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Seroepidemiology of human brucellosis in nomads in a rural area of Iran

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting research in which authors have studied the incidence of a zoonosis disease in rural region, and risk factors among nomads have been highlighted. Details on Page 335 ABSTRACT

Objective: To survey the seroprevalence of brucellosis among nomads in Shoulabad, Lorestan province, Iran.

Methods: In this descriptive study, a total of 5847 person took part by randomized cluster sampling and 312 of them with clinical feature of brucellosis were selected. The diagnosis of brucellosis was made with a standard tube agglutination test (STAT), Coombs test, and 2-mercaptoethanol (2ME) test, and the results with titers equal 1:80 or more were considered positive.

Results: The seroprevaluce of anti–*Brucella* titer was 29.5% for STAT, 29.9% for Coombs test and 21.1% for 2ME test. Overall, 92, 93 and 66 patients had titers of 1:80 or higher in STAT, Coombs test and 2ME test, respectively. Seroprevalence was more among males (54.3%) than females (45.7%). There is statistical difference between seroprevalence of infection and age (P<0.05). Based on age group, 10–19 years age group was more affected (37%).

Conclusions: This results confirmed the necessity of conducting comprehensive and scheduled program of seroprevalence survey, particularly in nomad area, which aims at reducing the incidence of brucellosis as well as to guide planning programs by decision makers to improve community health.

KEYWORDS

Seroprevalence, Brucellosis, Lorestan, Nomads, standard tube agglutination test

1. Introduction

Brucellosis as a global zoonotic disease remains an significant public health problem in many regions around the world, especially those in the Asia and Middle East. In Iran, brucellosis is endemic and uninterruptedly reported from different part of Iran^[1,2]. Its prevalence in Iran ranges from 0.5% to 10.9% in different reports^[3]. Brucellosis is usually transmitted from infected animals to humans through direct contact with animals or use of their products,

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mostly dairy products made from unpasteurized milk. In Iran, traditional eating habits including the consumption of unpasteurized milk and fresh cheese and butter, are particularly common in the nomadic areas. These products are the primary causes of the spread of brucellosis^[4,5].

Brucellosis is diagnosed either by isolation of *Brucella* organism in culture or by a combination of serological tests and clinical findings consistent with brucellosis. Isolation of the *Brucella* organism is the definitive means of diagnosis but in practice, it is difficult due to the early tissue

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localization, exacting culture requirements of the organism and also prolonged time required for isolation. In practice, blood cultures are positive in 10%–30% of brucellosis and the remainders are diagnosed serologically^[6]. Therefore, in the absence of bacteriological confirmation, a presumptive diagnosis can be made based on a single high rising titer of specific antibodies. Among a variety of serological tests, standard tube agglutination test (STAT) or Coombs test are the most widely used^[7]. According to epidemiological status of brucellosis in Iran, diagnosis of active brucellosis is based upon positive results for Coombs and 2–mercaptoethanol (2ME) tests (*Brucella* antibodies) with titers equal or higher than 1:80^[8].

Brucellosis can have a considerable impact on human and animal health as well as a socioeconomic status, especially in nomadic areas that largely rely on livestock breeding and dairy products for their livelihood^[9]. Hence, attempt should be applied to screen the seroprevalence of *Brucella* antibody, especially in nomadic area, for planning more efficient health promoting programs in the affected areas. For the same reason, in the present study, seroprevalence of *Brucella*–antibody titer, using STAT, Coombs and 2ME test, was evaluated in nomadic population of Shoulabad in Lorestan province, Iran.

2. Material and methods

This was a descriptive study which was done in Shoulabad nomads, Lorestan province, Iran during 2008 to 2009. The study protocol was approved by the ethical committee of Khorramabad University of Medical Sciences. After giving informed consent according to local ethic committee guidelines, participants were enrolled in the study. The present survey including 5847 of nomads who were living in western Iran was carried out, using a semi-structured questionnaire. Out of these participants, 312 patients with clinical picture of brucellosis such as fever, sweating, lethargy, malaise, weakness, arthritis and weight loss were selected. The name, address, age and sex were noted. Then, blood samples were collected in vacuum tubes (to prevent freezing) and were taken into a cool box filled with the icepack/ice cubes to the Laboratory of Communicable Disease Center and Prevention, Lorestan University of Medical Sciences. Blood samples were centrifuged at 3000 r/ min for 10 min, and serum was stored at -20 °C until assayed.

All serum were routinely diluted from 1:20 to 1:1280 and STAT was performed on serum dilutions using *Brucella abortus* standard antigen obtained from the reference central lab in Khorramabad, Iran. Serial dilutions of serum were mixed with the standard tube agglutination antigen and then incubated at 37 °C for 24 h. Each batch of the test included a positive and negative control. The anti-human globulin (Coombs) test and 2ME test were performed on all serum.

Titration of 1:80 was accepted as exposure to Brucella, and

1:160 was accepted as brucellosis infection. Therefore, the titrations of 1:80 and over were accepted as positive. The results were analyzed by descriptive-analytic statistical methods with SPSS 16 software.

3. Results

Screening of all serum specimens by STAT gave positive results in 29.5% (92/312) serum, of which 23.9% (22/92) were positive at a titer of 1:80 and remain samples showing a titer of more than 1:80. The most of seroprevalnce of anti–*Brucella* antibody was related to titer 1:320 in STAT. The overall distributions of STAT titer found in the surveyed patients are shown in Table 1.

Table 1

Results of serological test in 312 samples.

	Serological tests			
Titer	STAT [*]	Coombs	2ME	
	No. (%)	No. (%)	No. (%)	
1:20	0 (0.0)	0 (0.0)	9 (10.6)	
1:40	0 (0.0)	0 (0.0)	10 (11.8)	
1:80	22 (23.9)	23 (24.7)	25 (29.4)	
1:160	12 (13.1)	12 (13.1)	28 (32.9)	
1:320	29 (31.5)	29 (31.5)	10 (11.8)	
1:640	22 (23.9)	22 (23.9)	3 (3.5)	
1:1280	7 (7.6)	7 (7.6)	0 (0.0)	

*STAT: standard tube agglutination test.

The results of Coombs test with a titer \geq 1:80 was positive in 93 patients. The results of 2ME test was also positive in 66 patients (1:80–1:640). The results of Coombs and 2ME tests are shown in Table 1.

Out of 92 positive serum, 50 (54.7%) of samples were related to male and remain samples (45.7%) were related to female. We found significant difference between seroprevalence of infection and age groups. The highest and lowest seroprevalence occurred in the age groups of 10–19 years old (37%) and 0–9 years old (3.3%), respectively (P<0.05) (Table 2).

Table 2

Seroprevalence of brucellosis in Shoulabad nomads' based on gender and age.

Age	Gender				
(year)	Male No. (%)	Female No. (%)	Total No. (%)		
0-9	1 (2)	2 (4.8)	3 (3.3)		
10-19	20 (40)	14 (33.3)	34 (37.0)		
20-29	7 (14)	7 (16.7)	14 (15.2)		
30-39	4 (8)	4 (9.5)	8 (8.7)		
40-49	7 (14)	8 (19.1)	15 (16.3)		
50-59	8 (16)	4 (9.5)	12 (13.0)		
≥60	3 (6)	3 (7.1)	6 (6.5)		

4. Discussion

Brucellosis has remained an important public health challenge in the developed and the developing countries particularly located between Europe, Northern Africa and Southwestern Asia (Mediterranean region). Despite all efforts to control of brucellosis in Iran, it is still endemic and has been reported in different province including in the south west of Iran, Lorestan^[10,11].

There are thousands of nomads living in undeveloped areas. More than 1.5 million nomads live in Iran, and some of them living in the Lorestan province located in the west of Iran. Nomads living in this region, because of their life style and close contact to domestic animals such goats, are highly infected^[8]. Due to their lifestyle, their persistent migration and lack of health service facilities, zoonosis like brucellosis is frequently seen in this population^[8]. Since there are no reliable basic data about these parameters and their roles in *Brucella* infection rates, authors conducted this study. The control of this zoonotic disease depends mostly upon the use of efficient diagnostic procedures^[12]. Although a number of techniques have been developed for measuring *Brucella* antibodies, STAT is probably the most widely used^[13].

In the present study, the seroprevalence of *Brucella* antibody titer using STAT, Coombs and 2ME test were 29.5%, 29.9% and 21.1%, respectively, which was higher than studies conducted in Turkey and Iran^[8,14]. In Iran, brucellosis represents a major health problem and is continuously reported with increasing frequency from various parts of the country^[7]. In a study performed among nomads in Khuzestan province (southwest of Iran), the reported seroprevalence of *Brucella* was 6.3%^[8]. In another study conducted on blood donor in Boushehr province (south of Iran), of 10500 samples, only six samples with different titers (1:20–1:40), were detected^[15].

Data from developing countries in the Mediterranean basin, particularly the Middle East, reported that the seroprevalence rates are 1% in Jordan^[16], 6.26% in Egypt^[17], 16% in Iraq^[18], 18% in Turkey^[19] and 40% in Libya^[20]. Limited report has been recorded on seroprevalence of brucellosis antibody in nomadic areas in Iran. Seroprevalence of brucellosis in nomadic population in Khuzestan^[8], Guilan^[21] and Fars^[22] were reported to be 8%, 9.8% and 10%, respectively.

In this study, men compared to the women were more infected with brucellosis. In a study in Uganda, females were significantly more likely to be seropositive than males^[23]. In another study in Macedonia, the ratio of the male to female patients was calculated as 1.96:1[24]. A report from Rezaee et al. conducted among Kurdish people founded that gender distributions of patients were significantly different^[25]. Ebrahimpour et al. pointed out that in Mazandaran, northern Provience of Iran, about 45.6% of patients were female^[26]. Brucellosis is more frequently isolated in young patients, and with a less frequency in the elderly^[11]. In the study performed in Georgia^[27], 68.7% of patients were in the age group 10-50 years, while in the study of Ebrahimpour^[26] the highest infection rates (38.4%) were found in the age group above 50 years. So this disease is more related to nomad young men who work in ranch and contact with animals. We can justify that brucellosis is more prevalent in age group of 10-19 years because there are lots of young people and nomad have direct contact with animals, while Zeinali et

al. gained that most patients were in the age group of 20–30 years^[28]. Bosilkovski *et al.* reported that most patients were 15–30 years old^[29]. Generally, high prevalence of brucellosis in middle–age and young people can be due to consumption of unpasteurized dairy products and a more contact with cattle.

The incidence rate of this disease can be reduced by continuous educational programs, enhancing knowledge and attitude of persons, prompt diagnosis, appropriate treatment and health care of patients. We conclude that in the western region of Iran, brucellosis seroprevalence is high in nomad populations. Our data highlights the need for future research, including the isolation and characterization of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Infectious disease prevention and control has been one of the top public health objectives during the last century. Brucellosis is considered as a neglected zoonosis; however, it is not regarded as a priority by health systems in many endemic areas.

Research frontiers

The present study evaluated the seroprevalence of *Brucella*-antibody titer using STAT, Coombs and 2ME test in nomadic population of Shoulabad in Lorestan province, Iran.

Related reports

According to the authors, some related studies were carried out in Iran and other regions of the world, and they show similarities with the present study.

Innovations & breakthroughs

The present research work makes an attempt to investigate

the prevalence and epidemiology of brucellosis in nomads western Iran.

Applications

Results can provide critical guidelines for initiating control strategies for policy makers to monitor and predict the disease.

Peer review

This is an interesting research in which authors have studied the incidence of a zoonosis disease in rural region, and risk factors among nomads have been highlighted.

References

- Shakurnia A, Qasemzadeh A, Afra M, Kohshour MO. Seroprevalence of brucellosis among blood donors in Ahvaz, Southwest Iran. *Asian Pac J Trop Dis* 2014; 4: S307–S310.
- [2] Akhtardanesh B, Ghanbarpour R, Babaei H, Nazeri M. Serological evidences of canine brucellosis as a new emerging disease in Iran. Asian Pac J Trop Dis 2011; 1: 177–180.
- [3] Sofian M, Aghakhani A, Velayati AA, Banifazl M, Eslamifar A, Ramezani A. Risk factors for human brucellosis in Iran: a case– control study. *Int J Infect Dis* 2008; 12: 157–161.
- [4] Hartady T, Saad MZ, Bejo SK, Salisi MS. Clinical human brucellosis in Malaysia: a case report. *Asian Pac J Trop Dis* 2014; 4: 150–153.
- [5] Khalili M, Sami M, Aflatoonian MR, Shahabi-Nejad N. Seroprevalence of brucellosis in slaughterhouse workers in Kerman city, Iran. Asian Pac J Trop Dis 2012; 2: 448–450.
- [6] Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. *Indian J Med Microbiol* 2007; 25(1): 28–31.
- [7] Rajaii M, Naghili B, Pourhassan A. Comparison of ELISA and STA tests in diagnosis of brucellosis. *Iran J Clin Infect Dis* 2006; 1(3): 145–147.
- [8] Alavi SM, Rafiei A, Nikkhooi A. The effect of lifestyle on brucellosis among nomads in Khuzestan province of Iran. *Pak J Med Sci* 2007; 23(3): 358–360.
- [9] Al-Majali AM, Talafha AQ, Ababneh MM, Ababneh MM. Seroprevalence and risk factors for bovine brucellosis in Jordan. J Vet Sci 2009; 10: 61-65.
- [10] Kassiri H, Amani H, Lotfi M. Epidemiological, laboratory, diagnostic and public health aspects of human brucellosis in western Iran. Asian Pac J Trop Biomed 2013; 3: 589–594.
- [11] Heidari M, Nazer M, Kheirollahi A, Birjandi M, Zareie H. Frequency of epididymo-orchitis in hospitalized patients with acute scrotum at Shohadaye Ashayer Hospital, Khorramabad, Iran. J Pak Med Assoc 2012; 62(1): 44–46.
- [12] Samaha H, Mohamed TR, Khoudair RM, Ashour HM. Serodiagnosis of brucellosis in cattle and humans in Egypt. *Immunobiology* 2009; **214**: 223–226.
- [13] Ertek M, Yazgi H, Ozkurt Z, Ayyildiz A, Parlak M. Comparison of the diagnostic value of the standard tube agglutination test and the ELISA IgG and IgM in patients with brucellosis. *Turk J Med*

Sci 2006; **36**(3): 159–163.

- [14] Vancelik S, Guraksin A, Ayyildiz A. Seroprevalence of human brucellosis in rural endemic areas in eastern Turkey. *Trop Doct* 2008; **38**: 42–43.
- [15] Khorasgani MR, Esmaeili H, Pourkarim MR, Mankhian AR, Salehi TZ. Anti-brucella antibodies in blood donors in Boushehr, Iran. *Comp Clin Pathol* 2008; **17**: 267–269.
- [16] Abo-Shehada MN, Abu-Halaweh M. Seroprevalence of Brucella species among women with miscarriage in Jordan. East Mediter Health J 2011; 17(11): 871–874.
- [17] Hassanain NA, Ahmed WM. Sero-prevalence of brucellosis in Egypt with emphasis on potential risk factors. World J Med Sci 2012; 7(2): 81–86.
- [18] Yacoub AA, Bakr S, Hameed AM, Al-Thamery AA, Fartoci MJ. Seroepidemiology of selected zoonotic infections in Basra region of Iraq. *East Mediter Health J* 2006; 12(1-2): 112-118.
- [19] Arvas G, Akkoyunlu Y, Berktas M, Kaya B, Aslan T. The prevalence of brucellosis in adults in northeastern region of Turkey. Jundishapur J Microbiol 2013; 6(3): 262-264.
- [20] Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM, Ibrahim A, et al. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. *Euro Surveill* 2010; **15**(30): pii: 19625.
- [21] Nikokar I, Hosseinpour M, Asmar M, Pirmohbatei S, Hakeimei F, Razavei MT. Seroprevalence of brucellosis among high risk individuals in Guilan, Iran. J Res Med Sci 2011; 16: 1366–1371.
- [22] Kazerouni P, Ataollahi M, Moghadami M, Gooya M, Rezaei F. Prevalence of brucellosis in nomadic areas of the province in summer 1391. In: 21st Iranian Congress of Infectious Disease and Tropical Medicine; 2013 Jan 19–23; Tehran, Iran.
- [23] Makita K, Fèvre EM, Waiswa C, Kaboyo W, Eisler MC, Welburn SC. Spatial epidemiology of hospital-diagnosed brucellosis in Kampala, Uganda. Int J Health Geogr 2011; 10: 52.
- [24] Donev D, Karadzovski Z, Kasapinov B, Lazarevik V. Epidemiological and public health aspects of brucellosis in the Republic of Macedonia. *Prilozi* 2010; **31**: 33–54.
- [25] Rezaee MA, Rashidi A, Motaharinia Y, Hossaini W, Rahmani MR. Seroprevalence study of brucellosis among high-risk groups in comparison with other people of the population in Sanandaj (West of Iran). *Afr J Microbiol Res* 2012; 6(9): 1985–1989.
- [26] Ebrahimpour S, Youssefi MR, Karimi N, Kaighobadi M, Tabaripour R. The prevalence of human brucellosis in Mazandaran Province, Iran. Afr J Microbiol Res 2012; 6(19): 4090– 4094.
- [27] Akhvlediani T, Clark DV, Chubabria G, Zenaishvili O, Hepburn MJ. The changing pattern of human brucellosis: clinical manifestations, epidemiology, and treatment outcomes over three decades in Georgia. *BMC Infect Dis* 2010; **10**: 346.
- [28] Zeinali M, Shirzadi MR. Effective ingredient in accretion and reduction of brucellosis incidence in human in Iran in 1985–2005.
 In: Proceedings of 15th Veterinary Congress; 2008; Iran.
- [29] Bosilkovski M, Krteva L, Dimzova M, Vidinic I, Sopova Z, Spasovska K. Human brucellosis in macedonia–10 years of clinical experience in endemic region. *Croat Med J* 2010; 51(4): 327–336.