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# Using PCR technique and culture to diagnose bull brucellosis in Karbala province

# استخدام تقنية تفاعل سلسلة البلمرة والزرع لتشخيص اصابة الثيران بالبروسيلا في محافظة كربلاء

**Hussain jawad Kadhum/College of Veterinary Medicine**
**University of Karbala**

**Abstract**

Present study aimed to isolate brucella from testes of bull, 40 testes samples were collected from Karbala abattoir then withdraw sample from epidedimys after that stained with modified ziehl neelsen, samples were positive for stain were cultured on brucella selective media and tested with PCR. Results showed 8 samples were positive for modified ziehl neelsen(MZN) , 3 samples were positive for culture and 5 samples were positive for PCR. Concluded that PCR is more effective in diagnose bull brucellosis.

**المستخلص**

هذه الدراسة هدفت إلى عزل جرثومة البروسيلا من بربخ الثيران . تم جمع 40 عينة بعد ذالك سحب السائل المنوي من البربخ 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 8 عينات كانت موجبة للصبغة وان 3 عينات من العينات 8 الموجبة للتصبيغ بصبغة الزيل نلسن المحورة كانت موجبة للزرع 5 منها كانت موجبة لتقنية تفاعل سلسلة البلمرة.

## Introduction

The disease is usually asymptomatic in non pregnant 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. Even in the absence of abortion, profuse excretion of the organism occurs in the placenta, fetal fluids and vaginal discharges(1).

Bovine brucellosis is usually caused by Brucella abortus, less frequently by *B. melitensis*, and occasionally by *B. suis*. Infection is widespread globally. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent(2).

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*(3).

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 both sexes(4).

The mammary gland and associated lymph nodes may also be infected, and organisms may be excreted in the milk. Subsequent pregnancies are usually carried to term, but uterine and mammary infection recurs, with reduced numbers of organisms in cyetic products and milk(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).

**Materials and Methods**

Signs of orchitis were observed during collected 40 suspected brucellosis testes samples were obtained from bull carcasses from Karbala slaughter house, sperms were withdraw directly from epidedimys with sterile syringe and stained with modified ziehl neelsen , 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. The primer set for IS711 genomic region of *Brucella spp.* as used by Bricker& 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 of DNA extracted and 26.5 µl of DNase free deionized water. Each sample was tested in triplicate. The tubes containing the mixture were subjected to 35cycles of amplification in a thermocycler. During each cycle the sample of DNA was denatured at 95ºC for 35 seconds annealed at 64ºC for 30 seconds, and extended at 72ºC for 30 seconds. Prior to the cycling the mixture was subjected to incubation at 94ºC for a period of 4minutes. PCR product was then analyzed at 1.5% of agarose gel electrophoresis. The bands of *Brucella spp* DNA were detected by using gel documentation system and gave an applicant size of 208bp.

**Results**

*Brucella* organisms first recognized in smears obtained from epidedimys showed 8 (20%) samples were positive for (MZN) stained with (MZN) stain, which appeared red clumps against a blue background (Fig.1), from results showed 3 isolates were obtained from epidedimys , *Brucella* recognized on the basis of colonial morphology which appeared round translucent pale honey color on *Brucella* selective media (Fig.2), and results of biochemical test were positive for nitrate reduction, catalase, oxidase nitrate reduction, urease and H2S production, negative for Citrate utilization, gelatinase, MR-VP and indol production H2S production (Fig.3), 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 5 positive result from 40 samples (Fig.4)

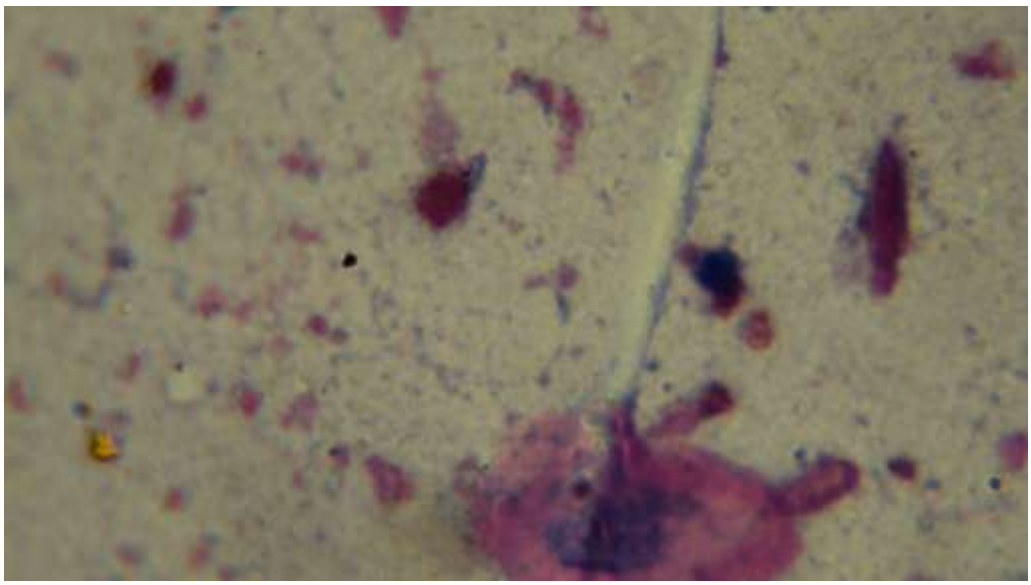

Fig. 1. Microscopic examination using modified ziehl neelsen stain for epidedimys samples

Fig. 2. Brucella colonies on *Brucella* selective media

Fig 3. H2S Productionby *Brucella abortus* isolates

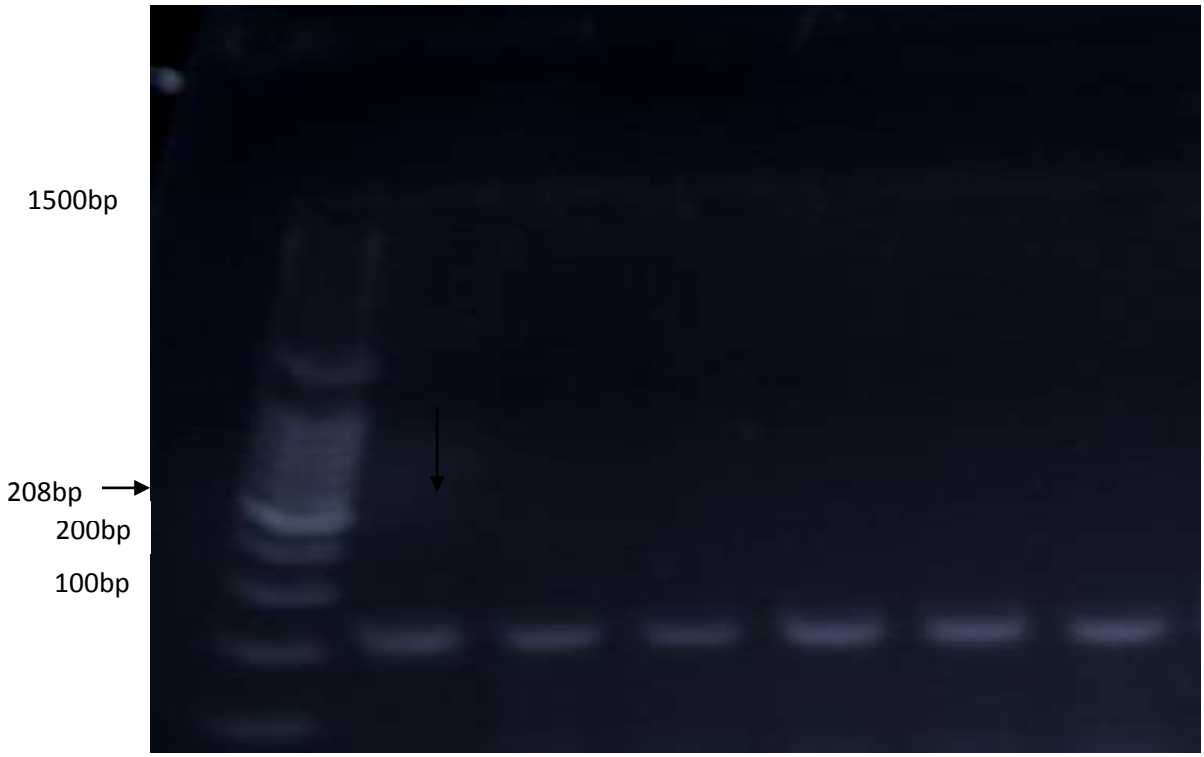


Fig.4. Gel electrophoresis for PCR products where 208bp showed positive for *Brucella spp.*

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

| | Test | +VE | % |
|---|---|---|---|
| NO of samples 40 | MZN | 8 | 20 |
| | Culture | 3 | 7.5 |
| | PCR | 5 | 12.5 |

**Discussion**

*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. From results showed 8 samples were positive for MZN this results same with that reported by OIE (1), 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. 3 samples were positive for culture and all isolates were *Brucella abortus*, this same that mentioned by (10) who said that Although eight biovars of *B. abortus*, the causative agent of bovine brucellosis, have been identified, , in countries where biovar prevalence has been studied Outbreaks of bovine brucellosis are associated with abortion during the last trimester of gestation, biovar 1 is most frequently isolated from cattle, 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. The DNA extracted from milk harbor *Brucella*'s DNA results of PCR were the same as that obtained by Baily (11) 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*. because then many studies described the PCR process for finding of the *Brucellae* in human and animals from special specimens, 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 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(12).

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