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Prevalence of antibodies to *Brucella* spp. and risk factors related to high-risk occupational groups in Eritrea

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SUMMARY

In a study of three high-risk occupational groups using Rose Bengal and complement fixation tests, the highest prevalence (7·1%) was found among dairy farm workers and owners in randomly selected dairy-cattle farms, followed by veterinary personnel (4·5%) and inhabitants in pastoralist areas (3·0%). There was no evidence for significant differences between the three populations. Among dairy farm workers, a higher risk was associated with the presence of sheep in the farm (OR = 13·2, CI = 2·2–76·7). In the pastoral area, a high risk was linked to having close contact with animals (OR = 6·32, CI = 0·88–∞), while a reduced risk was seen for contact with cattle (OR = 0·18, CI = 0–1·30). Symptoms suggestive of brucellosis were more commonly observed among the dairy farm workers, mainly found in the highlands, than among the pastoralist area inhabitants, where malaria is prevalent. The study documents not only the presence of serological and clinical evidence of human brucellosis, but also risk factors related to it in Eritrea, for the first time.

INTRODUCTION

Brucellosis is a zoonotic disease of worldwide distribution. The main sources of humans infection are domesticated food animals: cattle, sheep, goats, swine, and in some countries, buffaloes, yak and camels [1]. Human beings are susceptible to infection with *Brucella abortus*, *B. melitensis*, *B. suis* and *B. canis*, but infections caused by *B. melitensis* are known to cause more severe clinical and pathological effects and to be responsible for most worldwide morbidity, particularly in developing countries [1–3]. However, *B. abortus* infection can also be severe and life threatening, especially when resistance is low due to pre-existing disease or malnutrition [2]. Brucellosis is transmitted from animal sources to man through direct contacts, inhalation of infectious aerosols and ingestion of infected materials [4].

The prevalence of brucellosis among human populations is largely influenced by the prevalence of the disease among domestic animals and local traditions regarding the proximity of animal housing and human habitations and the consumption and processing of milk products. Other factors which may influence the prevalence of disease in human beings include the type of *Brucella* species present in the region, the type of the domestic animals kept near human dwellings, the climatic conditions, and standards of personal and environmental hygiene [2].

Brucellosis is a systemic disease clinically characterized by fever, diaphoresis, lymphadenopathy, an-

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orexia, malaise and general fatigue; sometimes the disease may have more localized manifestations such as pancarditis, glomerulonephritis, orchitis and arthritis [3]. The disease is often treated as a fever of unknown origin and frequently misdiagnosed as other, more common, febrile diseases such as malaria.

The presence of brucella infections in different animal species in Eritrea has been described in previous studies [5–9]. A recent study [10] showed that brucellosis was prevalent in cattle in the intensive dairy-cattle farms in the capital Asmara (8·2%) and in those kept by pastoralists in the lowlands (5% seroprevalence). The disease was less common in communal cattle kept in the highlands, but 3·8% of goats kept by the pastoralists were seropositive.

There is scanty information about the prevalence of brucellosis among human populations in Eritrea. However, the first author has found seropositive sera among individuals with a febrile disease of unknown origin. Most of these patients had been in contact with seropositive animals or have been professionally associated with animals or animal products. Furthermore, there is anecdotal evidence of brucellosis in patients with febrile disease initially diagnosed as malaria, which failed to respond to anti-malaria treatment but had a good response to antibiotics (Tewolde Yohannes, personal communication) and brucellosis has been reported in neighbouring countries [11–16].

The population of Eritrea is estimated at about 3·5 million, of which about 80% live in rural areas and are mainly engaged in some form of farming or livestock production. There are three main livestock production systems in Eritrea: intensive system of production consisting mainly of dairying around the capital city, Asmara; a pastoral system of production in the lowlands (0–600 m) where mobile, predominantly female herds, of cattle, goats, sheep and camels are kept, and a mixed crop-livestock system in the highlands (1500–3000 m) where goats, sheep and mainly work oxen are kept in sedentary villages.

Most milk sold in the country is not pasteurized and the drinking of raw milk, the consumption of untreated milk products, and the eating of raw liver is a common practice, particularly among pastoralists. These habits pose a potential increased risk to infection in man.

The aims of the present study were to study the prevalence of brucella antibodies in groups with high level of animal contacts and to identify and describe the risk factors involved, as a preliminary step in understanding the disease situation in humans in the country.

METHODS

Study population and survey design

The study was conducted between May 1998 and February 2000 among three groups assumed to have a high risk for brucella infection: veterinary personnel, dairy-cattle farm workers and pastoralists.

Asmara dairy farm workers

Ninety-five farms were randomly selected from a sampling frame of 394 dairy cattle farms in the capital city Asmara and surrounding areas. Only 79 farms actually participated, as the workers of 16 farms were not willing to be sampled. Between May and August 1998 all dairy farm workers and owners ($n = 140$), present at the time of the study were interviewed and serum samples collected. Of the 79 farms, herd status (brucella seropositivity) was known from previous studies [10, 17]. A dairy-cattle farm was regarded as positive if it had at least one animal seropositive in both RBT and CFT. A questionnaire was designed to collect information about the farm (farm site, type of animals present); personal data (age, consumption of non-pasteurised milk, handling of animal products, handling calvings/abortions). The presence of symptoms suggestive of brucellosis was based on literature [2, 18–21], suggesting an infection lasting for more than 3 days during that year. These included fever, chills, sweating, headache, abdominal pain, loss of appetite, insomnia, fatigue, arthralgia, low back pain and weight loss. The questionnaire was pre-tested and administered by two trained personnel. Each farm worker was clinically examined by a qualified physician and information was collected on physical findings related to brucellosis (lymphadenopathy, hepatomegaly, splenomegaly, skin rash, abdominal and joint tenderness).

All cases that showed high antibody titres and had clinical symptoms were treated for brucellosis using tetracycline HCl (500 mg p.o. QID) for 21 days and streptomycin (1 gm i.m. QD) for 7–14 days [22]. The cases were followed up over a period of 6 months.

Veterinary personnel

This study was conducted between January and February 1999. From 22 out of 25 veterinary clinics

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distributed throughout the country, one veterinary personnel staff from each clinic took part in the study. Each participant was interviewed and the questionnaire completed as for the farm workers, with slight modifications, and blood samples collected.

Western Lowlands pastoral area

This study was conducted during October 1999–February 2000. An average of 20 patients each (n = 201) from all 10 operational human health centres in the Gash-Barca Administrative Zone in the western lowlands of Eritrea took part in the study. Each medical centre was visited for 1 day and the first 20 patients attending the clinic that day were interviewed and a blood sample taken. The human health centres staffed by health assistants mainly provide treatment to sick patients based on symptoms and basic diagnosis. They also offer vaccinations to children. Cases regarded as serious are referred to nearby hospitals staffed with general practitioners and with better diagnostic facilities. Cases that cannot be handled at the regional hospitals are referred to a Central National Hospital.

Each participant was interviewed for place of residence, ownership of animals, types of animals owned, nature of animal contact, consumption of raw milk and meat, presence of symptoms suggestive of brucellosis, previous treatment for anti-malarial drugs and antibiotics.

Collection and handling of blood samples

From each person about 7 ml of blood was collected in evacuated silicone-coated tubes (Becton Dickinson, Cockeysville, NJ). Blood samples were left overnight to clot and the sera sent to the Central Veterinary Laboratory, Asmara in iceboxes and stored at −20 °C until tested.

Serological tests

Rose Bengal test (*RBT*)

The RBT test was carried out according to the method described by Alton [24] with *Brucella abortus* antigen obtained from the Veterinary Laboratories Agency (VLA), Weybridge, UK. Briefly, 25 μl of antigen were mixed, in flat plates, with an equal volume of human sera and checked for agglutination after 4 min. According to the degree of agglutination, the result was visually graded on a scale from 0 to 3. Presence of any trace of agglutination was regarded as positive.

Complement fixation test (*CFT*)

All the RBT-positive samples and 155 randomly selected RBT negative samples were re-tested by CFT using *Brucella abortus* (S99) antigen obtained from VLA. The CFT was performed according to the method of Alton [24] using human sera inactivated at 56 °C for 30 min. The tests were carried out in U-shaped wells of 96 well micro-titre plates (Bibby Sterlin, Stove, UK), using 25 μl of twofold dilutions of inactivated sera, 1 in 10 dilution of S99 antigen, 5 international units of guinea-pig complement (Sigma, Steinheim, Germany), and 3% of sensitized sheep red blood cells in veronal buffer (Sigma). The antibody titre of each serum was the reciprocal of the highest dilution showing 25% or more fixations.

Sera with antibody titres of 128 or higher were regarded as positives; those with 64 were regarded as suspicious. A person was defined as brucella infected if positive in both RBT and had a titre of 128 or higher in CFT and showed clinical symptoms suggestive of Brucella infection. For practical purposes sera were tested in CFT up to T:264 dilution.

Statistical analysis

Descriptive analyses of the various variables were carried out using the statistical software Statistix (Student edition 1.0, Analytical Software, Tallahassee, FL). Univariate analysis was done using χ^2 or Fisher's exact test for categorical variables and Kruskal–Wallis test for continuous variables. Variables with a P-value $< 0{\cdot}20$ in univariate analysis were further tested in a multiple logistic regression using LogExact for Windows (Cytel Software Corp., Cambridge, MA). Before variables were entered in the multiple models, associations between variables were assessed using the χ^2 test in Statistix. The model was built using a forward-selection process [25]. Variables with a likelihood ratio test P-value $< 0{\cdot}10$ were kept in the model. Confidence intervals for prevalences were calculated using EpiCalc 2000 (Version 1.02, Brixton Books, UK).

RESULTS

The distribution of sampled and positives across groups and gender are shown in Table 1 and the main symptoms of seropositives and seronegatives recorded in the different groups are shown in Table 2.

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Table 1. Brucella *complement fixation test seroprevalence by sex in three groups in Eritrea assumed to be at high-risk for* brucella *exposure*

	CFT$^+$			CFT$^-$			Prevalence	
Group	Male	Female	Total	Male	Female	Total	%	95% CI
Dairy farm workers	9	1	10	123	7	130	7·1	3·7–13·1
Veterinary personnel	1	0	1	13	8	21	4·6	0·24–24·9
Pastoralist area	3	3	6	105	90	105	3·0	1·2–6·7

Table 2. *Main clinical symptoms recorded in by the different groups – broken down by CFT$^+$/CFT$^-$ status*

	Dairy farm workers		Veterinary personnel		Pastoralist area	
Main symptoms	CFT$^+$ ($n = 10$)	CFT$^-$ ($n = 130$)	CFT$^+$ ($n = 1$)	CFT$^-$ ($n = 21$)	CFT$^+$ ($n = 6$)	CFT$^-$ ($n = 195$)
Abdominal pain	6	34	1	7	4	135/192
Back pain	7	41	1	7	5	139/192
Arthralgia	5	30	1	11	6	130/192
Chills	7	24	0	7	1	99/192
Fever	5	33	1	6	6	169/191
Fatigue	6	42	1	11	6	161/192
Appetite loss	7	24	1	2	4	145/192

Table 3. *Distribution of variables possibly linked to individual exposure to* brucella *for dairy farm workers*

Variable	CFT$^+$	CFT$^-$
Contact with sheep*	4/10	6/130
Contact with goat	0/10	2/130
Contact with horse	9/10	114/130
Contact with dogs	5/10	76/130
Handles animal product	10/10	123/130
Consumes non pasteurized milk	10/10	127/130
Sex (female)	1/10	7/130
Sex (male)	9/10	123/130

* $P < 0{\cdot}20$ in univariable analysis and offered to the multiple model.

Table 4. *Distribution of variables possibly linked to individual exposure to* brucella *for pastoral area group*

Variable	CFT$^+$	CFT$^-$
Sex (female vs. male)	3/6	90/197
*Direct contact with livestock	6/6	107/197
*Contact with goats	6/6	108/197
*Contact with cattle	0/6	82/197
Contact with camels	1/6	25/197
Contact with sheep	1/6	50/197
Owns any animal	6/6	154/197
*Consumption of raw milk	6/6	124/197
Consumption of raw liver	2/6	86/197

* $P < 0{\cdot}20$ in univariable analysis and offered to the multiple model.

Dairy farm workers

Of the dairy farm workers, 132 (94·3%) were males, showing that it was a male dominated occupation. All the 10 positive cases (7·1%) were treated for brucellosis, and 9 of these patients showed a marked clinical improvement, though their antibody titres remained high after 6 months. One of the patients continued to complain about clinical symptoms and had high antibody titres 6 months after treatment. Within this group, the following symptoms were highly related to seropositivity: arthralgia, back pain, fever, chills, fatigue, abdominal pain, loss of appetite and loss of weight (Table 2).

The distribution of the variables related to individual exposure is shown in Table 3. Based on results from univariate analyses of variables listed in Table 3, only two variables were selected for the multiple logistic regression model, the presence of sheep at the farm and the positive/negative serostatus of a farm. There was no difference in seropositivity related to age. Only the presence of sheep on a farm

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Table 5. *Results from multiple regression for variables from Table 3 and 4 for dairy farm workers and pastoralist group. Results from Exact Logistic Regression using LogExact*

Group	Variable	OR	95% CI
Dairy farm workers	Contact with sheep	13·2	2·16–76·6
Pastoral area	Occupation directly related with livestock	6·32	0·88–∞
	Contact with cattle	0·18	0–1·30

remained in the final model (OR = 13·2, 95% CI = 2·16–76·7).

Veterinary personnel

Of the 22 individuals tested, only one (4·5%) was seropositive, showing severe clinical symptoms. After treatment most of the clinical symptoms subsided but the high antibody titre continued to be detected up to 6 months after treatment. No statistical evaluation was possible for this group.

Pastoral area

Of the 201 individuals sampled in the pastoralist areas, 156 (77·6%) were pastoralists, and only 4 individuals of the rest owned some type of livestock. Nine (4·5%) were seropositives in RBT but only 6 (3·0%) were confirmed as positives by the CFT. All the 6 confirmed cases were pastoralists (half of which were females). The pastoralists kept goats (114 of 201, 56·7%) cattle (82, 40·8%), sheep (51, 25·4%) or camels (26, 12·9%). Of all the individuals interviewed, 168 (83·6%) were treated for malaria during the last year and 198 individuals (98·5%) had also taken antibiotic treatment, which may have been effective against brucella infections during the same year. The symptoms recorded are shown in Table 2.

Descriptive statistics for this group is shown in Table 4. In univariate analysis of these variables, a higher risk was linked to close contact with animals, contact with goats and no contact with cattle, and consumption of raw milk. There was a strong association between being a pastoralist and contact with goat/consuming raw milk, but only being a pastoralist and contact with cattle could be entered in the multiple models. Table 5 shows the results from the multiple model, where being a pastoralist (OR = 6·3, CI = 0·88–∞) and absence of contact with cattle (OR = 0·18, CI = 0–1·29) both were important predictors (Table 5).

Of 155 negative sera randomly selected, the majority 150 (96·7%) had CFT titres $\leqslant$ 16, 4 (2·5%) had CFT titres of 32 and one had 64. Of the 17 sera regarded as positives, all had CFT titres $\geqslant$ 128.

DISCUSSION

In rural areas, the incidence of brucellosis in man is supposed to reflect the incidence of animal disease [2]. The highest group prevalence group was found among dairy farm workers (7·1%). In a previous study, the highest individual seroprevalence in animals (8·2%) was also found among dairy cattle farms in Asmara [10]. It has also been reported that among those who work or live on dairy farms undulant fever seems to be almost (but not entirely) limited to those who handle and milk the cows [26]. The presence of high titres in the sera of dairy farm workers confirms the impression that intimate contact with the animals is more important than consumption of infected milk [27]. Our results support these views. However, in our study, among the dairy farm workers, a high risk was linked to the presence of sheep at a farm. Univariate analysis also showed a lower risk linked to working in a positive herd. As all workers were heavily exposed to cattle, it was not possible to study the relative importance of sheep and cattle. Seroprevalence of brucellosis in Eritrean sheep was reported to be low [10], but in the light of the present findings further studies are needed. The sheep factor may also reflect a double source of *B. abortus* from cattle and *B. melitensis* from sheep. It was reported that different breeds of sheep show varying susceptibility to brucellosis, for example Maltese sheep are very resistant while the fat-tailed sheep of South-West Asia are very susceptible [2]. Despite the dispute on the optimum antibiotic therapy and although FAO/WHO have recommended the combined use of rifampicin and

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doxycycline, the treatment we offered to the clinically ill dairy farm workers is still considered to give the fewest relapses [23]. The second highest prevalence was found among veterinary personnel (4·5%). The only veterinary staff found seropositive was also working in area where there are dairy-cattle farms and he had handled calvings during that year. Though the prevalence of brucellosis can vary among populations from different geographic locations and countries, mostly due to variation in risk factors and type of test used [19], higher prevalence among veterinarians was reported in different countries [19, 28–30]. The low seroprevalence we found among the veterinary staff is a reflection of the nature of the veterinary services in Eritrea. Outside the capital city, Asmara, animals are mainly kept under the traditional system and the main services provided to herds are vaccinations against the classical infectious diseases. The staffs are rarely involved in carrying out individual treatments and handling of calvings and abortions, an activity that may cause a higher risk to infection.

The lowest seroprevalence (3%) was found among those sampled in the pastoralist areas. This reflects the seroprevalence in animals in the same area which was reported as been 5% in cattle and 4·3% in goats [10]. Human infection in areas of nomadic animal husbandry is common, where close contact between animals and man are part of the ecology [1]. Although presence of goats and consumption of raw milk were linked to higher risks in pastoral areas, positive cases were found only among pastoralists, indicating that the main factor was having direct contact with animals [2]. Though it was reported in Iraq that human brucellosis is mostly acquired by the consumption of non-pasteurised milk and milk products of sheep and goats [31], this was not the case in our study. Higher risk was also related to absence of cattle in the herd, which may be an indication that brucellosis in the area may be linked to *Brucella melitensis* rather than to *B. abortus*.

It is well known that symptoms attributed to brucellosis are vague and non-specific and no definite conclusions can be drawn; although the relationship between high titre levels and symptoms may be helpful in deciding to treat or not to treat an individual patient [27]. In our study, symptoms suggestive of brucellosis were more manifested among the dairy farm workers in Asmara, which is situated at an altitude of 2400 m above sea level and the area was until recently regarded free of malaria. At present only few cases of malaria are recorded and some of the symptoms were related to seropositivity. While among the pastoralists who inhabit the lowland areas where malaria is endemic, symptoms were not related to seropositivity and may not be of use in the diagnosis of brucellosis.

Although, this is the first study in Eritrea that not only documents the presence of serological and clinical evidence of human brucellosis, but also looks into risk factors of importance in the transmission of the disease, it had its own shortcomings. The power of the study was low as the number of positive cases was small, and some important risk factors may have been missed. There may have been some potential biases. The study among the pastoralists was not representative for the general population, as sampling was done among patients that attended clinics for whatever complaint they have had. Some of the cases may have been missed and so the prevalence under estimated. Owners and workers of 16 dairy farms did not participate in the study, as they were not willing to be sampled. As the level of awareness of the transmission of the disease from animals to man is low, we think that their lack of participation was not because they may have known their farms to be infected but rather the people are generally not willing to give blood samples. The data for the veterinary personnel were too small to allow risk factor analysis.

It is hoped that this study will stimulate further research and raise awareness among medical professionals to consider brucellosis when symptoms suggestive of brucellosis are evident related to high-risk professions. There is also a need for more representative epidemiological studies and research to isolate and identify species and biovars involved in human brucellosis in Eritrea.

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