

Seroprevalence of Brucellosis among High Risk People in Northern Jordan

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Background. Brucellosis is considered the most important zoonosis in Jordan with high prevalence among man and livestock.

Methods. This study was carried out on high risk people in 1992 in order to assess the seroprevalence of brucellosis in northern Jordan. The sera of 1236 individuals (636 at high risk and 600 controls) were evaluated using the Rose Bengal plate agglutination test (RBPT) and enzyme-linked immunosorbent assay (ELISA-IgG) tests.

Results. A significantly ($P < 0.05$) higher seroprevalence of brucellosis among high risk people (8.2%) compared to the control sample (0.5%) was found. The overall seroprevalence was significantly higher among sheep farmers and meat handlers than in other occupations tested. The seroprevalence increased with age and years at work, but was not influenced by sex or locality. The results indicated a higher seroprevalence among veterinarians in northern Jordan, compared to central Jordan but the difference was not significant. Seroprevalence was present only among veterinarians working in clinics especially in the working age group (34–43 years).

Conclusion. The results of this study emphasized the importance of contact infections, namely contact with infected animals and their products, as a method of transmission of brucellosis compared to ingestion of contaminated animal products.

Keywords: Brucellosis, veterinarians, food handlers, animal handlers, epidemiology, zoonosis, Jordan

Brucellosis remains an important zoonotic disease which persists in regions where infection in animals, especially man's livestock, has not been brought under control and where, consequently, transmission of the infection to humans frequently occurs. The disease in humans actually reflects widespread disease in animals. There are tremendous differences in the yearly incidence of the infection in different countries, depending on the extent of animal brucellosis. In Jordan, the first strains of *Brucella melitensis* in humans were cultured in September 1985 but *B. abortus* has not been isolated. Awareness of human brucellosis began in late 1985 and this disease has been documented for the years 1986–1992, leading to the conclusion that brucellosis was widespread in Jordan. Reported cases in the studied area increased from 87 in 1985 to 159 in the first half of 1992. The prevalence of brucellosis among sheep and goats in 1992 was 9% and in cattle 2.8%. Evidence prior to 1990 suggested that there was no

decrease in prevalence in response to the control measures undertaken, namely health education and small ruminant vaccination programmes using the Rev. 1 vaccine.^{1,2}

This study was a preliminary attempt to determine the seroprevalence of brucellosis among high risk groups namely sheep farmers, meat handlers, veterinarians, cattle farmers, and milk handlers using the Rose Bengal plate agglutination test (RBPT) and enzyme-linked immunosorbent assay (ELISA). The effects of occupation, age, years at work, sex, and location on the seroprevalence of brucellosis among individuals were also determined.

MATERIALS AND METHODS

The study area is located in northern Jordan and has a population of about one million. About 17 500 livestock holders reside within the area keeping 43 500 cattle, 953 400 sheep and 286 500 goats.³ During the months April–October 1992 a random sample of 636 individuals, stratified by geographical location and occupation, and considered at high risk of contracting brucellosis mainly from sheep, goats and cattle, was identified. In addition to livestock owners, meat

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handlers, milk and milk product handlers and veterinarians were included and the stratified random sample was drawn from Irbid, Mafrq, Ramtha, Ajloun and Jerash including their satellite villages. A list of farms in the study area was obtained from the Ministry of Agriculture. From 246 cattle farms with more than five animals, 94 were picked at random using tables. Lists of sheep farms were not available, but individuals were sampled at random from 129 farms with more than 100 animals. According to information from the municipalities in each area, there were 132 meat processing plants and 80 dairy factories, from which 67 and 31 respectively were picked at random. A control group of 600 healthy subjects was picked by stratified random sampling from Ramtha, Mafrq, Irbid, Ajloun and Jerash. This group was similar to the study group with respect to age, sex and socio-economic status, but had no occupational contact with animals, milk and meat. In Jordan there are 406 registered veterinarians, 250 of whom are practising. A sample of the latter was picked at random to include veterinarians in the main areas of practice, of different age groups and in different geographical areas. Forty-six veterinarians from central Jordan were involved in order to compare their results with results obtained from veterinarians from northern Jordan.

Blood samples were collected and sera was separated from clotted blood by centrifugation and stored at -20°C until tested for the presence of *Brucella* antibodies using RBPT (bioMérieux, France). Samples seropositive with RBPT were confirmed by the ELISA method according to Magee⁴ to determine *Brucella*-IgG antibodies at a dilution of 1:100. An absorbance of ≥ 0.35 was considered positive. The cutoff value was determined by the mean absorbance value of four standard negative sera (+3SD). Test samples were measured photometrically at 405 nm.

Information was collected by personal interview. Questionnaires recorded the following information for each subject: age, sex, residence, occupation, time spent in the occupation, consumption of milk and milk products, contact with animals specifying the species of animal and duration of contact, past clinical disease that may have been brucellosis. Data were analysed using the Fisher's Exact Test and correlation analysis.

RESULTS

Of the total seropositive samples examined by RBPT, 97% had a positive absorbance (>0.35) in ELISA. The ELISA results confirmed a significantly ($P < 0.05$)

TABLE 1 *The seroprevalence of brucellosis among human individuals in different high risk occupations*

Occupation	No. examined	No. seropositives (%)
Vets + associates	58	8 (13.8)
Sheep farmers	192	24 (12.5)*
Cattle farmers	164	10 (6.1)
Meat handlers	82	4 (4.9)*
Cattle + sheep farmers	61	3 (4.9)
Milk handlers	79	3 (3.8)
Total	636	52 (8.2)

* Significant ($P < 0.05$) compared to other high risk occupations.

TABLE 2 *The seroprevalence of brucellosis among different age groups (male and female) from northern Jordan*

Age group (years)	Sex	No. examined	No. seropositives	M:F ratio
<20	M	93	5 (5.4)	2.3:1.0
	F	42	1 (2.4)	
21-30	M + F	135	6 (4.4)	1.0:1.4
	M	168	9 (5.4)	
	F	40	3 (7.5)	
31-40	M + F	208	12 (5.8)	12:00.0
	M	102	7 (11.8)	
	F	19	0 (00.0)	
41-50	M + F	121	12 (9.9)	1.0:1.0
	M	74	8 (10.8)	
	F	19	2 (10.5)	
>51	M + F	93	10 (10.8)	1.0:1.2
	M	56	8 (14.2)	
	F	23	4 (17.4)	
Total	M + F	79	12 (15.2)	1.1:1.0
	M	493	42 (8.3)	
	F	143	10 (7.7)	
	M + F	636	52 (8.2)	

higher overall brucellosis seroprevalence of 8.2% (52/636) among high risk people in northern Jordan compared to a seroprevalence of 0.5% (3/600) in the control group. Seroprevalences among sheep farmers (12.5% [24/269]) and meat handlers (4.9% [4/82]) were significantly higher ($P < 0.05$) than other occupations (Table 1). Veterinarians working as clinicians in Jordan had a prevalence of 24.5% (13/53). None of the veterinarians working in other veterinary areas were seropositive (the number examined was 22).

Seroprevalence of brucellosis increased with age (Table 2) showing a significant positive linear relationship ($P < 0.05$, correlation coefficient = 0.95) (Figure 1). No significant ($P < 0.05$) difference was found between the sexes (Table 2). The relationship between

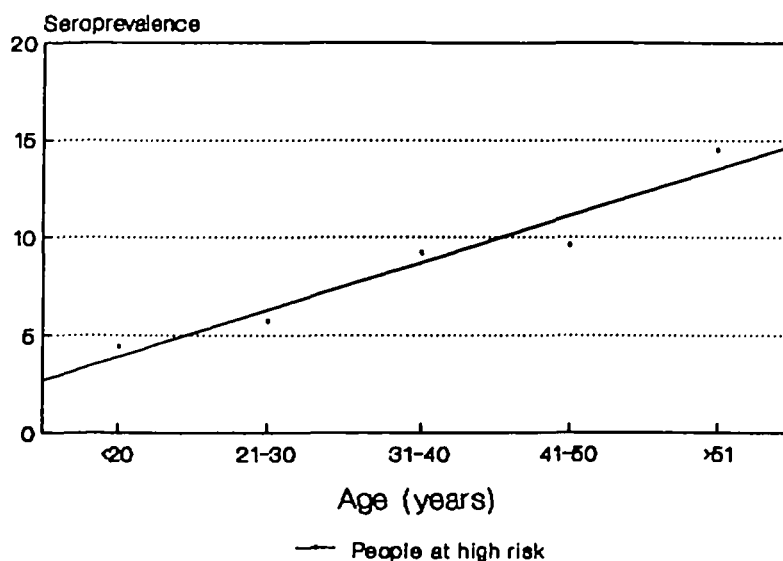


FIGURE 1 The trend in seroprevalence of brucellosis according to age in northern Jordan

TABLE 3 The seroprevalence of brucellosis among high risk people according to area

Area	No. examined	Total seropositives (%)
Ramtha	65	9 (13.8)
Mafraq	195	18 (9.2)
Ajloun	84	6 (7.1)
Irbid	214	15 (7)
Jerash	78	4 (5)
Total	636	52 (6.2)

age and the seroprevalence of *Brucella* antibodies stratified by sex showed a significant positive linear relationship (correlation coefficient 0.97, $P < 0.05$). Veterinarians of the age group 34–43 years had the highest ($P < 0.05$) seroprevalence among veterinarians (26.3% [11/38]). The seroprevalence of brucellosis was significantly higher ($P < 0.05$) among people working in high risk occupations for ≥ 22 years (15% [18/120]) compared to other groups working < 22 years (6.8% [34/516]). Ramtha and Mafraq had a relatively higher seroprevalence of brucellosis compared to Jerash and Irbid, though not significantly so ($P > 0.05$) (Table 3). Seroprevalence among veterinarians in northern Jordan (20.7% [6/29]) did not differ significantly ($P < 0.05$) from veterinarians in central Jordan (17.4% [8/46]). A result also found in the city of Irbid (17.6% [3/17]) when compared to the city of Amman (15.3% [4/26]).

DISCUSSION

The present study showed a significantly higher ($P < 0.05$) seroprevalence of *Brucella* antibodies, 8.2%, among people at high risk, including veterinarians, farmers, meat and milk handlers, compared to the control sample who had a seroprevalence of 0.5%. Among those at high risk, questioning ascertained that infection was mainly due to contact with animals and their products. In the control sample, infection was mainly due to ingestion of contaminated food as this group had no contact with animals. All seropositive cases in the control sample had developed a previous (not current) infection through consumption of products from infected sheep and goats purchased from bedouins.

Shepherds had the highest seroprevalence among all occupations, which can be explained by the widespread *B. melitensis* infection in sheep compared to other animals. Sheep are the organism's most significant host in Jordan and they constitute 66% of animals in Jordan.³ Current sheep husbandry methods facilitate the spread of the disease. During lambing and when abortion is rife, especially in spring and summer, the environment is heavily contaminated with *Brucella* creating favourable conditions for the transmission of the infection to other animals and to man.³ The poor hygiene practices employed by farmers further encourages transmission. Handwashing is not usual following contact with infected material in part because the shortage of water suffered by farmers, especially sheep farmers, is not conducive to regular washing and water is saved at

the expense of hygiene. Controlling the disease in sheep is difficult because of the high number and uncontrolled movement of flocks within Jordan and across its borders. These problems are not encountered with cattle explaining the significantly ($P < 0.05$) lower seroprevalence of the disease among cattle farmers compared to sheep farmers. These results agree with other reports estimating the level of infection at about 9% in sheep and 2.8% in cattle.² The significantly ($P < 0.05$) higher seroprevalence among sheep farmers was not found where cattle and sheep are raised together. This is probably the result of the small numbers of both animals in such herds and confirms the known effect of flock size on the spread of infection, i.e. larger flocks are at higher risk of brucellosis infection.⁵

The significantly ($P < 0.05$) high seroprevalence among meat handlers compared to other occupations is indicative of the importance of contact infections⁵ among high risk people, especially as cases of brucellosis from meat ingestion are rare.⁶ Meat handlers considered in this study included butchers and abattoir workers who are in direct contact with raw meat and the carcasses of infected animals and infection probably occurred through cuts and wounds to bare hands or through splashing of infected blood or other fluids into the conjunctiva. More importantly, hygiene regulations in slaughterhouses are not strictly adhered to, e.g. clean protective clothing is not used and all carcass processing is performed in one room. In addition, a large proportion of animals are slaughtered outside abattoirs (e.g. in yards adjacent to butchers' shops).

The results also emphasize brucellosis as an occupational disease among veterinarians in northern Jordan, indicating that contact with sheep and goats during labour and abortion is an important method of transmission.

Seroprevalence among milk handlers is explained by sheep and goat milk being the source of infection especially during cheese processing.⁴ Health education campaigns in Jordan concentrate mainly on milk handling which increases the awareness of milk handlers to the problem. Safety measures encourage hygiene practices such as the careful handling and heating of milk, procedures that are easily performed. The current results do not exclude milk handling as an important source of infection but it is not ranked equally with animal contact in agreement with Al-Sekait.⁸

The prevalence of brucellosis increased with age which is consistent with observations made in Saudi Arabia.⁸ The lower prevalence found in children compared with adults may be the result of increased exposure of adults to livestock. This is in agreement with the result showing that people working for ≥ 22

years had a significantly higher seroprevalence than those who had worked for < 22 years, since older age groups were usually found to be at high risk for longer periods than younger age groups in this study. The level of risk of brucellosis for people at high risk depends very much on whether the individual has been in a job for a long time or has recently taken it up. In the former case he is likely to have some immunity to *Brucella*.⁵

No significant difference in seroprevalence was found between males and females in general, or in age-specific groups. This is probably because the study was limited to males and females equally exposed to infection in high risk occupations. This observation indicates that gender does not influence the immune response to *Brucella*.

Statistical analysis showed no significant effect of area on seroprevalence of brucellosis. The relatively high seroprevalence in the Ramtha area can be explained by its location on the border where uncontrolled movement of flocks may have its highest effect especially on villagers and bedouins travelling and living in close contact with animals. Most people in the Ramtha area, including the town of Ramtha, are dependent in one way or another on raising animals, usually sheep. This problem is compounded by poor hygiene. High seroprevalence was also found in the Mafraq area, probably due to similar conditions. This was not found to be the case in the Irbid area where people have a more urban lifestyle. In Irbid villages cattle are the main livestock rather than sheep which may also explain the relatively low prevalence compared to Ramtha. The low seroprevalence in Ajloun and Jerash can be explained by high risk people depending mainly on arable farming in addition to raising a small number of animals. They live mostly in villages with less travelling and trading and therefore have less chance of encountering the infection.

Seroprevalence was found to be significantly higher ($P < 0.05$) among veterinary clinicians compared to those with other occupations. This result supports similar findings that there is a higher seroprevalence of the infection among clinicians who are frequently in contact with infected sheep and goats in labour.⁹ The seroprevalence among veterinarians in 1986 was 54.5% (36/66), significantly higher ($P < 0.05$) than among veterinarians in 1992 (18.7% [14/75]). This is probably due to the preventive measures now undertaken by veterinarians in response to increasing awareness of the disease as an occupational hazard. The results showed that there was no seroprevalence among veterinarians 24–33 years old, probably because most veterinarians of this age group in Jordan are not in areas of the occupation which expose them to the infection e.g. the

poultry industry. Prevalence increased significantly ($P < 0.05$) among veterinarians 34–43 years old, this may be because many veterinarians of this age have been long established in practices which regularly bring them into close contact with sheep and goats. Low prevalence was found among the age group 44–53 years. This group are usually in administrative positions which may reduce exposure to new *Brucella* infections.

In conclusion the seroprevalence of brucellosis among high risk people is significantly ($P < 0.05$) higher than the control group emphasizing the importance of contact infection. Although brucellosis is a notifiable disease, reported human cases do not reflect the actual prevalence, as revealed by this study, leading to underestimation of the extent of the disease.¹⁰ Brucellosis in animals remains a major public health hazard due to its transmissibility to man. The only effective way to control the disease in man is by the elimination of infected animals and vaccination of healthy ones in order to render those in regular contact with animals at lower risk and to produce brucellosis-free animal products.¹¹ The effectiveness of vaccination programmes can be evaluated by investigating incidence rates in humans, especially people at high risk, before and after vaccination. It is recommended that surveillance of brucellosis should be strengthened in the population at risk and organized information systems established. Cooperation between the Ministries of Health and Agriculture, and cooperation with neighbouring countries should be encouraged in order to effect brucellosis control programmes. These should include health education programmes that aim at stopping the further

spread of infection among animals and then to humans. Regulations concerning the adoption of hygiene measures among high risk people, especially in slaughterhouses and among farmers and veterinarians, should be strictly adhered to in order to minimize the spread of infection.

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(Revised version received June 1995)