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Full Length Research Paper

Seroprevalence study of brucellosis among high-risk groups in comparison with other people of the population in Sanandaj (West of Iran)

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Brucellosis is a zoonosis disease caused by *Brucella*. The burden of human brucellosis in Kurdistan province was 83.5 cases per 100.000 people; occupying the second place of point of infection rate in Iran. This study aims at evaluating the seroepidemiology of brucellosis among high-risk groups in comparison with the rest of population in Sanandaj city. The data is collected using a questionnaire. Blood samples were randomly obtained from 1541 persons (130 persons of the high-risk groups and 1411 persons of the remaining population). After serum extraction, standard agglutination test (SAT), anti-human globulin (Coombs') test (AHGT) and 2-Mercapto ethanol (2-ME) test were performed. Agglutination tests in high-risk groups and other people of the population was negative in 80.7 and 85.9% of cases respectively, and positive antibody in two population categories were found in 19.3 and 14.1% respectively. This study showed that the high-risk groups exposed to direct contact with livestock are vulnerable to the infection. In addition, to regular vaccination of livestock, proper sanitary methods should be seriously followed by the people who are in direct contact with animals. Administrating of bio-safety measures is recommended, and precautions considerations in personal vaccine should be designed and used.

Key words: Brucellosis, Serology, Ranchers, Butchers, Slaughterhouse workers, High-risk groups.

INTRODUCTION

Brucellosis is an infectious disease common in the Middle East due to human contact with infected animals or consumption of contaminated animal products. The infection occurs in different routes including the consumption of contaminated dairy products, microbial inoculation through cuts or abrasions in the skin surface, the conjunctiva inoculation, inhalation of infectious aerosols, accidental human contact with infected animals and consumption of contaminated meat (Fosgate et al., 2002). These responsible micro-organisms are coccobacilli, Gram-negative, non-spore-forming, non-motile, facultative anaerobes; an important characteristic of is that they act as facultative intracellular parasites (Acosta-González et al., 2006) and in some cases, brucellosis may cause death (Rahman et al., 2001). *Brucella* often penetrate the endothelial reticulum and can infect macrophages (Zavala et al., 1994). Antibody profiles do not have specific clinical correlations, and titers often remain high for a protracted period. The asymptomatic patient has an isolated positive titer of IgG and IgA antibodies (Ariza et al., 1992). IgM against lipopolysaccharide appeared during the first week of infection, followed by IgG as early as the second week.

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Both classes of immunoglobulin peaked during the fourth week and the use of antibiotics was associated with a decline in both IgM and IgG titers. IgM titers persisted at levels that were higher than those of IgG titers for more than six months, and both classes were present for almost a year. The appearance of IgA in conjunction with IgG for longer than six months was consistent with the presence of chronic disease. Antibody response in brucellosis, although extremely useful diagnostically, plays a limited part in the overall host response (Bravo et al., 2003).

There are common clinical symptoms including weakness, lethargy, chill, fever, sweating, decreased appetite, arthralgia, myalgia, weight loss, headache, back pain and psychological symptoms. According to World Health Organization (WHO), the number of known patients reported is 10 to 25 times less than the actual frequency of the disease in the community. Therefore, every year 500.000 people are affected (World Health Organization, 2003). In the USA, 4 to 10% of cases are diagnosed and reported (Moniri, 2001). The prevalence rate of brucellosis in different provinces of Iran varies from 1.5 up to 107.5 per 100.000 persons in 2003. The highest levels of infection appeared in Hamedan with 107.5, Kurdistan with 83.5, West Azerbaijan with 71.4 and Zanjan with 67.1 per 100.000 people (World Health Organization, 2003). Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals and personnel in microbiologic laboratories (Perez-Avraham et al., 2001; Vizcaino et al., 1997, 1994; Almuneef et al., 2004). The most important factor for control and treatment of the disease is accurate diagnosis of brucellosis (Elfaki et al., 2005). Bacteriological detection of brucellosis in humans depends on isolating the bacteria from blood and bacteriological tests and its biotype, which takes at least seven days (Jay et al., 2004). Today, there are several serological tests used to diagnosis brucellosis in different laboratories (Navarro et al., 2000) including the standard agglutination test (SAT), anti-human globulin (Coombs') test (AHGT) and enzyme-linked immunosorbent assay (ELISA) (FAO/WHO Expert Committee on Brucellosis, 1986). Since contact with a living infected organism is risky and the most important of all, the microbial cultures are not capable of detecting the bacteria rapidly and accurately, the initial isolation of bacteria is a current problems in diagnostic laboratories (Leal- Klevezas et al., 1995). As the symptoms of brucellosis are non-specific, the diagnosis can be done only on the basis of laboratory findings (Guler et al., 2003).

Kurdistan province has the second rank in Iran with respect to the seroprevalence of brucellosis, and the region is considered an endemic area for brucellosis (World Health Organization, 2003).

In recent years, few studies have been performed on Brucella antibody levels among high-risk individuals (Perez-Avraham et al., 2001). Therefore, the purpose of the current study is to evaluate the seroepidemiological study of brucellosis among high-risk groups in comparison with other people of the population in Sanandaj city.

MATERIALS AND METHODS

The study area/city

The study and sampling were mainly performed in Sanandaj, Kurdistan province, west of Iran. In this region, people are mostly employed in agriculture and animal husbandry sector, and direct contact with animals and animal products is common. In the area, taking unpasteurized dairy products is usual. Furthermore, keeping, slaughtering, and taking of dairy and non-dairy products of livestock are carried out traditionally. The main livestock of the region are sheep and goat.

Demographic and epidemiologic properties and questionnaire

The study was performed from June 2010 to August 2011. The samples (n=1541) were taken from individuals randomly selected from the registry of high-risk group including ranchers, butchers and slaughterhouse workers and other people of the population in Sanandaj (n=1411). A questionnaire was designed to collect information related to personal, epidemiologic, and sociodemographic profile (age, sex, occupation and history of disease in individuals and their families), current eating habits, and clinical information about symptoms suggestive of brucellosis (fever, sweating, arthralgia, myalgia and weakness) lasting for more than 5 days in the previous 5 years (Lulu et al., 1988). In the high-risk groups, most individuals were male. Therefore, for a better comparison, we tried to mostly include men from the general population.

Collection and handling of blood

At the beginning, all participants signed a fully informed written consent on testing and blood sampling. Then, they were provided some instructions about the processes of interview and filling out the questionnaire. The questionnaire was filled out and blood samples were obtained by qualified nurses. From each participant, a 7-ml blood sample was obtained. Within one hour after sampling, sera were separated in Faculty of Medicine, Kurdistan University of Medical Sciences. Then, the sera were transported (in a cool box) to the Serology Unit at the Kurdistan University of Medical Sciences, where they were stored at - 20℃ until tested .

Brucella standard agglutination test (SAT), anti-human globulin (Coombs') test (AHGT) and 2- Mercapto ethanol (2-ME)

The SAT was carried out on doubling dilution of serum from 1:20 to 1:2560, essentially as reported earlier by Young (1991). *Brucella abortus* antigen and positive and negative control sera (Pasteur Institute, Tehran, Iran) was used according to the instruction of the manufacturer. Positive reactions were determined by an agglutinoscope and the titres given indicated the highest dilution in which 50% or more agglutination occurred in tube. Sera showing negative agglutination (that is, titre <) were tested continuously by Brucella anti-human globulin (Coomb's) test. AHGT was subsequently performed as extension of the SAT for detection of 'blocking' or 'non-agglutinating' immunoglobulin G (IgG) antibodies (Young, 1991). The 2-ME, a modification of the SAT, was

performed as described by Alton et al. (1975a). A reduction in titre of at least one complete tube compared with the control was arbitrarily taken to indicate that the serum contained a significant proportion of IgM antibody (Alton et al., 1975).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 12.0. The significance of differences between groups was determined using *Chi-square* and *t* tests. A value of P ≤ 0.05 was considered statistically significant.

RESULTS

Demographic findings

Totally, total of 1541 individuals 130 in the high-risk groups and 1411 from the remaining population were interviewed and tested: among the high-risk occupations, 24.6% (130 individuals) were butchers, 15.4% were ranchers, 46.2% were abattoir workers and 13.8% were unpasteurized milk products sellers. Out of the individuals studied, 96.93% were male, and overall positive titres were found in 92% of the male individuals. In the other people of the population (n=1411), 13.2% were housekeeper, 0.3% unemployed, 13.4% office clerks, 2.8% farmers, 12% college students, and 55.2% with other jobs (workers, shopkeepers, drivers, military men, barbers, tailors, etc.).

In the high-risk occupations, the mean age of the patients was 39.54±12.4 years, where in other people of the population, it was 35.5±11.54 years. Although the number of males was higher than females, in all serological tests, the difference was not significant (P≥0.05). Considering seropositive brucellosis cases among high-risk occupations, ranchers and slaughter-house workers had the highest seroprevalence for brucellosis (P≤0.05) (Table 1).

Findings in standard agglutination test, 2-Mercapto ethanol (2-ME) and anti-human globulin (Coombs') test (AHGT)

The overall distribution of SAT, 2-ME and AHGT titres found in the surveyed individuals and the percentages of individuals with a SAT or AHGT titres as shown in Table 2.

In high-risk occupations, negative titres (≤) in SAT and AHGT were 6.91 and 14.61% of participants, where in other people of population in Sanandaj the rates were 99.7%, respectively. In high-risk occupations, the percentage of individuals showing a titre of ≥ in SAT and AHGT were and 14.61% respectively, however, in the other population of people the rates were and 0.3% respectively.

Findings of serologic tests in individuals with and without symptoms

Among 1541 individuals whose questionnaires were completed for symptoms in high-risk groups, 87 (66.93%) had symptoms while in the other population of the people, 418 (29.6%) had symptoms that may be associated with brucellosis, and the difference was not significant in the both groups (P 0.05).

DISCUSSION

Brucellosis is a zoonotic infection associated with reproductive failure in animals and febrile disease in humans. It represents one of the most widespread zoonosis and remains an important public health and an economic problem worldwide. The situation in Iran is improving, according to data from the National Commission on Communicable Diseases Control. In 1989, the annual incidence exceeded 1000 cases per million people (Regional Animal Disease Surveillance and Control Network, Food and Agriculture Organization of the United Nations, 2005); while, the annual incidence in 2003 had fallen to 238.6 cases per million people. Nevertheless, human brucellosis still remains a huge burden for the country (World Organisation for Animal Health, 2005). The current study represents the first report on the seroprevalence of brucellosis in high-risk groups in comparison with other people of the population in Sanandaj. The overall exposure rate of 19.3 and 14.1% by SAT, and 19.22 and 0.8% by AHGT found in this study is suggestive of the high frequency of *brucella* exposure in the high-risk groups and other people of the population in Sanandaj, respectively. The frequencies are comparable to those reported from other countries.

The seroprevalence rates of brucellosis in studies conducted in Iran are different. For instance; in a study conducted by Moniri and Dastegoli (1997) on 2376 individuals in Kashan, the rate of the infection has been shown by SAT and Rose Bengal test (RBT) to be 0.9% in general the population, and 3.9% in high-risk groups (Moniri and Dastegoli., 1997). Based on the results of another study performed by Karimi et al. (2003) on healthy slaughterhouse workers, butchers, and general population in Shiraz, the prevalence of brucellosis by serologic agglutination was reported 20, 4, and 2, in the three mentioned groups, respectively (Karimi et al., 2003).

In the studies of Araj and Azzam (1996), Moniri and Dastegoli (1997) and Cetinkaya et al. (2006) (on 1850 villagers in Turkey), consumption of unpasteurized milk products or milk with significant higher frequency of positive serology was reported *(*P<0001) but it was not the case in our study (P>0.05).

In the study by Bokaie et al. (2009), housekeepers (29.53%) and farmers (18.75%) were at high risk of brucellosis among different occupations, while our data

Table 1. Demographic and epidemiologic characteristics and common clinical symptoms of high-risk occupations in comparison with other people of the population in Sanandaj city and their positive titres of Brucella antibody.

	High-risk occupations			Other people of the population		
		Individuals (%)	Individuals with positive titre (%)		Individuals (%)	Individuals with positive titre (%)
Gender	Male	126 (96.93)	23 (18)	Male	1170 (83.4)	155 (13.2)
	Female	4 (3.07)	2 (50)	Female	234 (16.6)	42 (17.9)
Occupation	Butcher	32 (24.6)	5(15.6)	Housekeeper	185 (13.2)	30 (16.2)
	Rancher	20 (15.4)	6(30)	Unemployed	42 (3)	5 (11.9)
	Slaughter house workers	60 (46.2)	12(20)	Employee	189 (13.4)	22 (11.6)
	Milk product sellers	18 (13.8)	2(11.11)	Farmer	40 (2.8)	8 (20)
				College students	169 (12)	28 (16.5)
				Other jobs	779 (55.2)	103 (13.2)
History of disease in persons	+	8 (6.2)	4(50)		28 (2)	5 (17.85)
	-	122 (93.8)	21(17.21)		1377 (98)	191 (13.8)
History of disease in family	+	10 (7.69)	3(30)		Not was studied	
	-	122 (93.8)	22(18.03)			
Common clinical symptoms of brucellosis	Night sweats	30 (23.08)	8(26.7)		36 (2.6)	4 (11.11)
	Orchitis	4 (3.08)	2(50)		1 (0.1)	1 (100)
	Back pain	49 (37.69)	9(18.36)		127 (9)	27 (21.25)
	Headache	32 (38)	7(21.87)		115 (8.2)	20 (17.39)
	Fever	9 (6.9)	2(22.2)		3 (0.2)	1 (33.33)
	Myalgia and joints pain	34 (26.15)	4(11.76)		136 (9.9)	30 (22.05)

showed that dairy product sellers (11.11%), and office clerks (11.6%) were at the lowest risk of brucellosis and ranchers (30%), farmers and slaughterhouse workers (20%) were at highest risk of brucellosis among the different occupations.

In the studies by Cetinkaya et al. (2006) and Jordi et al. (2000) (on 347 persons in Jussa), significant correlation was found between positive antibody and history of brucellosis in persons or their families. In our study, history of brucellosis in persons was positive in 6.2 and 2% of high-risk groups and other people of the population in Sanandaj, respectively, while 50 and 17.21% of them had anti brucella antibody, respectively.

The *Brucella* antibodies detected in asymptomatic individuals could be related to a history of exposure, inactive brucellosis, or repeated exposure to antigenic stimuli, as was reported earlier in veterinarians and abattoir workers (Cooper, 1992).

Conclusion

The results of this study showed that in Sanandaj, as an endemic area for brucellosis, preventive measures is necessary in high-risk groups and persons who are in contact with contaminated animals. For instance, vaccination of livestocks

Table 2. The distribution range of negative and positive titres of SAT, AHGT and 2-ME among high-risk occupations and other people of population in Sanandaj.

Result	Test					
	High-risk occupations (n=130)			Other people of population (n=1411)		
	SAT (%)	AHGT (%)	2-ME (%)	SAT (%)	AHGT (%)	2-ME (%)
Negatie	105 (80.7)	105 (80.7)	127 (97.7)	1214 (86)	1401 (99.2)	1407 (99.6)
1/20	10 (7.69)	-	1 (0.76)	194 (13.7)	7 (0.5)	1 (0.1)
1/40	6 (4.61)	6 (4.61)	-	1 (0.1)	-	-
1/80	7 (5.38)	4 (3.07)	1 (0.76)	-	1 (0.1)	1 (0.1)
1/160	-	5 (3.84)	1 (0.76)	1 (0.1)	1 (0.1)	1 (0.1)
1/320	2 (1.53)	10 (7.7)	-	-	-	-
1/640	-	-	-	1 (0.1)	1 (0.1)	1 (0.1)
Total	130 (100)	130 (100)	130 (100)	1411 (100)	1411 (100)	1411 (100)

appropriately, to equip slaughterhouses and pasteurization of dairy and milk products etc.

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