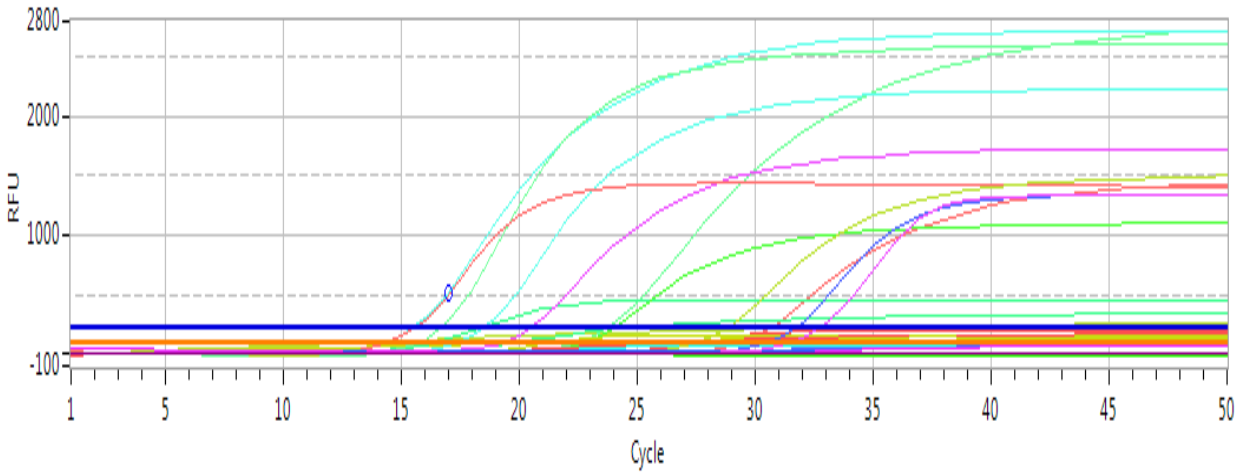
positive prediction value = 23 / 23+0 = 100%

Negative prediction value = 14 / 14+13 = 51.8 %

Sensitivity = 23 / 23 + 13 = 63% Specificity = 14 /14 + 0 = 100%



Figure, 2: Colony of Brucella on Brucella agar showing the pearly white colony(Real time Polymerase Chain reaction (RT-PCR)



Figure, 3: Real-time polymerization reaction curves for Brucella genus amplification (Sample that Cross the threshold in FAM are Shown with Ct are POSTIVE and sample that not break the threshold are shown as No C. these sample are considered negative) (º= Positive Control).

Table 4. Number of positive samples.

Test	Number of positive samples
Bacterial Culture	23
RT-PCR	36

Total sample	50

Table (5) shows the analysis of these results

	PCR result		
	Positive	Negative	**Total**
Result of Bacterial Cultur	True positive:23	False positive: 0	23
	False Negative 13:	True Negative:14	27
	Total	14	50

References

1-Dadar, M., and Alamian, S. (2020). Isolation of Brucella melitensis from seronegative camel: potential implications in brucellosis control. Prev. *Vet. Med, 185:*105194.

2- Whatmore, A. M.; Davison, N.; Cloeckaert, A., Al Dahouk, S.; Zygmunt, M. S.; Brew, S. D and Schlabritz-Loutsevitch, N. E. (2014). Brucella papionis sp. nov., isolated from baboons (Papio spp.). Inter. *J. Sys. Evol. Microbiol. 64*(12): 4120.

3- French agency for food,environmental and occupational heath & safety (2014). Brucella spp. Data sheet on foodborne biological hazard, june

4- World Health Organization Terrestrial Manual. (2016). Brucellosis (*B. abortus*, *B. melitensis* and *B.suis*) infection with *Brucella abortus*, *B. melitensis* and *B.suis*. , *2*.1.4:1-44.

5- Al-Sherida, Y., El-Gohary, A. H., Mohamed, A., El-Diasty, M., Wareth, G., Neubauer, H. and Abdelkhalek, A. (2020). Sheep Brucellosis in Kuwait: A Large-Scale serosurvey, identification of Brucella species and zoonotic significance. *Vet. Sci. 7*(3): 132.

6- Constable, P.D.; Hinchcliff, K.W. Done, S.H. & Grunberg, W. (2017). Veterinary medicine, textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th edition, Saunders Elsevier, China.

7- Radostits, O. M; Henderson, J. A; Blood, D. C; Arundel, J. T. and Gay, C. C. (2007). "Veterinary Medicineد A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses". 11th Ed W.B. Saunders Elsevier. UK. Chapter 18: 966-993

8- Alemneh, T. and Akeberegn, D. (2018). A Review on Small Ruminants Brucellosis. *J. Med. Res. 18*(2):40-54.

9- Poester, F.P., Nielsen, K.B., Samartino, L. and Yu, W.L. (2010). Diagnosis of brucellosis. *Open. Vet. Sci. J. 4:* 46–60.

10- Pereira, C. R.; Cotrim de Almeida, J. V. F.; Cardoso de Oliveira, I. R.; Faria de Oliveira, L.; Pereira, L. J.; Zangerônimo, M. G. and Dorneles, E. M. S. (2020). Occupational exposure to Brucella spp.: A systematic review and meta-analysis. *PLoS neglected tropical diseases, 14*(5): e0008164.

11- Kaltungo, B. Y.; Saidu, S. N. A.; Sackey, A. K. B. and Kazeem, H. M. (2014). A review on diagnostic techniques for brucellosis. *African J. Biotechnol. 13*(1):1-10.

12- Khurana, S. K., Sehrawat, A., Tiwari, R., Prasad, M., Gulati, B., Shabbir, M. Z. and Chaicumpa, W. (2020). Bovine brucellosis–A comprehensive review. *Vet. Quarter.* 1-46

13- Corbel, M.J. (2006): Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organisation for Animal Health.

14- Yagupsky, P., Morata, P., & Colmenero, J. D. (2019). Laboratory diagnosis of human brucellosis. *Clinical Microbiol. Rev. 33*(1): e00073-19.

15- Kadri, K. (2019). Polymerase chain reaction (PCR): principle and applications. In Synthetic Biology-New Interdisciplinary Science. IntechOpen.

16- Bashari, M. A. (2020). Development of polymerase chain reaction knowledge. *Inter. Res. Sci. Develop. J. 1*(3): 38-64.

17- World Health Organization (2013). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2013. Chapter 1.1.1. Collection, submission and storage of diagnostic specimens (NB): Version adopted in May 2013.

18- Al-abdali, A. B. A. (2005). Infection with Brucellosis in Nineva district and some biochemical aspects. PhD dissertation. University of Mosul.

19- Al-Hankawi, Omar Khazal Sel (2006). Sheep and goats in Nineveh Governorate using the ELISA test with other serological tests. Master Thesis, College of Veterinary Medicine, University of Mosul.

20- Leyla, G., Kadri, G. and Ümran, O. K. (2003). Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. *Vet. Microbiol. 93*(1), 53 -61.

21- Al-Nima, M.; A. Al-Badrani, B. and I Al-Farwachi, M. (2010). Detection of Brucella antigen in the aborted ovine fetal

stomach contents using a modified ELISA test. *Iraqi J. Vet. Sci. 24(*1): 1-4.

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

23- Hamdy, M. E. and Amin, A. S. (2002). Detection of Brucella species in the milk of infected cattle, sheep, goats and camels by PCR. *Vet. J. 163*(3), 299-305.

24- Shakerian, A., Deo, P., Rahimi, E., Shahjavan, A. R. and Khamesipour, F. (2016). Molecular detection of Brucella melitensis in sheep and goat milk in Iran. Trop. J. Pharma. Res. 15(5):913-918.

25-Samadi, A.; Ababneh, M.; Giadinis, N. D. and Lafi, S. Q. (2010). Ovine and caprine brucellosis (Brucella melitensis) in aborted animals in Jordanian sheep and goat flocks. *Vet. Med. Inter*.

استخدام اختبار تفاعل البلمرة المتسلسل في الوقت الحقيقي لتشخيص الحمى المالطية في الأغنام في محافظة الأنبار

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الخلاصة

هدفت الدراسة إلى تحديد نسبة الإصابة بمرض البروسيلا في النعاج المجهضة في محافظة الأنبار بتقنية تفاعل البلمرة المتسلسل في الوقت الحقيقي (RT PCR) والزرع البكتيري وتقييم كفاءة الاستزراع البكتيري عند استخدام تفاعل البلمرة المتسلسل في الوقت الحقيقي (RTPCR) كاختبار تقييم. تم جمع محتويات المعدة من الاجنة المجهضة والمسحات المهبلية من 50 نعجة مجهضة. وكانت نسبة الإصابة بمرض البروسيلا في النعاج في محافظة الأنبار 72٪ في اختبار PCR و46٪ في الزرع البكتيري. كانت قيمة التنبؤ الإيجابي وقيمة التنبؤ السلبي وحساسية وخصوصية الزرع البكتيري (100٪ ، 51.8٪ ، 63٪ ، 100٪) على التوالي .الاستنتاجات: محافظة الأنبار متوطنة في داء البروسيلا ، خاصة في الأغنام ، اختبار تفاعل البلمرة المتسلسل (RTPCR) هو اختبار سريع وسهل ودقيق في الكشف حتى عن أعداد صغيرة من البكتيريا في العينة ، تفوق كبير لتكنولوجيا تفاعل البلمرة المتسلسل على تقنية الزرع البكتيري .

الكلمات المفتاحية: داء البروسيلا في الأنبار ، النعاج ، تفاعل البلمرة المتسلسل في الوقت الحقيقي ، الزرع البكتيري .