

# Diagnosis of Clinical and Laboratory Findings of Brucellosis in Isfahan

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## Abstract

**Aims:** Brucellosis is one of the important infectious factors in most areas of Iran and other developing countries. Brucellosis has different clinical manifestations and should be considered in the differential diagnosis of infectious and noninfectious diseases. The aims of this study was to determine the epidemiological, clinical findings of the brucellosis in patients based on blood culture and serological tests. **Materials and Methods:** In this cross-sectional study, 190 brucellosis patients admitted in Isfahan Hospital in 2016–2017 that were studied based on positive immunological tests such as Wright, 2-mercaptoethanol (2ME), and enzyme-linked immunosorbent assay. Samples of blood were cultured (BACTEC) and incubated at 37°C for 5 days and then on *Brucella* agar. In addition, clinical and laboratory characteristics of brucellosis were done. The patients who had brucellosis (Coombs test  $\geq 1/80$  and 2ME  $\geq 1/40$ ) were selected. Data were analyzed using SPSS statistical package. **Results:** About 62.5% of patients were female with mean age of 37.5% years. 54.8% of cases were living in urban and 45.2% in rural areas. The most contagious seasons were spring. The most common transmission way was consuming of contaminated dairy products (59.3%); however, some of the patients had a history of animal contacts. The most common symptoms were fever (65.2%), arthralgia (68.1%), sweating (32%), malaise and fatigue (37.2%). The most common clinical signs were fever and peripheral arthritis. High leukocytosis and elevated erythrocyte sedimentation rate ( $>20$  mm/h) were reported in all of the cases. Elevated C-reactive protein was detected in 72.1% of patients. **Conclusion:** Brucellosis should be considered in the differential diagnosis of patients with prolonged fever, spondylitis, or peripheral arthritis in endemic areas.

**Keywords:** Brucellosis, epidemiology, Iran

## INTRODUCTION

Brucellosis or undulant fever is widespread zoonosis disease that caused by Gram-negative bacteria, *Brucella* spp. accidentally transmitted to humans during occupational contact with infected animals and then transmitted from infected animals to humans from different ways such as consumption of contaminated dairy products or even direct contact with animals. Human brucellosis is endemic in all parts of Iran and symptoms such as fever, arthralgia, and sweating are the most important clinical manifestations of the disease.<sup>[1-3]</sup> It is a major public health subject throughout the world and one of the most socioeconomic problems in many developing countries, especially in Iran.<sup>[4,5]</sup> Brucellosis is a systemic disease with wide spectrum of clinical manifestations; moreover, it may involve various organ systems including the liver, GI, nervous system, lungs, blood vessels, heart, skin, eyes, and joints.<sup>[1,2,6]</sup> The most common local involvement of

the disease is seen in the musculoskeletal system.<sup>[7,8]</sup> It is a very serious and destroying complication, so early diagnosis and proper treatment of brucellosis may be prevent from the following disabilities.<sup>[9]</sup>

The real outbreak of brucellosis in the world is unknown because of inadequate reportable systems and inaccessible to valid diagnostic tests in some developing countries. Annually, 500,000 cases with brucellosis are reported to the WHO in the world; the most of them are from the developing countries.<sup>[10]</sup> According to the annual report of the Center for

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Disease Control in Iran, the incidence rate of brucellosis was 39/100,000 and 30/100,000 populations in 2005 and 2007, respectively.<sup>[8,9]</sup> In addition, the incidence rate of brucellosis has increased to 130/100,000 populations in the west of Iran in last year.<sup>[11,14]</sup>

One of the important reasons of increasing the rate of brucellosis is incapacitation in control of brucellosis in animals.<sup>[15]</sup> In addition, different clinical manifestations of brucellosis in humans and incapacitation in definite diagnosis of the disease with laboratorial tests caused the incidence rate to be underestimated.<sup>[16,17]</sup> Indeed, brucellosis is prevalent in rural populations because of living near cattle places and direct or indirect contact with infected animals or animals' products.<sup>[1-3]</sup> Infected patients diagnosed by doing blood culture in Castaneda media or serologic laboratory tests such as Wright, Coombs Wright, and 2-mercaptoethanol (2ME) or polymerase chain reaction (PCR).<sup>[13]</sup>

The aims of this study were to determine epidemiological, clinical manifestations, complicated and noncomplicated brucellosis in infected patients and analysis of laboratory findings of patients with brucellosis in Isfahan hospital in 2016–2017. The molecular methods with special primers that amplify a 223-base pair fragment from the conserved region of the gene, which encodes an immunogenic membrane protein of 31 KDa of *Brucella abortus*, specific to the *Brucella* genus.

## MATERIALS AND METHODS

In this cross-sectional study, all patients with brucellosis who had Wright (standard tube agglutination test)  $\geq 1/80$  or Coombs test  $\geq 1/80$  with 2ME  $\geq 1/40$ ,<sup>[19]</sup> admitted in Isfahan hospital in 2016–2017 that enrolled. All demographic characteristics of patients such as age, sex, education, region of living, potential risk factors, clinical manifestations, and laboratory findings of all admitted patients with brucellosis were extracted and recorded in the questionnaires. Acute brucellosis is defined as a patient who has clinical manifestations compatible with brucellosis for at least 3 months and in the chronic form of brucellosis, the symptoms and signs lasting  $>1$  year.<sup>[12]</sup> In addition, laboratory data of all patients including complete blood count, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were recorded.<sup>[13-15]</sup> The patients with clinical features compatible with brucellosis and positive serologic tests, Wright (standard tube agglutination test)  $\geq 1/80$  or Coombs Wright test  $\geq 1/80$  with 2ME  $\geq 1/40$ , or positive blood culture for brucellosis were included, and patients with incomplete data in their files were excluded. Data were analyzed using SPSS statistical package, version 15.<sup>[14-16]</sup>

### DNA extraction and polymerase chain reaction

In addition, PCR was used for precise identification of isolated bacteria. A PCR assay with primers B4 (5'-TGG CTC GGT TGC CAA TAT CAA-3') and B5 (5'-CGC GCT TGC CTT TCA GGT CTG-3') (MWG-biotech, Germany) was used to detect *Brucella* DNA. The molecular methods PCR with

especial primers that amplify a 223-base pair fragment from the conserved region of the gene, which encodes an immunogenic membrane protein of 31 KDa of *B. abortus*, specific to the *Brucella* genus. Figure 1 shows agarose gel electrophoresis and ethidium bromide staining.<sup>[13-16]</sup>

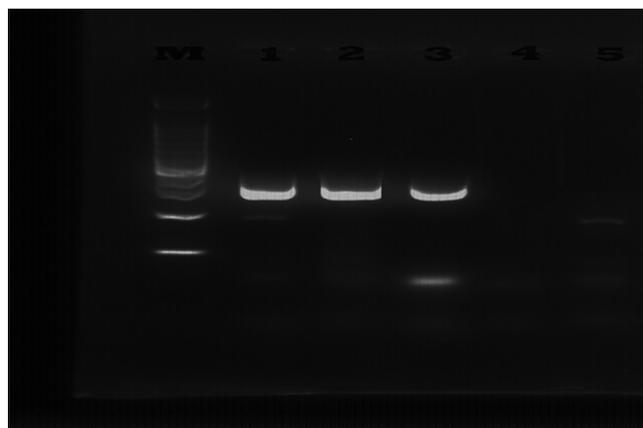
## RESULTS

Totally, 190 patients with brucellosis, 119 (62.5%) females with the mean age of  $37.5 \pm 2$  years ranged from 9 to 87 years were enrolled. (75%) of patients had a history of contact with infected animals, and (69.5%) had a history of consuming of unpasteurized dairy products. Table 1 shows the sign and symptom of patients.

The distribution of brucellosis in the summer, autumn was (28.2%). Twenty-four percent of the patients had acute brucellosis, and the duration of their disease was  $<3$  months. The most patients, (70.8%), were in subacute stages of brucellosis and rare patients (4.1%) had chronic brucellosis. The most common clinical manifestations were back pain, fever, and arthralgia that were shown in Table 1. Moreover, the white blood cell (WBC) counts and leukopenia. Elevated ESR ( $>20$  mm/h) and elevated CRP were shown in Table 2.

## DISCUSSION

Brucellosis is one of the most important zoonotic diseases around the world and still an important public health problem in many developing countries.<sup>[1-3]</sup> In the present study about 62.5% of patients were female with mean age of 37.5 years. 54.8% of cases were living in urban and 45.2% in rural areas. The most contagious seasons were spring. The most common transmission way was consuming of contaminated dairy products (59.3%); however, some of the patients had a history of animal contacts. The most common symptoms were fever (65.2%), arthralgia (68.1%), sweating (32%), and malaise and fatigue (37.2%). The most common clinical signs were fever and peripheral arthritis. High leukocytosis and elevated ESR ( $>20$  mm/h) were reported in all of the cases. Elevated CRP was detected in 72.1% of patients.



**Figure 1:** Sign and symptom of patients gel electrophoresis and ethidium bromide staining

**Table 1: Sign and symptom of patients**

Symptom	Percentage of patients
Fever	65.2
Sweating	32
Back pain	78.2
Headache	62.1
Diarrhea	35.2
Fatigue	42.5
Malaise	35.1
Arthralgia	68.1

**Table 2: Results of laboratory tests**

Symptom	Percentage of patients
ESR (>20)	58.1
CRP	64.8
Lymphocytosis	21.2
Leukocytosis (WBC >10,000)	52.9

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, WBC: White blood cell

Brucellosis can occur in both sexes and any age group.<sup>[17-19]</sup> The most common age group was between 9 and 87 years that were similar to other studies.<sup>[20-25]</sup> 75% of patients had a history of contact with infected animals, and (69.5%) had a history of consuming of unpasteurized dairy products, Table 2 shows sign and symptom of the patients that were the same as some researches with 67.9% and 66.7% cases.<sup>[26,27]</sup> However, in some studies, an expenditure of unpasteurized dairy products was reported in 76.4% or 88.3% of patients.<sup>[21,22]</sup> But in three studies from Iran, it was reported between 17.1% and 34.25%.<sup>[22,27,28]</sup> However, in some studies, in other countries, a history of contact with animals was showed from 32% to 71%.<sup>[20,21,26]</sup>

Brucellosis is a common disease in the spring and summer seasons,<sup>[28,29]</sup> and the results of our study showed the same pattern. About 54.8% of cases were living in urban and 45.2% in rural areas; however, Haj Abdolbaghi *et al.*<sup>[22]</sup> and Haddadi *et al.*<sup>[27]</sup> reported 15.67% and 40.5% of patients from rural areas, respectively, which were less than our result. In contrast to other studies, Isfahan has high percentage of population who live in rural.<sup>[22,27]</sup> In Tehran, the most population live in urban areas. Hashemi *et al.* reported that 190 (77.6%) of 245 patients were from rural areas and 152 (62%) were male which were similar to our study.<sup>[30]</sup>

About 50.7% of patients had middle school or lower education, so low education can be a predisposing factor of catching the disease. The most common signs and symptoms of the patients were fever, arthralgia, sweating, and malaise, which were similar to a study by Haddadi *et al.*<sup>[27]</sup> However, Ranjbar *et al.* reported fever, sweating, bone pain, back pain, and headache were the most common clinical features in brucellosis.<sup>[23]</sup>

The results are similar to our findings. Hashemi *et al.* reported arthralgia (78.4%) and fever (76.7%) as the predominant clinical manifestations in patients with brucellosis and

70 (28.6%) of them had osteoarticular involvements included sacroiliitis (75.7%), spondylitis (21.4%), and peripheral arthritis (8.6%).<sup>[30]</sup> In the present study, leukocytosis and lymphocytosis were observed in 52.9% and 21.2% of cases, respectively and also elevated ESR and positive CRP were reported in 58.1% and 64.8% of cases, respectively. However, Haddadi *et al.* reported normal CBC in the most patients.<sup>[27]</sup> In a study by Roushan *et al.*, 84.5% of cases had normal WBC and 60.4% had elevated CRP, but ESR and positive CRP in our study were reported in 58.1% and 64.8% of cases, respectively.<sup>[28]</sup>

## CONCLUSION

Brucellosis is an important zoonotic disease that can involve different organs of humans with various clinical manifestations in infected patients, and early diagnosis and treatment of brucellosis may help to prevent from early complications and relapse of disease. Different manifestations of brucellosis, it should be considered in the differential diagnosis of prolonged fever, spondylitis or peripheral arthritis, especially in endemic areas in Iran.

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## Conflicts of interest

There are no conflicts of interest.

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