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Original Article

The relationship between some risk factors and seroconversion to brucellosis in Gachsaran, Iran

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Summary
Brucellosis, or Malta fever, is one of the common diseases in humans and livestock that causes many health problems and economic losses. Therefore, this study investigated the frequency of seroconversion of persons with brucellosis symptoms in Gachsaran, Iran from 2016 to 2017. In this study, 1379 people (age range of 10-70 years) with clinical symptoms of brucellosis were monitored by standard agglutination test (SAT, Wright's Agglutination Test) who referred to the pathobiology laboratory from the beginning of 2016 to the end of 2017. In this monitoring, the individuals with a titer greater than or equal to 1: 320 (based SAT) and greater than or equal to 1: 160 (based 2ME) were selected. Using questionnaires, data on gender, contact with livestock, and dairy consumption behavior were collected. The study variables were analyzed using a *chi*-square test ($p \leq 0.05$).There was no significant difference in the incidence of brucellosis between men and women ($p = 0.16$), but direct contact with livestock ($p = 0.001$), history of unpasteurized dairy consumption ($p = 0.001$), occupation ($p = 0.001$) and location ($p = 0.001$) had a significant effect on infectivity to brucellosis. Based on the results of this study, apart from gender, other factors are the most important causes of seroconversion of persons to brucellosis.
Keywords: Epidemiology, Zoonotic disease, Brucellosis, Risk factor, Iran.

Introduction

Brucellosis, or Malta fever, is one of the major health problems in many countries (McConnell, 2016). Brucellosis is a zoonosis disease caused by *Brucella* spp. (*Gram*-negative bacilli) (Kashfi et al., 2018). The disease is mainly transmitted to humans through consuming contaminated dairy products or skin scrapes and direct contact with the flesh or blood of the infected animals (Heymann, 2008). The incidence of the disease is more than 122,000 cases annually, with various distribution in different regions (Seleem et al., 2010).

In Iran, brucellosis was firstly reported in a human patient in 1932 (Amirzargar et al., 2009). The prevalence of this disease increases during spring and summer as the breeding and lactation seasons in livestock and specifically is observed in housewives, ranchers, and farmers (Dastjerdi et al., 2012). Brucellosis is an endemic disease with a higher prevalence in rural areas than urban areas

(Taheri et al., 2016). Several factors such as the long borders of the country and lack of supervision on livestock imports, large numbers of nomadic populations, traditional livestock breeding, insufficient supervision of production and distribution of dairy products, and lack of regular vaccination in livestock have the main role in brucellosis prevalence in Iran (Mostafavi and Asmand, 2012).

The clinical symptoms of brucellosis are nonspecific, and the diagnosis is based on microbiological (microbial culture) or serological (detecting antibody) tests (Pakzad et al., 2012). The incubation period of the disease is between one to three weeks, and humans usually show flu-like symptoms, including fever, sweating, headache, low back pain, and weakness (Mostafavi and Asmand, 2012). The disease develops in both acute and chronic forms and has various clinical features that make it difficult for physicians to diagnose. The disease generally starts in acute form and continues with persistent or regular fever with intermittent periods, frequent sweating (especially at night), fatigue, anorexia, weight loss, headache, muscle aches, and general body pain. The symptoms of brucellosis depend on the severity of the disease (acute, sub-acute, chronic, and topical). Symptoms of brucellosis can disappear and relapse again after a few weeks or months. In some people, brucellosis can be chronic, and the symptoms could persist for several years even after treatment (Hashtarkhani et al., 2015). The long-term symptoms include fatigue, fever, arthritis, and spinal cord edema. The brucellosis could be treated with antibiotics. Treatment may take several weeks to several months; however, relapse periods are very common (Hashtarkhani et al., 2015; Mostafavi and Asmand, 2012). Brucellosis is a multi-systemic disease that appears as a (sudden or gradual) fever in severe and acute cases, but if the disease is not diagnosed and treated, it develops and progresses to a chronic, debilitating disease with severe and sometimes fatal complications (Shoraka et al., 2010). The livestock brucellosis has either been eradicated or is in the process of eradication in many developed countries (Blasco and Molina-Flores, 2011). However, it is still a major public health problem worldwide (Shahnavazi et al., 2016). Conducting epidemiological studies that evaluate the importance and risk factors of brucellosis in different regions and provide effective results in controlling and preventive actions are very critical. Despite many studies regarding the prevalence of brucellosis, there is still limited information about the impact of gender on brucellosis and the relationship between the risk factors in men and women. Therefore, the present study investigated the relationship between gender and risk factors of infection with *Brucella* spp. by studying the frequency of seroconversion in persons suspected of brucellosis in Gachsaran, Iran form 2016 to 2017.

Materials and Methods

Study population

The present study is a descriptive-analytical study in 1379 patients (age range: 10-70 years) suspected to brucellosis, referred to Gachsaran Health network, Kohgiluyehva Boyer Ahmad Province, (southwestern Iran), from 2016 to 2017.

Epidemiological study

The epidemiological data (comprising all diagnostic criteria for brucellosis) were collected from persons suspected to brucellosis. Suspicious brucellosis was considered as having a positive history of contact with animals or animal products, having a positive history of contact with animals, or animal products or positive standard agglutination test (SAT, Wright's Agglutination Test) (equal or greater than 1:320) and for 2ME (equal or greater than 1:160)., In addition to having clinical symptoms, all patients had a positive serology test (Burning 1.320 and 2ME more than 1.160) for brucellosis.

Since brucellosis is not commonly mentioned as urgently reported disease, diagnosis is performed by a specialist and with laboratory examination each month. Thus, all data for this infectious cases are frequently documented in the provincial health care center archive. In the present study, the information considering all reported cases of

brucellosis formearly 2016 to the end of 2017 was collected and categorized. It should be noted that the patient's subsequent referrals data were not included in our study. Comparison of the demographic data of the affected people and the frequency of the disease in various age groups, as well as the frequency of the disease based on the population of urban, rural, and nomad areas in 2016-2017 were analyzed.

Statistical analysis

Descriptive statistics were expressed as numbers, mean, and percentages. Since the data of this study was non-parametric, data were analyzed using the chi-square test. Pearson correlation coefficient test was used to investigate the relationship between the incidence of the disease and research variables in men and women, and the quota correlation coefficient test was used to eliminate the effect of gender and to examine the relationship between the variables and brucellosis. Data were analyzed by SPSS software version 22, and the level of significance was considered at $p \leq 0.05$.

Results

The demographic characteristics of people with brucellosis are presented in Table 1. Totally, 116 (51 male and 65 female, respectively) out of 1379 were positive for SAT serological test. According to the result, 7.32% of all suspected individuals (regardless of gender) had a 1:160 titer. 5.1% of males and 10.75% of females were positive for the serological brucellosis test. Among females, 26.19% had a 1:160 titer, while 15.25% of males had a 1: 160 titer. Considering the 1:320 titer as a conventional indicator for diagnosing brucellosis in endemic areas, 3.77% of females and 8.96% of males had positive titers for brucellosis.

Table 1. Demographic characteristics of people with brucellosis based on serological testing, in the form of a number (%)

Risk factor		Male	Female	*P-value*
Ages (years)	10-19	14.2	16.4	0.042
	20-29	28.3	23.1	
	30-39	14.2	11.9	
	40-49	22.5	11.2	
	50-59	15.8	21.6	
	60-70	5	15.7	
Occupation	Farmer	13.3	7	0.033
	Livestock	57.5	47.3	
	Housewife	8	39.5	
	Freelance	21.2	6.2	
Location	Urban	5.8	9.7	0.035
	Rural	66.7	58.2	
	Nomad	27.5	32.1	
Livestock contact	With contact	93.3	92.5	0.001
	Without contact	6.7	7.5	
Consumption of unpasteurized dairy products	With consumption	99.2	99.3	0.001
	Without consumption	0.8	0.7	

The 2ME test was reported positive in all cases. Also, the results of the chi-square testshowed there was no significant difference between males and females with brucellosis ($p = 0.16$) (Table 2). The number of positive cases of the individuals who had direct contact with livestock (91.5%) was significantly higher than those who did not have direct contact with livestock (7.7%) ($p = 0.001$). Moreover, the number of positive cases of the individuals who had a history of consumption of unpasteurizeddairy products (99.2%) was significantly higher than those who did not

consume unpasteurizeddairy products with brucellosis (p = 0.001). There was a significant difference in the number of individuals with a titer above 1:32 in agriculture (10.4%) (p = 0.001), animal husbandry (52.2%) (p = 0.001), housekeeping (0.30%) (p = 0.001) and freelance (7.3%) (p = 0.001). Also there was a significant difference in the number of individuals with a titer above 1:320 in people living in cities (12.5%) (p = 0.001), villages (64.5%) (p = 0.001) or nomads (23.0%) (p = 0.001) (Table 2).

Table 2. Descriptive statistics and nonparametric single sample t-test results to investigate gender differences, direct contact with livestock, and history of consumption of unpasteurized dairy products in brucellosis

Risk factor		Number	percent	*P-value*
Gender	Male	51	43.6	0.16
	Female	65	55.6	
Direct contact with livestock	With	107	91.5	0.001
	Without	9	0.5	
Consumption of the unpasteurized dairy products	With	115	98.3	0.001
	Without	1	0.7	
Occupation	Farmer	6	10.4	0.001
	Livestock	72	52.2	
	housewife	29	30.0	
	Freelance	9	7.3	
Location	Urban	13	12.5	0.001
	Rural	97	64.5	
	Nomad	44	23.0	

Discussion

Here, the Wright test findings were observed to be 1.320 in the majority of patients. In a previous study, the result of 1.320 was reported more than the other titer, which was in agreement with the idea that most of the Wright test results were in endemic areas of the 1.320 titer (Kassiri et al., 2013). On the other hand, regarding the results of the 2ME test, titer 1.80 was reported more than other titers, which was in agreement with a previous study in Khorasan Razavi (Hashtarkhani et al., 2015). Therefore, in the current study, there was a positive correlation between the results of wright and 2ME assays and vice versa.

There are varies reports about the incidence of brucellosis in different genders. Most studies reported that the prevalence of brucellosis is higher in males (Hosseini et al., 2016; Seleem et al., 2010). However, several studies stated that the prevalence of brucellosis was greater in females (Mostafavi and Asmand, 2012).

Based on the present findings, people (men and women) who lived in the village and their jobs were ranchers, have a higher risk to develop and present the disease.In a similar study in Turkey, 49% of females and 51% of males were affected by the disease, which agree with the current study (Gür et al., 2003). But,in an older study in Spain, 38.2% of patients were female and 66.27% were male (Gonzalez et al., 1997). In another previous study from Iran, 60% of patients were male and 40% were female in Arak (Farahani et al., 2012). Taken to gather, it seems that women who live in the city are involved in most rancher work, and they have a high level of contact with livestock, they made up a large percentage of patients.

This could be related to some factors like climate and even cultural differences in the studied areas. For instance, in some cultures, local women are more engaged in dairy products and the milking processes than men.

The average age for infectivity in men and women did not show any significant. In this study, the most

infectivity was found in the age group of 20-29. The average age difference between men and women was not statistically remarkable ($P > 0.05$), meaning that the age of brucellosis infection in males and females was almost the same in this city. Although, a recent study in Greece reported that the age distribution of patients with brucellosis was considerably different between men and women (Fouskis et al., 2018). In this regard, a similar study in Babol, Iran, presented the average age of females and males were 38.5 and 35.9, respectively (Roushan et al., 2004). The results of the study conducted in the west of Iran showed that there was no significant difference between men and women with brucellosis disease (Taheri et al., 2016).

Our results showed that approximately 93% and 99% of patients had a history of contact with the livestock and non-pasteurised dairy consumption, respectively, which was statistically notable. Regarding two important ways of brucellosis transmission in this city are, including contact with livestock and non-pasteurised dairy products; probably,the traditional animal husbandry and using traditional dairy products in these areas play an important role in this line.Of note, in Pakistan, contact with livestock and raw milk consumption were the powerful ways of brucellosis transmission (Ali et al., 2018). However, the use of non-pasteurised dairy products was the most important transmission way in Turkey (Guler et al., 2014). The association between the consumption of unpasteurized dairy products and brucellosis is confirmed in many studies. However, it is still one of the risk factors for the prevalence of brucellosis. Moreover, exposure to infected livestock is also considered an important risk factor (Heymann, 2008). The findings of the previous study indicate that in developing countries such as Iran, women are as much in contact with animals as men and direct contact with livestock and consumption of unpasteurized dairy products are the most important causes of brucellosis (Taheri et al., 2016). Due to the lack of awareness among the general population about the chronic symptoms of brucellosis, one of the limitations of the study was that the results of this study might not show the exact prevalence of brucellosis in Kohgiluyeh-va-Boyer Ahmad province. Future studies should explore the larger population and perform various diagnostic tests. One of the limitations of the present study was that the literacy level, health awareness and knowledge were not measured in the studied subjects. Therefore, it is suggested that future studies investigate the level of knowledge, education, as well as conditions, and healthy attitudes toward livestock. In addition, geographical locations (due to differences in cultures) are reported to have a different prevalence of brucellosis, so it is suggested that future studies should study several geographical locations in parallel.

Few cases with brucellosis are diagnosed, so the actual incidence of brucellosis is much lower than the clinically suspected cases. The results of this study demonstrated that apart from gender, direct contact with livestock, history of unpasteurized dairy consumption, Occupation and location are the most important risk factor in seroconversion to *Brucella* sp.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This research was approved by the Research Ethics Committee of the Behbahan Branch, Islamic Azad University of Medical Sciences by the Research Ethical Code IR.BIAU.REC.1877.99.

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