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RESEARCH ARTICLE

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Evaluation of Human Brucellosis Prevalence in the Studied Regions of Iran, in Patients with Clinical Symptoms Compatible with this Disease

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Abstract

Background: Brucellosis is an ecumenical problem and irresistible illness that can influence a sizably voluminous population in creating nations and common between creatures and people. There are many procedures for diagnosing and recognition of *Brucella* spp. such as microbiological, serological, and molecular assays. This consider looked for to identify *Brucella* disease in human serum by both serological and molecular methods. In the intense and sub-acute stages of the infections, to access a corroborate diagnosis, it is needed to the confinement of *Brucella* from clinical tests which is the foremost delicate assay. When the titers of the patient's antibody are lower than 160 or antibodies that cross-react with other bacteria have existed or the illness comes to chronic localized cases, nucleic acid amplification tests (NAATs) such as PCR are more sensitive and specific than serology methods.

Materials and Methods: For the determination of *Brucella* spp. the sensitivity and specificity of the B4-B5 primers and designed IS711 primers were assessed and contrasted with the outcome of the 2ME test within the clinical specimens of 49 suspected patients.

Results: The comes about uncovered that from 49 serum sample in 2ME test only 45(91.83%) samples were positive, the amplicon of BCSP31-PCR was 223 bp and all the 49 (100%) serum samples, and the amplicon of the designed IS711-PCR was 448 bp length and 46 of 49 (93.87%) serum specimens were positive. The outcomes showed that the slightest number of both *Brucella melitensis* and *Brucella abortus* 0.05 CFU/reaction could be recognized by the B4-B5 primers.

Conclusion: The sensitivity of the designed primers of IS711 is 94%, whereas the sensitivity of B4-B5 primers is 100%, and the specificity of the 2 primer pairs is 100%.

ARTICLE HISTORY

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KEYWORDS

Human brucellosis, 2ME, PCR, *Brucella melitensis*, Blood and Serum samples.

INTRODUCTION

Brucellosis caused by the intake of unpasteurized dairy items or exchanged from contaminated creatures to people by creature exudates. Human brucellosis has been rated among the top seven carelessness zoonosis by The World Health Organization (WHO) (1, 2).

Within 500,000 patients are reported yearly by WHO reports, and a predominance rate surpassing 10 cases per 100,000 in a few nations.

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Most of these cases have a place for Iran, China, Iraq, Saudi Arabia, and the Middle East countries (3). In Iran, the prevalence of the cases is 34 per 100,000 each year (4). Furthermore, two regions with the most elevated prevalence of brucellosis are the Mediterranean locale and Latin America (5).

The genus *Brucella* is a facultative anaerobic, non-motile, gram-negative and intracellular bacillus, which has many species such as *Brucella melitensis, Brucella pinnipediae, Brucella canis, Brucella abortus, Brucella microti, Brucella suis, Brucella ceti, Brucella inopinata, Brucella ovis, and Brucella neotomae* (2, 6). Moreover, given this reliance on creature supplies, the veterinarians, agriculturists, representatives of butcher houses, and meat handling endeavors are the most debilitated populace. (7). Human brucellosis is relegated into three forms: (a) acute brucellosis characterized by weakness, undulant fever, headaches, myalgia, fine red rash, splenomegaly, hepatomegaly, and gastrointestinal symptoms. The acute phase may end in death, curing, transition into a sub-acute or chronic form; (b) sub-acute brucellosis characterized by almost all symptoms typical of the acute course but milder; (c) chronic brucellosis in which prolong signs and symptoms may include weariness, repetitive fevers, arthritis, endocarditis, and spondylitis (7,8, 9). , *Brucella* disease appears a strong tissue inclination for the regenerative system, bone, and fetal ectoderm. In humans, early manifestations of brucellosis comprise of fever, sweating, joint torment, side effects of harming, unremitting spine joint pain, testis or ovarian irritation, and neurological complications (12). Due to the need for legitimate medications and solid conclusion strategies, brucellosis truly debilitates the human wellbeing and could be a worldwide open wellbeing concern (12). Bone marrow/ blood culture is still “gold standard” however, required at the slightest 5 days to detect the genus *Brucella* spp. However, Microbiological, molecular, and serological techniques are efficacious for the detection and identification of *Brucella* spp. All things considered, the affectability of blood culture has been detailed by approximately 15% to 70% (10, 11). The forecast of brucellosis tends to be destitute and can result in incapacity in the event that an early conclusion was missed and/or no convenient treatment was started. Hence, an early conclusion and treatment are required for accomplishing palatable results. (12). *Brucella* contamination in people actuates an antibody response by the generation of both IgM and IgG antibodies at 1–2 weeks after contamination, which continues for 1 year or longer. Hence, antibody discovery cannot successfully recognize current or repetitive disease from settled or passive contamination (12). Besides, the actuated antibodies can cross-react with *Escherichia coli* O157:H7, *Stenotrophomonas maltophilia, Pseudomonas, Vibrio cholerae, Yersinia enterocolitica*, and *Bartonella* after *Brucella* contamination which affected the specificity of serological techniques (3, 14, 15). The identity based on blood-based strategy likely leads to misdiagnosis when a fever is caused by these pathogens (12). Many different serological assays have been used for the detection of *Brucella* spp. (10). Serological tests are first-line screening methods used for the diagnostic of brucellosis in endemic areas. They provide titer ranges that are adapted to the region where people are highly exposed to *Brucella* pathogen. The patients having chronic brucellosis, the specificity of the serological tests are low for them. (13). Be that as it may, the molecular approaches including nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR) is an imperative strategy that's utilized to identify brucellosis quickly and classify species effectively. Its sensitivity and specificity are much higher than serology assays. Also, PCR expends a brief sum of time and little amounts of materials have asserted to be quick (16), and used to identify brucellosis prevents direct bacterial contamination (17). The BCSP31 or the IS711 amplicons allows the determination of *Brucella* DNA but does not provide identification of the involved species (*Brucella melitensis, Brucella abortus*, etc.) (18). A tall caliber of genetic parallelism (up to 99.9%) in human pathogens and other species of *Brucella* have been shown by sequencing the whole genome of *Brucella abortus, Brucella melitensis, Brucella suis* (19), Most of the molecular diagnostic methods for brucellosis have a sensitivity ranging from 50% to 100% and specificity between 60% and 98%. so PCR can surmount the constraint of formal technique (20).

Subsequently, inside the show ponder, the PCR has been utilized to determine various species of *Brucella* DNA in blood/serum specimens of dubious people with clinical manifestation of brucellosis, after that, B4-B5 primers and novel IS711 primers have assessed and contrasting them with each other and compared with the outcomes of 2ME test.

MATERIALS AND METHODS

Description of time and region of the survey

The persistent examples were accumulated from different territories in Iran, inside 10 months from October 2018 to July 2019. Babol had two specimens of patients, 2 from Shiraz, 6 patients from Mashhad, 4 from Borujerd, 4 from Urmia, 3 from Makoo, 24 from Khoy, and 4 from Tabriz (Figure 1).

Fig 1: Map of IRAN showing the location of brucellosis cases

Subject description, and accumulation of data

This study included 49 subjects that Serum/blood samples have accumulated from doubtful patients who had the clinical manifestation of brucellosis and alluded to clinical centers, hospitals, and medical labs in various regions in Iran, during 10 months. At the same time as blood accumulation, serum specimens were organized. Afore patient samples amassment, the engraved advised assent was gotten and the survey which included age, class, work, home zone, essential clinical indications were filled for each understanding. All patients had clinical manifestations compatible with brucellosis.

Serological test

The 2 Mercaptoethanol method as a serological test was the primary test performed on the patient samples (sera). At that point, taking after standard strategies, a positive titer of 2ME test was characterized as either rise to or more noteworthy than 1:80 and a positive titer of Coombs- Wright was considered as either rise to or more vital than 1:80 (21). Clinical tests have been collected from doubtful patients which their titer of the 2ME test was break even with or higher than 1/20, and serial diluted serum arranged as takes after 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. The negative and positive control samples have been checked by the 2 Mercaptoethanol test (21).

Extrication of bacterial DNA

The standards were prepared using the cultured *Brucella melitensis* and *Brucella abortus,* and *Escherichia coli* (ATCC 35218) from the bacterial

culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran (FVM-UT). The extraction of DNA from the aforesaid bacteria as a positive control and *Escherichia coli* as a negative control has been performed following with the kit catalog and guideline the commercial kit (GTP. Tehran, Iran). After that, a serial diluted DNA extracted was arranged and stored at -20° C.

DNA Extrication of serum samples

The disconnected patients' serum tests were independently kept in sodium citrate tubes. A 200-µL volume of serum was used for DNA extraction. At that point, in understanding with the unit catalog and rule (GTP. Tehran, Iran) (www.irgtp.com).

DNA amplification, Bioinformatics analyses, and primer design

After bioinformatics analysis, two types of primer pairs with distinctive target genes have been utilized and molecular detection of the genus *Brucella* was done.

The First one was B4-B5 primer pairs (Table1), that encoding a *Brucella* cell surface protein (BCSP31) 31-kDa *Brucella abortus* antigen which all species of *Brucella* have this preserved sequence (22). The reaction mixture with an include up to the volume of 25 µL has arranged for the B4-B5 PCR strategy which contains taking after ingredients: 12.5 µL of 2X PCR master mix (Amplicon, Denmark), 5 µL of DNA template, 0.5 µL of each primer (B4-B5 forward and reverse primer), and nuclease-free water up to 25 µL. Techne, touch gene gradient PCR machine utilized to amplification of the DNA. (Model: Techne TC-512) (WWW.Techne.com). The PCR amplification cycle comprised of: 95°C utilized for beginning denaturation at 5 min. 94°C for 1 min utilized to template denaturation at 35 cycles, 60°C at 30 Sec utilized for primer annealing and 72°C for 60 Sec utilized for primer extension and the final extension was at 72 °C for 7 min. A 223 bp fragment of DNA has been amplified by the B4-B5 PCR.

Table 1: Primers used in this project

Target	Gene target	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)
Genus *Brucella*	BCSP31	B4: TGG CTC GGT TGC CAA TAT CAA	B5: CGC GCT TGC CTT TCA GGT CTG	223
Genus *Brucella*	IS711	F: CGC TCG CTG CCA TAC TTG CA	R: CTG AAC AAG CCG GGC CTG AT	448

The Second one was IS711 specific primer which was stored within the GenBank designed based on the sequence of *Brucella melitensis*. By utilizing Codoncode Aligner software (V.7.1.2) the novel IS711 primer pairs have been designed (Table 1). A 448 bp fragment of DNA has been amplified by the novel IS711 PCR and these sequences of the IS711gene were unique in different species of *Brucella*.

All species of *Brucella* at least, have one copy of repetitive IS711 gene fragments as a common locus. (6). The mixture with an add up to the volume of 25 µL has prepared for the IS711 PCR assay as the same blend which was utilized for the B4-B5 PCR strategy. The PCR amplification cycle comprised of: 95°C utilized for beginning denaturation at 5 min. 94°C for 1 min utilized to template denaturation at 35 cycles, 63°C at 60 Sec utilized for primer annealing and 72°C for 60 Sec utilized for primer extension and the final extension was at 72 °C for 7 min.

Brucella melitensis Rev. 1 and *Brucella abortus* S19 were positive control samples and *E. coli* (ATCC 35218) was negative control samples that extraction of DNA was performed for all of them.in each PCR strategy, a positive control extracted DNA from *Brucella melitensis* Rev. 1 and *Brucella abortus* S19 and extracted DNA from *E. coli* (ATCC 35218). All the control items were connected to guarantee a precise and sharp execution of the running handle and the nonappearance of impurity. For anticipating from any plausible defilement the control items were rechecked (23). Each handle of these tests was performed two times. After obtaining the amplicon fragments, these samples were transferred to 1.2% agarose gel (Sigma), and then ethidium bromide (1 µg/ml) was utilized to recolor the gel and performed the electrophoresis. Ultimately, the visualized bands of DNA could be distinguished in the DNA Gel Documentation Transilluminator system (UVP-Analytik Jena).

DNA sequencing has been provided for some of the amplified products (both BCSP31 and IS711 genes) and standard nucleotide blast has been done in NCBI. DNA sequences were edited by Chromaspro Version 2.1.3 (Technelysium Pty Ltd, Australia) and BioEdit Version 7.0.5.3.

Sensitivity assessment

Within the current consider, suspensions of *Brucella melitensis* and *Brucella abortus* that had been cultured 48 hours prior been prepared in sterile PBS, at that point successive dilutions of 10^{-1} to $10^{-10}$of both bacteria were arranged and after that 0.1 ml of each dilution was cultured on *Brucella* agar culture medium and incubated at 37°C for 72 hours and the number of colonies grown in each culture medium was counted to calculate the colony-forming unit (CFU) (24). The calculated esteem of CFU for both bacteria was equal to 5×10^8 CFU/ml. A while later, for performing the PCR tests from each dilution five microliters were utilized as a DNA template. The extracted DNA of *E. coli* as negative control had not any amplification in the PCR test. Each process of these tests was performed two times.

RESULT

Epidemiologic information

At the current venture, the geological spread of patients in numerous cities was as takes after: Khoy: 48.97%, Mashhad: 12.24%, Tabriz, and Urmia, and Borujerd: 8.1%, Makoo: 6.1%, Shiraz, and Babol: 4.08 % (Figure 2).

The comes about appeared, In terms of gender, the infected male patients appeared to be 83.67% and the female patients were 16.37% separately (Figure 2). The groupage of the patients varied from 18 to 70 years old with an average of 33.02 years old. Bunches age of the patient composed of 1 quiet beneath 20, 14 patients between 20-30, 27 patients between 30-40, 4 patients between 40-50, 2 patients between 50-60, and 1 persistent over 60 years old (Figure 4).

The occupational classification of patients was as follows: husbandry 32 (65.30%), dairy business personnel 5 (10.20%), the clinic nurse 1 (2.04%), and various employments 11 (22.44%) (Figure 5).

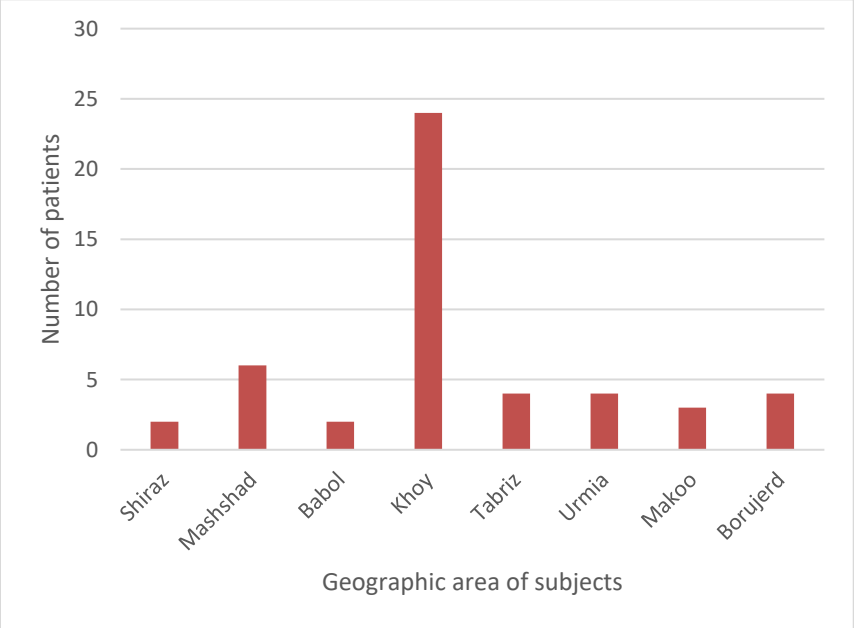

Fig. 2: Distribution of studied patients in different cities. The total number of patients; 49. Shiraz:2 cases, Mashhad:6 cases, Babol:2 cases, Khoy:24 cases, Tabriz:4 cases, Urmia:4 cases, Makoo:3 cases, Borujerd:4 cases.

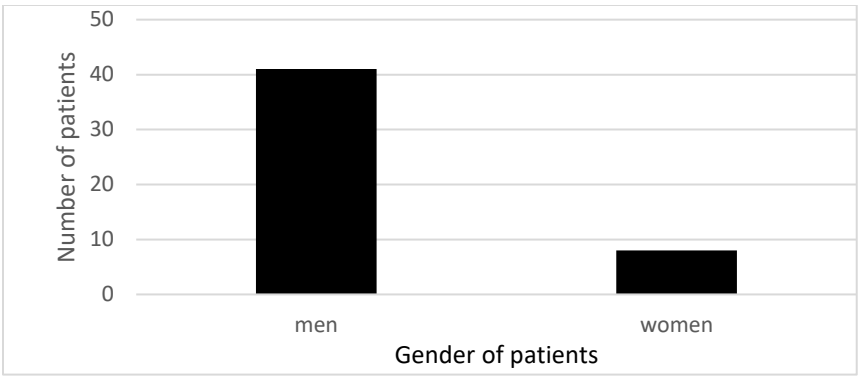

Fig. 3: Gender distribution of the studied subjects

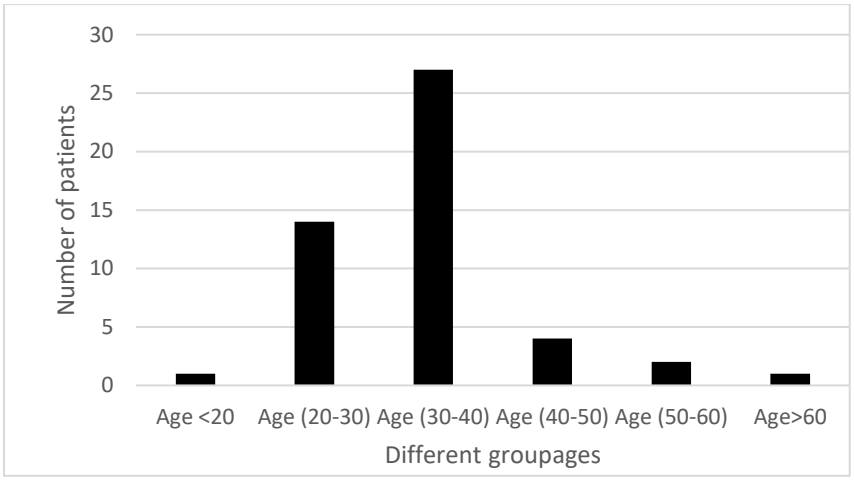

Fig. 4: Age distribution of the studied patients. Total number of patients; 49, Age <20:1, 14 subjects were between 20 and 30 years old, 27 subjects were between 30 and 40 years old, Age 4 subjects were between 40 and 50 years old, 2 subjects were between 50 and 60 years old, and One subject was over 60 years old

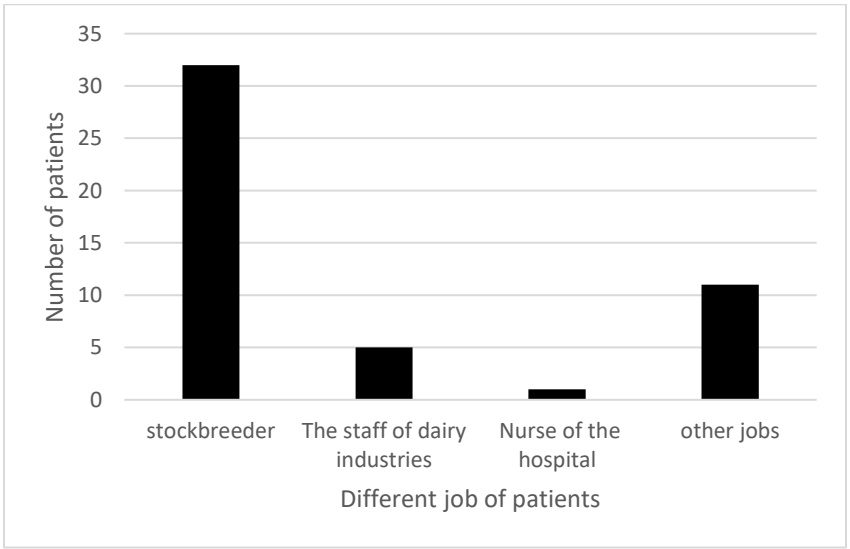

Fig. 5: Occupations of the studied patients. Total number of patients; 49, stockbreeder: 32, The staff of dairy industries: 5, Nurse of the hospital: 1, other jobs: 11.

The outcomes of the serological test

Serum tests were diluted with a titer of 1/20 to 1/1280 for performing the 2 mercaptoethanol test. The outcomes of the 2ME test in 49 serum tests were as take after:3 (6.12%) quiet with a titer of 1: 20, 1 (2.04%) persistent with a titer of 1: 40, So these four cases have been negative, 29 (59.18%%) quiet with a titer of 1: 80, 9 (18.36%) persistent with a titer of 1: 160, 1 (2.04%) persistent with a titer of 1: 320 and 4 (12.24%) patients with a titer of 1: 640 titer, 2(4.08%) patients with a titer of 1/1280, (Figure 6).

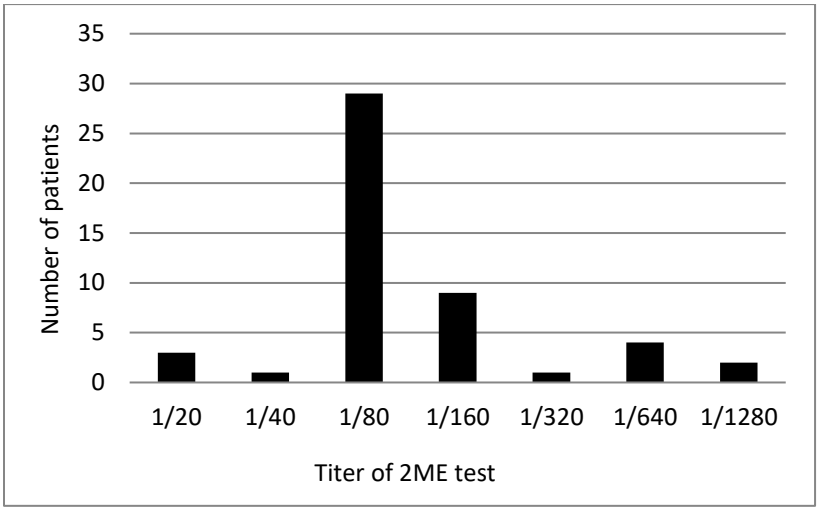

Fig. 6: Results of 2ME test; Titer 1/20:3 patients, Titer 1/40:1, Titer 1/80:29, Titer 1/160:9, Titer of 1/320:1, Titer 1/640:6, Titer 1/1280:2.

Conventional PCR determination of DNA

The outcome of the B4 -B5 PCR test for recognizing the genus *Brucella* appears in Figure 7. As anticipated, the amplicon size of the BCSP31 gene was 223 bp, and Within the B4-B5 PCR test, all 49(100%) samples from the patients were positive. However, with 2ME test 45 samples were have been positive.

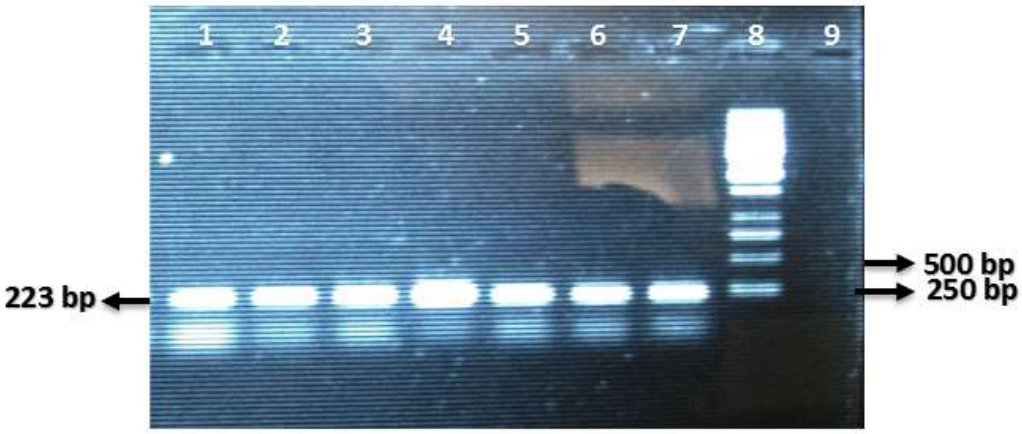

Fig 7: Detection of DNA amplified fragments with B4-B5 specific primers by staining of ethidium bromide and running on 1.2% agarose gel electrophoresis. The obtained size of an amplicon was 223 bp in the gel. Lanes: 1-6, serum samples; 7, positive control (*B. melitensis*); 8, 1 kb ladder (MBI-Fermentas); 9, *E. coli* as a negative control.

Testing of subjects with novel IS711–PCR

Designed IS711 PCR could diagnose *Brucella* DNA in serum specimens, the IS711 sequences are particular to the various genus of *Brucella*. Figure 8, reveals positive outcomes. The size of the IS711 amplicon is 448 bp. The three samples negative by the IS711 PCR

have been negative as same as the 2ME test, and the 49 (93.87%) samples have been positive by the IS711 PCR test from all 49 samples.

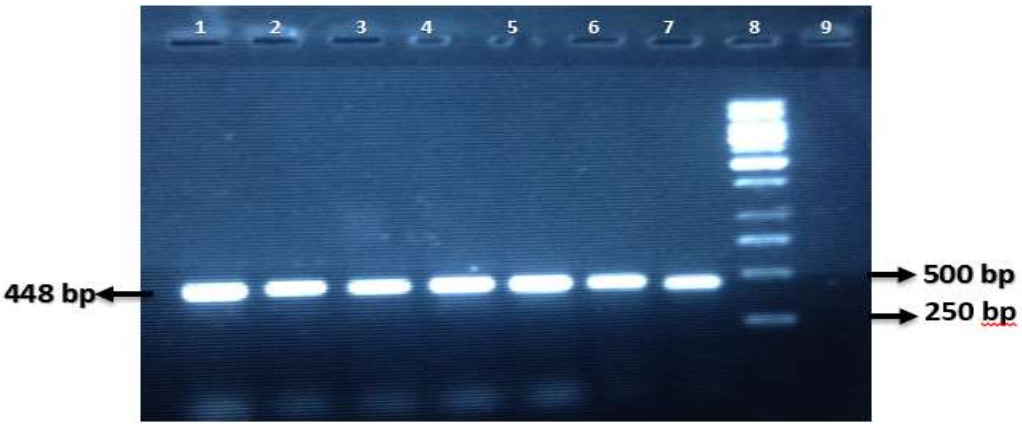

Fig 8: Detection of DNA amplified fragments with novel IS711 specific primers by staining *of* ethidium bromide and running on 1.2% agarose gel electrophoresis. The obtained size of an amplicon was 448 bp. Lanes: 1-6, serum samples; 7, positive control (*B. melitensis*); 8, 1 kb ladder (MBI-Fermentas); 9, *E. coli* as a negative control.

Sensitivity assessment of applied PCR tests

Extracted DNA from the *B.melitensis* and *Brucella abortus* has been diluted from 10^{-1}-10^{-10}, after that the sensitivity of the used B4-B5 and IS711 PCR test have been assessed. Figures 9, and 10 showed the relative values of cognate tests. The number of bacterial cells of *B. melitensis* and *B. abortus*, which have been determined by The B4-B5 PCR test was 0.05 CFU/reaction, while the novel IS711 PCR test could diagnose 2 CFU/reaction.

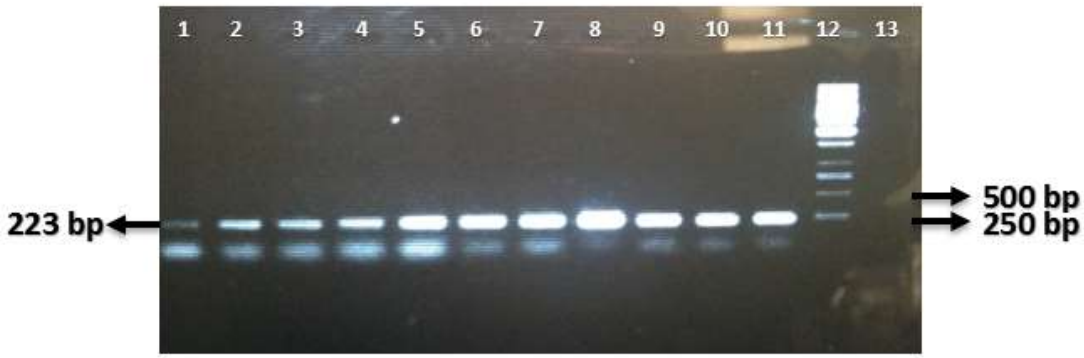

Fig 9: PCR products derived from10- fold serial dilutions of *Brucella* spp. DNA from 10^{-1}-10^{-10} and B4-B5 primers. .Lanes: 1-10 diluted DNA from 10^{-10}-10^{-1}, respectively; 11, positive control (*B. melitensis*); 12, 1 kb ladder (MBI-Fermentas) ;13, *E. coli* as a negative control.

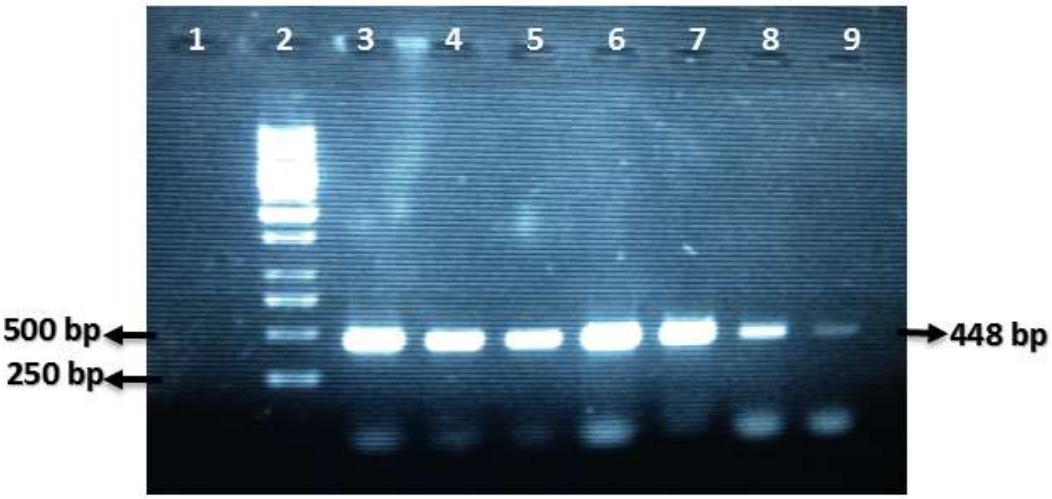

Fig 10: PCR products derived from10- fold serial dilutions from 10^{-1}-10^{-6} of *Brucella* spp. DNA and IS7II primers by 1.2% agarose gel electrophoresis and ethidium bromide staining. Lanes: 1, *E. coli* as negative control; 2, 1kb ladder (MBI-Fermentas); 3, positive control (*B. melitensis*); 4-9, diluted DNA from 10^{-1}-10^{-6} .

DISCUSSION

A person with clinical manifestation and epidemiologic risk factors for infection is expressed as a suspect patient. Clinical manifestation of brucellosis may occur between 5 days to 5 months following infection and may vanish and return several weeks or months later. The culture of *Brucella* stays to be the foremost specific diagnostic strategy for brucellosis determination, and it is the foremost sensitive strategy within the acute and subacute stages of the infection (12).

Generally, the conventional classic diagnostic methods such as serology strategies have low sensitivity and specificity, particularly for the discovery of brucellosis which could play an important role in erroneous maltreatment (25, 26). Whereas, progressed nucleic acid amplification techniques (NAAT) are a dependable procedure to choose up exact comes about the shortest possible time. Amid diverse sorts of nucleic acid amplification techniques (NAAT). PCR may be an exact demonstrative procedure that has tall affectability. But PCR may be more sensitive than culture only for localized forms of brucellosis (e.g., liver abscess). Hence, PCR as a nucleic acid amplification technique (NAAT), ought to make the best or most effective utilization to reach a precise and exact consequence (25, 26). There could be a part of wind coordination between all the components of each research facility procedure. These adjustments should be made between how the persistent is inspected, how the test is performed, the strategy, and how the result is gotten, so that a redress conclusion can be made. Every procedure had possessed impediments (26). Furthermore, concurring to inquire about nucleic acid amplification techniques (NAAT)such as PCR are the most perfect way for the disclosure and recognizing confirmation of particular organisms like *Brucella* In nations like Iran, Syria, Saudi Arabia, India the foremost common cause of human brucellosis between distinctive species of *Brucella*, is *Brucella melitensis*. (27, 28).

Agreeing to outcomes of this consider, the number of males (83.67%) contaminated with brucellosis was higher than females (16.33%).

The highest rate of illness was watched within the age groupage of 30- 40 years (55.1%) and the highest elevated rate of infection was seen within the occupation of husbandry, (65.30%). Khoy (48.97%) had the most elevated rate of malady. Some people in Khoy city consumed a type of traditional cheese denominated Koozeh cheese, which is made from unpasteurized milk.

In the current research, the results of the 2ME test revealed that 45(91.83%) samples of 49 were positive. For the discovery of diverse species of *Brucella* in 49 doubtful tests, the PCR was utilized. Two sorts of primer pairs containing B4-B5 and IS711 were evaluated and the results indicated that the 2ME titers minor than 1: 80 ought to not be overlooked without besides follow-ups, and titer equal 1: 80 is not indicating of extreme malady, particularly in endemic districts (29, 30, and 31). For anticipating the progression of the illness a 2ME test can be utilized and conjointly demonstrated valuable to screen anti-microbial treatment (32, 33). For the determination

of *Brucella* DNA in human specimens like blood and serum, the affectability, and specificity of distinctive PCR conventions utilizing the B4-B5 primer pairs was altered between 50% and 100%, separately (22,34,35,36, 37, 38). In the current research, the affectability and specificity of the 2ME test and PCR test that performed by 2 goal genes counting the BCSP31 and novel IS711 were compared. In this consider, the affectability and the specificity of the B4-B5 PCR test, were 100% agreeing to past comes about. Ciftchi et al. In a ponder conducted in Turkey were able to distinguish 5×10^2 CFU/ml of *Brucella* in serum tests by utilizing of IS711 PCR test whereas Ours comes about demonstrated that The novel IS711 PCR test could identify slightest 0.2×10^1 CFU/ ml bacteria within the specimens, which has been almost 2.5×102 as delicate as the inquire about done by Ciftchi (26). PCR comes about that were detailed by Khosravi in Iran and Elfaki in Saudi Arabia has been significantly comparable to these conclusions (39, 40). Within the performed ponder by Garshasby et al, a huge number of *Brucella abortus* DNA was identified by utilizing the IS711 PCR test, and the affectability was moo. But, our discoveries are altogether diverse from the detailed comes about by Garshasby et al. In this study, an expansive number of *Brucella -melitensis* DNA was recognized by utilizing the novel IS711 PCR test (18). To this extend, for running the PCR test, Serum samples utilized to bacterial DNA extraction instead of whole blood samples. The extraction of DNA from serum samples are more proficient from whole-blood samples. There are numerous blocking agents of the PCR tests in whole-blood samples than in serum samples. One of the materials which are inhibitor in the PCR test is heparin which binds to Taq DNA polymerase, the other one is EDTA which chelating to Magnesium ions, after that both of them were suspended inside the PCR mixture. (38).

In this ponder, the variables that can influence the affectability of PCR tests were assessed and one of these variables was the provision of consecutive dilutions of *Brucella* DNA in the PCR tests from 1-10 and it was found that this factor as an endorsed program, could not have any impact on the affectability of the over tests. Also, the sensitivity of the B4-B5 PCR test and the IS711 PCR tests were 100% and 93.87% separately, and the B4-B5 PCR test for the discovery of *Brucella* DNA was limited to 0.05 CFU / reaction and the IS711 PCR test restricted to 2 CFU/reaction. The presence of 3 negative results in the IS711 PCR test for diagnosing of *Brucella* could be due to the lacking amount of bacteria that could be identified in serum samples by this test.

CONCLUSION

As it turned out in this survey, in arranges to recognize and definitively diagnose diverse species of *Brucella*, the utilize of the IS711 PCR test and the B4-B5 PCR test to the power of a hopeful strategy and a program for a clinical demonstrative method ought to be utilized in microbiological research facilities., for diminishing the hazard of taking care of with infective microorganisms within the research facilities, Utilizing nucleic acid amplification techniques NAATs based on human serum tests is more down to earth (18). The comes about of this ponder appeared that the affectability of both PCR tests is 100%, but the specificity of the B4-B5 PCR test was 100% and the IS711 PCR test was 94%. Planning a modern NAAT strategy to distinguish and diverse species of *Brucella* was one of the most objectives of this think about. In any case, the microbiological culture of *Brucella* is still known as the golden test for the conclusion and distinguishing proof of *Brucella* from different human and creature examples.

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Faculty of Medicine, Tehran Islamic Azad University of Medical Sciences, Research Ethics Committee, with approval ID: IR. IUA. TMU. REC: 1397. 245.

The present study involved the clinical samples of suspected patients with brucellosis symptoms who were referred to diagnostic microbiology laboratories in the studied geographical areas.

The written informed consent was obtained prior to taking blood and the questionnaire which included age, genus, job, residence area, primary clinical symptoms was filled for each patient.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

The accession numbers of *Brucella* used for the design of the primers in November 2017 are as follows: AM040246 – AF036614 – AF047478 – DQ845343 – JF939171 – KF730265 – HM598413 – JN561159 – JN561158 – GQ443747 – GQ479519 – HM598412.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

PH and MRR conceived and designed the study**; PH and MRR** performed the experiments; **MS and AAS** advised the research**; PH, MRR, and AAS** analyzed the data; **PH** wrote the manuscript; **MRR** revised the manuscript. All authors read and approved the final manuscript.

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