Original Article

Evaluation of Different Primers for Detection of *Brucella* in Human and Animal Serum Samples by Using PCR Method

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Abstract

Background: The Polymerase Chain Reaction (PCR) method can overcome the limitations of conventional methodology. The aim of this study is to evaluate three primer pairs broadly used B4/B5, F4/R2 and JPF/JPR, for detection of *Brucella* by PCR, in human and animal serum samples and to determine analytic sensitivity of primers.

Methods: Total of 68 serum samples were collected during the acute phase of brucellosis. 10-fold serial dilutions were prepared from bacterial suspension and serum suspension using *Brucella abortus* S19. DNA was isolated using boiling. The best dilution from DNA was determined for PCR with three primer pairs. PCR was performed using primer pairs on all bacterial dilutions, serum dilutions, 1/200 dilutions and serum samples. Comparison of sensitivity between three primer pairs was performed with statistical analysis.

Results: The best DNA dilution was 1/200. From 68 serum samples, 54 cases (79.41%), 44 cases (64.70%) and 35 cases (51.47%), were positive by PCR with B4/B5, F4/R2 and JPF/JPR respectively. B4/B5, F4/R2 and JPF/JPR were able to identify 9×10^2, 9 and 9×10^5 bacteria in 1 ml of bacterial suspension and 9×10^4, 9×10^5 and 9×10^7 bacteria in 1 ml of dilution 1/200 of Serum dilutions respectively. The differences between primers by statistical analysis were significant for human, animal and total samples.

Conclusion: No band was observed in dilutions one of DNA isolated from serum. Therefore, to decrease the effects of inhibitors, DNA was diluted. When DNA isolation is boiling, F4/R2 and B4/B5 have the greatest sensitivity for purified bacteria and serum in the detection of *Brucella* respectively. DNA isolation by boiling can decrease the PCR costs.

Keywords: *Brucella,* primer, PCR, serum

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Introduction

Brucellosis remains a major zoonosis in some communities[1] and according to the World Health Organization (WHO), each year more than half a million new cases are reported worldwide.[2, 3]

Currently, diagnosis of this zoonosis is based on microbiological and serological laboratory tests.[4] Nucleic acid amplification methods such as PCR can overcome the limitations of conventional detection methods as they are rapid, sensitive, high specific and low cost.[1,5,6] Detection of *Brucella* DNA by PCR is more sensitive than blood culture and more specific than serological tests for acute disease.[3] PCR is a useful method for *Brucella* in detection of acute, follow-up, recurrent and chronic stages.[6] Using the different *Brucella* genes in cases which serological titers is higher than 1/160, has different results.[4,7] PCR can be used in clinical laboratories,[6] but complexity of this method, makes PCR low using in laboratories routinely.[8,9,10] Also the presence of factors such as immunoglobulin G (IgG),[11,12] proteins and polysaccharides in serum inhibit DNA amplification.[5,7,13] These inhibitors are important in clinical samples such as serum where concentration of DNA is low.[11,14]

Analytical sensitivity of PCR is determined by purified organism or cloned DNA targets.[15] The sensitivity of PCR is affected by different factors such as DNA extraction method, and the presence of the foreign DNA and inhibitors.[13] Despite the high speed, high sensitivity and specificity of PCR,[16] sometimes inhibitors may decrease the sensitivity of this method.[17]

Several regions of the *Brucella* genome has identified and used in PCR techniques.[18,19] DNA is released into bloodstream during bacteremia.[12,20] Although the concentration of DNA in serum is lower than whole blood, but because of severe decreasing in PCR inhibitors, this sample is the preferred specimen for diagnosis of brucellosis.[1] According to previous studies, the diagnosis of brucellosis using serum is rapid, safe and suitable.[6] The successful isolation of nucleic acid from clinical samples is very important in detection of infection.[21] The boiling method for DNA isolation process is simple, reproducible, fast and effective, and does not require sophisticated equipments.[12,22,23]

Three pairs of primers including B4/B5,[24] F4/R2[25] and JPF/JPR[26] have already used for detection of *Brucella*. B4/B5 has been often used for diagnosis of brucellosis and amplifies a 223 bp fragment of the gene encoding 31-kDa antigen of *Brucella abortus* (*BCSP31* gene).[4,13] F4/R2 amplifies a 905 bp fragment of gene encoding *16s rRNA* sequence of *Brucella abortus* and JPF/JPR amplifies a 123 bp fragment of gene encoding *omp2* (Table 1).[3,4,13] Based on many genetic researches, these three genes are largely preserved in all *Brucella* species and biovars, and these three primer pairs are able to identify almost all *Brucella* species and biovars.[24,25]

Direct detection of *Brucella* by PCR in clinical samples with the origin of human and animal, has been investigated in a few

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studies.[4,27,28] Further research should be done to compare the sensitivity of primer pairs.[4,22,29,30] The results of these investigations depend on the clinical samples, the DNA isolation method and the stages of disease.[31]

The aim of this study was to evaluate and compare the sensitivity of three primer pairs broadly used (B4/B5, F4/R2 and JPF/JPR) for detection of *Brucella* genus by PCR, in human and animal serum samples with DNA isolation using the boiling method. The analytical sensitivity of primer pairs on bacterial and serum suspension was also determined, using the same DNA isolation method.

Material and Methods

Serum samples

In this study, 38 human and 30 sheep serum samples were collected. The human samples were prepared from patients with acute brucellosis from one hospital and four private laboratories in Hamedan Province Iran (Nahavand). Antibody titers with Wright, 2ME and Coombs Wright tests were determined higher than 1/40 for human samples.

Animal samples were also collected with help of Department of Veterinary of Hamedan Province from aborted sheeps. None of them had the history of vaccination. Antibody titers with Wright and 2ME tests were 2/80 to 4/80 for animal samples.

Determination of analytical sensitivity

Brucella abortus strain S19 was cultured on blood agar with CO_2 and was incubated for three days. After observation of the pure and needle shape colonies on culture media, *Brucella* was confirmed by microbiological tests, including catalase, oxidase and gram staining. Then a pure culture of bacteria was prepared. Using sterile distilled water, a cell suspension of bacteria with a concentration equivalent to 3 McFarland (9×10^8 bacteria in 1 ml) was prepared and then 10 serial dilutions 10-fold (dilutions 10^0 to 10^{-9}) were prepared. A total of 200 µl of each dilution was poured into separate tubes. Also, 150 µl from suspension with 150 µl of *Brucella* negative serum in a 1.5 ml tube was mixed, and vortexed. Then, five serial dilutions 10-fold (from 10^0 to 10^{-4}) were prepared. The serum dilutions were incubated overnight and processed for DNA isolation.

DNA isolation from purified bacteria, serum suspension and serum samples

The boiling method was selected for DNA isolation from pure bacteria and serum because it is a cheap and easy method, without the need for sophisticated equipments and extraction stages in kits. DNA isolation by boiling method was performed as follows: first 200 µl of serum (or bacterial suspension or serum suspension) poured into a 1.5 ml tube and then centrifuged for 15 minutes at 15000 × g. The supernatant was discarded and then 200 µl of sterile distilled water was added, vortexed and centrifuged for 10 min at 15000 × g. The supernatant was discarded and 40 µl of sterile distilled water was added, vortexed and placed in a water bath at 95 – 97˚C for 10 minutes until the bacterial cell wall lysed and cell contents released. Immediately, tubes were placed in the freezer at -20˚C for 2 minutes. Then, tubes were centrifuged for 10 seconds at 15000 × g and were placed for later uses in the freezer -20˚C. The DNA of each serum dilution also was diluted 1/200. The DNA isolated from dilution 1 of *Brucella abortus* strain S19 was used as positive control in all PCR protocols. To ensure the presence of DNA in serums, nine DNA samples with different serological titers were assessed by Nanodrop, and these samples were taken on the gel electrophoresis using loading buffer.

PCR assays

In this study, three pairs of primers including B4/B5 (modified in this study), F4/R2 and JPF/JPR were used for detection of *Brucella*. Primer pairs were studied using the bioinformatics softwares Allele ID7, Primer premier 5 and Base stacking Tm Online. The DNA sequence comparisons with GenBank database were searched and assessed for species or genus assignment using BLAST (basic local alignment search tool; National Center for Biotechnology Information). Small modifications were performed on the sequences B4 and B5. Profiles of genomic sequences of primer pairs are shown in Table 1.

To prepare the main solution for PCR with primers B4/B5, F4/R2 and JPF/JPR, ingredients were mixed according to table 2 in tubes 0.2 ml DNAse free in total volume 25 µl**.** Premix Master Mix was ordered from Pars Tus Iran. The contents of this Master Mix include Taq polymerase enzyme, PCR buffer, Protein Stabilizer, loading solution blue 2x and dNTPs of four types with equal ratios. The primers were ordered from Denazist Asia, Iran and synthesized by Macrogene Korea. From solution 10 pmol primers were used in all experiments.

To resolve the problem the low DNA in serum, PCR cycles were raised from 35 to 40 cycles, and to stop or decrease the effects of inhibitors, DNA extracted from one serum sample that was positive by PCR, was diluted to ratios 1/100, 1/200, 1/300 and 1/500 and with three primer pairs PCR was performed to determine the best dilution for PCR. PCR was performed using three primer pairs on all bacterial dilutions, serum dilutions, 1/200 dilutions and serum samples.

The PCR program for each primer pair in the articles had a little difference in stages and cycles; therefore it was needed to select proper programs in order to provide the best results. The program chosen for the primers B4/B5 include: initial denaturation at 90˚C for 5 min, denaturation at 90˚C for 60s, annealing at 53˚C for 60s, extension at 72˚C for 60s with 40 cycles and finally the final extension at 72˚C for 10 min. The program chosen for primers F4/R2 includes: initial denaturation early at 95˚C for 5 min, denaturation at 95˚C for 30s, annealing at 54˚C for the 90s, extension at 72˚C for 90s with 40 cycles, and finally the final extension at 72˚C for 6 min. The program chosen for primers JPF/JPR included: initial denaturation at 94˚C for 4 min, denaturation at 94˚C for 60s, annealing at 58˚C for 60s, extension at 72˚C for 60s with 40 cycles and finally the final extension at 72˚C for 10 min.

All serum DNAs were diluted 1/200 with sterile distilled water (the best dilution for test). To ensure the absence of contamination and false-positive results, a positive control and a negative control were also set up along with each PCR. Reactions were placed in a thermal cycler (Biorad German) without mineral oil. The B4/B5, F4/R2 and JPF/JPR products were electrophoresis on 1.6%, 1.4% and 1.6% gel agarose respectively, stained using ethidium bromide and were observed under UV light, using transuliminator. TAE buffer for gel preparation was ordered from the Denazist Asia Company.

Comparison of results

Comparison of sensitivity between three primer pairs was performed using (a) serial dilutions of purified bacterial suspensions (b) serial dilutions of serum suspension (c) dilutions 1/200 of

Table 1. The primers and genes used in PCR

Primer	Sequence 5′ → 3′	Gene	Product
B4	TGGCTCGGTTGCCAATATC	*BCSP31*	222 bp
B5	CGCTTGCCTTTCAGGTCTG		
F4	TCGAGCGCCCGCAAGGGG	*16s rRNA*	905 bp
R2	AACCATAGTGTCTCCACTAA		
JPF	GCGCTCAGGCTGCCGACGCAA	*OMP2*	193 bp
JPR	ACCAGCCATTGCGGTCGGTA		

Table 2. Materials for preparation of PCR main solution

Materials/primers	B4/B5	F4/R2	JPF/JPR
Master Mix 1X	10 µl	10 µl	10 µl
Primer (10 pmol)	0.7 µl for each	1 µl for each	0.9 µl for each
Twice sterile distilled water	8.6 µl	8 µl	8.2 µl
DNA (< 250 ng/ml)	5 µl	5 µl	5 µl
Total volume	25 µl	25 µl	25 µl

Table 3. The results of PCR on human and animal serum samples with three different primer pairs

Samples	n	B4/B5		F4/R2		JPF/JPR	
		P	N	P	N	P	N
Human samples	38	33 (86.84%)	5 (13.16%)	27 (71.05%)	11 (28.95%)	22 (57.89%)	16 (42.11%)
Animal sample	30	21 (70%)	9 (30%)	17 (56.67%)	13 (43.33%)	13 (43.33%)	17 (56.67%)
Total samples	**68**	**54 (79.41%)**	**14 (20.59%)**	**44 (64.70%)**	**24 (35.30%)**	**35 (51.47%)**	**33 (48.53%)**
P: positive, N: negative, n: number							

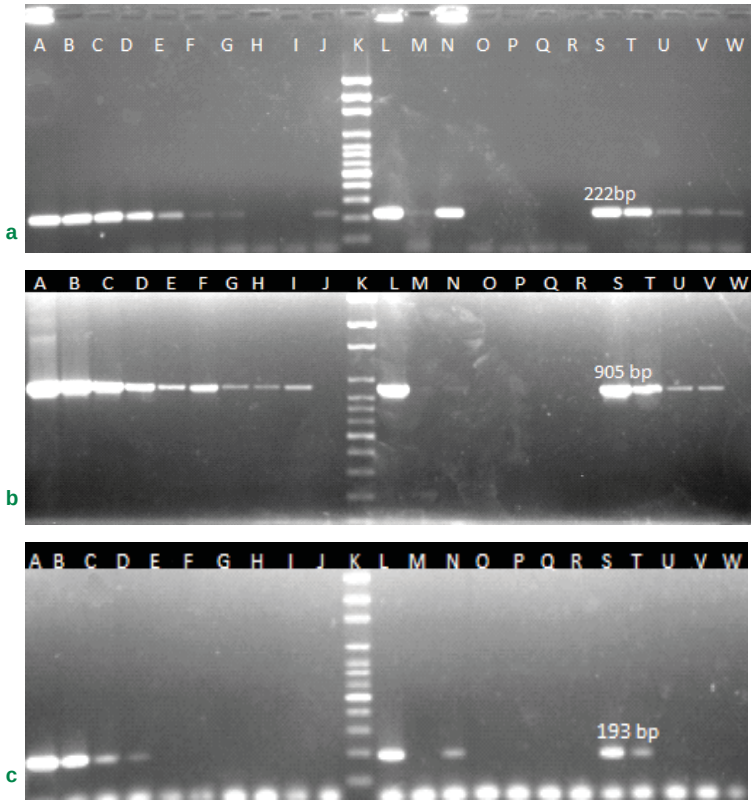

Figure 1. The results of determination analytical sensitivity in detecting *Brucella*, **a)** using primer pair B4/B5; **b)** using primer pair F4/R2; **c)** using primer pair JPF/JPR. Columns A, B, C, D, E, F, G, H, I, J: serial dilutions of purified bacterial suspension from 1 to 10^{-9} respectively, column K: ladder 100 bp, column L: positive control, column M :negative control, columns N, O, P, Q, R: serial dilutions of serum suspension from 1 to 10^{-4} respectively, columns S, T, U, V, W: dilution 1/200 from DNA isolated from serum serial dilutions from 1 to 10^{-4} respectively.

DNA isolated from serum dilutions and (d) serum samples. Then sensitivity for each pair of primers was determined by detection of limitations for the number of bacteria in PCR. Also, statistical analysis was performed by SPSS20 using method of Chi-Square, on human and animal serum samples separately and together. $P < 0.05$ was accepted as significant.

Results

Results of determination analytical sensitivity

As regard each ml of 3 McFarland contains 9×10^8 bacteria, following results were obtained (Figure 1): B4/B5 was able to identify dilution 10^{-6} of bacterial suspension, 10^0 of serum suspension

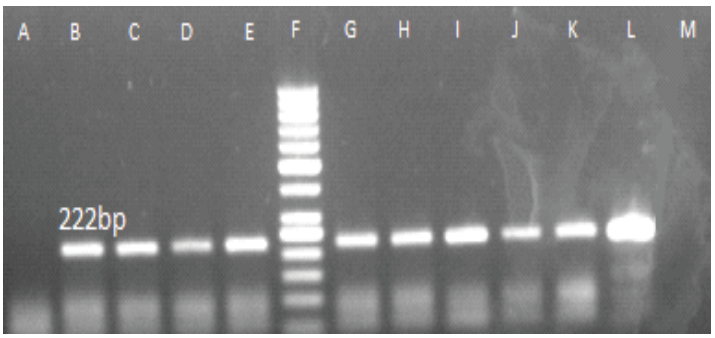

Figure 2. The results of PCR using primers B4/B5 on serum samples. Column F: ladder 50 bp, column L: positive control, column M: negative control, columns B, C, D, E, G, H, I, J, K: positive samples, column A: negative sample.

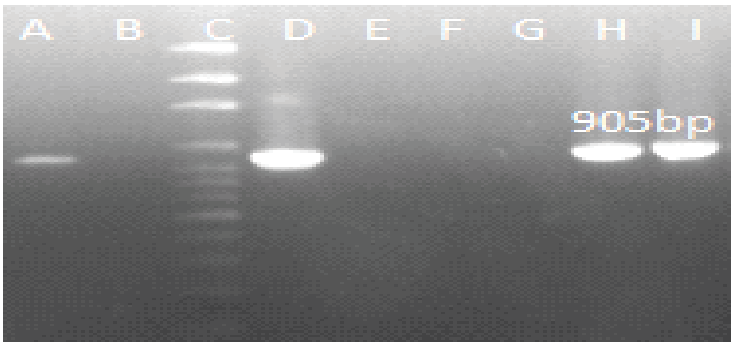

Figure 3. The results of PCR using primers F4/R2 on serum samples. Column C: ladder 100 bp, column D: positive control, column E: negative control, columns A, H, I: positive samples, columns B, E, F, G: negative samples.

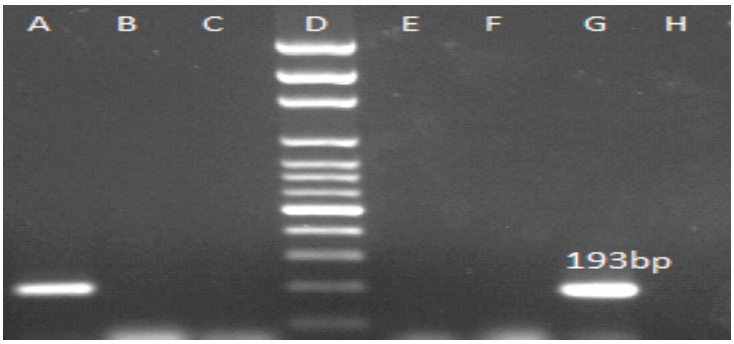

Figure 4. The results of PCR using primers JPF/JPR on serum samples. Column D: ladder 100 bp, column G: positive control, column H: negative control, column A: Positive sample, columns B,C,E,F: negative samples.

and 10^{-4} of dilutions 1/200. Therefore, this primer pair was able to identify 900 bacteria in 1 ml of purified bacterial suspension, 9×10^8 bacteria in 1 ml of serum suspension and 9×10^4 bacteria in 1 ml of dilutions 1/200. F4/R2 was able to identify dilutions 10^{-8} of bacterial suspension, 10^0 of serum suspension and 10^{-3} of dilution 1/200. Therefore, this primer pair was able to identify nine bacteria in 1 ml of purified bacterial suspension, 9×10^8 bacteria in 1 ml of serum suspension and 9×10^5 bacteria in 1 ml of dilution 1/200. JPF/JPR was able to identify dilution 10^{-3} of bacterial suspension, 10^0 of serum suspension and 10^{-1} of dilutions 1/200. So, this primer pair was able to identify 9×10^5 bacteria in 1 ml of purified bacterial suspension, 9×10^8 bacteria in 1 ml of serum suspension and 9×10^7 bacteria in 1 ml of dilutions 1/200.

Results of determination proper dilutions of DNA isolated from serum for PCR

Examination of nine DNA by Nanodrop showed that concentration of DNA was good, but the numerical value of A260/A280 was less than one. The smeared DNA bands were also observed on gel electrophoresis. PCR results on DNA isolated from serum in dilution 1 using each three pairs of primers were negative, but for DNA diluted with different ratios, bands were observable. The best dilutions for B4/B5 to create clear bands were 1/100 and 1/200. Bands with dilutions 1/300 and 1/500 were also detectable. The best dilutions for F4/R2 were 1/100, 1/200, 1/300 and 1/500, and the best dilution for JPF/JPR was 1/200. Dilution 1/100 was also detectable, however dilutions 1/300 and 1/500 were not detectable. It was also found that dilution 1/200 was suitable for all primers and in all tests with three primer pairs on the serum; dilution 1/200 was used.

Results of PCR on DNA serum samples

In total, from 68 serum samples (38 human samples and 30 animal samples) which were tested by PCR using three primer pairs,

following results were obtained (Table 3): In PCR using primers B4/B5, 54 samples (79.41%) were positive, 33 samples (86.84%) of these were in relation with human and 21 samples (70%) were in relation with animal (Figure 2). PCR results from the use of primers F4/R2 include: 44 positive samples (64.70%), 27 samples (71.05%) of these were in relation with human and 17 samples (56.67%) were in relation with animal (Figure 3). In PCR using primers JPF/JPR, 35 samples (51.47%) were positive, 22 samples (57.89 %) of these were in relation with human and 13 samples (43.33%) were in relation with animal (Figure 4).

Statistical analysis by Chi-Square

The results of analysis showed significant differences between primer pairs, so primers B4/B5 in comparison with F4/R2 ($P = 0.001$ in human samples, $p < 0.001$ in animal samples and $p < 0.001$ in total samples), primers F4/R2 in comparison with JPF/JPR ($p < 0.001$ in human samples, $p < 0.001$ in animal samples and $p < 0.001$ in total samples) and primers B4/B5 in comparison with JPF/JPR ($P = 0.009$ in human samples, $P = 0.002$ in animal samples and $p < 0.001$ in total samples), were significant for human serum samples, animal serum samples and total samples. In total, all comparisons were statistically significant.

Discussion

In this study, primers B4/B5, F4/R2 and JPF/JPR were used. These primer pairs encode fragments 222 bp, 905 bp and 193 bp of the genes *BCSP31*, *16SrRNA* and *OMP2* respectively and are able to identify almost all species and biovars of *Brucella*. The aim of this study was to evaluate and compare three pairs of primers, which are broadly used for diagnosis of brucellosis, in human and animal serum samples.

In the present study, examination of DNA isolated from serum with different serological titers for brucellosis by Nanodrop, showed that concentration of DNA was good, but A_{260}/A_{280} which its standard should be ≥ 1, was less than 1. The results showed that other substances are present in DNA samples and are probably inhibitors for PCR. Also, in examination of DNA on the gel electrophoresis, no band was observed that implies the presence of other substances along with DNA in samples. In PCR on DNA, no band was observed in dilutions 1. In dilutions 1/100 to 1/500 bands was observed, so the best result was obtained with DNA dilution with sterile distilled water. This method dilutes inhibitors and decreases its effects on PCR. For DNA isolation from serum samples, boiling was selected and results only depended on the sensitivity of primers. In this study for standard comparison of primers, all conditions were the same except primers and PCR protocols.

The sensitivity of PCR using different clinical samples has not been well studied; therefore the diagnosis is not yet standardized. Still, many investigations in routine laboratory tests for diagnosis of brucellosis are needed.[20] In a study by Navarro and colleagues for determination the analytical sensitivity of primers, F4/R2, B4/B5 and JPF/JPR amplified 8 fg, 5 pg and 20 pg of purified *Brucella melitensis* Rev 1 DNA respectively. They reported that from three primer pairs mentioned above, F4/R2 has the most sensitivity for detection of *Brucella* from purified bacteria, which is consistent with our study. The sensitivity of B4/B5 and F4/R2 was affected by human DNA, but with JPF/JPR was not affected.[4] In a study by Romero, the limit of detection of F4/R2 for *Brucella abortus* 2308 viable cells was 170 CFU/ml, and for *Brucella melitensis* 115 the detection limit was 10-fold concentrated. However, in their previous study the threshold sensitivities of the PCR assay determined by testing serial DNA dilutions were similar for *Brucella abortus* 2308 and *Brucella melitensis* 115 (50 to 60 fg of DNA, corresponding to approximately 15 to 20 cells).[25,28]

In our study 9 bacteria was detectable in pure culture, which according to studies is less than 20 fg. In a study by Queipo-Ortuño, The detection limit of primers B4/B5 in 200 µL of serum spiked with serial dilutions of *Brucella abortus* B19 was one bacterial cell (equivalent to 5 fg of DNA).[10] In our study, B4/B5 was able to detect 9×10^8 bacteria in 200 µL of serum. Mukherjee, analyzed the sensitivity and specificity of three genus-specific PCR for *Brucella* with goals *BCSP31*, *16s rRNA* and *Omp2* on blood samples. Mukherjee investigation showed that B4/B5 had the highest sensitivity (92.72%). JPF/JPR and F4/R2 had the sensitivity of 61.81% and 0 respectively.[30] In a study by Baddour and Alkhalifa, to evaluate above primer pairs on human blood samples, the sensitivity of F4/R2, F4/R2 and JPF/JPR was 98%, 53.1% and 83.1% respectively. F4/R2 had the lowest sensitivity and requires the greatest cells to create positive results.[32] Kanani compared three primer pairs mentioned above for detection of brucellosis from semen cow samples which B4/B5, F4/R2 and JPF/JPR had the highest sensitivity respectively.[19]

In the above-mentioned studies, the best results are in relation with blood samples and purified bacteria. No research has been done to compare the primers in serum using the boiling method. The sensitivity of primers is depending on the clinical sample. In our study, serum samples were used. The highest sensitivity was in relation with B4/B5 with 79.41%. The sensitivity of F4/R2 and JPF/JPR was 64.70% and 51.47% respectively. In PCR using primers B4/B5 and F4/R2, in dilutions 1/100 and 1/200 of extracted DNA, bands were observed. In dilutions 1/300 and 1/500 bands were observed weakly. In PCR using primers JPF/JPR bands were observed only in dilution 1/200 of extracted DNA. In conclusion, the sensitivity of primers JPF/JPR is lower than two other primer pairs.

In a study by Queipo-Ortuño, for diagnosis of brucellosis from serum by a Real time Light Cycler and DNA isolation using boiling method, 91.9% of the samples were positive with primers B4/B5.[10] In another study by Queipo-Ortuño for diagnosis of brucellosis with primers B4/B5 from the serum by Real time, boiling was reported as a rapid, inexpensive and reproducible method which requires no sophisticated equipments.[12]

Some cases were positive in serological tests and negative in PCR. This can be due to the presence of inhibitors in DNA isolated by boiling, the short time of a *Brucella* bacteremia or cross-reaction in serology with other organisms. In PCR for detection of *Brucella,* boiling can be used for DNA isolation and with using this method; costs associated with PCR would be decreased to a considerable amount. B4/B5 had the highest sensitivity in the diagnosis of brucellosis in serum samples, when boiling was used for DNA isolation.

In human samples, the percentage of positive cases by PCR was more than animal samples. So, the clinical diagnosis of human brucellosis is easier than animal brucellosis, because the symptoms suggest acute brucellosis, which in animals has not clear symptoms. In such situations, the diagnosis of chronic cases using PCR is somewhat doubtful. Therefore, more studies are required for diagnosis of brucellosis in acute and chronic phases by

PCR. Using serum rather than whole blood, decreases the effect of inhibitors greatly. Using serum, DNA presented in the sample will be decreased. To compensate these effects, cycles should be increased[8,20] or two times PCR should be performed. In addition, working with serum is simple and detection sensitivity is higher.

In the present study, to decrease formation of primer dimer, the concentration of primers used in reactions was decreased. Also, cycles were raised from 35 to 40 cycles to enhance detection sensitivity of primers. In this study, investigation of primer specificity was not performed on other important bacteria, because these studies have conducted previously.

In the present study, with modifications that were performed by using bioinformatic softwares in B4 and B5 sequences, the sensitivity of primers was greatly increased theoretically. It seems the sequences of this primer pair, in comparison with origin sequences designed by Baily are more sensitive and more specific for diagnosis of brucellosis. So, further studies are needed to evaluate this primer pair and compare it with the reference sequences and clarify which can be more efficient for the diagnosis of brucellosis. B4/B5 had the highest sensitivity using serum samples. So, B4/B5 is preferred for detection of *Brucella* in serum samples and probably other samples such as blood or buffy coat, which have more inhibitors for PCR. Also, it can be used in the detection of *Brucella* in serum when DNA isolation is boiling method. The lower sensitivity of F4/R2 and JPF/JPR is probably because of the low conservation related genes in species of *Brucella*. F4/R2 could detect the minimum number the bacteria in serial dilutions from purified bacteria (1.8 bacteria in 200 µl bacterial suspension). Also, B4/B5 could detect all the serum dilutions 1 to 10^{-4} of dilution 1/200 of serum dilutions. So F4/R2 for detection of *Brucella* in purified bacteria and B4/B5 for detection of *Brucella* in serum samples, have the greatest sensitivity.

Brucellosis in Iran each year imposes huge financial losses on the country's regulatory system. Animals usually do not show clinical signs of the disease. Therefore, the laboratory diagnosis of disease is very important along with the clinical diagnosis to decrease the costs associated with this disease. Detection of *Brucella* DNA by PCR is more sensitive than blood culture and more specific than serological tests for acute disease. Also, PCR can decrease the risk of infection in the laboratory. Diagnosis of brucellosis by PCR assay routinely has not yet been standardized. If DNA isolation optimize, the sensitivity of PCR will increase. This method can be used for diagnosis of brucellosis in clinical laboratories routinely. Also, this method can be a proper substitution for risky culture method and nonspecific serological methods. So, further studies are needed to standardize the molecular diagnosis of brucellosis and be used along with the clinical diagnosis of this disease.

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