

SEROEPIDEMIOLOGICAL SURVEY OF BRUCELLOSIS ANTIBODIES IN SAUDI ARABIA

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Background: Brucellosis is considered the most important zoonosis in Saudi Arabia, with a high prevalence among man and livestock.

Patients and Methods: A natural survey of 23,613 subjects was conducted in 1997, in order to assess the seroprevalence of brucellosis in Saudi Arabia. Investigations included interviews, clinical examination and blood sampling for antibody determination, using the standard tube agglutination test (STAT).

Results: The result of the study revealed that the seroprevalence of brucellosis was 15%. The seroprevalence increased by age, and was higher in rural areas, and among people in high-risk occupations.

Conclusion: Direct contact with domestic animals and consumption of raw products of animal origin were identified as the main risk factors.

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Key Words: Brucella, seroprevalence.

Brucellosis is an infectious disease transmitted to humans through contact with infected animals or animal products.^{1,2} The incidence of the disease has decreased markedly in industrialized countries,²⁻⁴ however, it remains a major public health problem in many developing countries.⁵⁻²⁰ Saudi Arabia is one of those countries where control measures are either lacking or difficult to implement. Diagnosis of human brucellosis relies on serological tests, such as the standard tube agglutination test (STAT), Coombs' test, and enzyme-linked immunosorbent assay (ELISA).²¹⁻²⁴

The purpose of this study was to investigate the seroprevalence of brucellosis antibodies in the general population in different regions of Saudi Arabia.

Materials and Methods

Saudi Arabia covers an area of about two million square kilometers, and has an estimated population of about 16 million, with almost 70% living in urban areas. About 7% of the population lead a nomadic lifestyle in tent settlements (National Population Atlas, Kingdom of Saudi Arabia, 1998).

A cross-sectional survey was carried out in Saudi Arabia in 1997 by a random multi-stage cluster sampling.

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Based on earlier prevalence estimates of 2.5%,⁵⁻⁹ and assuming equal distribution between sexes, a sample size of 24,000 people was determined as adequate for achieving a reasonably precise estimate of the seroprevalence of brucellosis. The sampling procedure used was proportional to the population size (PPS), with cluster sampling and urban/rural satisfaction. Saudi Arabia is divided into 13 administrative districts, therefore, the estimated sample size of 24,000 was obtained by selecting a number that was proportionate to the population of each district. In the first stage of sampling, several cities, towns and villages were randomly selected in each region. Maps of selected cities and towns and villages were obtained, and depending on the population density, one to several primary segments of 100 houses were chosen by a random procedure (second-stage sampling). Finally, 20 households were randomly drawn from each randomly selected primary segment (third stage of sampling). In each randomly selected household, all members were examined.

Data Collection

There were 26 medical teams (two in each region), and each team consisted of a general physician, nurses, and a laboratory technician. Each member of the team participated in a training session prior to the survey, and performed repeat examinations on 10% of the subjects to ensure a high level of consistency and reliability. A standard questionnaire was administered to each household member. The information recorded included age, sex, nationality, residence, educational level, occupation, type of housing, history of previous

TABLE 1. Distribution of the tube agglutination titers in the human subjects positive for brucella antibodies.

Standard tube agglutination titer	Healthy individuals (%)	Individuals with symptoms (%)	Total (%)
1:20	386 (2.4)	107 (1.5)	493 (2.1)
1:40	365 (2.2)	251 (3.4)	616 (2.6)
1:80	464 (2.8)	289 (3.9)	753 (3.1)
1:160	477 (2.9)	213 (2.9)	690 (2.9)
1:320	413 (2.5)	186 (2.5)	599 (2.5)
1:640	110 (0.7)	147 (2.0)	257 (1.1)
1:1280	2 (0)	50 (0.7)	52 (0.3)
1:2560	0	38 (0.5)	38 (0.2)
1:5120	0	32 (0.4)	32 (0.1)
1:10,240	0	28 (0.4)	28 (0.1)
Total positive	2217 (13.6)	1341 (18.2)	3558 (15.0)
Total negative	14,038 (86.4)	6017 (81.8)	20,055 (85.0)
Total	16,255 (100)	7358 (100)	23,613 (100)

TABLE 2. Distribution of seropositivity by administrative region.

Variable	Total number of samples	Seropositive cases
Central region	5597	816 (14.6)
Eastern region	2939	410 (14.0)
Western region	7131	824 (11.6)
Northern region	3152	630 (20.0)
Southern region	4794	878 (18.3)

brucellosis, whether or not the subjects drank raw milk or other milk products, and whether they have contact with animals.

Blood samples were collected and sera were separated from collected blood by centrifugation and stored at -20°C , until tested for presence of brucella antibodies. Serum specimens were analyzed in two phases, using suspension of *B. abortus* and *B. melitensis* (Wellcome Laboratories, UK). In the first phase, all specimens were screened by the microplate agglutination test (MAT).²¹ A titer of 1:80 or greater was considered to represent the presence of specific agglutination brucella antibodies (seropositive).²¹⁻²² In the second phase, seropositive specimens were analyzed by the standard tube agglutination test (STAT). A titer of 1:20 or greater was taken as an index of seropositivity.^{23,24}

Statistical Analysis

The collected data and the results of laboratory tests were analyzed by Statistical Package for Social Science (SPSS), to determine those variables that were significantly associated with seropositivity to brucella. Relative risk (RR) and 95% confidence intervals (CI) were calculated according to established methods.²⁵

Results

In all, 23,613 out of 24,000 subjects (98% compliance) participated in this study, of whom 7887 (33.4%) lived in

rural and 15,726 (66.6%) in urban areas. Initial screening of all serum specimens by MAT gave positive results in 11,491 (48.7%) sera, of which 4952 (21%) were positive at a titer of 1:80 and above. When these specimens were retested with STAT, 3558 specimens (15.0%) gave a positive reaction of 1:20 and above. The percentage of individuals showing a titer of less than 1:160 by STAT was 10.8%, while the percentage showing a titer of 1:160 or greater was 4.2%. The overall distributions of STAT titer found in the surveyed individuals are shown in Table 1.

Table 2 shows that seropositivity was higher in the northern and southern regions compared to other regions. Seropositivity was strongly associated with increasing age, residence, occupation, and low socioeconomic status (Table 3). The seroprevalence of antibody of brucella increased significantly with age ($P<0.001$), was higher in rural compared with the urban areas (26.2% vs. 9.5%), and was also significantly higher ($P<0.05$) in people of low socioeconomic status when compared to people of high socioeconomic status (24.4% vs. 6.3%). No significant difference was found between the sexes. The seroprevalence of brucellosis was significantly higher ($P>0.05$) among people working in high-risk occupations. The distribution of seropositivity with respect to other risk factors is also shown in Table 4. There were significant associations in seroprevalence between those with the risk factors compared to those without the risk factors.

Discussion

Brucellosis is diagnosed either by isolation of brucella organism in a 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 localization and the exacting culture requirements of the organism. In practice, blood cultures are positive in 10%-30% of brucellosis,²² and the remainder is diagnosed serologically. Although no single test provides 100% sensitivity and specificity, STAT still remains the test of choice in diagnosis. In the presence of appropriate signs and symptoms, a presumptive diagnosis of brucellosis is usually defined serologically as a standard tube agglutination titer of 1:160 or greater.²²⁻²⁴ It is, however, time-consuming and costly for seroepidemiological studies, where a large number of samples have to be processed. Therefore, the microplate agglutination test (MAT) was developed as screening test and successfully utilized in population surveys.^{18,19} MAT is probably the ideal method for population surveys because it has the advantage of being more rapid, economical, and highly sensitive.

Recent serological data on brucellosis in developed countries are not available. Indeed, brucellosis has been brought under control in these countries through rigorous diagnostic and control procedures at the animal production level, as well as through elimination of brucella in

livestock and proper pasteurization of milk.² Therefore, there are very few reports of indigenously acquired human cases of brucellosis, while acute imported human infections continue to occur, often associated with the consumption of raw milk or cheese.²⁶⁻²⁷ Data from developing countries in the Mediterranean basin, particularly the Middle East, report seroprevalence rates ranging from 8% in Jordan¹² to 12% in Lebanon and Kuwait.^{13,17} Even higher seroprevalence rates have been reported in sub-Saharan countries, with percentages of 18% in Uganda¹⁹ and 13% in Nigeria.²⁰ Seroprevalence data are not available for most regions in Saudi Arabia, but it is worth noting that our results agree with earlier reports,^{5,6,9} which showed the seroprevalence rate of brucellosis to be 24% in the Northern region in 1991, and 19% in the Southern region in 1993.

The seroprevalence of brucellosis increases with age, a result which is consistent with observations made in Iran, Jordan, Lebanon, and Kuwait.^{11-13,16} The lower seroprevalence rates found in children, as compared to adults, may be the result of increased exposure of adults to livestock. In children, morbidity depends largely on the pathogenicity of the infected brucella species.^{11,16} In contrast to other studies, we found no significant difference in the seroprevalence between male and female in any age group. The result of our study indicates that gender does not influence the immune response to brucella.

Statistical analysis showed that the area of residence (northern or southern region) has a significant effect on seroprevalence. Border locations where there are uncontrolled movement of animals may have a high prevalence rate, especially in villages and settlements where Bedouins live in close contact with animals. Furthermore, most people in rural areas are dependent in one way or another on farms animals, usually sheep and goats.

The result of the study revealed a high seroprevalence of brucella among persons in high-risk occupations, such as farmers, meat handlers and animal dealers. The high seroprevalence among meat handlers, compared with other occupations, shows the significance of contact infections. Meat handlers considered in this study include butchers and abattoir workers who are in direct contact with raw meat and carcasses of infected animals, and through whom infection probably occurred through cuts and wounds to bare hands, or through splashing of infected blood or other fluid to the conjunctiva.² Hygiene regulations in slaughterhouses are not strictly adhered to, and furthermore, large proportions of animals are slaughtered outside abattoirs.

Shepherds had the highest seroprevalence among all occupations, which explains the widespread infection of *Brucella melitensis* in sheep, compared to other animals.²⁸⁻³⁰ Current sheep husbandry methods facilitate the spread of the disease. During lambing, the environment is heavily contaminated with brucella, creating favorable conditions

TABLE 3. *Distribution of seropositivity by demographic characteristics.*

Variable	Total # of samples	Seropositive cases (%)	Relative risk (95% CI)
Age			
0-14	7381	716 (9.7)	1.0*
15-29	7826	1252 (16.0)	1.7 (1.2-2.2)
30-44	5522	1033 (18.7)	1.9 (1.3-2.5)
≥45	2884	557 (19.3)	2.0 (1.5-2.5)
Sex			
Male	11,665	1831 (15.7)	1.0*
Female	11,948	1727 (14.5)	0.9 (0.4-1.4)
Residence			
Urban	15,726	1494 (9.5)	1.0*
Rural	7887	2064 (26.2)	2.8 (1.3-4.3)
Social class			
Professional	4987	314 (6.3)	1.0*
Intermediate	6372	651 (10.2)	1.6 (1.2-2.0)
Skilled	4216	717 (17.0)	2.7 (1.3-3.1)
Semi-skilled	3861	857 (22.2)	3.5 (2.6-4.3)
Unskilled	4177	1019 (24.4)	3.8 (2.7-4.9)
Occupation			
Clerk	3126	401 (12.8)	1.0*
Student	8150	1090 (13.3)	1.0 (0.6-0.4)
Housewife	6112	984 (16.1)	1.3 (0.9-1.7)
Farmer	653	188 (28.3)	2.2 (1.4-3.0)
Abattoir worker	269	96 (35.7)	2.8 (2.6-3.6)
Shepherd	216	90 (41.8)	3.3 (2.5-4.1)
Other	4987	709 (14.2)	1.1 (0.7-1.4)
Total	23,613	3558 (15.0)	

for the transmission of the infection to other animal and to man.²⁸ In Saudi Arabia, poor hygiene practices employed by farmers further encourage transmission. Handwashing is not done routinely following contact with infected animals or materials, partly because of the shortage of water supply.²⁸

The seroprevalence of brucellosis among the Saudi population is significantly higher than that of neighboring countries. Despite the fact that brucellosis is a notifiable disease, reported human cases do not reflect the actual prevalence, as indicated by this study, leading to an underestimation of the extent of the disease.²⁸ Brucellosis in animals remains a major public health hazard due to its transmissibility to man.³¹⁻³² The only effective way to control the disease in man is by the elimination of the infected animals, and vaccination of healthy ones in order to reduce the risk of those in regular contact with animals, and to produce brucellosis-free animal products.^{28,29} The effectiveness of vaccination programs can be evaluated by investigating incidence rate in humans, especially people at high risk, before and after vaccination. It is recommended that surveillance of brucellosis should be strengthened. Cooperation and joint supervision between the Ministries of Health, Agriculture, and Municipality and Rural Affairs, and cooperation with neighboring countries, should also be encouraged. Furthermore, we should adopt health education programs that aim at stopping the spread of infection among animals and then to humans. Regulations concerning the adoption of hygiene measures among high-risk population, especially

TABLE 4. Risk factors associated with seroprevalence of brucellosis in Saudi Arabia.

Variable	Total # of samples	Seropositive cases (%)	Relative risk (95% CI)
Drinking raw milk	4109	2792 (67.9)	5.5 (3.8-7.2)
Heating raw milk	3132	1610 (51.4)	1.0 (0.4-1.7)
Consumption of milk products	10,744	3991 (37.1)	0.9 (0.8-1.2)
Animal contact	4028	2665 (66.2)	4.8 (2.2-7.4)
Milking animals	2517	1774 (70.5)	6.2 (3.3-8.4)
Breeding animals	3130	2124 (67.9)	9.3 (4.3-13.7)
Parturient animals	2393	1782 (74.5)	13.5 (6.5-20.5)
Contact membrane placenta	1713	1367 (79.9)	12.9 (5.8-20.2)
Cutting raw meat	3167	2215 (69.9)	5.3 (3.4-7.2)

in slaughterhouses and among farmers, abattoir workers and veterinarians, should be strictly adhered to in order to control the spread of brucellosis.

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