Journal of Infection and Public Health 12 (2019) 337–342

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Comparison of multiplex real-time polymerase chain reaction with serological tests and culture for diagnosing human brucellosis

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ARTICLE INFO

Article history:
Received 5 June 2018
Received in revised form
13 November 2018
Accepted 18 November 2018

Keywords:
Brucellosis
Multiplex real-time PCR
Serological diagnosis
Culture

ABSTRACT

Objective: Brucellosis is a zoonotic disease with various clinical presentations and early diagnosis is crucial to avoid severe complications. Due to limitations of conventional diagnostic methods, polymerase chain reaction (PCR) based approaches have gained importance in diagnosis.We aimed to evaluate diagnostic value of multiplex real time-PCR (mRT-PCR) in serum samples collected from brucellosis suspected patients by comparision sensitivity of mRT-PCR with those of conventional diagnostic methods.
Material and Methods: A total of 249 serum samples collected from the suspected brucellosis patients admitted to the hospitals in three different provinces were analyzed by serological tests, culture and mRT-PCR. In laboratories of the participating hospital, serum samples were tested for the *Brucella* specific antibody by commercial serological kits including standart tube agglutination test (STAT), Coombs' test, and immunocapture test (ICT). Blood culture was performed for 153 of the patients in the participating hospital. All serum samples were analyzed for the presence of *Brucella* DNA by mRT-PCR.
Results: According to laboratory test results, 215 of the 249 suspected cases having comparible clinical data were identified as brucellosis cases. Of the 215 brucellosis cases, 36 were diagnosed as definitive cases, the remaning 179 patients were presumptive cases. Sensitivity of mRT-PCR in the samples that were positive by ICT, STAT, Coombs' test, and blood culture was 70.2%, 77.3%, 83%, and 97.2%, respectively. By using mRT-PCR, additional 17 suspected patients were diagnosed as presumptive cases. Among the mRT-PCR positive serum samples, *Brucella abortus* was detected in 3 samples (1.9%), the remaining 156 samples (98.1%) had *B. melitensis* DNA.
Conclusion: Our results indicate that mRT-PCR can be considered a useful diagnostic tool in patients who have negative serologic test results, and in detection of *Brucella* species.

Introduction

Brucellosis is the most common zoonotic diseases caused by *Brucella* species that are facultative intracellular pathogens. Annualy, about 500 000 new human cases have been reported over the world [1]. The disease is highly prevalent in Central Asia, Middle East, the Mediterranean region, Africa, and Latin America [2,3]. Brucellosis leads to abortion, sterility, mastitis in animals, and leads to great economic losses [4].

Transmission from animals to humans can occur by consumption of infected unpasteurized milk and dairy products, inhalation of infected aerosolized particles, and direct contact with infected animal parts [5,6].

Among *Brucella* species, *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Brucella canis*, and *Brucella pinnipedia* can infect humans [6]. In Turkey brucellosis is still a public health problem with an incidence of 12–50/100,000 [7]. The studies carried out in Turkey indicate that almost all human brucellosis are caused by *B. melitensis* with a ratio ranging from 97.9% to 100%, and the rate of *B. abortus* is very rare ranging from 0% to 2% [8–11].

Brucellosis has acute, subacute, localized, and chronic forms and can cause long-lasting clinical symptoms, and focal complications

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https://doi.org/10.1016/j.jiph.2018.11.008

[4,6,12]. Despite treatment, relapse rates range from 6% to 11% [13]. Because of heterogeneous and nonspecific symptoms, early and accurate diagnosis of brucellosis is difficult. Although serological tests and culturing methods are available for diagnosis of brucellosis [3,13–15], the diagnosis and treatment monitoring of brucellosis patients are still problem. Culture that is still excepted as 'gold standard' takes time and it has potential risk for laboratory-acquired brucellosis during the identification of growing bacteria [1,16,17]. Although serological tests have been commonly used in rutine laboratories, each has some limitations. In endemic regions, they are associated with a broad range of susceptibility and a lack of specificity. Evaluation of these tests is problematic in persistant infections, recurrent cases, patients with cross-reacting antibodies [14]. Recently many researchers have begun to use polymerase chain reaction (PCR) for diagnosis of brucellosis [18–21]. DNA amplification methods are alternative methods giving results in a short time with a high sensitivity and specificity. They also reduced laboratory acquired brucellosis [16,18,19]. The most important issues in these methods are the false negative results because of inhibitors in clinical samples and limited analytical sensitivity of classic PCR requiring electrophoresis [18,22]. To overcome of these limitations, real-time PCR methods have been used in most laboratories [16,22,23]. In our laboratory, we optimized a multiplex RT-PCR approach for diagnosing of human brucellosis in the serum samples having spiked *Brucella melitensis* with a concentration of 10^2-10^4 cfu/ml. Its sensitivity was found to be 93.3% and this method was able to give positive results in the samples with the bacterial level of 10^2 cfu/ml [16]. In present study, we aimed to evaluate diagnostic value of of the optimized mRT-PCR for the accurate diagnosis of brucellosis in serum samples of patients with suspicion of brucellosis.

Material and methods

Patients and clinical specimens

In this study, a total of 249 serum samples were collected from individuals with suspected brucellosis living in Kars, Erzurum, or Erzincan province in Eastern Anatolia Region with high brucellosis insidence in Turkey. All available information such as age and sex of patients, clinical and laboratory data were retrospectively recorded. The suspected cases had clinical symptomes such as fever, arthralgia, night sweating, weight loss, and malaise which are compatible with brucellosis, and they were admitted to hospital for rutine laboratory diagnosis of the brucellosis. The patients with clinical symptoms that lasted less than two months were classified as acute cases and those having symptoms more than six months were considered as chronic brucellosis [24]. Serum samples were tested for the presence of *Brucella* specific antibody in laboratories of participating hospitals by commercial serological tests such as Rose-Bengal (RB) slide agglutination test (Seromed Laboratory product, Istanbul/Turkey), standart tube agglutination test (STAT) (Croma test Linear Chemicals, Barcena/Spain), Brucella Coombs' test (Plasmatec laboratories limited United Kingdom), and immunocapture test (ICT) (Master Lab Biological Products, Istanbul/Turkey). Blood culture was done for 153 of the patients after draw of blood samples at the particpating centers, which were Erzurum and Erzincan, by using BD Bactec Plus Aerobic/F medium (Becton, Dickenson and Company, NJ, USA) or BacT/ALERT® (BioMérieux, Marcy l'Etoile, France). The inoculated blood culture vials were incubated in instrument and monitored for 21 days or until getting positive signal. *Brucella* were identified by using conventional methods such as Gram staining, oxidase, catalase, and urease tests, and positive agglutination reaction with *Brucella* specific antiserum [25].

Table 1
Oligonucleotid primers and probes used in multiplex RT-PCR [16,28].

Targets	Primers and probes
Brucella spp.	Forward:5′-GCTCGGTTGCCAATATCAATGC-3′ Reverse:5′-GGGTAAAGCGTCGCCAGAAG-3′ Probe: 5′-Joe-AAATCTTCCACCTTGCCCTTGCCATCA-Tamra-3′
B. melitensis	Forward: 5′-AACAAGCGGCACCCCTAAAA-3′ Reverse: 5′-CATGCGCTATGATCTGGTTACG-3′ Probe: 5′-Fam-CAGGAGTGTTTCGGCTCAGAATAATCCACA-Tamra-3′
B.abortus	Forward:5′-GCGGCTTTTCTATCACGGTATTC-3′ Reverse: 5′-CATGCGCTATGATCTGGTTACG-3′ Probe: 5′-HEX-CGCTCATGCTCGCCAGACTTCAATG-BHQ1-3′
GAPDH	Forward:5′-CCACCCATGGCAAATTCC-3′ Reverse:5′-TCGCTCCTGGAAGATGGTG-3′ Probe: 5′-Rox-TGGCACCGTCAAGGCTGAGAACGT-BHQ2-3′

All serum samples were stored at −20 °C in local laboratories until they were sent to Ankara Yıldırım Beyazıt University (AYBU) Medical Faculty Molecular Microbiology Laboratory for mRT-PCR study. The patient's information forms were also sent to AYBU together with the serum samples.

Patients having positive culture for *Brucella* spp. were evaluated as definitive cases [6]. Patients having comparible clinical data together with the presence of *Brucella* total antibody titer of ≥1/160 by STAT, a Coombs' or immunocapture titer of ≥1/320, or presence of *Brucella* DNA in a clinical specimen by mRT-PCR assay were classified as presumptive cases [6,18,26].

Ethical consideration

This study was approved by the ethics committee of Ankara Yıldırım Beyazıt University, Ankara/Turkey (Protocol code: 2017-125).

Molecular microbiological studies

Serum samples were analyzed for brucellosis by multiplex real time-polymerase chain reaction (mRT-PCR) following the protocol optimized previously [16]. DNA extraction from serum samples were performed using a Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Germany) in accordance with the manufacturer's instructions [27]. DNA concentration was measured by using NanoDrop Spectrophotometer (Thermo Fisher Sci, Waltham, MA, USA). The primers and probes described by Probert et al for amplification of *Brucella* genus, *B. melitensis,* and *B abortus* were used [28]. The target of primers and probe used for *Brucella* genus was *Brucella* cell surface 31 kDa protein (bcsp31) gene, which is highly conserved in *Brucella* genus and presents its all biovers [28,29]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was performed as internal control [16]. Primers and flourescent labelled probes used in this study were shown in Table 1. Multiplex RT-PCR tests were carried in a total volume of 25 μl using CFX-96 Real-time system (BioRad, Hercules, CA, USA). PCR reaction tube contained 12.5 μl 2X Ampliqon multiplex PCR master mix (Ampliqon, Odense, Denmark), 0,8 μl of each primers (10 pmol/μl) and probes (5 pmol/μl) for *Brucella* spp, *B. melitensis*, *B. abortus*, and GAPDH and 3 μl template DNA. Thermal-cycling conditions were as follows: an initial denaturation at 95 °C for 10 min, followed by 44 cycles at 95 °C for 20 s, at 60 °C for 50 s, and at 72° for 50 s. Positive and negative controls were used for each experimental study [16].

Statistical analysis

The data were statistically analyzed using SPSS program (software version 17.0). Differences in detection rates of brucellosis in

Table 2
Clinical and demographic data of the 215 brucellosis patients analyzed.

	Number of the patients (%)
Gender	
Female	92 (42.8)
Male	123 (57.2)
Age	
Children (<18 age)	76 (35.3)
Adult (18-60 age range)	101 (47.0)
Elder (≥60 age)	38 (17.7)
Clinical data	
Acute brucellosis	210 (97.7)
Chronic brucellosis	5 (2.3)
Arthritis	71 (33.0)
Arthralgia	164 (76.3)
Fever	117 (54.4)
Malaise	98 (45.6)
Weight loss	75 (34.9)
Night sweating	59 (27.4)

different age groups, gender, and the tests used were evaluated using the chi-square (χ^2) test. A p-value of <0.05 was considered statistically significant.

Results

The clinical and demographic data of the patients

According to laboratory test results, 215 of 249 suspected patients with clinical symptoms comparible with brucellosis were diagnosed as brucellosis cases. Age of the brucellosis patients ranged from 33 months to 88 (mean age of 30 years) years. The most of the patients (47%) was in the 18–60 years of age group, followed by patients younger than 18 years old (35.3%), and patients older than 60 years old (17.7%). Of the patients, 123 (57.2%) were male, 92 (42.8%) were female. There was not a significant difference according to genders ($p < 0.05$). The most common symptom was the arthralgia observed in 76.3% of the patents followed by fever (54.4%), malaise (45.6%), weight loss (34.9%), and night sweating (27.4%) (Table 2). Of 215 brucellosis patients, 210 (97.7%) had acute brucellosis, and 5 (2.3%) had chronic brucellosis.

Blood culture and serological test results

Blood culture yielded positive results in 36 (23.5%) of the 153 suspected patients whose culture results were available. These patients were considered as "definitive brucellosis" cases. *B. melitensis* was identified from all of the positive cultures.

At least one serological test result was available in 249 samples collected from suspected patients. Ninety-six samples from Kars province had ICT and RB test results, 56 samples from Erzurum province had RB, STAT and Coombs' test results, and 96 samples from Erzincan province had RB, STAT, and ICT results. RB slide agglutination test used as screening test was positive in 239 (96%) samples. Ten samples from Erzurum province yielded no agglutination by RB test, but six of these sera were positive by STAT and/or Coombs test. Positivity rate of STAT, Coombs' tests, and ICT was 57.5%, 83%, and 73%, respectively (Table 3). The 162 suspected patients whose blood culture negative or not available, but STAT antibody titer was ≥1/160, ICT and/or Coomb's antibody titer was ≥1/320 were evaluated as a "presumptive case".

Multiplex real time-PCR results

Among the 249 serum samples of the suspected cases, 159 (63.9%) were PCR positive for *Brucella* spp and 90 (36.1%) were

Table 3
Blood culture and serological test results in the serum samples tested.

Test/Result	Blood culture n (%)	STAT n (%)	Coombs' test n (%)	ICT n (%)
Positive	36 (23.5)	88 (57.5)	47 (83)	141 (73)
Negative	117 (76.5)	65 (42.4)	9 (17)	53 (27)
No of samples tested	153	153	56	193

STAT: Standard tube agglutination test, ICT: Immunocapture test. STAT antibody titers ≥1/160 was considered as positive [30]. Coombs' test and Immunocapture antibody titers ≥1/320 was considered as positive [18,25].

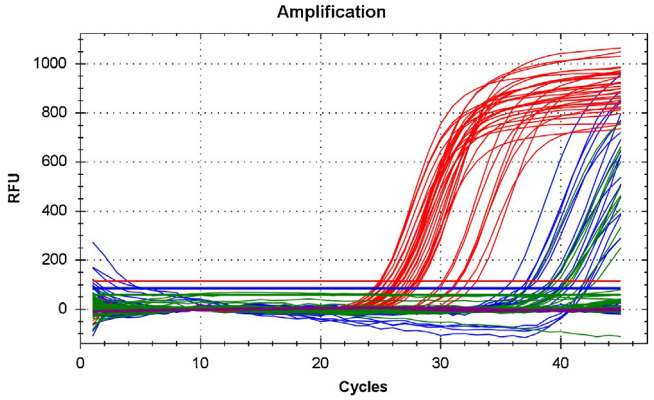

Fig. 1. Amlification curve of mRT-PCR. Blue amplification curve represented *Brucella* spp., green amplification curve represented *B. melitensis* and red amplification curve represented GAPDH.

negative. Mean DNA concentration of the samples having PCR positive results was 1.642 ± 0.280 ng/μL. It was 1.363 ± 0.190 ng/μL for PCR negative samples. Out of the 159 mRT-PCR positive samples; 156 (98.1%) were positive for both *Brucella* spp. and *B. melitensis*, and 3 (1.9%) were positive for only *B. abortus*. The internal control GAPDH was positive in both *Brucella* mRT-PCR postive and negative samples (Fig. 1).

Diagnostic efficacy of mRT-PCR method was evaluated in the serum samples yielding positive results by blood culture or in the samples having significant antibody titres that were STAT score of ≥1/160, and an ICT or Coombs' test score of ≥1/320. Of the 36 patients with positive blood cultures, 35 (97.2%) were mRT-PCR positive. Only one culture positive sample having also high antibody titer by STAT (1/1280) and Coombs' test (1/640) yielded negative results by mRT-PCR repeated two times. No PCR inhibitor was detected in this sample. Multiplex RT-PCR was also positive in 61 samples yielding negative results with culture. Of the 61 mRT-PCR positive samples, 49 (80.3%) had also significant antibody titers by STAT, Coomb's test and/or ICT. PCR positivity was significantly higher in blood culture positive samples than those of culture negative (97.2% versus 52.1%, respectively; $P < 0.05$). Multiplex RT-PCR positivity rates were also significantly higher among the serologically positive sera than those of seronegative. Its positivity rates in the serum samples with positive results by STAT, Coombs, and ICT were 77.3%, 83%, and 70.2%, respectively (Table 4). Multiplex RT-PCR detected *Brucella* specific DNA in 17 samples that had *Brucella* specific antibody titers less than significant titers. Sixteen of these samples were culture negative, but RB was postive. Multiplex RT-PCR increased number of presumptive cases from 162 to 179.

Evaluation of mRT-PCR results according to antibody titers indicated that PCR positivity steadly increase by the increasing of antibody titers. Multiplex RT-PCR yielded positive results in 28%, 31.8%, 68.2%, 87.5% of the sera with STAT antibody titers of <1/20, 1/40, 1/160, and 1/640, respectively. The positivity of mRT-PCR increased from 22.2% in the samples with Coomb's test antibody titer of ≤1/160–88.9% in serum samples having antibody titer of

Table 4
Comparison of mRT-PCR positivity by blood culture and serological test results.

	Multiplex RT-PCR positivity n (%)
Blood culture (n = 153)	
Negative (n = 117)	61 (52.1)
Positive (n = 36)	35 (97.2)
Immunocapture (n = 193)	
Negative (n = 52) (<1/320)	20 (38.4)
Positive (n = 141) (≥1/320)	99 (70.2)
Coombs' test (n = 56)	
Negative (n = 9) (<1/320)	2 (22.2)
Positive (n = 47) (≥1/320)	39 (83)
STAT (n = 153)	
Negative (n = 65) (<1/160)	24 (37)
Positive (n = 88) (≥1/160)	68 (77.3)
STAT and ICT/Coombs'test	
Negative (n = 42)	17*(31)
Positive (n = 79)	64 (81)

* Only one sample was culture positive.

Table 5
Multiplex RT-PCR positivity rates according to titers of serological test results.

	Antibody titers	Multiplex RT-PCR positivity: n (%)
STAT (n = 153)	<1/20 (n = 25)	7 (28)
	1/20 (n = 1)	1 (100)
	1/40 (n = 22)	7 (31.8)
	1/80 (n = 17)	9 (52.9)
	1/160 (n = 22)	15 (68.2)
	1/320 (n = 24)	16 (66.7)
	1/640 (n = 24)	21 (87.5)
	1/1280 (n = 18)	16 (88.9)
Coombs test (n = 56)	<1/160 (n = 3)	0 (0)
	1/160 (n = 6)	2 (33.3)
	1/320 (n = 11)	7 (63.6)
	1/640 (n = 35)	31 (88.6)
	1/1280 (n = 1)	1 (100)
Immunocapture (n = 193)	<1/40 (n = 3)	0 (0)
	1/40 (n = 10)	2 (20)
	1/80 (n = 8)	5 (62.5)
	1/160 (n = 31)	13 (41.9)
	1/320 (n = 27)	14 (51.9)
	1/640 (n = 23)	14 (60.9)
	1/1280 (n = 42)	35 (83.3)
	1/2560 (n = 24)	16 (66.7)
	1/5120 (n = 24)	20 (83.3)

≥1/640. Similarly, while mRT-PCR positivity was 15.4% in the sera having antibody titers of ≤1/40 detected by ICT, it raised to 78.9% at antibody titer of ≥1/1280 (Table 5).

Discussion

Brucellosis often indicates atypical and nonspecific symptoms. Clinical data and history of the patient are important for the diagnosis; however, diagnosis by clinical signs alone is not advised and it relies predominantly on laboratory testing [1,6,14]. Although it is more prevalent in males, its incidence is not directly related to gender [12,30]. In our study, 57.2% of the patiens were male. In contrast to our result, a study carried on 1922 brucelosis patients in Erzincan province indicated higher positivity rate in female than male (53% versus 47%, respectively) [31]. In concordance with the literature, there was not an important difference between genders. Our study showed that brucellosis mainly observed in patients aged 18–60 years (47%), followed by the patients younger than 18 years old (35.3%). In agreement with the previous studies performed in Turkey [12,32,33], the current study indicates that brucellosis is a problematic disease in the young and middle-aged population. Brucellosis may present with acute, subacute, and chronic forms [1]. The most predominat form is acute form [1,12]. Brucellosis generally presented with high fever, myalgia and arthralgia of the large joints [1,12,30]. In agreement with the literatures, in our study the fever (54.4%) and arthralgia (76.3%) were the common clinical manifestations. and acute brucellosis was predominant (97.7%).

The "gold standard" for the diagnosis of brucellosis is the isolation and identification of the *Brucella* spp. from blood or bone marrow specimens [6,15,17]. However, its positivity showed big variation. A study investigating the performance of the two automated blood culture systems reported that BACTEC 9240 system detected 52.9% and the BacT/ALERT detected 82.3% of brucellosis patients [34]. In another study, the positivity of BACTEC 9120, VITAL systems, and the Hémoline biphasic flasks were detected as 73.7%, 94.7%, and 47.4%, respectively [35]. In a study performed in 91 patients from Turkey, blood culture positivity was found to be 31% [12]. In the current study, among 153 patients having blood culture results, 23.5% were blood culture positive and they were considered as "definitive brucellosis". In aggrement with the literature, the current study indicated that even culture is still accepted as 'gold standart' its positivity rate can be very low, in addition to its long incubation period and laboratory-acquired brucellosis risk [1,17,34,35].

Although there are many commercial serological tests that are commonly used for diagnosis, they have important limitation such as false positive and negative results, disconcordance between each other [14,18,36]. Because of these limitations in commercial tests, in brucellosis suspicious patients, RT-PCR can be an alternative to make a final decision. It does not require post amplification handling of PCR products and reduce laboratory contamination risk and false positivity [14,37]. In a study, Mohseni et al compared the performance of ELISA, classic PCR and STAT methods on 100 serum samples of suspected patients. They found that 50% of the samples were positive by STAT; 45% was positive by PCR. In their study, the samples with STAT positive (≥1/160 titers) were also PCR positive [20]. Purwar et al evaluated performances of conventional serological techniques and classic PCR on the 400 acute undifferentiated fever patients with elevated base line antibody titers. STAT, PCR, and culture gave positive result in 34, 32, and 20 patients, respectively. In their study, all patients having STAT antibody titer of ≥160 IU/ml were also positive by PCR [21]. A Syria study compared the performance of RT-PCR with conventional methods and positivity rate of agglutination, culture, *Brucella* IgG, and RT-PCR tests was found as 53%, 35%, 35%, and 29% of the 34 samples collected from suspected brucellosis, respectively [23].

In the current study, mRT-PCR and conventional tests were compared in the serum samples collected from the brucellosis suspected patients. The highest positivity rate (83%) was obtained by Coombs' test, followed by ICT (73%), mRT-PCR (64%), and STAT (57.5%). Multiplex RT-PCR positivity rates significantly increased to 97.2% in the culture positive samples. Its sensitivity in the samples with significant antibody titers by STAT, ICT, Coombs' test, and both STAT and ICT was 77%, 70%, 83%, and 81% respectively. In aggrement with previous studies [18,19,38], mRT-PCR also yielded positive results in some samples that were negative by either culture (52%) or any of STAT (37%), ICT (38%), Coombs tests (22%), and both STAT and ICT/Coombs' test (31%). Ditealed analysis of the results indicated that majorty of these mRT-PCR positivities was confirmed by another serological test or culture. For example, 49 (80.3%) of 61 mRT-PCR positive/culture negative samples were also serologically positive. Three of the 20 mRT-PCR positive/ICT negative samples were STAT positive. One of the two mRT-PCR positive/Coombs' negative samples were positive by culture and other was positive by STAT. Of the 24 mRT-PCR positive/STAT negative samples, 16 were positive by ICT and/or Coombs' test and three were culture positive. These results showed that multiplex RT-PCR positivity together

with corresponding clinical findings in the samples having negative results by conventional methods should be considered as true positive.

As it was expected, there was well correlation between mRT-PCR positivity and antibody titers. For example, mRT-PCR positivity increased from 36.9% at STAT titer of <1/160–88.8% at STAT titer of 1/1280. While mRT-PCR was negative in all samples with Coomb's antibody titer of <1/160, its positivity increased to 88.5% at antibody titer of 1/640. Similarly, mRT-PCR was negative all serum samples with antibody titers of ≤1/40 with ICT, it was positive in 83.3% of the sera with antibody titer of 1/5120. In the current study, mRT-PCR detected *Brucella* DNA in 17 serum samples having *Brucella* specific antibody less than significant titers. Detailed analysis of these 17 serum samples showed that one of these sera was culture positive. Of the remaining 16 sera, 10 had ICT antibody titer of 1/160, 4 had ICT antibody titer of 1/80, 2 had ICT antibody titer of 1/40 and all these sera gave agglutination by RB test. These results indicate that using mRT-PCR can increase number of presumptive cases. In parallel to our study, Garshasbi et al. found PCR positive in 95.5% of the sera having STAT antibody titer of ≤1/160 [19]. Alsayed et al found that 3 (9%) of 34 patients were positive by RT-PCR but negative by agglutination tests or culture [23]. From this data, one can speculate that negative titers of the serological tests mainly 1/160 titer of ICT and 1/80 titer of STAT might be considered as positive.

On the other hand, mRT-PCR method could not detected *Brucella* specific DNA in one culture positive and 45 seropositive samples. The false negativity of PCR method is mainly resulted from presence of a number of *Brucella* below the analytical sensitivity of the test used and presence of inhibitor in amplification tube [18]. In the current study, we used GAPDH gene as an internal control and it was amplified in all mRT-PCR reaction tubes, indicating there was no PCR inhibitors. The analytical sensitivity of the mRT-PCR method used in this study was previously estimated as 10^2 cfu/ml, at this concentration it gave positive results in 80% of the tested samples [16]. From these results, we can supeculate that the mRT-PCR negativity in brusellosis cases is most probable coming from the presence of low level *Brucella* ($\leq 10^2$) in samples. As a proof for this speculation, we measured DNA concentration for the culture positive, PCR negative sample as 1.410 ng/μL, which was lower than the mean DNA concentration (1.642 ± 0.280 ng/μL) which yielded positive PCR results in our study.

The current study indicated that *B. melitensis* was the causative agent of 98.1% of brucellosis cases in our study population; *B. abortus* was only detected in limited cases. This result is in aggrement with the previous data reported from various regions in Turkey [8–11]. Similarly, in Greece which is neighbor country, it was found that *B. melitensis* was responsible for all human cases [33]. In contrast to this result, a current study from Iran that is another neighbour country indicated that majority of the human brucellosis cases were resulted from *B. abortus* [19].

Although the current study provided very useful data in diagnosis of brucellosis, it has some limitations. First, since molecular study was performed on the sera previously collected from three different health centers, follow up study could not be done on our cases. Second we were not able to do blood culture for all samples. Third, it would be more value if all serum samples could be tested by three serological tests that were STAT, ICT, and Coombs' test.

In conclusion, brucellosis is an infection with multiple presentations and early diagnosis is needeen for avoiding complications. Simultaneous use of serology and mRT-PCR can overcome limitations of diagnosis. Multiplex RT-PCR is a useful tool to identify more brucellosis cases from suspected patients and to reduce isolation time and contamination risks in culture methods. It can be preferred as a rapid and sensitive diagnostic method in patients living high endemic regions, in patients with negative serologic test results and in the detection of the *Brucella* species from clinical samples.

Funding

This study was partly supported by Ankara Yıldırım Beyazıt University, Reseach Foundation [Grand number:4759].

Competing interests

None declared.

Ethical approval

Not required.

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