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RESEARCH ARTICLE

Detection of *Brucella melitensis* in Milk of Hair Goat *(Capra hircus)* by Polymerase Chain Reaction (PCR) [1]

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Summary

The aim of this study was to investigate to *B. melitensis* in Hair Goat in the Çine district of Aydın province. *B. melitensis*, is a significant problem for public and animal health in Turkey. In this study, 222 milk samples from 116 goats were collected from 10 flocks in the villages of Kavşit, Mutaflar, Tatarmemişler and Elderesi. Hair goat of 2-7 years of age that no history of vaccination against brucellosis. In this study, oligonucleotide primers specific for IS711, *B. melitensis* and *B. abortus* were used. After DNA extraction from the milk samples, PCR was used to detect *B. melitensis* and 50 positive results (22.5%) of milk samples of Hair Goat produced a 731 bp PCR band specific for *B. melitensis*.

Keywords: *Brucella melitensis, IS711, PCR, Hair Goat, Milk*

Kıl Keçisi *(Capra hircus)* Sütlerinden Polimeraz Zincir Reaksiyonu (PZR) ile *Brucella melitensis* Saptanması

Özet

Bu çalışmanın amacı, Aydın'ın Çine ilçesindeki Kıl Keçilerinde *B. melitensis*'in yaygınlığını araştırmaktır. Türkiye'de *B. melitensis* insan ve hayvan sağlığı yönünden önemli bir sorundur. Bu çalışmada Kavşit, Mutaflar, Tatarmemişler ve Elderesi köylerinden 10 sürü ve116 keçiden 222 süt örneği toplanmıştır. Örnek alınan keçilerin yaşları 2-7 arasındadır ve brusellozise karşı aşılama yapılmamıştır. Bu araştırmada IS711, *B. abortus* ve *B. melitensis* için spesifik oligonükleotid primerleri kullanılmıştır. Süt örneklerinden DNA (Deoksiribonükleikasit) ekstraksiyonu yapıldıktan sonra, *B. melitensis*'i saptamak için PZR kullanılmıştır. 50 Kıl Keçisi süt örneği (%22.5) *B.melitensis* için spesifik olan PZR 731 bp band aralığında pozitif sonuç vermiştir.

Anahtar sözcükler: *Brucella melitensis, IS711, PZR, Kıl Keçisi, Süt*

INTRODUCTION

Brucellosis is a zoonosis and an important bacterial disease of domestic animals and humans. The disease causes economic losses, and potentially threatens human health. *Brucella* can be transmitted to human from infected animals, especially consumption of contaminated milk or milk products more less contact with their carcasses [1]. *Brucella* genus, six species of *Brucella* recognized currently, *B. abortus* and *B. melitensis* are responsible for diseases characterized by placentitis, abortion and infertility in cattle and sheep, respectively [2]. Human infections are often due to *B. melitensis*, with only a few instances of *B. abortus* infection [3]. *B. melitensis* is considered a major risk to human health due to the high virulence of the agent to man and traditional consumption of raw

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milk and milk products [4]. Human brucellosis, especially resulting from *B. melitensis* is an important public health problem in rural areas and also ıt has been reported that *B. melitensis* is frequently observed in goats [5]. Polding [6] first reported the isolation of *B. melitensis* in goats. *B. melitensis* is the major cause of abortion in sheep and goats in many countries including Turkey [7,8]. Therefore, it is difficult to diagnose Brucella species, classical detection methods such as serological, cultural and biochemical tests [9]. However, these methods are not wholly satisfactory. Bacteriological isolation is time consuming procedure, and handling the microorganism is hazardous. Serological methods are not conclusive, because not all infected animals produce significant levels of antibodies and because cross-reaction with other bacteria can give false negative results [10]. Recently, detection studies based on PCR (Polymerase Chain Reaction) have become widespread [10]. PCR is mostly preferred due to its sensitivity and rapidity [10,11]. PCR technology has been applied to the detection of *Brucella* contamination in food products, such as milk and cheese [12-19].

Despite the fact that many studies have been carried on Brucellosis in different regions of Turkey [7,8,20-25]. There is no study on PCR detection of *B. melitensis* during goat breeding in rural flocks. This paper describes the detection of *B. melitensis* in hair goat milk by PCR

MATERIAL and METHODS

The materials of the research consisted of 222 milk samples collected from 116 goats from 10 flocks in Kavşit, Mutaflar, Tatarmemişler and Elderesi villages. Hair goat of 2-7 years of age that no history of vaccination against brucellosis. The collected samples were brought from the villages to the laboratory in cold conditions. The milk samples collected from Hair goats were identified using their numbered earrings.

Reference strain

B. melitensis Rev. 1 vaccine and *B. abortus* S19 vaccines were used as positive control.

Oligonucleotide primers

B. abortus and *B. melitensis* primer sequences were used as previously described by Bricker and Halling [26].

DNA extraction from milk samples

Extraction and purification of *Brucella* DNA were applied according to the method proposed by Leal-Klevezas et al.[27]. DNA extraction was performed using 400 µl of the fatty top layer of raw milk. Four hundred microliters of lysis solution (2% Triton X-100, 1% Sodium Dodecyl Sulphate (SDS), 100 mM NaCl 10 mM Tris-HCl and 10 µl of 10 mg/L Proteinase K were added to the tubes with 400µl raw milk samples, and the contents were mixed thoroughly and incubated for 30 min at 50ºC. Four hundred microliters of saturated 0.2 2%-Mercapto Ethanol (ME) was added, and the contents were mixed and centrifuged at 8.000g for 5 min. The aqueous layer was transferred to a fresh tube, and an equal volume of chloroform-isoamyl alcohol (24:1) was added; the tubes were mixed and centrifuged at 8.000 g for 5 min. The upper layer was again transferred to a fresh tube. Two volumes of 95 % ethanol were added, the contents were mixed, and the tubes were stored at -20ºC. DNA was recovered by centrifugation of the samples at 8.000 g for 5 min, and the pellets were rinsed with 1 ml of 70% ethanol, dried and re-suspended in 20 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, and the samples were stored at -20°C until they were processed.

PCR assay

In our study, previously reported oligonucleotide primers specific for IS71128, *B. melitensis* and *B. abortus* were used. Assay was performed in a final volume of 25 µl mixture containing PCR buffer [60 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)2SO_4$, 1.5 mM MgCl] (MBI Fermentas), 250 µm (each) of the four deoxynucleotide triphosphate (MBI Fermentas), a primer cocktail consisting of three primers (1 µm IS711-specific primer, 0.1 µm each of the *B. melitensis* and *B. abortus*-specific primer), 1.25 U Taq polymerase and 2.5 µl template DNA. Amplification reactions were performed in Mastercycler Personal Thermal Cycler (Eppendorf AG, Germany) in the following steps: initial denaturation at 95ºC for 3.0 min, cycling at 95ºC for 2.0 min for denaturation, at 55.5ºC for 2.0 min for annealing, at 72ºC for 2.0 min for 35 cycles with the a final extension at 72ºC for 4 min [27].

Following PCR, the products were analyzed by electrophoresis through a 1.5% agarose gel after

which the gel was stained with ethidium bromide and photographed after visualized on an UV transilluminator.

RESULTS

In our study, 50 milk samples of hair goat produced a 731 bp PCR band specific to *B. melitensis*. Some PCR amplification-products are shown in *Figure 1*.

Out of 222 samples, *B. melitensis* was isolated from 50 (22.5%) samples of 116 Hair Goats in 10 flocks. The percentages of *B. melitensis* in hair goat samples have been displayed in *Table 1*.

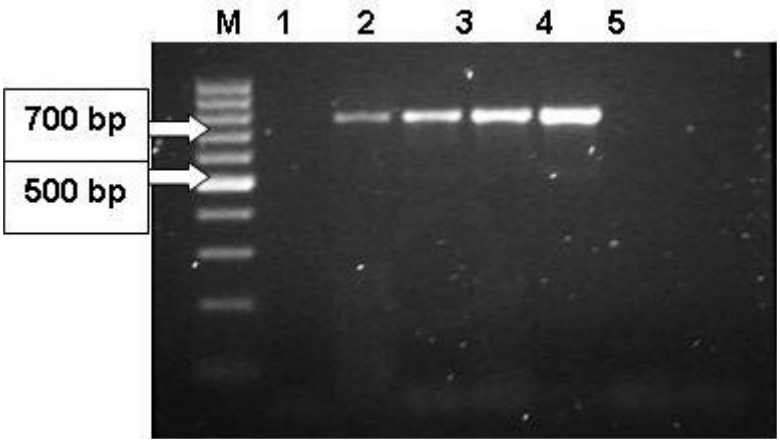

Fig 1. The results of PCR analyses for *B. melitensis* (DNAs of *B. melitensis* specific amplification of 731 bp) Lane M, 1-bp DNA ladder (MBI Fermentas); lane 1, negative control (distilled water), lane 2, *B. melitensis* Rev. I vaccine; lane 3-5 i positive samples

Şekil 1. *Brucella melitensis* için PZR analiz sonuçları (*Brucella melitensis*'in DNA'larından 731 baz çifti aralığının spesifik amplifikasyonu) Sütun M, 1-kb DNA işaretleyicisi (MBI Fermentaz); sütun 1 negative kontrol; sütun 2, *Brucella melitensis* RevI aşı suşu; sütun 3-5 pozitif örnekler

Table 1. *Percentages of B. melitensis in hair goat milk samples*
Tablo 1. *Kıl keçisi süt örneklerindeki B. melitensis yüzdelerinin dağılımı*

Name of villages	Number of hair goat	Number of milk samples	Number of right udder positive	Number of left udder positive	Number of each villages breeding doe	Number of total positive hair goat
Kavşit	81	157	19 (12.1%)	16 (10.2%)	350	26 (16.6%)
Mutaflar	12	21	2 (9.5%)	1 (4.8%)	150	2 (9.5%)
Tatarmemişler	10	19	2 (10.5%)	7 (36.8%)	180	8 (42.1%)
Elderesi	13	25	2 (8.0%)	1 (4.0%)	25	38 (12.0%)
Total (4 villages)	116	222	25 (11.26%)	25 (11.26%)	655	39 (17.6%)

Determination of the detection sensitivity of PCR

Detection limit of the PCR assay was eveluated for goat milk samples. To determine colony forming unit (cfu), a concentrated culture of the *Brucella melitensis* was prepared in sterile saline and $5x10^5$ cfu/ml dilution was made. $5x10^5$ cfu/ml dilution was serially diluted 2-fold to 5 cfu/ml. From the all dilutions, 0.1 ml suspensions onto two tryptose soy agar plates and incubated at 37ºC for 48 hours. Then colonies on plates were counted. Twenty microliters of each dilution was boiled for 10 min and added directly to the PCR mixture. The bacterial concentration of the positive PCR results were obtained with different aliquots containing at least 10^3 cfu of *B. melitensis* organisms were detected per milliliter of goat milks. However, some aliquots containing at least 100 cfu of *B. melitensis* organisms were detected per milliliter of goat milks.

DISCUSSION

This study was conducted in the mountainous area of Çine district of Aydın. A total of 10 hair goat flocks were investigated for detection or prevalence of B. melitensis in milk samples. Fifty (22.5%) of the 222 milk samples were detected with B. melitensis. PCR-based methods have the

potential to be fast, accurate and efficient in detecting *Brucella* [28]. Because of this, PCR has an important role in the diagnosis. In this study, PCR method has been applied to hair goat milk samples for the first time in the Turkey. Due to the fact that there have been no studies on the detection of *B. melitensis* by PCR on hair goats in Turkey, but in direct detection of *B. melitensis* from soft cheese was examined Öngör et al.[24]. In our study, some aliquots containing at least 100 cfu of *B. melitensis* organisms were detected per milliliter of goat milks. In PCR technique, non-viable microorganisms, contamination of the sample with other micro-organisms or delays in the analysis do not affect the results. The same index was reported by Tantillo et al.[18], who studied the presence of *Brucella* spp. in 46 cheese samples (goat and sheep cheese) by means of microbiological culture and PCR [18]. Tantillo et al.[18] reported that PCR method is of remarkable epidemiologic interest because it is an indirect test indicating the sanitary quality of milk used in dairy industries and this method showed good sensitivity, specificity, faster and less expensive than the conventional bacteriological assays.

The results of this study have been compared to serological studies. According to serological studies, the result of our study is similar to the result of Muz et al.[23]. Gupta et al.[10] used the same method as ours, but the result of our study related to the *B. melitensis* produced a lower result than the result obtained by Gupta et al.[10]. The reasons for these differences have emerged due to contamination of infection among the goat flocks, regional differences, breeding conditions and studies conducted in aborted goats. The rate of Brucellosis in sheep and goats changes as reported by Robert [29]; Mohsen [30] and Gupta et al.[10] in different regions and different methods.

Infections of *B. melitensis* are widespread in Latin America, some part of the Africa, the Mediterranean, and the Middle East [2]. Studies carried out in different areas have reported that *B. melitensis* is one cause of abortion in goats in Turkey [24]. In this study, detection of the *B. melitensis* in the goat milk samples was found to relatively higher to 22.5%. Primarily the species and the used methods, the management conditions, the region, the type of material (blood or milk, aborted or not aborted), and variation within the flock could possibly account for the differences in Brucellosis rates observed in previous studies [27-30]. The majority of studies related to detection of Brucellosis have been limited to aborted animals and serological methods [19,31-32].

In conclusion, the fact that the rate of Brucellosis in Hair Goats happened to be high in the present study, could be attributed to several factors such as some primitive, social and management conditions of breeders and their goat flocks, such as the absence of vaccination and health protection programmes. According to the result of this study, Brucellosis is one of the problems in the Hair Goat flocks of Çine district of the Aydın region and *B. melitensis* can cause economical losses as a result of abortion in the flocks. The results of this study indicate that in order to successfully control *B. melitensis* in rural conditions, some precautionary measures have to be made. Therefore, in order to decrease the prevalence of this zoonosis in a wide range, applicable recommendations for breeders and several investigations are needed. These investigations should be performed in various rural farm conditions and aim to determine the factors affecting the rates of *B. melitensis*. The disease risks and economical losses resulting from *B. melitensis* may be prevented to improve animal health protection and breeding conditions of the flocks.

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