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Seroprevalence of brucella antibodies among persons in high-risk occupation in Lebanon

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SUMMARY

Prevalence of brucella-specific antibodies was measured in 597 persons in high-risk occupations living in 10 regions of Lebanon using the standard agglutination test (SAT), anti-human globulin (Coombs') test (AHGT) and enzyme-linked immunosorbent assay (ELISA) for measuring immunoglobulin G (IgG), IgM and IgA. The study population consisted of butchers (54%), farmers (35%), laboratory technicians (8%), abbatoir workers (2%) and veterinarians (1%), with 82% males and 18% females. The overall seroprevalence based on SAT and AHGT titres of $\geqslant 80$ was 1·7% and 15%, respectively, but seroprevalence varied by region from 0–5% in SAT and from 3·4–34% for AHGT. The overall seroprevalence based on ELISA IgG (OD $\geqslant$ 0·6), IgM (OD $\geqslant$ 0·6) and IgA (OD $\geqslant$ 0·3) was 57, 61 and 26%, respectively. The highest seroprevalence was noted in Biqaa (34%), Kisrwan (24%), Shouf (21%), Sidon (16%) and Aley (12%) regions. Nineteen percent of those surveyed reported symptoms that could be associated with brucellosis. We conclude that exposure to brucellosis is high among persons in high-risk occupations from all surveyed regions in Lebanon. Such findings should be used to design control measures especially now that the 17 years of civil strife is over.

INTRODUCTION

Many countries have reported a high incidence of adult and childhood brucellosis with serious economic and public health sequelae [1–5]. To date, human infection by brucella organisms has been caused by four species: *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis* [3, 6]. In Lebanon and the Middle East, *B. melitensis* accounts for most human cases [7–10]. The major route of human infection in endemic areas is ingestion of unpasteurized milk or its products. In non-endemic areas, occupational exposure through direct contact with infected livestock, or brucella culture represents the major route of transmission, via the respiratory tract, conjunctiva, skin abrasion and transfusion of blood [4, 6, 12].

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Diagnosis of human brucellosis relies on serological tests such as the standard agglutination test (SAT), anti-human globulin (Coombs') test (AHGT) and enzyme-linked immunosorbent assay (ELISA) [3, 6, 12, 13]. In the Middle East, countries like Kuwait, Jordan and Saudi Arabia have reported a high brucella incidence of 69, 46 and 40, respectively, per 100000 population [7, 12, 14]. Seroprevalence of brucellosis is higher in individuals working in livestock and meat processing industries, veterinary services and laboratory workers handling brucella culture [3, 11, 15, 16].

In Lebanon and over the last 40 years only three studies on brucellosis have been reported: two on animals [17, 18] and one on six human cases with brucellosis in pregnancy [19]. Moreover, the dearth of information on brucellosis from Lebanon, mostly due

to the civil conflicts, was evident in a 1983 report on the epidemiological status of brucellosis in 16 Mediterranian countries which did not show any data from Lebanon but stated that 'this country seems to have low sporadic incidence of *B. abortus* and moderate incidence of *B. melitensis*. Considering the country's small population we must admit that it has a serious brucellosis problem...' [2].

Such limited information constituted the rationale for the present study which was undertaken to determine the current status of seroprevalence of brucella-specific antibodies among persons in high-risk occupational exposure from different geographic regions in Lebanon.

METHODS

Study population, geographic regions and questionnaire

This study was conducted between 1 April and the end of September 1994. The study population was randomly selected from the registry of the Ministry of Public Health (MPH) and consisted of persons in high-risk occupations: butchers, farmers, laboratory technologists, abbatoir workers and veterinarians from different geographical regions in Lebanon. These regions cover the majority of the administrative areas in Lebanon as follows: South (Tyre and Nabatieh and Sidon), Mount Lebanon (Shouf and Iqliem, Aley and Matten, Beirut Suburb and Kisrwan), North (Tripoli, Zgharta and Bshari and Accar) and Biqaa area. Due to difficulties in logistics the capital city (Beirut) was not included.

A questionnaire was designed to collect information related to personal and sociodemographic status (age, sex, profession, years in practice and location), current eating habits, and clinical information about symptoms suggestive of brucellosis (fever, sweating, anthralgia, myalgia and weakness) lasting for more than 5 days in the previous 5 years, is reported earlier [12].

The questionnaire and blood sampling was carried out by professional teams from the Ministry of Public Health who were instructed about the process of interview, filling out the questionnaire and the collection of blood prior to their field work.

Collection and handling of blood

Around 7 ml of blood was collected in a sterile plain tube. The tubes were transported to regional government laboratories where sera was separated within 12 h. Then sera were transported (in a cooler) to the serology section at the American University of Beirut Medical Center (AUBMC) where they were stored at −20 °C until tested, within 3 months of collection.

Brucella Standard Agglutination Test (SAT)

The SAT was carried out on doubling dilution of serum from 1:20 to 1:2560, essentially as reported earlier [20]. *Brucella abortus* antigen (Immunostics Inc., New Jersey, USA) was used according to the instruction of the manufacturer. Positive reactions were determined by an agglutinoscope and the titers given indicate the highest dilution in which 50% or more agglutination occurs in tube. Sera showing negative agglutination (i.e. titre < 20) were continued to be tested by brucella anti-human globulin (Coomb's) test.

Anti-human globulin (Coombs') test (AHGT)

AHGT was subsequently performed as extension of the SAT for detection of 'incomplete', 'blocking' or 'non-agglutinating' immunoglobulin G (IgG) antibodies.

All tubes showing negative SAT were centrifuged at 3000 rpm for 15 min, the supernatant decanted and 1 ml physiologic saline added. The pellet was resuspended by mechanical agitation. This washing procedure was repeated three times. Anti-human globulin reagent (Anti-IgG, Ortho Diagnostic Systems, New Jersey, USA) was added (100 μl) to the last pelleting. The pellet was resuspended, incubated in a waterbath at 37 °C for 24 h. Agglutination was determined using an agglutinoscope and the titre given indicate the highest dilution in which 50% or more agglutination occurs in tube [20]. In our study, a titre of $\geqslant 80$ was arbitrarily chosen as the cut-off positive finding in SAT and AHGT.

Brucella ELISA

The determination of brucella-specific IgG, IgM and IgA in the serum specimen by ELISA was essentially carried out as previously described [21]. Briefly, 96-well microtitration plates were coated with 100 μl of predetermined *B. abortus* antigen (Immunostics Inc.). After incubation and washing, a 100 μl of 1:100 dilution of serum was added. Then, after incubation and washing a predetermined dilution of alkaline-

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phosphatase conjugated anti-human IgG, IgM or IgA (Sigma Chemical Co., St. Louis, USA) was added to the designated wells. The plates were then incubated, washed and P-nitrophenyl phosphate (Sigma) added. The reaction was stopped after 45 min incubation at 37 °C by addition of 3N sodium hydroxide and the optical densities (OD) of the wells were read at 405 nm in a Titertek Multiscan (Flow Laboratories, Scotland). Known positive and negative control sera were included in each run. In addition, background activity of conjugates was tested in each run using phosphate buffered saline instead of serum in designated control wells. For this study and based on our previous findings in ELISA [12, 21] the OD cut-off values of seropositive antibodies were chosen to be $\geqslant 0{\cdot}6$ for IgG and IgM and $\geqslant 0{\cdot}3$ for IgA.

Statistical analysis

Computer programs of Epi Info 5 and SPSS were used for statistical analysis. The significance of differences between groups were determined using a χ^2 test.

RESULTS

Demographic findings

A total of 597 persons were interviewed and tested from the 10 geographic locations: 54% were butchers, 35% were farmers, 8% laboratory technicians, 2% abbatoir workers and 1% veterinarians. 82% were men.

Findings in SAT and AHGT

The overall distribution of SAT and AHGT titres found in the surveyed individuals are shown in Table 1. Negative titres (< 20) in SAT and AHGT were found in 96% and 74%, respectively, of tested individuals. The percentages of individuals showing a titre of $\geqslant 80$ by SAT and AHGT were 1·7% and 15%, respectively, while those showing a titre of $\geqslant 160$ were 0·7% and 11%, respectively.

The percentages of individuals with a SAT or AHGT titre of $\geqslant 80$ according to regions are shown in Figure 1. Generally, the SAT titres in individuals from different regions were low ($\leqslant 5\%$): Biqaa (5%), Beirut suburbs (5%), Shouf (3·7%), Tripoli (2·3%) and Sidon (1·3%) with zero findings in the remaining regions. The AHGT titres of $\geqslant 80$ were found in individuals from all regions but in variable percentages (3·4–34%), the highest being in Biqaa (34%) followed by Kisrwan (24%), Shouf (21%), Sidon (16%), Aley (12%) and Beirut suburbs (10%).

Table 1. *Distribution of the 597 surveyed persons in high-risk occupation according to SAT and AHGT titres, Lebanon 1994*

Titres	Percent* of persons with titres in SAT	AHGT
< 20	96	74
20	0·7	6
40	1·2	5
80	1·0	4
160	0·5	5
320	0	2
640	0	2
1280	0	1
$\geqslant 2560$	0·2	1

* Rounded for the fractions.

The percentages of AHGT titres of $\geqslant 80$ or $\geqslant 160$ according to individuals profession are presented in Table 2. The highest percentages were found in veterinarians followed by farmers, butchers and laboratory technologists.

Findings in brucella-ELISA IgG, IgM and IgA

The overall distribution of the surveyed individuals based on brucella ELISA OD readings for IgG, IgM and IgA is illustrated in Table 3. This distribution is scattered along a wide range of OD readings. Considering the positive cut-off values in ELISA at OD $\geqslant 0{\cdot}6$ for IgG or IgM and at $\geqslant 0{\cdot}3$ for IgA the percentages of seropositive individuals by these criteria are presented according to region in Table 4. Overall, the percentages of individuals with positive IgG, IgM and IgA were 57, 61 and 26%, respectively. Variation in the percentages of positive findings among different regions is observed for all tested classes of Igs. The seroprevalence was almost similar for males and females in all the tests except for ELISA IgM whereby the percentages of positive males was lower than females, 57% and 81%, respectively.

Serologic tests findings in individuals with and without symptoms

Among 568 individuals whose questionnaires were completed for symptoms, 107 (18·8%) had symptoms

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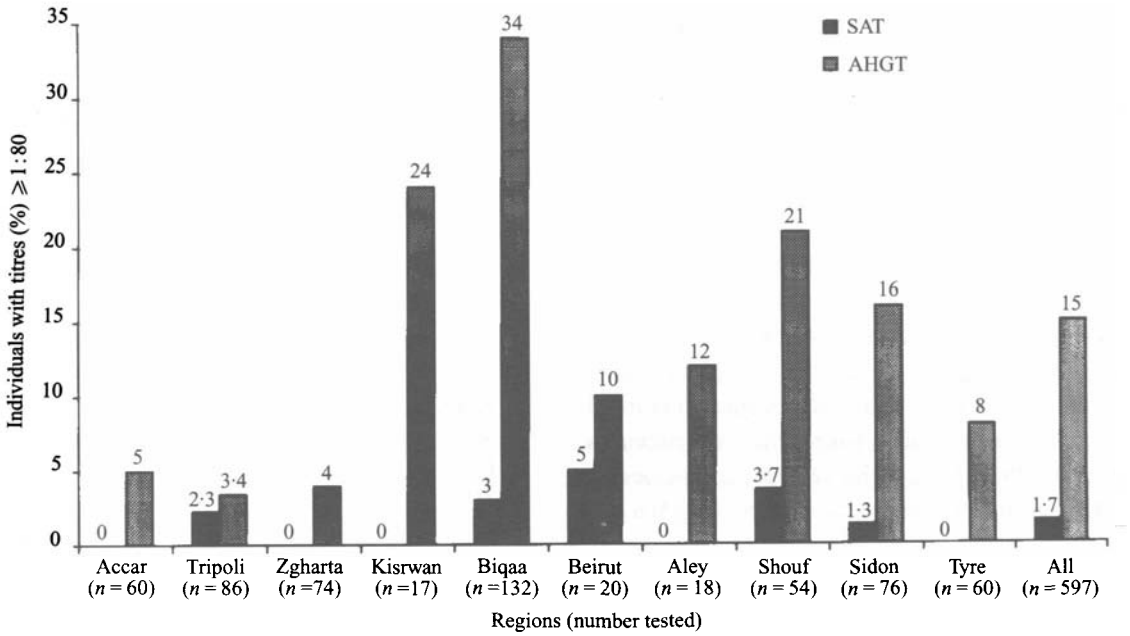

Fig. 1. Brucella SAT and AHGT titres of ⩾ 80 among persons in high-risk occupations in different regions in Lebanon, 1994.

Table 2. *Distribution of AHGT titres and ELISA IgG findings according to known profession of surveyed persons*

Profession	Number tested	Percent* of persons showing titres ⩾ 80	Percent* of persons showing titres ⩾ 160	ELISA IgG OD ⩾ 0·6
Butchers	306	9·2	5·2	47
Farmers	197	23·9	19·2	45
Laboratory technicians	43	4·7	0	41
Abbatoir workers	10	0	0	50
Veterinarians	8	50	50	50

* Rounded for the fractions.

while 461 (81·2%) did not have symptoms that could be associated with brucellosis. Generally, individuals with symptoms showed higher percentages of elevated (⩾ 80) SAT and AHGT titres. In ELISA, however, this elevation was more pronounced with IgA than IgG or IgM (Table 5). Those persons with symptoms were mostly from Biqaa valley (36%), Tripoli (15·8%), Shouf (11·2%), Sidon (11·2%) and Accar (10·2%). People reporting symptoms suggestive of brucellosis had significantly higher proportion with elevated IgG ($P = 0{\cdot}002$), IgM ($P < 0{\cdot}0001$) and IgA ($P < 0{\cdot}0001$) titres.

People reporting consumption of fresh cheese or milk had significantly ($P < 0{\cdot}001$) higher proportion with positive serology. Consumption of raw meat or home made 'labneh' was not associated with elevated titres.

DISCUSSION

The present study represents the first report on the seroprevalence of brucellosis among persons in high-risk occupational exposure in Lebanon. The overall exposure rate of 1·7% by SAT, 15% by AHGT and 26–61% by ELISA found in this study is suggestive of the high frequency of brucella exposure in the surveyed population and is comparable to those reported from other countries in this region [7, 22]. Although the study population was mostly males, overall, the prevalence of antibodies did not show sex differences. Among the screened population, the majority (81%) were asymptomatic while 19% reported symptoms that could be associated with brucellosis and would require clinical follow-up as was suggested by others [23]. The brucella antibodies detected in the asymptomatic persons could be

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Table 3. *Distribution of the 597 surveyed persons in high-risk occupation according to brucella ELISA OD readings for IgG, IgM and IgA, Lebanon, 1994*

OD readings (nm)†	Percent* of persons showing OD for IgG	IgM	IgA
0·1	0	0·3	8·5
0·2	9	1·2	66·5
0·3	13·7	8·5	12·6
0·4	10·6	14·7	4·0
0·5	10·2	13·4	1·0
0·6	8·0	15·2	1·7
0·7	8·9	15·2	1·0
0·8	5·7	10·9	0·3
0·9	6·2	8·9	0·5
⩾ 1·0	27·7	10·9	4·5

* Rounded for the fractions.

† Positive cut-off values are considered at OD ⩾ 0·6 for IgG or IgM and ⩾ 0·3 for IgA. OD values (mean ± S.D.) of PBS controls included in each run were for IgG = 0·12 ± 0·02, IgM 0·13 ± 0·03 and IgA 0·12 ± 0·02.

Table 4. *Distribution of seropositive findings in persons from different regions according to ELISA test*

Regions	Number tested	Percentage* of individuals with seropositive results in ELISA with OD for IgG ⩾ 0·6	IgA ⩾ 0·3	IgM ⩾ 0·6
Accar	60	28	10	42
Tripoli	86	49	15	63
Zgharta	74	65	32	67
Kisrwan	17	35	18	12
Biqaa	132	64	36	78
Beirut	20	75	40	40
Aley	18	50	26	55
Shouf	54	61	30	68
Sidon	76	56	22	55
Tyre	60	67	12	58
All	597	57	26	61

* Rounded for the fractions.

ascribed to a history of exposure, inactive brucellosis or repeated exposure to antigenic stimuli, as was reported earlier in veterinarians and abbatoir workers [6, 15, 24].

In Lebanon, earlier studies on animals have shown high prevalence of positive reactors; 0–36% in 1957 and 16–27·7% in 1982 [17, 18]. Brucellosis in animals was also found in different countries in our region

Table 5. *Distribution of individuals with and without symptoms according to seropositive (elevated) results in tests*

Test/titre or OD	Cut-off values	Percent* of elevated results in individuals With symptoms (n = 107)	Without symptoms (n = 461)
SAT titre	⩾ 80	5	0·9
	⩾ 160	3	0·2
AHGT titre	⩾ 80	32	11
	⩾ 160	26	6
ELISA IgG OD	⩾ 0·6	61	56
IgM OD	⩾ 0·6	66	59
IgA OD	⩾ 0·3	38	22

* Rounded for the fractions.

such as Saudi Arabia, Yemen, Libya, Sudan and Jordan with reported seroprevalence in goats, sheep, cows, cattles and camels ranging between 0·3% and 7% [25–30].

The prevalence of brucellosis can vary among populations from different geographic locations and countries, mostly due to variation in risk factors and the type of test use. For example, among 66 veterinarians in Jordan subclinical and clinical brucella infections were reported in 44% and 10·6%, respectively, based on SAT cut-off titres of ⩾ 80 [30]. In Saudi Arabia, on the other hand, positive antibodies were reported in 7·14% of veterinarians and 2·67% of butchers [31]. In our present study, 50% of the veterinarians and 9·2% of the butchers had positive antibodies based on AHGT ⩾ 80. In addition, brucella antibodies titres were also present among other professions, such as farmers which constituted the second highest population, in this study, who had a seroprevalence of 23·9%. These farmers in Lebanon share similar exposures to those in Jordan villages who often live in close proximity with their livestock, consume raw milk and make white cheese from sheep and goat using unhygienic methods [7]. These factors also accounted for the majority of reported cases in Kuwait [12] and Saudia Arabia [10, 22].

The laboratory diagnosis of brucella infection relies mostly on serologic testing since culture is of low yield and takes a long time [3, 6, 21]. However, in order to have proper interpretation of serologic findings, in this study and others, one has to understand the immune response after infection as well as the diagnostic and prognostic values of brucella-specific

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Ig classes, as was studied earlier in different types of individuals with brucellosis [6, 12, 21, 32]. Initially, IgM antibodies are produced followed within a few weeks by a switch to IgG synthesis. In patients with acute brucellosis, however, it was reported that only 2% of the cases had IgM alone, the rest had also IgG and IgA, indicating the insidious onset of the disease and the delay in seeking medical consultation [12, 21]. Gradual decline in titres can be observed starting around 5 months of initiating successful treatment [6, 12]. IgM, however, was reported to persist for several years despite clinical recovery [6, 23] and such a finding can explain IgM levels in our study population. Others have also reported that IgM persistence was mostly recognized among persons in high-risk exposure such as veterinary surgeons, possibly due to repeated exposure to antigenic stimulus [23, 33]. Moreover, this IgM persistence was observed among asymptomatic individuals in the general population in endemic area, such as, Saudi Arabia, whereby IgM to brucella was found among 11% of 216 asymptomatic well women (antenatal patients) and 4·9% of 107 males using 2-mercaptoethanol treatment of sera [23].

In addition to their diagnostic values, IgG antibodies seem to be good prognostic markers in brucellosis. A rapid fall in IgG antibodies has been associated with successful therapy, and lack of IgG decline (possibly due to repeated or prolonged exposure) suggests the need for close monitor of clinical status while resurge in the level of IgG antibodies can indicate reinfection or relapse [15, 23, 34]. Thus, it is important to follow up individuals serologically and clinically. This is especially needed because in the absence of symptoms it is not possible based on any serologic test result to distinguish healthy people exposed to brucella from those with symptoms consistent with chronic brucellosis [12, 33].

Among the several serologic tests, the demonstration of brucella-specific immunoglobulin (Ig) classes can be easily and directly displayed by tests such as ELISA and IFA [21, 35, 36]. In the present study, the seroprevalence based on ELISA brucella-specific IgG, IgM and IgA were detected with higher frequency than in SAT or AHGT, most likely due to differences in test sensitivity. For example, Bettelheim and colleagues [37] reported that among 307 healthy blood donors who were negative by SAT, 20·1% had positive brucella antibodies by AHGT. The sensitivity of ELISA was shown to be remarkably higher than that of SAT especially in detecting chronic and complicated cases of brucellosis [12, 13]. Moreover, since differences in decline in antibodies among different tests have been reported [23] this may also be the case with ELISA compared to SAT or AHGT. However, because there are no commercially available ELISA kits for the serodiagnosis of brucellosis, the reliable interpretation of its results has to take into consideration the optimization and standardization of its components, especially in relation to the antigen used and the established cut off limits in the surveyed community [21, 38]. In patients with different types of brucellosis (acute, subacute or chronic), the ELISA, used in the present study, was previously reported to have a sensitivity, specificity, predictive positive and negative values of $\geqslant 97\%$, 98%, $\geqslant 85\%$ and $\geqslant 97\%$, respectively [13, 21].

The question pertaining to the discriminative titres or positive cut-off values in various serological tests in relation to determining exposure, subclinical and symptomatic infections or those treated and cured remains controversial [6, 21, 39]. This is so because the cut-off values for positive or negative results may vary with different factors such as specimen type (e.g. serum or CSF), antigen used, type of test and the population studied in relation to geographic location of endemic or non-endemic areas. For example, in non-endemic areas, the diagnostic level of SAT has generally been considered at 80–160 [3, 32]. In endemic areas, however, conclusive correlations between SAT titres and patients with active brucellosis was uncommon below a titre of 640 [39]. In USA, analysis of 214 cases by agglutination tests showed that although most patients with active brucellosis had SAT titres of $\geqslant 160$, no single titre was always diagnostic [6]. For the purposes of studying levels of exposure, as in this study, agglutination titres of $\geqslant 80$ or $\geqslant 160$ seems satisfactory [3, 32]. Moreover, although Moyer and colleagues [32] noted the lack of availability of a single agglutination test for brucellosis in high-risk group, the simultaneous use of combined tests (e.g. agglutination and ELISA), as in this study, can be helpful in providing information about the status of brucellosis especially when this information is integrated with clinical information.

The possibility of detecting cross reacting antibodies in serologic testing for brucellosis especially in using agglutination tests cannot be overlooked. Cross-reactions were reported to occur in the brucella SAT test with serum from patients infected with or immunized against the organisms cholera, tularemia

and yersiniosis. Common antigens have also been shown between *Brucella* sp. and *E. coli* serotypes 0:116 and 0:157, Salmonella serotypes of Kaufmann–White group N (0:30), *Pseudomonas maltophilia*, and *Yersinia enterocolitica* serotype 0:9 [32, 40]. These cross-reactions were reported to be due to the lipopolysaccharide (LPS) fraction. However, no cross-reactions between soluble proteins of these organisms has been reported indicating that tests using such antigens rather than LPS, as in RIA and ELISA, are superior to the SAT [38, 41, 42]. For example, Goldbaum and colleagues [42] reported that among 15 patients with cholera, anti-brucella antibodies were detected in 20% and 0% of these patients when using brucella LPS and protein antigens, respectively. In the present study, it is difficult to determine the magnitude of cross reaction in SAT or AHGT and its influence on the serologic findings or subclinical infection in the surveyed population. Although vaccination against cholera, tularemia and yersiniosis is not carried out in Lebanon and isolation of their aetiologic agents is very rarely reported from clinical specimens, the possibility of their contribution to the anti-brucella antibodies detection in the present study cannot be excluded.

This study revealed a high prevalence of exposure to brucella among persons in high-risk occupation in Lebanon. Such findings could be used as means to locate cases of human brucellosis [43], stimulate further investigations, initiate public education and design measures focused at controlling brucellosis, especially in countries that still suffer from this disease.

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