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Detection of *Brucella* in infected bull testis by using PCR technique

الكشف عن جراثيم البروسيلا في خصية الثيران المصابة باستخدامتقنية تفاعل سلسلة البلمرة

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Abstract

Brucella infect male animals and cause orchitis and infertility, present study aimed to isolate brucella from testes of bull, 50 testes samples were collected then withdraw sample from epidedimys after that stained with modifiedziehl neelsen, samples were positive for stain were cultured on brucella selective media and tested with PCR. Results showed 6 samples were positive for modified ziehl neelsen , 4 samples were positive for culture and 6 samples were positive for PCR,from our research showed that PCR is more effective in diagnose *Brucella* infection.

الخلاصة

جرثومة البروسيلا تسبب التهاب الخصية والعقم في الثيران لذلك هدفت الدراسة إلى عزل جرثومة البروسيلا من خصية الثيران . تم جمع 50عينة بعد ذالك سحب السائل المنوي من الاسهر باستخدام محاقن طبية نبيذة ثم صبغت بصبغة الزيل نلسن المحورة وتم زرع وفحص العينات الموجبة لصبغة الزيل نلسن المحورة باستخدام تقنية تفاعل سلسلة البلمرة . اظهرت النتائج بان 6عينات كانت موجبة للصبغة وان 4عينات من العينات الست الموجبة للتصبيغ بصبغة الزيل نلسن المحورة كانت موجبة للزرع وست منها كانتموجبةلتقنيةتفاعلسلسلةالبلمرة. اظهرت نتائج بحثنا بان تقنية تفاعل سلسلة البلمرة كانت الافضل في الكشف عن الاصابة بجراثيم البروسيلا.

Introduction

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and occasionally by *B. suis*. Infection is widespread internationally. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent(1), (2).The disease is usuallyasymptomatic in nonpregnant females. Following infection with *B.abortus* or *B.melitensis*, pregnant adult females develop a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. In acute infections, the organism is present in most major body lymph nodes. Adult male cattle may develop orchitis and brucellosis may be a cause of infertility in male (3),(4). Hygromas, usually involving leg joints, are a common manifestation of brucellosis in some tropical countries and may be the only obvious indicator of infection; the hygroma fluid is often infected with *Brucella*(5).Presumptive evidence of *Brucella* is provided by the demonstration, by modified acid-fast staining of organisms, of *Brucella* morphology in abortion material or vaginal discharge, especially if supported by serological tests(6). The polymerase chain reaction methods provide additional means of detection. Whenever possible, *Brucella* spp. should be isolated using plain or selective media by culture from uterine discharges, aborted fetuses, udder secretions or selected tissues, such as lymph nodes and male and female reproductive organs. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria, Polymerase chain reaction (PCR) can provide both a complementary and biotyping method based on specific genomic sequences(7). To the best of our knowledge there is no similar study in Iraq about orchitis in bull infected with Brucella.

Materials and Methods

A total of 50 suspected brucellosis testessamples were obtained from bulls carcasses with signs of orchitis(enlargement in testis, fever and hygroma in knee joints) were collected from Al-Samawa slaughter houses, sperms were withdraw directly from epidedimys with sterile syringe and stained withModified Ziehl Neelsen (MZN) , then cultured directly on brucella selective media then

certified *Brucella* growth with biochemical tests and monospecific antisera according to Alton(8),samples were positive for MZN were used for DNA extraction , was performed by using Promega DNA Isolation kit by using theprimer set for IS711 genomic region of *Brucella spp.* asdirected byBricker& HallinG (9) was commercially prepared and the sequences were as follows:
F: CAATCTCGGAACTGGCCATCTCGAACGGTAT
R: ATGTTATAGATGAGGTCGTCCGGCTGCTTGG
The PCR was performed in 50µl reaction mixture1X Taq Buffer, 0.2mM dNTPs mixture, 1.5mM, MgCl2,2.5U/µl Taq Polymerase, 4µM of each primer, 4µl ofDNA extracted and 26.5 µl of DNase free deionized water. Each sample was tested in triplicate. The tubescontaining the mixture were subjected to 35cycles ofamplification in a thermocycler. During each cycle thesample of DNA was denatured at 95ºC for 35 secondsannealed at 64ºC for 30 seconds, and extended at72ºC for 30 seconds. Prior to the cycling the mixturewas subjected to incubation at 94ºC for a period of 4minutes. PCR product was then analyzed at 1.5% ofagarose gel electrophoresis. The bands of *Brucellaspp* DNA were detected by using gel documentation system and gave an applicant size of 208bp.

Results

Results showed 6 (12%) samples were positive for modified ziehl neelsen stain,*Brucella* organisms first recognized in smears obtained from epidedimys stained with Modified Ziehl Neelsen stain, which appeared red clumps against a blue background (Fig.1), from results showed 4 isolates were obtained from epidedimys ,*Brucella* recognized on the basis of colonial morphology which appeared round translucent pale honey color on *Brucella* selective media,the results of biochemical test were positive for oxidase, catalase, nitratereduction, H2S production and urease, negative for MR-VP, gelatinase,citrate utilization and indol production, all isolates were agglutinate with monospecific antisera for A.PCR was used to detect *Brucella.spp* in testes sample using the primers for IS711 genetic element and gave an applicant size of 208bp. The ladder used was 1500bp. PCR gave 6 positive result from 50 samples (Fig.2)

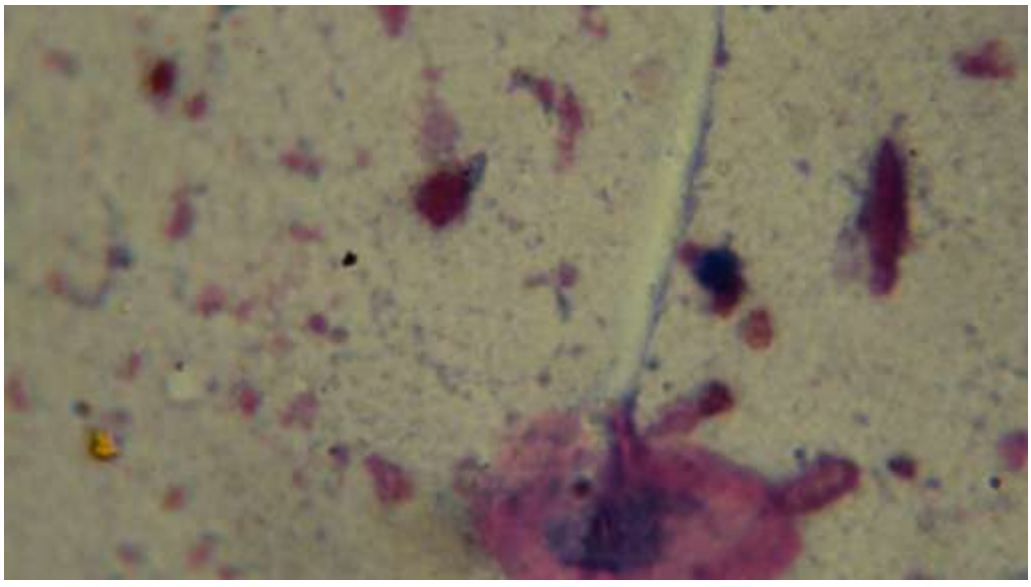

Fig. 1. Microscopic examination using Modified Ziehl Neelsen stain for epidedimys samples

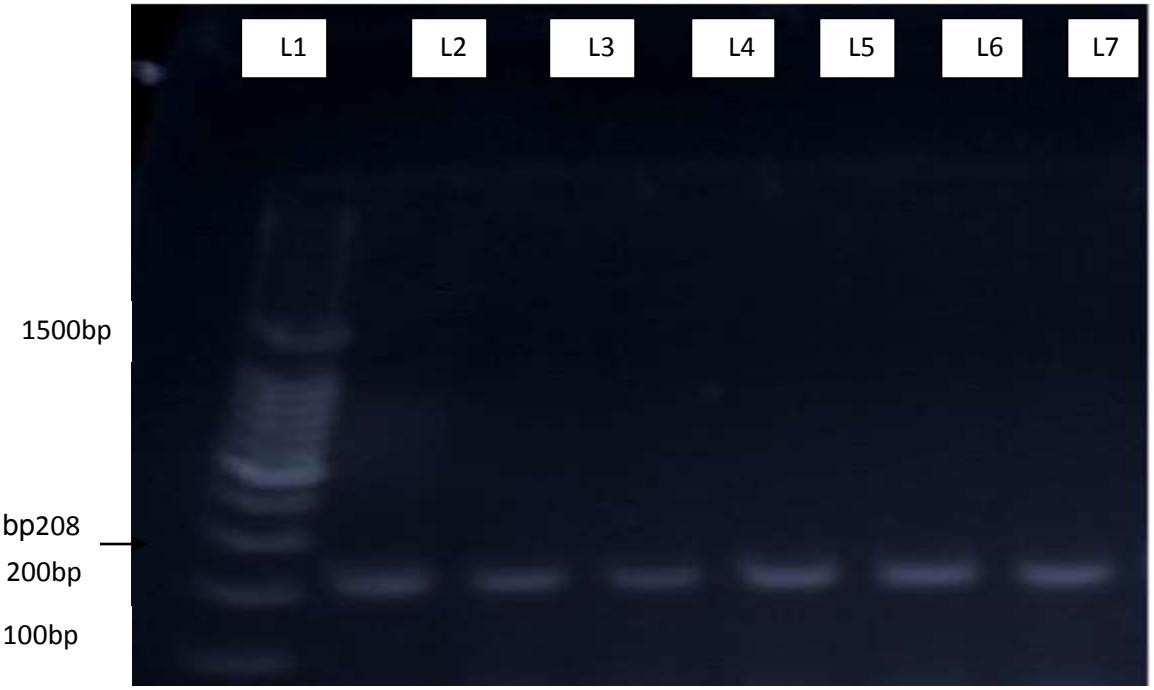

Fig.4. Gel electrophoresis for PCR products where 208bp showed positive for*Brucella spp.*
Lane 1= marker, lane 2= *Brucella* isolate positive, lane 3= *Brucella* isolate positive,
lane 4= *Brucella* isolate positive, lane 5= *Brucella* isolate positive, lane 6= *Brucella* isolate positive,
lane 7= *Brucella* isolate positive,

Table(1). Positive samples for MZN, Culture and PCR assays.

	Test	+VE	%	-VE
NOof samples 50	MZN	6	12	44
	Culture	4	8	46
	PCR	6	11	44
	Total	16	31	

Discussion

The results showed that 6 samples were positive for MZN these results same with that reported by OIE, (1),was reported that *Brucella* are not truly acid-fast, but are resistant to decolorisation by weak acids and thus stain red by the Stamp's modification of the Ziehl–Neelsen's method. This is the usual procedure for the examination of smears of organs or biological fluids that have been previously fixed with heat or ethanol, and by this method, *Brucella* organisms stain red against a blue background. Only 4 samples were positive for culture and all isolates were *Brucellaabortus*, this same that mentioned by (10),(11) whomconcluded that biovar 1 is most frequently isolated from cattle, in countries where biovar prevalence has beenstudied Outbreaks of bovine brucellosis are associated with abortion during the last trimester of gestation, and produces weak newborn calves, and infertility in cows and bulls. The primer pair used in this study succeeded in the amplification of a 208-bp fragment from epidedimys samples were studied. in the meantime, the DNA extracted from milk harbor *Brucella*'s DNA results of PCR were the same as that obtained by Baily (12), (13) who certified that the PCR amplification contained a single pair of oligonucleotide primers designed to amplify a 223 bp product and reported that the assay was sensitive and specific for *B.melitensis* and *B.abortus*. PCR was used in the diagnosis of brucellosis and demonstrated it as an extremely specific, sensitive and easy and could become an usual diagnostic test for brucellosis. because then many studies described the PCR process for finding of the *Brucellae* in human and animals from special specimens. PCR process practical to human blood samples provide superior results than the conventional culture techniques for the diagnosis of together primary infection and relapses, as well as for focal complication of the disease(14),(15).

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