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Short Communication

Seroprevalence of Brucellosis among high risk individuals in Guilan, Iran

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Abstract

BACKGROUND: Brucellosis is a major public health problem in developing countries and has remained endemic in Iran. The aim of this study was to investigate the Seroprevalence of Brucellosis among high risk individuals in Guilan, Iran.

METHODS: In a cross-sectional study, 478 blood samples from people living in rural areas (n = 292) and 186 slaughterhouse workers were screened by slide agglutination and microplate agglutination tests. Seropositive specimens were analyzed with Elisa for IgG and IgM antibody.

RESULTS: Seroprevalence of brucellosis among slaughterhouse workers and the people living in rural areas were 9.8% (n = 18) and 5.5% (n = 16), respectively (p = 0.04). A significant association was observed between the seropositivity and type of abattoir (p = 0.04) and contact with animals (p = 0.02) among slaughterhouse workers as well as consumption of unpasteurized milk products (p = 0.02) in people living in rural areas. IgG antibodies titer was higher than IgM in seropositive cases of the slaughterhouse workers and the people living in rural areas.

CONCLUSIONS: Seroprevalence of brucellosis in slaughterhouse workers was higher than people living in rural areas. Consumption of raw products and direct contact with domestic animals were found to be significant risk factor for brucellosis. High titer of IgG antibody among the two study groups indicated that most seropositive subjects were in chronic phase of brucellosis.

KEYWORDS: Brucellosis, Seroprevalence, Agglutination Tests, Elisa.

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Brucellosis as a worldwide zoonosis disease remains an important public health problem in many countries around the world, especially those in the Middle East.[1] In Iran, human brucellosis is endemic and continuously reported from various part of the country.[2] The prevalence of brucellosis in Iran has been reported from 0.5% to 10.9% in different provinces.[3] This disease is usually transmitted from infected animals to man by direct contact or by consumption of raw milk that was infected with *Brucella* organisms.

Consumers of unpasteurized dairy products especially from areas of endemic infection are at a significant risk of food-borne brucellosis.[4] In Iran, traditional eating habits including the consumption of unpasteurized milk and fresh cheese and butter, is particularly common in the rural areas. These products are the primary causes of the spread of brucellosis.[2] Brucellosis is also an occupational hazard. Slaughterhouse workers and others involved in animal handling are at a higher risk of direct inoculation by skin abrasion, mucous membranes and inhalations.[5, 6]. Isolation of Brucella bacteria from clinical sample is the gold standard for diagno-

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sis of brucellosis. In the absence of bacteriologic method, a variety of serologic tests can be made on the basis of high or rising titers of specific antibodies for diagnosis of brucellosis. The serum agglutination test (SAT), which is the most commonly used test, will detect antibodies against B.abortus, B.suis, and B.melitensis. False negative reactions due to blocking antibodies are seen and therefore dilutions of serum should be made to avoid the prozone phenomenon. Micro plate agglutination test (MAT) can be used for detection of Brucella antibody by a serial dilution method and avoiding prozone phenomenon. The enzyme-linked immunosorbent assay (ELISA) test for Brucella is an important method to assess specific antibody titer in brucellosis. Early in infection, antibodies of IgM class predominate; followed shortly by a switch to IgG antibodies. The IgG antibody has a delayed appearance, although it is found together with IgM 4 weeks after the initial antigenic stimulus.[7] Determination of the seroprevalence of brucellosis and the major risk factors of it among high risk groups are very important for understanding of the nature of the disease and eradication of brucellosis. The aim of this study was to determine the seroprevalence of brucellosis among people living in rural areas as well as slaughterhouse workers and evaluation of specific antibodies in seropositive subjects by ELISA method.

Methods

A cross-sectional epidemiological study was carried out from May to October 2009 to determine the seroprevalence and identify risk factors among people living in the rural areas and slaughterhouse workers in Guilan province, north of Iran. A total of 478 blood samples were collected voluntarily from all male slaughterhouse workers (n = 186) and 292 people living in rural areas (105 male and 187 female) by quota sampling method. Subjects from rural area that participated in this study lived in the region of Roudsar, a city in the east of Guilan province. This area is divided into 2 agro-climatic zones, sub mountain region (3 villages) and plain region (4 villages). Slaughterhouse workers that took part in this study were staffs of two slaughterhouse, an industry in the center (Rasht region) and traditional abattoir in the eastern of Guilan province (Langeroud region) which were the only active slaughterhouses in the this province. All participants were given informed written consents to participate in this study. For serology assay, blood samples were centrifuged (3000×*g* for 10 min) and each serum was divided into aliquots and stored at –20°C until tested for presence of Brucella antibodies.

All sera were screened using slide agglutination test and the micro plate agglutination test. A titer of 1:160 or greater was considered positive for specific agglutination *Brucella* antibodies. Seropositive specimens were analyzed by ELISA tests for detection of IgG and IgM antibodies. ELISA kite purchased from a commercial company (IBL, Hamburg, Germany) for human sera and was used based on the manufacturer's instructions. Based on ELISA kite the Optical Density (OD) cut-off values of seropositive samples were chosen to be > 0.5 for IgG and IgM.

Statistical analysis: The analysis was performed using SPSS version 12 for Windows. Chi-square and Fisher exact tests were used to compare categorical variables. P value less than 0.05 was considered as statistically significant. Categorical variables were shown by number and percentage.

Results

Out of 186 slaughterhouse workers, 18 (9.8%) subjects showed presence of antibodies against Brucella antigen by SAT and MAT methods. Likewise, 5.5% (16 out of 292) of people living in rural areas showed presence of antibodies. A significantly higher seroprevalence of brucellosis among slaughterhouse workers was found compared to the people living in rural areas (p = 0.04). In the slaughterhouse workers, a significant difference of seropositivity was observed in two types of abattoir (p = 0.04), age groups (p = 0.001), duration of work

categories (p = 0.001) and contact with animals (p = 0.02) (Table 1). All positive cases in rural areas had a history of unpasteurized and fresh milk products consumption especially chesses (p = 0.02). Seropositivity was significantly associated with sex and were higher among men (p = 0.04). Seroprevalence was found to be insignificantly higher among people living in sub-mountain areas compared to plain regions. The analysis of ELISA technique indicated that all sera that showed positive agglutination by SAT and MAT were positive by ELISA method except for 4 cases (25%) in people of rural areas and 3 cases (16.6%) of slaughterhouse workers.
Table 2 shows the frequency of individuals with antibody concentration higher than determined cut-off values. Among the six rural subjects that showed both IgG and IgM antibodies, in the 4 (66.7%) subjects higher level of IgG and 2 (33.3%) subjects higher level of IgM were detected. In the one slaughterhouse workers that showed both IgG and IgM, IgG titer was more than IgM.

Discussion

Brucellosis has remained an important public-health problem and zoonotic infection in many developing countries particularly in Mediterranean region. In Iran, despite all efforts to control this disease, it is still endemic and has been reported in different province including in the north of Iran, Guilan.[3] All Brucellosis infections in humans are due to the direct or indirect contact with infected animals and eating habits. Brucellae are found in a great numbers in the milk and abortive products of infected animals and thus brucellosis has become an occupational disease especially in slaughterhouse

Table 1. Characteristics of study subjects and distribution of seroprevalence of brucellosis

	n	No. seropositives (%)	P-value
A) Slaughterhouse workers			
Age(years)			0.001
<25	33	0(00.0%)	
25-35	62	1(1.6%)	
36-45	57	6(10.5%)	
>45	36	11(30.5%)	
During of work(years)			0.001
<10	103	2(1.9%)	
10-20	46	7(15.2%)	
>20	37	9(24.3%)	
Contact with animal			0.02
Sheep	32	7(21.8%)	
Cattle	69	3(4.3 %)	
Sheep+Cattle	85	8(9.4%)	
Type of abattoir			0.02
Traditional	46	8(17.3%)	
Industrial	140	10(7.1%)	
B) People living in rural areas			
Sex			0.04
Male	105	9(8.75%)	
Female	187	7(3.4%)	
Region of living			0.141
sub mountain	76	6(7.89%)	
Plain	216	10(4.63%)	
consumption of milk products			0.02
unpasteurized	**236**	16(6.7%)	
pasteurized	**56**	0 (0%)	

Table 2. Prevalence of Brucella IgM and IgG antibodies among high risk group individuals

	n	IgG	IgM	IgG and IgM	P-value
Slaughterhouse workers	15	13(86.6%)	1(6.6%)	1(6.6%)	0.0001
People living in rural areas	12	5(41.6%)	1(8.4%)	6(50%)	0.07

workers. The present study was conducted to determine the seroprevalence of brucellosis in high risk groups including the abattoir workers and people living in rural areas. In Iran, few studies have been carried out on brucellosis especially on slaughterhouse workers and rural areas.

In this study, seroprevalence of *Brucella* antibodies were found 5.4% among people living in rural areas. Higher seroprevalence rates were reported in rural areas of other countries, such as 26.2% in Saudi Arabia,[9] 18% in Uganda[10] and 13% in Nigeria.[11] Data from developing countries in the Mediterranean basin, particularly the Middle East, reported seroprevalence rates ranging from 8% in Jordan[12, 5] to 12% in Lebanon and Kuwait.[13, 14] All of the seropositivite subjects in rural areas had a history of infected cheese and milk consumption. In the other studies, consumption of fresh cheese and milk produced from unpasteurized milk was reported to be a significant risk factor for brucellosis.[4, 15, 16] According to our findings similar to another study in rural areas[15] seropositivity was higher in males. A study in the urban areas showed a higher incidence of brucellosis in females.[17] The higher rate of seroprevalence of brucellosis in males compared to females was probably due to an increased involvement of men in farming domestic animals and handling their products in rural areas. We found no significant difference of seroprevalence between the people living in submountain than plain region**.** The variation in prevalence rates of brucellosis among population in different geographical locations and countries may be due to variation in existence of disease among animals, occupational contact and social habits of different population.[14]

In the present study, seropositivity was found to be 9.8% among slaughterhouse workers. In Iran, very few studies have been carried out on slaughterhouse workers. In the study of Karimi et al.,[18] a higher rate in slaughterers was reported (20%). The prevalence of brucellosis shows marked variation between countries. The seroprevalence of brucellosis among slaughterhouse workers was reported to be 35% in Saudi Arabia,[9] 37.6% in Algeria[19] and 4.1% in Brazil.[8] In the present study, a higher seropositivity was observed in the older age group. A maximum percentage (30.5%) was seen in the age group of above 45 years. The study of Abo-Shehada *et al.* also showed an increase in seroprevalence with advancing age.[20]

A significant association between the seropositivity and duration of job of was observed in the current study. Karimi *et al.* also highlighted a strong association between brucellosis and duration of occupational exposure.[18] The result of the study revealed a high seroprevalence of Brucella among slaughterhouse workers who were in contact with sheep. In abattoir workers who are in direct contact with raw meat and carcasses of infected animals, infection probably occurs through cuts and wounds to the bare hands or through splashing of infected blood or other fluid to the conjunctiva. In these cases infection was mainly due to the direct contact with domestic animals and their products specially sheep. Contact with animals has been reported as one of the important risk factors for brucellosis in other studies.[9, 21] Higher seropositivity was found among slaughterers working in traditional abattoir compared to industrial one**.** To our knowledge, this study was the first report on the seroprevalence of brucellosis among workers of the two type abattoir**.** However, Mukhtar and Kokab[22] reported opposing results. They found no relationship between the workers of two abattoir types (government and army) and seroprevalence of brucellosis.

Results by Elisa method indicated that 86% of sera from slaughterhouse workers had significantly elevated IgG levels. In the rural areas, IgG antibodies were positive in 41.6%

while IgM were found in 8.4% and both IgM and IgG positivity was in 50% of subjects. Among the subjects that showed both IgG and IgM antibody, IgG titer was more than IgM. In many studies that ELISA was used, it was determined that IgG positivity and increase in of antibody titers were considerably valuable in cases with chronic infections.[23, 7] Moreover, in countries where the disease is highly endemic, a large proportion of the population may have persistent Brucella specific IgG antibodies.[24]

Conclusion

Based on the findings of our study, we conclude that seroprevalence of brucellosis is high in slaughterhouse workers owing to their close contact with animals specially sheep. This study confirmed the endemicity of brucellosis among people living in rural areas and consuming raw products with animal origin were identified as the main risk factors. High titer of IgG antibody was shown among slaughterhouse workers and people living in rural areas that indicats more subjects were in chronic phase. In the few cases, IgM antibodies were detected from sera of subjects which is important to diagnose brucellosis in acute phase. Evaluation of IgG and IgM antibody by ELISA method can be important for recognizing brucellosis in different stages.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

IN carried out the design and statistical analysis of study, participated in most of the experiments and prepared the manuscript. MH provided assistance for all experiments. MA developed the concept and design of the study and edited the text. SP collected blood samples and provided assistance for experiments. FH collected blood sample and provided assistance for experiments. MTRT provided assistance for blood sample collection. All authors read and approved the content of the manuscript.

References

1. Araj GF, Kattar MM, Fattouh LG, Bajakian KO, Kobeissi SA. Evaluation of the PANBIO Brucella immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays for diagnosis of human brucellosis. Clin Diagn Lab Immunol 2005; 12(11): 1334-5.
2. Heydari F, Ozaffari NA, Tukmechi A. A comparison of standard seroagglutination tests and ELISA for diagnosis of brucellosis in west Azerbaijan province, Iran. Research Journal of Biological Sciences 2008; 3(12): 1460-2.
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(2): 157-61.
4. Gwida M, Al DS, Melzer F, Rosler U, Neubauer H, Tomaso H. Brucellosis - regionally emerging zoonotic disease? Croat Med J 2010; 51(4): 289-95.
5. Al-Majali AM, Talafha AQ, Ababneh MM, Ababneh MM. Seroprevalence and risk factors for bovine brucellosis in Jordan. J Vet Sci 2009; 10(1): 61-5.
6. Yoo SJ, Choi YS, Lim HS, Lee K, Park MY, Chu C, et al. [Seroprevalence and risk factors of brucellosis among slaughterhouse workers in Korea]. J Prev Med Public Health 2009; 42(4): 237-42.

7. 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): 159-63.
8. Ramos TR, Pinheiro Junior JW, Moura Sobrinho PA, Santana VL, Guerra NR, de Melo LE, et al. Epidemiological aspects of an infection by Brucella abortus in risk occupational groups in the microregion of Araguaina, Tocantins. Braz J Infect Dis 2008; 12(2): 133-8.
9. Al-Sekait MA. Seroepidemiology survey of brucellosis antibodies in Saudi Arabia. Ann Saudi Med 1999; 19(3): 219-22.
10. Ndyabahinduka DG, Chu IH. Brucellosis in Uganda. Int J Zoonoses 1984; 11(1): 59-64.
11. Sixl W, Rosegger H, Schneeweiss H, Withalm H, Schuhmann G. Serological investigations in Nigeria for anthropozoonoses in human sera: brucellosis, echinococcosis, toxoplasmosis, chlamydial diseases, listeriosis, rickettsiosis (Coxiella burneti and Rickettsia conori). J Hyg Epidemiol Microbiol Immunol 1987; 31(4 Suppl): 493-5.
12. Dajani YF, Masoud AA, Barakat HF. Epidemiology and diagnosis of human brucellosis in Jordan. J Trop Med Hyg 1989; 92(3): 209-14.
13. Araj GF, Azzam RA. Seroprevalence of brucella antibodies among persons in high-risk occupation in Lebanon. Epidemiol Infect 1996; 117(2): 281-8.
14. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR, Fenech FF. Human brucellosis in Kuwait: a prospective study of 400 cases. Q J Med 1988; 66(249): 39-54.
15. Bikas C, Jelastopulu E, Leotsinidis M, Kondakis X. Epidemiology of human brucellosis in a rural area of northwestern Peloponnese in Greece. Eur J Epidemiol 2003; 18(3): 267-74.
16. Cetinkaya Z, Aktepe OC, Ciftci IH, Demirel R. Seroprevalence of human brucellosis in a rural area of Western Anatolia, Turkey. J Health Popul Nutr 2005; 23(2): 137-41.
17. Cooper CW. The epidemiology of human brucellosis in a well defined urban population in Saudi Arabia. J Trop Med Hyg 1991; 94(6): 416-22.
18. Karimi A, Alborzi A, Rasooli M, Kadivar MR, Nateghian AR. Prevalence of antibody to Brucella species in butchers, slaughterers and others. East Mediterr Health J 2003; 9(1-2): 178-84.
19. Habib A, Near A, Qamor J, Azrot R. Prevalence of brucellosis: a serological study in Tiaret, Western Algeria. Arab Gulf J Scientific Res 2003; 21(4): 244-8.
20. Abo-Shehada MN, Odeh JS, Abu-Essud M, Abuharfeil N. Seroprevalence of brucellosis among high risk people in northern Jordan. Int J Epidemiol 1996; 25(2): 450-4.
21. Sumer H, Sumer Z, Alim A, Nur N, Ozdemir L. Seroprevalence of Brucella in an elderly population in mid-Anatolia, Turkey. J Health Popul Nutr 2003; 21(2): 158-61.
22. Mukhtar F, Kokab F. Brucella serology in abattoir workers. J Ayub Med Coll Abbottabad 2008; 20(3): 57-61.
23. Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F. Specific antibody profile in human brucellosis. Clin Infect Dis 1992; 14(1): 131-40.
24. Hussein AA, Sayed AS, El Feki MA. Seroepidemiological study on human brucellosis in Assiut Governorate. Egypt J Immunol 2005; 12(1): 49-56.