



Diagnosis of *Brucella melitensis* infection in goats milk by milk ring test & Polymerase chain reaction

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Abstract

B. melitensis is a more infectious zoonotic agents infect human and animals, 120 suspected brucellosis milk samples from goats with history of abortion were collected from Al-Samawa city tested with Milk ring test and Polymerase chain reation technique, results showed 11(9.16%) samples were positive for MRT, while 5(4.16%) samples were positive for PCR technique.

Introduction

Brucellosis, a bacterial disease caused by members of the genus *Brucella*, is an main zoonosis and a significant cause of reproductive wounded in animals. Brucellosis is usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs(1). Abortions, placentitis, epididymitis and orchitis are the most frequent cost, although other syndromes are also reported(2). The major contact is economic; deaths are rare except in the fetus and neonate. Some *Brucella* species are also maintained in wildlife populations. Wildlife reservoirs including feral pigs, bison, elk and European hares complicate eradication efforts for *B. abortus* and *B. suis* (3). Marine mammal isolates of *Brucella* have recently been recognized in many species of pinnipeds and cetaceans, and there are concerns that these organisms might have a detrimental impact on some species (4). *Brucella melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis. Sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats(5). *Brucella melitensis* is endemic in the Mediterranean region, but infection is widespread world-wide(6).

Materials and Methods :

A total of 120 suspected brucellosis milk samples from goats with history of abortion were collected from Al-Samawa city .

Milk Ring Test :

Milk ring test was conducted on milk samples as described by Blythman et al (6). The positive samples were differentiated on the basis of blue ring present on the top of milk after overnight reaction.

DNA extraction of serum and milk samples was performed by using Promega DNA Isolation kit. The primer set for IS711 genomic region of *B. melitensis* as used by Bricker& HallinG (7) was commercially prepared and the sequences were as follows:

5-CAG-GCA-AAC-CCT-CAG-AAG-C-3 (Forward)

5-GAT-GTG-GTA-ACG-CAC-ACC-AA -3 (Reverse)

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 95°C for 35 seconds annealed at 64°C for 30 seconds, and extended at 72°C for 30 seconds. Prior to the cycling the mixture was subjected to incubation at 94°C for a period of 4minutes. PCR product was then analyzed at 1.5% of

agarose gel electrophoresis. The bands of *Brucella melitensis* DNA were detected by using gel documentation system.



Fig(1).

Showed MRT for goats milk samples.

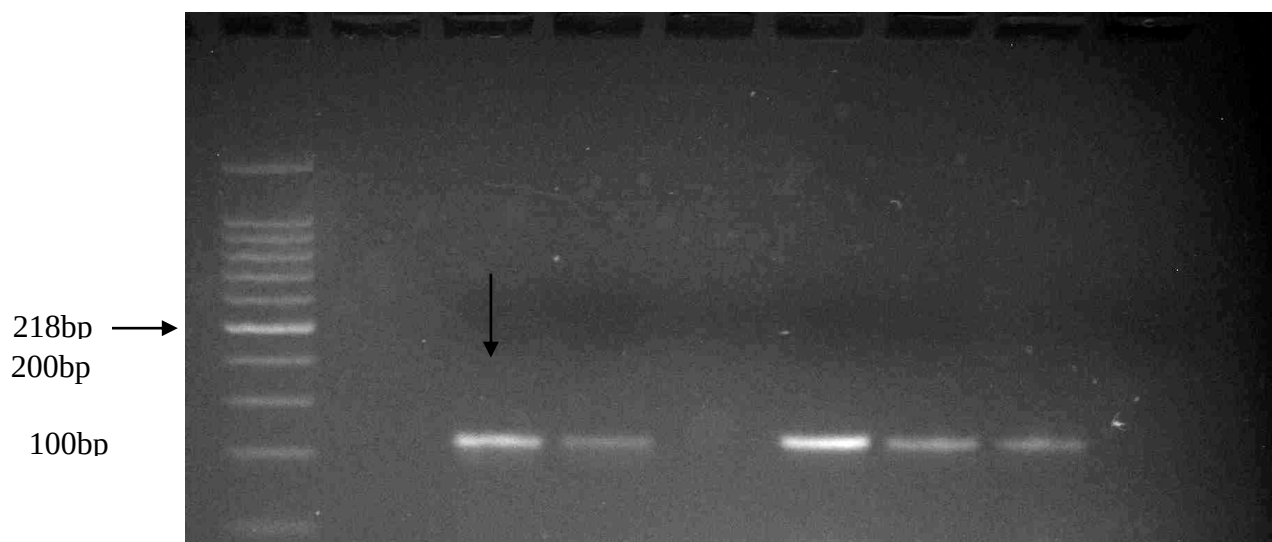
Results:

Results showed 11 (9.16%) samples were positive for MRT (Fig.1). PCR was used to detect *B.melitensis* in milk samples using the primers for IS711 genetic element and gave an applicant size of 218bp. The ladder used was 100bp. PCR gave 5 positive result from 120 milk samples (Fig.2) (table1).

Table(1). Positive milk samples for Milk ring test and Polymerase chain reaction assays.

Test	+VE	%	-VE	%
MRT	11	9.16	109	90.8
PCR	5	4.16	115	95.83
Total	17		224	

*positive for MRT , positive for PCR



Fig(2). Gel electrophoresis for PCR products where 218bp showed positive for *Brucella melitensis*.

Lane m= marker, Lane 1= + milk sample, Lane 2+ milk sample, Lane 3 - milk sample, Lane 4 + milk sample, Lane 5 + milk sample, Lane 6= + milk sample

Discussion:

Our results revealed that MRT was good in diagnose *brucella* infection in goats but this test may be detected the non-specific antibodies produced by the other phylogenetically related bacteria such as *Yersinia*, *E.coli*, *salmonella* and *vibrio*.(8) also this test don't differentiate between vaccinated and infected animals(9). Also gave false negative in infected animals especially after birth and abortion(10). The primer pair used in this study succeeded in the amplification of a 218-bp fragment from milk 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 et al., (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*. 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. Because of the high specificity and strict sensitivity (12), PCR is the only one which was able to detect the occurrence of *Brucella* organisms in all milk samples (13) and (15).

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تشخيص إصابة حليب الماعز بالبروسيللا المالطية بواسطة اختبار حلقة الحليب وسلسلة تفاعل البلمرة

الخلاصة :

البروسيللا المالطية واحدة من أهم المسببات المرضية المعدية والمشاركة التي تصيب الإنسان والحيوانات , تم جمع 120 عينة حليب ماعز مجهضات سابقا , اجري فحصي حلقة الحليب وتفاعل سلسلة البلمرة على عينات الحليب , اظهرت النتائج بان 11(9,16%) عينة موجبة لفحص حلقة الحليب وان 5(4,16) عينة موجبة لفحص تفاعل سلسلة البلمرة .