Use of PCR technique for direct detection of *Brucella spp.* from milk of sheep and cattle

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Summary

Brucella spp are important food pathogen those can be infected the human-being during consumption of contaminated milk and milk products from sheep, goats, and cattle with *Brucella* spp. In this study the polymerase chain reaction (PCR) for direct detection of *Brucella* spp. from milk of sheep and cattle were employed to amplify 233bp product of highly conserved regions of *BCSP31* gene encoding a 31-KDa cell surface protein in *B. melitensis* and *B. abortus*. The results showed that the sheep were more frequent for shedding of *Brucella* spp in their milk, where appeared (6/50 samples) at (12%). Whereas the cattle appeared less frequency for shedding of *Brucella* in their milk, which showed (2/50 samples) at (4%). It can be concluded that PCR technique is highly sensitive and specific technique for direct detection of *Brucella* from milk and the sheep and cattle can be shedding the *Brucella* in their milk. Therefore, the contaminated milk with *Brucella* spp may have dangerous effect on public health, when consumed by human.

Keywords: Milk, *Brucella* spp, PCR, Sheep, Cattle.

Introduction

Brucella is small coccobacillus Gram-negative, aerobic, nonmotile, nonsporeforming, and facultative intracellular bacterium, which infected human and animals (1). The genus *Brucella* consists of 8 species, of which *Brucella abortus*, *Brucella melitensis*, *Brucella canis*, and *Brucella suis* are pathogenic for humans. *Brucella microti*, *Brucella inopinata*, *Brucella ceti*, and *Brucella pinnipedialis* were isolated from animals but can occasionally cause disease in man (2 and 3). Among these species, the main species in humans are *B. abortus* and *B. melitensis*. They caused brucellosis, also known as Malta fever (4). Brucellosis is one of most contagious bacterial infection of livestock and continues to be of great health concern and economic importance worldwide especially in Mediterranean countries (5). Acute infection with *Brucella* spp. is initiated by the entrance of the bacteria into the bloodstream after which they are engulfed by circulating polymorphonuclear cells and macrophages, evading the bactericidal systems of the cells. Due to the bacterium's predilection for cells producing erythritol, *Brucella* spp. localise in the pregnant uterus of ruminants, inducing abortions in late pregnancy and premature births (6). After the initial acute phase, brucellosis in the primary host usually becomes latent, although abortions in the subsequent gestation may happen. Females may shed the bacteria periodically through milk and uterine and vaginal discharges in subsequent parturitions, as described in ewes (7). The human become infected by brucellosis through direct or indirect contact by ingestion of animal products as after drinking raw milk or eating unpasteurized cheese (8). Microbiological, serological, and molecular techniques are commonly used for diagnosis of brucellosis (9-11). Microbiological tests such as the isolation of bacteria from host tissue or blood cultures following by bacteriological characterization remain important, although they are tedious and time consuming (10). Among molecular techniques, polymerase chain reaction (PCR) is one of the most useful tools for the diagnosis of brucellosis. It has been reported to identify of the genus *Brucella* level but not species with in the genus, it can be performed by PCR using primers targeting highly conserved regions such as the *BCSP31* or 16SrRNA (12 and 13). This study was performed for direct detection of

Brucella spp. from milk of sheep and cattle by PCR assay.

Materials and Methods

One hundred samples of milk were collected from healthy 50 sheep and 50 cows, from different farms in Al-Diwanyia city at a period approximately for six months from January to June. The milk samples were collected in 25ml sterile containers after clean and washing the quarters of udder by disinfectant solution, then the milk samples transported into laboratory and stored in a refrigerator until use for genomic DNA extraction.

The whole bacterial genomic DNA was extracted from milk according to method described (14) by using (Genomic DNA extraction kit. Geneaid. USA). 1ml aliquot of milk was centrifuged at 6,000 × *g* for 10 min, then the clear portion was pipetted and discarded. The remaining milk solids and butterfat were used for DNA extraction in which it has been done according to kit instruction using DNA purification spin column. After that, the purified DNA eluted in elution buffer provided with kit and store at -20°C, then, used for prepared of PCR master mix.

Polymerase chain reaction was carried out by using specific primer which was designed by Baily (12) to amplify a 223-bp product targeting of highly conserved regions of BCSP31 gene encoding a 31-KDa cell surface protein in *B. melitensis* and *B. abortus*. The forward primer (TGGCTCGGTTGCCAATATCAA) and Reverse primer (GCGCTTGCCTT TCAGGTCTG) were provided by Bioneer. Korea. Then PCR master mix was prepared by using standard PCR premix tube kit provided from Bioneer. Korea. The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, $MgCl_2$ 1.5mM, stabilizer, and tracking dye) and prepared according to kit instruction by added 5uL of purified genomic DNA and 1.5ul of (10 pmol) of each primer, then complete the PCR premix tube by deionizer PCR water into 20ul and briefly mixed by vortex. The reaction was performed in a thermocycler (Techne TC-3000. USA) at a initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 55 °C for 30 s, and extension 72 °C for 30 s and then final extension at 72 °C for 7 min. The amplification products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Results and Discussion

The polymerase chain reaction (PCR) technique is found very specific and sensitive assay, and less time consumption, when using for direct detection of *Brucella* from milk, in contrast to other conventional diagnostic techniques. Our results showed that PCR is very important tool for the identification of *Brucella* spp from milk of sheep and cattle by using universal primers for BCSP31 gene that encoding a 31-KDa cell surface protein in *B. melitensis* and *B. abortus*. Where the positive results of PCR amplification was highly specific 233bp PCR product without nonspecific bands (figure, 1). These results were in agreement with (15) who referred that molecular diagnosis including both PCR and hybridization assays were fast and accurate diagnosis of brucellosis as compared with other conventional techniques such as Milk Ring Test (MRT), Bacterial isolation, and ELISA.

Accuracy of PCR assay that using in direct detection of *Brucella* which depend on universal primers from *BCSP31* gene that is designed by (12), who found that this gene has highly conserved regions that encoding a 31-KDa cell surface protein in *B. melitensis* and *B. abortus*. The results show that *Brucella* spp are found in milk of sheep and cattle, with differences in incidence between them, in contrast the sheep which appeared more frequent incidence as compared with cattle (Table,1). This result was coincide with (15) who observed that sheep was more frequency for shedding of Brucella in their milk (60.3%) as compared with cows, buffaloes, goats and camels those showed an incidence rate of 48.3, 53.9, 50.4 and 28.1%, respectively. In

conclusion, these results reveal that the application of PCR technique is highly sensitive, specific and less time consumption for direct detection of *Brucella* spp. from milk of sheep and cattle.

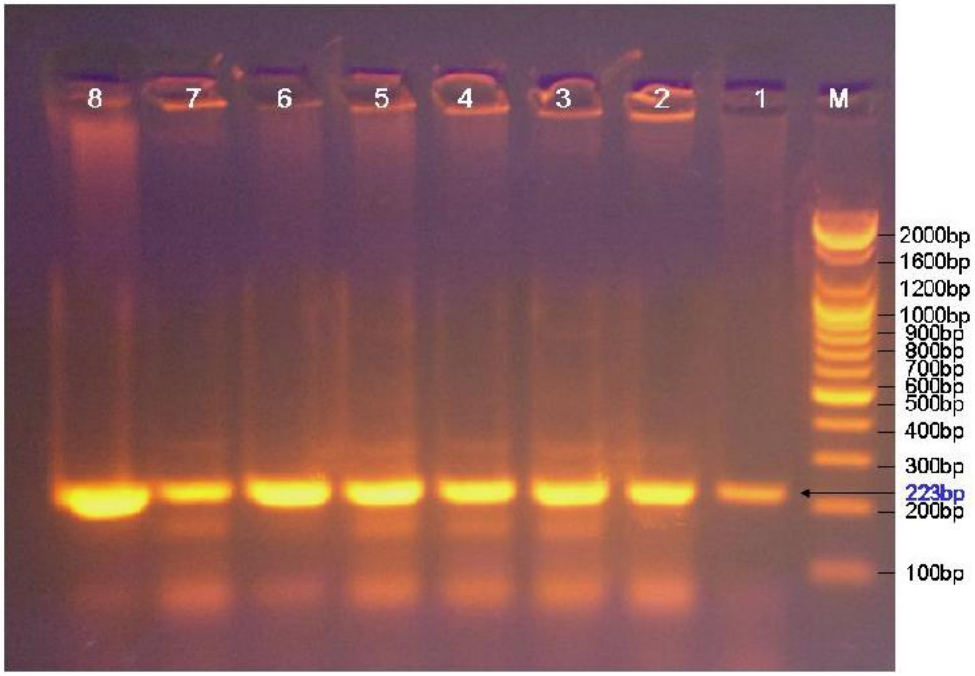

Figure ,1: Agarose gel electrophoresis image which appear the positive results of PCR amplification in BCSP31 gene at 233bp PCR product. Where (M: Marker 2000-100bp, 1-6 from sheep, 7-8 from cattle).

Table ,1: frequency of incidence of *Brucella* spp. in sheep and cattle.

Animals	No. of samples	Positive result	Percentage
Sheep	50	6	12%
Cattle	50	2	4%

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استخدام تقنية التفاعل سلسلة البلمرة في التشخيص المباشر لجرثومة البروسيلا من حليب الاغنام والابقار

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الخلاصة

تعد جرثومة البروسيلا من أهم ممرضات الغذاء التي يمكن ان تصيب الانسان من خلال استهلاك الحليب ومشتقاته الملوثة بالجرثومة من الابقار والاغنام. استخدم في الدراسة اختبار تقنية تفاعل سلسلة البلمرة وذلك للكشف المباشر عن جراثيم البروسيلا من حليب الاغنام والابقار باستخدام البادئات العامة والتي ستعمل لتضخيم (233bp) ناتج من المنطقة عالية الثبات لجين ال (*BCSP31*) الذي يشفر 31-KDa من البروتين السطحي لخلايا جرثومة البروسيلا المالطية والبروسيلا البقرية. أظهرت النتائج أن الأغنام هي أكثر تردد لطرح البروسيلا في الحليب، حيث ظهرت (50/6) عينة) بنسبة (12٪). في حين أن الأبقار أقل تردد لطرح البروسيلا في الحليب، حيث أظهرت (50/2 عينة) وبنسبة (4٪). من خلال هذا الدراسة يمكن ان يستنتج تقنية ال PCR هي تقنية عالية الحساسة ومتخصصة للكشف المباشر لجراثيم البروسيلا من حليب الأغنام والأبقار. لذلك الحليب الملوث بجراثيم البروسيلا له تأثير خطير على الصحة العامة عندما يستهلك من قبل الانسان.

الكلمات المفتاحية: الحليب، جرثومة البروسلا، إختبار تفاعل سلسلة البلمرة, الاغنام والابقار.