



## Brucella Antibody Levels in Preschool Children in the North East of Iran

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### Abstract

**Background:** Brucellosis is a zoonotic disease of humans and animals that has affected worldwide. Iran is one of the endemic areas infected with brucellosis. Early diagnosis of this disease may protect the affected children from disabilities and mortalities. This study aimed to evaluate the Brucella antibody levels in preschool children of the Shahroud city in Iran.

**Methods:** This cross-sectional study evaluated 850 participants, in particular, school children from the Shahroud city. General information was collected by interviewing the children's parents. Moreover, a 5 ml of blood sample was collected from all the children. The samples were studied using standard tube agglutination test (Wright) and 2-Mercaptoethanol. Thereafter, IgG and IgM levels were determined by the ELISA method.

**Results:** A total of 850 children were enrolled, of which 51.2% were boys and 48.8% were girls, with a mean age of  $5.17 \pm 1.55$  years. Of the all children evaluated, 839 (97.5%) children had a titer  $<1/80$  and 21 children (2.5%) had a titer  $\geq 1/80$ . A significant difference was observed between the antibody titers in terms of gender ( $P=0.012$ ), whereas no significant association was found among antibody titers with other variables such as age, history of nonpasteurized foods, exposure to animals, history of brucellosis disease, and parental occupation.

**Conclusions:** The antibody titer for suspected brucellosis in preschool children of Shahroud was very low. According to the results of our study, in particular, in the endemic areas, a Wright's titer of 1/80 in suspected cases for brucellosis can be considered as a diagnostic titer.

**Keywords:** Brucellosis, Antibody, Preschool children, Shahroud.

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## Introduction

Brucella species are small, Gram negative, nonmotile, nonspore-forming, rod-shaped (coccobacilli) bacterial organisms. Brucellosis is a zoonotic disease caused by the ingestion of raw unpasteurized milk from infected animals or by a close contact with their secretions.<sup>1</sup> There are different animal reservoirs for different Brucella spp. Although the disease has been eradicated in most European countries, its occurrence is still seen in the Mediterranean, the Arabian Peninsula, the Indian subcontinent, Asia, central and South America; Iran is an endemic area for brucellosis infection and the prevalence of 225 per 100,000 of the population has been

reported.<sup>1-2</sup> In the Islamic Republic of Iran, brucellosis is a major health problem, and its frequency is increasingly observed in various parts of the country, this disease being quite prevalent throughout the country.<sup>3</sup> The reason for the high prevalence of brucellosis in Iran (especially in Shahroud) is attributed, but not limited, to the following: the nomadic life style including animal raising, especially sheep and goat; the traditional belief of the benefit of ingesting raw milk, especially sheep milk; the high rate of animals imported from southeast Iran where the disease is endemic, with a lag in compliance with national and international policies of animals screening and quarantine rules; the mixing of different animal herds, such as raising sheep and cattle together; the low levels of public awareness about the seriousness of brucellosis as a human disease; and the resistance to slaughter infected animals.<sup>4</sup> The disease is more prevalent in younger children. Brucellosis caused by Brucella melitensis is more common among children in endemic countries.<sup>4-5</sup>

In Iran and most of the neighboring countries, the most prevalent species is B. melitensis, responsible for 70–90% of the brucellosis cases.<sup>6</sup> This disease can rarely spread by human interactions. The disease can cause serious morbidity, and it has important economic consequences. Early diagnosis and appropriate timely treatment can prevent mortality, morbidity, and disability in the affected patients. Diagnosis of brucellosis is based on clinical and laboratory findings.<sup>7</sup>

Diagnosis is occasionally confounded due to nonspecific clinical manifestations, and is confirmed only if the Brucella species are recovered from blood, bone marrow, cerebrospinal fluid (CSF) and urine. Brucellosis is usually associated with an intense humoral response. Isolation of the microorganism is possible only in a minority of the infected patients during the acute phase of the disease. Although most laboratories are now employing rapid isolation techniques (BACTEC, DuPont isolator, PCR methods, etc.), these techniques are not available in most developing countries and conventional methods of isolation are less effective in routine diagnosis.<sup>7-8</sup>

Therefore, in the absence of bacteriological confirmation, a presumptive diagnosis can be made on the basis of a single high rising titer of specific antibodies. Among a variety of serological tests, standard tube agglutination (STA) is the most widely used. Evaluation of various ELISA assays for IgG and IgM have shown that these techniques are generally more sensitive and specific than the conventional tests, while they are able to distinguish specific antibodies of IgM and IgG

classes associated with acute and chronic brucellosis.<sup>8</sup> The obtained results are easily interpreted, since they are specific for single immunoglobulin classes. Alternatively, these techniques are not routinely available in developing countries, especially in the rural areas. Hence, attempts to increase the sensitivity of available tests should be made.

A positive Brucella serology test of  $1:\geq 80$ , using the STA for patients presenting with symptoms suggestive of brucellosis. For screening purpose and in the absence of clinical indicators of active brucellosis, a titer of 1:160 or higher is more specific to detect the presence of the disease.<sup>9</sup> However, false positive serological tube agglutination test for diagnosis of brucellosis, may be create to cross-reactivity with microorganisms such as *Vibrio cholera*, tularemia or *Yersinia*, this test because of cheap and fast, is one of the most common test to detect brucellosis.<sup>10</sup> As the signs and symptoms are not pathogenic for brucellosis, serological and bacteriological tests are essential to confirm the diagnosis. Brucellosis is an important public-health problem in many in developing countries, such as Iran, especially in Shahroud (northeast Iran). The seroprevalence of brucellosis varies between 10% and 16% in Iran. In some high-risk groups, this rate may increase up to 22.5%. Children may constitute 20–30% of all brucellosis cases in the world, especially in the endemic regions.<sup>11</sup> Data on the seroprevalence of brucellosis in child population are very limited. Given the importance of serological tests, especially in cases with a history of antibiotic use and a low possibility of positive samples, this study was conducted to evaluate the Brucella antibody levels in preschool children in the northeast Iran (Shahroud).

## Materials and Methods

This is a cross-sectional study, which was performed on all kindergarten children (18 kindergarten children with 850 children) of the Shahroud city in 2014. Shahroud city is located in northeast Iran where brucellosis is endemic. All children from 4 to 6 years, who attended the kindergartens, were invited. After receiving parental consent to participate in the research, they were examined and blood samples were collected.

The main variables included age, gender, body mass index (BMI), and history of consuming unpasteurized dairy and parental occupation. The age range was 4–6 years.

Serum samples were obtained by centrifugation of peripheral blood and were preserved at  $-40^{\circ}\text{C}$  until the day of examination. All the serum samples were transported to the Shahroud University of Medical Sciences central lab, in standard conditions, and were studied with STA and 2 mercaptoethanol (2ME).

Wright's agglutination test included:

A—A two-fold serial dilution of serum samples (starting dilution of 1/20) was prepared.

B—0.5cc of antigen solution (*Brucella abortus*) was added to each tube.

C—the tubes were gently stirred and incubated at  $370^{\circ}\text{C}$  overnight.

D—the last tube that showed agglutination was considered as the titer of the serum antibody.

All sera were routinely diluted from 1.20 to 1.1280. A positive titer indicates a ratio of 1:160 or more. In endemic areas, there may be a persistent and continuous exposure to the source of infection; therefore, there may be a persistent low titer in the range of 1:80–1:160 in the absence of true infection. Each batch of the test included a positive control and a negative saline control. A definite agglutination of the suspension was considered as a positive reaction. Agglutination was not seen in the negative samples. For the positive samples, the lowest positive titre was determined. Titrations of 1.80 and above were accepted as positive for brucellosis. 2ME, a foul-smelling sulfhydryl compound that acts as a reducing agent, was used to differentiate between IgG and IgM in a mixture by disrupting the disulfide bonds of IgM so that only IgG was measurable.

2ME (2- mercaptoethanol): This test was performed similar to that of Wright's agglutination; however, the antigen solution was mixed with a reductive chemical agent (2-ME) that reduces the S=S bonds in IgM molecules. Thus, eventually acute, subacute, and chronic states of brucellosis could be distinguished.

Ethical consideration: The subjects and their parents were informed about the nature of the study, and written consents were obtained from the parents of the patients.

Descriptive statistics [mean standard deviation (SD), frequency], chi-square test, Fisher's exact test, and Mann-Whitney U test were used for analysis in this study. Data were analyzed using the Statistical Package for the Social Science (SPSS version 16). A P-value less than 0.05 were considered as statistically significant.

## Results

Of the 998 children, studying in kindergarten, only 850 who were willing to participate in this study after obtaining informed consent from their parents, were enrolled. The mean age of the study population was  $5.17\pm 1.55$  years (range 4–6 years). There were 435 (51.2%) boys with mean age of  $5.04\pm 1.64$  years and 415 (48.8%) girls with mean age  $5.23\pm 1.51$  years. No significant relationship was observed between the mean age and gender of the participants ( $P<0.105$ ). The mean BMI of the all children was  $19.25\pm 3.19$  kg/m<sup>2</sup> (mean BMI of the boys was  $19.27\pm 2.95$  and that of girls was  $19.18\pm 3.23$  kg/m<sup>2</sup>); no significant relationship was observed between the mean BMI and gender of participants ( $P<0.088$ ).

**Table 1. The frequency (Number (%)) of antibody titers based on gender**

	Antibody titer				Total
	<1/20	1/20	1/40	1/80 >	
Gender					
- Boys	396 (51.2)	20 (52.6)	8 (47.1)	11 (52.4)	435 (51.2)
- Girls	378 (48.8)	18 (47.4)	9 (52.9)	10 (47.6)	415 (48.8)
Total	774 (91)	38 (4.5)	17 (2)	21 (2.5)	850 (100)
P.V*			0.012		

\* Chi-square test

About 774 participants (91%) had an agglutination (Wright) titer of less than 1/20, 38 (4.5%) had titers equal to 1/20, 17 (2%) had titers equal to 1/40, and 21 (2.5%) had titers

equal to 1/80 and higher, respectively. Table 1 shows the frequency of antibody titers (Headline Wright) based on gender. These findings reveal that there is a significant difference between antibody titers in both genders ( $P=0.021$ ). For all children who wright test was 1.80 or higher, was done 2ME test. The frequency of the antibody titers (Headline Wright) based on the age group is shown in table 2. No significant relationship was observed between the antibody titers and age groups ( $P=0.185$ ).

**Table 2. The frequency (Number (%)) of antibody titers based on age group**

Age	Antibody titer				Total
	<1/20	1/20	1/40	1/80 >	
- 4 years	272 (35.1)	15 (39.5)	7 (41.2)	8 (38.1)	302 (35.5)
- 5 years	259 (33.5)	12 (31.6)	5 (29.4)	7 (33.3)	283 (33.3)
- 6 years	243 (31.4)	11 (28.9)	5 (29.4)	6 (28.6)	265 (31.2)
Total	774 (91)	38 (4.5)	17 (2)	21 (2.5)	850 (100)
P.V*	0.185				

\* Chi-square test

Among the 21 children who had Wright headline of 1/80 or higher, the parents of 3 students had jobs related to livestock and livestock products. No significant difference was observed between high antibody titers of children and the parental occupations ( $P=0.358$ ). Distribution of children with high antibody titer and parental occupations is shown in table 3.

**Table 3. The frequency (Number (%)) of children with high antibody titers based on parental occupations**

Father's occupations	High antibody titers	Low antibody titers	P.V*
Farm workers	3 (14.3)	85 (10.3)	0.358
Laborers	5 (23.8)	175 (21.1)	
Civil servant	4 (19.1)	294 (35.5)	
Engineer	2 (9.5)	129 (15.6)	
Executive	3 (14.3)	95 (11.5)	
Others	4 (19.1)	51 (6.2)	
Total	21 (100)	829 (100)	

\* Chi-square test

A total of 61 children (7.2%) had a past history of consumption of unpasteurized dairy, 122 children (14.4%) had a history of care and contact with animals, and 60 students (7.1%) had a past history of brucellosis. The frequency of the history of consumption of unpasteurized dairy, history of care and contact with animals, and previous history of brucellosis based on antibody titers are shown in table 4. No significant relationship was observed between the antibody titers and the history of consumption of unpasteurized dairy ( $P<0.059$ ), history of care and contact with animals ( $P<0.068$ ), and a history of brucellosis ( $P<0.075$ ).

**Table 4. The frequency (Number (%)) of history of consuming the unpasteurized dairy, history of care and contact with animals, and previous history of the disease brucellosis based on the antibody titers**

Antibody titer	History					
	History of consuming the unpasteurized dairy		History of care and contact with animals		Previous history of brucellosis	
	Positive	Negative	Positive	Negative	Positive	Negative
<1/20	43 (70.5)	731 (92.6)	106 (86.9)	668 (91.8)	44 (73.3)	730 (92.4)
1/20	10 (16.4)	28 (3.5)	8 (6.6)	31 (4.3)	9 (15)	29 (3.7)
1/40	5 (8.2)	12 (1.5)	4 (3.3)	13 (1.8)	3 (5)	14 (1.8)
1/80 >	3 (4.9)	18 (2.3)	4 (3.3)	17 (2.3)	4 (6.7)	17 (2.2)
Total	61 (7.2)	789 (92.8)	122 (14.4)	728 (85.6)	60 (7.1)	790 (92.9)
P.V*	0.059		0.068		0.075	

\* Chi-square test

## Discussion

Brucellosis is endemic in western Asia, Middle East Africa, Latin America and Mediterranean regions. In Iran, brucellosis has been one of the important public-health problems; hence, this infection is still a major health concern among Iranian population. Brucellosis is common in children, and people of all ages are susceptible to the disease. In our study, 21 (2.5%) of the children were positive for STA while the 2ME test had the all negative results, which were similar to those of another retrospective study by Bilgehan and Yentur Doni.<sup>11-12</sup> In our study, children with 4 years constitute the majority of childhood brucellosis cases (Wright test level  $\geq 1/80$ ). It was in contrast to the findings of Buzgan et al. that nearly 18% of children in the study were younger than 5 years. This is associated with a higher probability of contact with pet animals and consumption of unpasteurized milk outside home. It should be noted that method of doing this study, is different from our study.<sup>13-16</sup> In few studies, half of the patients belonged to the farmer families and rural areas, and occupational contact with animals was unavoidable; but in our study, the number of parents who butcher, or owned slaughterhouses or homes for the care of animals, worked very limited, and no relationship was found between the increase in antibody titer with a positive history using of nonpasteurized foods, exposure to animals, or the history of brucellosis.<sup>17-18</sup>

Occupational exposure is an important risk factor.<sup>19</sup> For instance, seropositivity in butchers, cattle dealers, and slaughter-house workers was higher than other people; however, in the present study, no significant differences were found between the high antibody titers and parents' occupation.<sup>20-21</sup> In endemic countries, the disease is not necessarily an occupational one and children are at a similar risk of contracting brucellosis as adults.<sup>22</sup> The medical history may not be given by the parents clearly or the child may be crying, therefore, be difficult to diagnose.<sup>23-25</sup> Therefore, the evaluation of the clinical features and laboratory findings of brucellosis in children is of great importance. Alternatively, prompt diagnosis and treatment of infection is another efficient strategy. Isolation of microorganisms from blood culture is a qualified monitoring technique, but requires relatively long time or laboratory expertise. So, it is that was used from easier and faster tests to detect the infection.<sup>24-26</sup>

Vaccination of ruminants (especially sheep and cows) against *Brucella* and use of pasteurized milk or milk products could decrease the rate of infection in the human societies. Brucellosis occurs mainly in school-aged children (50%). Childhood brucellosis remains a significant community health problem in Iran, despite the trend toward an overall decrease in the disease. The yearly incidence of brucellosis in Iran has declined over time.<sup>27-29</sup>

The prevalence of brucellosis decreased in Iran as a result of animal disease control programs directed by the Ministry of Agriculture, Rural Affairs and Veterinary Authority. With the implementation of vaccination campaigns, seropositivity decreased for both human and animal brucellosis.<sup>30</sup>

Due to the very low rate of wright titration in preschool students of Shahroud, it can be said in this sense, Shahroud have good status.

In conclusion, we emphasize that the eradication of brucellosis, which is a worldwide disease, can be achieved only by aggressive preventive measures, including elimination of the vector, elimination of infected animals, vaccination of newborn animals, education, and enforcement of control measures.

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## Conflict of Interest

The authors declared that they have no conflict of interest.

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