Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008

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Brucellosis is a global zoonotic disease, endemic in North African countries and around the Mediterranean. A prospective study of *Brucella* seroprevalence was conducted in north-western Libya (western mountains region). Blood samples collected over 13 months in the period December 2006 to January 2008 from 561 animals (goats, sheep, cattle and camels) and 546 human volunteers were tested for *Brucella* using the Rose Bengal test, tube agglutination test and ELISA assays. Amongst livestock, 31% of goats and 42% of cattle were seropositive. Human samples showed a high seropositivity of 40%, with 95 (43%) of the 221 positive samples positive for IgM, indicating active or recent infection. Control measures are needed to reduce this high prevalence of brucellosis in Libya.

Introduction

Brucellosis is a global zoonotic disease associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also in humans [1-6]. The disease is widely distributed throughout the developing world, considered to be a serious problem in at least 86 countries [7]. Brucellosis is a severe zoonosis in North African countries [8] and the Near East [9,10] causing economic and livestock losses and affecting industrial production [3,11]. In these regions *Brucella melitensis*, the predominant species in goats and sheep, has spread to other animals such as cattle and camels. *B. melitensis* is also the dominant type in humans [9]. Consumption of unpasteurised milk and milk products from cows, small ruminants or camels is considered to be the main route of infection as well as an occupational hazard [12]. In the North African region, as in sub-Saharan countries, social and economic factors play a major role in the spread of brucellosis [13]. Libya is considered to be endemic for brucellosis [2], although little information is available; previous studies are limited to food-producing animals such as cattle and goats [14-16] and reports of human brucellosis in Libya are limited to a few cases [9,17].

We investigated the seroprevalence of brucellosis in humans and several livestock species around eight cities in the Yafran municipality, Al Jabal al Gharbi district (Western mountains region), north-west Libya. This region is 9,310 km2, with a population of 153,000, and dependent on livestock production, mainly goats (750,000 sheep and goats and around 8,000 camels). The Yafran is well-known for its rural culture and traditional lifestyle, where different livestock species are kept together and people live in close proximity to their livestock. Brucellosis is the likely cause of economic losses for owners and companies through abortions in pregnant animals especially in goats.

Methods and materials

Study design

We investigated the seroprevalence of *Brucella* in the Yafran, both in livestock and in humans, from samples collected over a period of 13 months from December 2006 to January 2008. A total of 561 blood samples were collected from different animals (a convenience sample of 340 goats, 188 sheep, 19 cattle, 14 camels). Human blood samples were also collected from 546 selected individuals (332 male and 214 female) from the populations in the area surrounding the farms, who worked and/or had contact with the studied animals. Both human and animal populations are widely dispersed in this large area in Libya, and due to the socio-economic factors of the involved region a few small towns could not be included in this study.

Diagnostic methods

Animal and human samples were processed in various laboratories in Libya, as described [18,19] using the Rose Bengal test and ELISA for the detection of *Brucella* in serum (DRG *Brucella* IgM-ELISA and *Brucella* IgG ELISA). Whole blood samples of 5 ml were collected aseptically. Serum was stored at 2-8 °C until testing could be performed. Statistical analysis was performed using the two-proportion z-test. Lifestyle data on milk

consumption and animal contact were collected using a simple questionnaire.

Results and discussion

The prevalence of *Brucella* in different livestock species is summarised in Table 1. Rates of seropositivity were 1.6 times higher (p=0.05,) in goats (31%) than in sheep (24%). A local serological survey at the Al Jabal al Gharbi University in the western mountains region in 1997 found that 8.5% of sheep, 28.4% of goats, and 3.5% of camels were positive for brucellosis [20]. Our data indicate a substantial increase over the past ten years, with 24% of sheep, 31% of goats and 14% of camels testing positive for *Brucella* in our study. The increased prevalence in different animal species may be attributable to husbandry methods. Previous research has shown that controlling this disease in goats and sheep can be effective in reducing infection in other livestock [11]. The role of goats in perpetuating brucellosis and in disseminating the disease among humans has also been highlighted [4].

The overall prevalence of *Brucella* seropositivity among humans in the Yafran municipality was 40%, with Jado (47%) and Yifrin (46%) having the highest proportion of brucellosis brucellosis-positive people (Table 2). The prevalence of IgG and IgM antibodies in seropositive individuals was 57% (126/221) and 43% (95/221), respectively, suggesting that a substantial proportion of the population in this region were actively or recently infected. This compares with 8% seropositivity in Egypt [11] where control measures have been introduced.

We observed that significantly more seropositive humans had a history of raw milk consumption than had direct contact with livestock (71% and 58%, respectively, p<0.02). Similarly, a study of female brucellosis patients in Egypt found that more than 87% had a history of raw milk consumption [5], which has been identified as a risk factor also by other authors [10,12]. We noticed that seropositivity in the Yafran municipality, Libya, was higher among males than females, with 66% of samples from males and 34% of samples from females positive for *Brucella* infection in our study. This could be explained by the fact that in the culture and tradition of that region raw milk is consumed more frequently by men.

Effective eradication campaigns in the European Union have significantly reduced the incidence of brucellosis, with many countries being given brucellosis-free status [2]. Programmes and control measures have been undertaken in many countries in North Africa and the

Table 1

Prevalence of *Brucella* in domestic animal species assayed by the Rose Bengal test, Libya, December 2006–January 2008

Animal species	Animals tested	Seropositive animals	Proportion of positive animals
Goats	340	104	31%
Sheep	188	45	24%
Cattle	19	8	42%
Camel	14	2	14%
Total	**561**	**159**	**28%**

All animals were apparently healthy at sampling; those with a history of any recent disease or vaccination were excluded.

Table 2

Brucella prevalence in humans, immunoglobulin analysis, and possible risk factors among seropositive volunteers from eight cities in the Yafran municipality, Libya, December 2006–January 2008

Locality	Number of samples n	Positive samples n (%)	Analysis of *Brucella*-positive individuals			
			Drinking of raw milk n (%)	Direct contact with animals n (%)	IgG-positive n	IgM-positive n
Zentan	128	48 (38)	40 (83)	35 (73)	34	22
Jado	196	93 (47)	61 (66)	52 (56)	44	36
Rujban	29	11 (38)	8 (73)	6 (55)	8	4
Rehibat	50	15 (30)	11 (73)	7 (47)	8	7
Qasr Alhaj	12	4 (33)	3 (75)	2 (50)	1	3
Chkoke	15	2 (13)	2 (100)	2 (100)	1	1
Morgan	32	9 (28)	6 (67)	4 (44)	6	4
Yifrin	84	39 (46)	25 (64)	20 (51)	24	18
Total	**546**	**221 (40)**	**156 (71)**	**128 (58)**	**126**	**95**

Samples were assayed initially using the Rose Bengal test, then seropositive samples were further analysed by ELISA, in duplicate, for IgG and IgM.

Near East (e.g. Egypt and Kuwait) [11,21,22]. However, underreporting and underdiagnosis of other food-borne pathogens is a problem around the Mediterranean [23], particularly in North African countries, where communication with local authorities is problematic and most of the available information is unpublished or limited to seminars and workshops [9]. The high prevalence of *Brucella* seropositivity in the Yafran municipality in Libya might suggests that the size of the problem has been underestimated by the authorities and that action needs to be taken to control brucellosis in this region.

Conclusion

We conclude that in the north-western region of Libya, Brucellosis seroprevalence is high in animals and human populations. Our data highlights the need for further research, including the isolation and characterisation of the causative agents, reliable epidemiological studies and the need to implement a transparency policy and effective control measures in Libya.

References

1. Boschiroli ML, Foulongne V, O'Callaghan D. Brucellosis: A worldwide zoonosis. Curr Opin Microbiol. 2001;4(1):58-64.
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91-9.
3. Corbel MJ. Brucellosis: An overview. Emerg Infect Dis. 1997;3(2):213-21.
4. Banai M. Control of small ruminant brucellosis by use of Brucella melitensis rev.1 vaccine: Laboratory aspects and field observations. Vet Microbiol. 2002;90(1-4):497-519.
5. Sabah AA, Aly AM, Tawab AH, Arafa WA. Brucellosis in Egyptian female patients. J Egypt Soc Parasitol. 2008;38(2):671-8.
6. Corbel MJ, Food and Agriculture Organization of the United Nations, World Health Organization, World Organisation for Animal Health. Brucellosis in humans and animals. Geneva: World Health Organization; 2006.
7. World Health Organization, Office of World Health Reporting. The world health report 1996: Fighting disease, fostering development: Executive summary. Geneva: World Health Organization; 1996.
8. Aggad H, Boukraa L. Prevalence of bovine and human brucellosis in western Algeria: Comparison of screening tests. East Mediterr Health J. 2006;12(1-2):119-28.
9. Refai M. Incidence and control of brucellosis in the near east region. Vet Microbiol. 2002;90(1-4):81-110.
10. Jennings GJ, Hajjeh RA, Girgis FY, Fadeel MA, Maksoud MA, Wasfy MO, et al. Brucellosis as a cause of acute febrile illness in Egypt. Trans R Soc Trop Med Hyg. 2007;101(7):707-13.
11. Samaha H, Al-Rowaily M, Khoudair RM, Ashour HM. Multicenter study of brucellosis in Egypt. Emerg Infect Dis. 2008;14(12):1916-8.
12. Almuneef MA, Memish ZA, Balkhy HH, Alotaibi B, Algoda S, Abbas M, et al. Importance of screening household members of acute brucellosis cases in endemic areas. Epidemiol Infect. 2004;132(3):533-40.
13. Makita K, Fèvre EM, Waiswa C, Kaboyo W, De Clare Bronsvoort BM, Eisler MC, et al. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. Ann N Y Acad Sci. 2008;1149:309-11.
14. el Sanousi SM, Omer EE. Serological survey of brucellosis in Benghazi cow project (Libya). Int J Zoonoses. 1985;12(3):207-10.
15. Aboudaya MA. An evaluation of diagnostic methods for bovine brucellosis in Libya. Int J Zoonoses. 1986;13(4):282-5.
16. Gameel SE, Mohamed SO, Mustafa AA, Azwai SM. Prevalence of camel brucellosis in Libya. Trop Anim Health Prod. 1993;25(2):91-3.
17. Tiller RV, De BK, Boshra M, Huynh LY, Van Ert MN, Wagner DM, et al. Comparison of two multiple-locus variable-number tandem-repeat analysis methods for molecular strain typing of human Brucella melitensis isolates from the Middle East. J Clin Microbiol. 2009;47(7):2226-31.
18. Alton GG, Maw J, Rogerson BA, McPherson GG. The serological diagnosis of bovine brucellosis: An evaluation of the complement fixation, serum agglutination and rose bengal tests. Aust Vet J. 1975;51(2):57-63.
19. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis--a review of the literature. Part II: Serological tests for brucellosis. Clin Lab. 2003;49(11-12):577-89.
20. Elarbi A. [Serological study on brucellosis in farm animals in the Yafran municipality]. Al Jabal: al Gharbi University; 1997. [In Arabic].
21. al-Khalaf SA, Mohamad BT, Nicoletti P. Control of brucellosis in Kuwait by vaccination of cattle, sheep and goats with Brucella abortus strain 19 or Brucella melitensis strain rev. 1. Trop Anim Health Prod. 1992;24(1):45-9.
22. Alton GG. Control of Brucella melitensis infection in sheep and goats--a review. Trop Anim Health Prod. 1987;19(2):65-74.
23. Gargouri N, Walke H, Belbeisi A, Hadadin A, Salah S, Ellis A, et al. Estimated burden of human Salmonella, Shigella, and Brucella infections in Jordan, 2003-2004. Foodborne Pathog Dis. 2009;6(4):481-6.