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A Survey on Antibody Levels among Individuals at Risk of Brucellosis in Khorasan Razavi Province, Iran

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Abstract: Brucellosis is being reported with exceeding frequency in Iran. Serum antibodies in high-risk and general populations help to determine cut-off points and could be used as simple and fast diagnostic tests in involved areas. We have conducted the serum agglutination test, Combs' Wright and 2-mercaptoethanol titer determination on 908 healthy people. The analysis of our data shown that 275 out of healthy subjects, 30.3% were serum agglutination test and Combs' Wright test positive that 12% of them had titer more than 1:80 and 82.2% had titer less than 1:80. 56.3% of them were 2 mercaptoethanol titer positive. Basic and medium titers in whole of population were between 1:20-1:40. There are no high titers of these antibodies in endemic areas of Iran.

Key words: Brucellosis, serum level, antibodies, occupational status, Iran

Introduction

Brucellosis is an important public health problem in many developing countries. The disease is found globally, but is more common in the Mediterranean basin, the Arabian Peninsula, the Indian subcontinent and in parts of Mexico and Central and South America (Young *et al*., 2000). In the Islamic Republic of Iran, brucellosis represents a major health problem and continues to be reported with increasing frequency from various parts of the country (Panahi, 2000). Incidence of brucellosis in Khorasan province in the northeastern part of Iran is considered high in comparison with other infected provinces. However, according to data derived from active surveillance during 2001-2005, the incidence was between 120-400 per 100 000 people (Karimi, 2000). According to that active case finding, most of cases were among farmers, slaughterers and butchers or had an occupational risk factor (Karimi, 2000). Furthermore, one large study from 1986 disclosed that approximately 7.4% of cows were infected (Panahi *et al*., 2000). As 83% of cases in the country are in individuals less than 40 years old (Panahi *et al*., 2000), the importance of occupational exposure especially during adolescence and young adulthood can not be overemphasized (Young *et al*., 1998).

Diagnosis is confounded largely because of a non-specific clinical picture, so diagnosis is only made certainty when Brucella species are recovered from blood, bone marrow or other sites (Young, 2000; Young, 1998). Although most laboratories now employ rapid isolation techniques (BACTEC, Dupont isolator, polymerase chain reaction methods etc), these techniques are not available in most developing countries and conventional methods of isolation are too slow to use routinely for diagnosis (Young, 2000; Young, 1998; Sifuentes, 1997; Dabdoob and Abdulla, 2000). Therefore, in the absence of bacteriologic confirmation, a presumptive diagnosis can be made on the basis of a single high or rising titer of specific antibodies (Young, 2000; Young, 1998). A variety of serological tests has been applied to diagnosis of brucellosis, of which serum agglutination test (SAT) is the most widely used (Dabdoob and Abdulla, 2000; Hurtado, 2001, Al-Sekait, 1999). Evaluation of various enzyme-linked immunosorbent assays (ELISA) for IgG and IgM has shown that these techniques are generally more sensitive and specific than conventional tests (Hurtado, 2001; Gad El-Rab and Kambal, 1998), but these techniques are also not generally available for routine use in developing countries, particularly in rural areas. As the serum level of antibodies in high-risk and general populations were examined in our study, serum levels were investigated in order to avoid reducing the sensitivity of the SAT through the routine application of a predetermined titer (1:160) (Gad El-Rab, 1998) and because no single titre of *Brucella* sp. antibodies is always 'diagnostic' (Young, 2000). This enabled us to define a cut-off level that can be used as a simple and rapid diagnostic test in infected areas (Al-Sekait, 1999).

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Materials and Methods

In this cross-sectional study, the rate of *Brucella* sp. seropositivity was investigated from September 2005 to July 2006 in urban and rural regions of Khorasan Razavi province, Islamic Republic of Iran. The study population consisted of 10 groups: 120 slaughterhouse staff, 140 local butchers, 100 veterinarians and their assistants, 50 domestic animals seller, 70 local milkmaids, 50 animal husbandry staffs, 220 animal owners, 100 shepherds, 80 milk collectors, 20 staff of pasteurization manufactories and 100 people for control group from the general population. Whole of subjects are healthy people which have a contact with domestic animals in selling, slaughtering process or their products such as meat, diary and wool .The patients or suspects were excluded from the study. The control group selected from general population which have the following criteria:

- Whom had no any contact with domestic animals.
- And did not consumption of unpasteurized diaries.
- And no previously history of Brucellosis and diagnosed at present.

After randomized sampling, all groups were met at their workplaces and after a brief explanation about the aims of the project voluntarily agreed to participate. Those individuals with previous history of brucellosis or compatible signs or symptoms such as night sweating, prolonged fever, fatigue, anorexia, weight loss, headache or artheralgia at any time during the preceding two years were excluded. Also, after physical examination, those individuals with positive evidence of lymphadenopathy, hepatomegaly or splenomegaly were excluded from the study. As a result, 99 individuals including 20 butchers and 25 slaughterers were either excluded or chose not to participate.

A total of 908 asymptomatic people participated in this study (118 women and 789 men). Each completed a questionnaire in which data about sex, age, education and job, consumption of unpasteurized dairy products or raw meat, direct contact with domestic animals or handling of parturient domestics or placental membranes were recorded. General knowledge regarding the routes of transmission of the disease was also questioned. For each case, a blood sample (5 cc) was obtained by veno puncture. After agglutination process and centrifugation for serum separation, all samples were analyzed by the SAT, Combs Wright and 2-mercaptoethanol (2 ME) titres.

Chi-squared and T student tests were used as statistical methods in order to determine the correlation of epidemiological variables with serologic tests.

Results

The SAT and Combs Wright used for all ten groups in which were positive for slaughterhouse staff 65.3%, local butchers 30%, veterinarians and their assistants 31.8%, domestic animals seller 35.4%, local milkmaids 28.6%, animal husbandry staff 23.9%, animal owners, 33.3% shepherds 33.3%, milk collectors 16%, staff of pasteurization manufactories 8%. Overall, 30.3% of all participants were positive, with varying degrees of positivity. Moreover, 2ME test among those, whom had more than1:20 of SAT titre in mentioned groups were positive 66.7, 60, 25, 50, 30.5, 33.3 and 51.9%, respectively. Meanwhile, 2ME test was 43.7% positive among people with positive SAT more than 1:20, while, 50% of them had titer more than 1:20, 21% of them had 1:40 titre and 25% had 1:80 titre of SAT. There were only 2 people with titre more than 1;80 of SAT . More details have shown in Table 1-4. Most SAT determination was found 20.4% in veterinary technicians at 1:20 titre. But illegal and local slaughters had the most 2 ME positive test

Table 1: Serum levels of SAT according to occupation of subjects

		Wright titre							
		Without of titre	1:20	1:40	1:80	1:160	1:320	1:640	Total
Occupation status	Legal slaughter	101	13	7	2	0	0	0	123
		82.1%	10.6%	5.7%	1.6%	0	0	0	100%
	Illegal and local slaughter	96	19	7	7	1	0	0	130
		73.8%	14.6%	5.4%	5.4%	0.8%	0	0	100%
	Veterinarians	31	7	3	2	1	0	0	44
		70.5%	15.9%	6.8%	4.5%	2.3%	0	0	100%
	Veterinary technicians	19	10	14	2	4	0	0	49
		38.8%	20.4%	28.6%	4.1%	8.2%	0	0	100%
	Animal	35	7	4	2	0	0	0	48

Table 1: Continued

		Wright titre							
		Without of titre	1:20	1:40	1:80	1:160	1:320	1:640	Total
	seller	72.9%	14.6%	8.3%	4.2%	0	0	0	100%
	Local	55	7	6	2	0	0	0	70
	milkmaid	78.6%	10%	8.6%	2.9%	0	0	0	100%
	Animal	158	23	20	7	2	1	1	212
	owners	74.8%	10.3%	9.35%	3.4%	0.9%	0.5%	0.5%	100%
	Animal	37	6	2	1	0	0	0	46
	husbandry workers	80.4%	13%	4.3%	2.2%	0	0	0	100%
	Shepherds	70	13	7	6	3	0	0	99
		70.7%	13.1%	7.1%6.1%	6.1%	3%	0	0	100%
	Milk	65	6	2	0	2	0	0	75
	collectors	86.7%	8%	2.7%	0	2.7%	0	0	100%
	Staff of pasteurized	11	0	1	0	0	0	0	12
	milk Manufactories	91.7%	0	8.3%	0	00	0	0	100%
	Total	678	111	73	31	13	1	1	908
		74.7%	12.2%	8%	3.4%	1.4%	0.1%	0.1%	100%

Values expressed as percent and number

Table 2: Serum levels of 2 ME titre according to occupation of subjects

		2 ME titre						
		Without of titre	1:20	1:40	1:80	1:320	1:640	Total
Occupation	Legal	8	0	0	1	0	0	9
status	slaughter	88.9%	0	0	11.1%	0	0	100%
	Illegal and	6	7	1	0	0	1	15
	local slaughter	40%	46.7%	6.7%	0	0	6.7%	100%
	Veterinarians	2	2	1	1	0	0	6
		33.3%	33.3%	16.7%	16.7%	0	0	100%
	Veterinary	15	2	0	3	0	0	20
	technicians	75%	10%	0	15%	0	0	100%
	Animal	3	2	1	0	0	0	6
	sellers	50%	33.3%	16.7%	0	0	0	100%
	Local	5	1	2	0	0	0	8
	milkmaid	62.5%	12.5%	25%	0	0	0	100%
	Animal	15	9	3	3	1	0	31
	owners	48.4%	29%	9.6%	9.6%	3.2%	0	100%
	Animal husbandry	2	1	0	0	0	0	3
	workers	66.7%	33.3%	0	0	0	0	100%
	Shepherds	8	2	2	4	0	0	16
		50%	12.5%	12.5%	25%	0	0	100%
	Milk collectors	2	0	1	1	0	0	75
		50%	0	25%	25%	0	0	100%
	Staff of pasteurized	1	0	0	0	0	0	1
	milk Manufactories	100%	0	0	0-	0	0	100%
	Total	67	26	11	13	1	1	119
		56.3%	21.8%	9.2%	10.9%	0.8%	0.8%	100%

Values expressed as percent and number

Table 3: Serum levels of Combs Wright titre according to occupation of subjects

		Combs Wright titre					
		Without of titre	1:20	1:40	1:80	1:160	Total
Occupation	Legal	92	5	3	0	1	101
status	slaughter	91.1%	5%	3%	0	1%	100%
	Illegal and	91	3	2	0	0	96
	local slaughter	94.8%	3.1%	2.1%	0	0	100%
	Veterinarians	30	0	1	0	0	31
		96.8%	0	3.2%	0	0	100%

Table 3: Continued

	Combs Wright titre					
	Without of titre	1:20	1:40	1:80	1:160	Total
Veterinary	17	1	0	1	0	19
technicians	89.5%	5.3%	0	5.3%	0	100%
Animal	31	3	0	1	0	35
sellers	88.6%	8.6%	0	2.9%	0	100%
Local	50	4	1	0	0	55
milkmaid	90.9%	7.3%	1.8%	0	0	100%
Animal	69	2	1	0	0	72
owners	95.8%	2.7%	1.4	0	0	100%
Animal husbandry	35	2	0	0	0	37
workers	94.6%	5.4%	0	0	0	100%
Shepherds	66	2	2	0	0	70
	94.3%	2.9%	2.9%	0	0	100%
Milk collectors	63	1	1	0	0	65
	96.9%	1.5%	1.5%	0	0	100%
Staff of pasteurized	11	0	0	0	0	11
milk manufactories	100%	0	0	0	0	100%
Total	633	27	15	2	1	6 7 8
	93.4%	4%	2.2%	0.3%	0.1%	100%

Values expressed as percent and number

Table 4: Serum levels of Combs Wright and Wright titre according to occupation of subjects

	Wright and Combs Wright titre							
	Without of titre	1:20	1:40	1:80	1:160	1:320	1:640	Total
Legal	92	18	10	2	1	0	0	123
slaughter	74.8%	14.6%	8.1%	1.6%	0.8%	0	0	100%
Illegal and	91	22	9	7	1	0	0	130
Local slaughter	70%	16.9%	6.9%	5.4%	0.8%	0	0	100%
veterinarians	30	7	4	2	1	0	0	44
	68.2%	15.9%	9.1%	4.5%	2.3%	0	0	100%
Veterinary	17	11	14	3	4	0	0	49
technicians	34.7%	22.4%	28.6%	6.1%	8.2%	0	0	100%
Animal sellers	31	10	4	3	0	0	0	48
	64.6%	20.8%	8.3%	6.3%	0	0	0	100%
Local milkmaid	50	11	7	2	0	0	0	70
	71.4%	15.7%	10%	2.9%	0	0	0	100%
Animal owners	147	58	25	7	2	1	1	241
	60.9%	24.1%	10.4%	2.8%	0.9%	0.4%	0.4%	
Animal husbandry	35	8	2	1	0	0	0	46
workers	76.1%	17.4%	4.3%	2.2%	0	0	0	100%
Shepherds	66	15	9	6	3	0	0	99
	66.6%	15.2%	9.1%	6.1%	3%	0	0	100%
Milk collectors	63	6	4	0	2	0	0	75
	84%	8%	5.3%	0	2.7%	0	0	100%
Staff of pasteurized	11	0	1	0	0	0	0	12
milk manufactories	91.7%	0	8.3%	0	0	0	0	100%
Total	633	137	89	33	14	1	1	9 0 8
	69.7%	15.1%	9.8%	3.6%	1.5%	0.1%	0.1%	100%

Values expressed as percent and number

among people in which had SAT positive test at this titre ($p<0.01$). No higher titre was found in the general population.

There was also a strong correlation between occupational status and positive SAT titre 1:40 ($p = 0.001$). Lack of literacy and ignorance of routes of transmission were significantly correlated with the rate of seropositivity by 2 ME (with any titre) and SAT titre 1:80 ($p = 0.025$ and $p = 0.016$, respectively).

Discussion

Recreational and occupational exposure to *Brucella* sp. has been recognized as an important risk factor even in areas in which brucellosis has been

disappearing (Al-Sekait, 1999; Dajani *et al.*, 1989; Al-Ballaa, 1995). The aim of this study was to determine chance of contamination for healthy people in which at risk of Brucella infection and their serum antibodies titre cut off points of this infection antibodies.

This study showed that the positive SAT titre base is between 1:20-1:40 among people in which are at risk in endemic areas. There was a significant difference in the incidence of seropositivity to *Brucella* sp. between men and women. This recent finding may be due to the gender dependent for these occupations. Overall, SAT titre 1:40 could be used as a cut-off point in the general population in this region. Furthermore, there were the same results for 2 ME titres (i.e., 1:20-1:40). However, this cut off value may be acceptable titres, in this regions.

Rates of seropositivity among high-risk occupations vary greatly in various countries. For example, 35.7% of abattoir workers in Saudi Arabia were seropositive in one study (Al-Sekait, 1999). In Lebanon, 1.7% of persons in high-risk occupations were sero-positive based on SAT titre 1:80 (Araj and Azzam, 1996). In northern Jordan, the rate of seropositivity among high-risk people was reported to be 8.2% (Abo-Shehada *et al.*, 1996). Approximately 14% of asymptomatic, 'at risk' individuals screened in northern India were seropositive for *Brucella* sp. (Handa, 1998). These great differences may be due to cultural variations, especially poor hygiene practices employed by persons in high-risk occupations in various countries. This study also showed that the educational status have a main role for utilization of protective instruments and conducting of prophylactic processes.

Seroprevalence rates among the general population also vary greatly in the Middle East (Dabdoob and Abdulla, 2000; Al-Sekait, 1999; Dajani *et al.*, 1989; Al-Ballaa, 1995; Mousa, 1998; Luhi, 1998; Al-Shamahy, 1997; Idris, 1993). In a similar study in southern Saudi Arabia, 4900 subjects were randomly selected in a house-to-house survey. Investigations included interview, clinical examination and blood sampling for antibody titre determination by a microplate agglutination test. Standard tube agglutination and 2 ME tests further analyzed reactive sera. A significant proportion of the population (19.2%) in the southern region had serological evidence of exposure to *Brucella* sp. antigen (Al-Ballaa, 1995). A more recent study using SAT in various regions of Saudi Arabia (Al-Sekait, 1999) found the seroprevalence rate of brucellosis to be 20% in the northern region, 19% in the southern region and 11.6% in the western region. These rates of seropositivity were much lower than the rates reported from our study. Surprisingly, in the Republic of Yemen, a nearby country in the Arabian Peninsula, the rate of serologically positive samples was reported to range from 0% to 0.8% (Al-Shamahy, 1997). In Oman, the frequency of serologically positive sera in six locales ranged between 0 and 2% (Idris, 1993). In Iraq, based on rose Bengal screening test and SAT, approximately 6% of healthy randomly selected subjects were seropositive (Dabdoob, 2000). Data from Kuwait reported seroprevalence rates of approximately 12% (Al-Sekait, 1999). Higher seroprevalence rates have been reported in sub-Saharan countries, with percentages of 18% in Uganda and 13% in Nigeria (Al-Sekait, 1999). In a cross sectional study that has performed in Shiraz, one of southern cities of Iran, the cut off points for titre of SAT and 2 ME were 1:80 and 1:20, respectively among high risk people (Karimi, 2003). This difference with our study may be due to the less prevalence of infection in that province.

These data demonstrate the importance of regional variations of the disease in the endemic areas. However, high prevalence of lower titre seropositivity in this study may be a significant marker for probably and previous exposure, although only one investigated risk factor (direct contact with placental membranes of domestic animals) was significantly related to SAT titres of 1:40 ($p = 0.05$). A history of such contact should be considered when interpreting SAT titres in endemic areas. There was also a strong relation between illiteracy and ignorance of routes of transmission and positive titres 1:40 (for 2 ME, $p = 0.005$ and SAT, $p = 0.01$).

In the absence of an efficient and effective method for control of the disease, an educational programme, especially one regarding the routes of transmission, would be a cost-effective method for prevention and control. This is particularly so in this area as fresh white cheese, a popular food consumed daily by many people, is usually produced from unpasteurized sheep or goat milk.

Conclusion: A single titre of SAT 1:40 in the presence of 2 ME titre 1:20 can be diagnostic in the general population in this area of the country. Nonetheless, serological studies in high-risk individuals should be interpreted cautiously and confirmed only after a four-fold rising of titres or through bacteriological confirmation. Although the rising titre of antibodies to *Brucella* sp. is the most reliable serological method for accurate diagnosis, it is not always possible to postpone the diagnostic or therapeutic process.

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