Risk factors for human brucellosis in northern Jordan

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عوامل اختطار داء البروسيلات البشري في شمال الأردن

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الخلاصة: إن المعلومات حول عوامل اختطار داء البروسيلات في الأردن قليلة. وقد أجرى الباحثان دراسة للحالات والشواهد شملت 56 أردنيا عولجوا لإصابتهم بداء البروسيلات مع ثلاثة شواهد على الأقل في مقابل كل حالة، فبلغ عدد الشواهد 247، وكانت أوجُه التقابل هي الجنس والعمر والموقع (في نفس القرية)، والمعيار الاجتماعي والاقتصادي. وبالمجمل درس الباحثان 17 عاملاً من عوامل الاختطار تتعلق بالمخالطة لمختلف المواشي، واستهلاك اللبن الحليب ومنتجات الحليب، ومعالجة مياه الشرب والوعي بالمرض. وكانت أكثر المتغيرات ترتبط بداء البروسيلات في التحليل الوحيد المتغير، إلا أن النموذج اللوجستي النهائي يتضمَّن أربعة متغيرات فقط، هي: حَلْب النعاج والعنـزات (معدل الأرجحية 3.5)، واستهلاك جبن أبيض (فيتا) غير مطبوخ مصنوع من ألبان النعاج والعنـزات (معدل الأرجحية 2.8)، واستهلاك حليب البقر (معدل الأرجحية 0.4)، واستهلاك الجبن الأبيض (فيتا) المطبوخ (معدل الأرجحية 0.4). ويرى الباحثان ضرورة تدريب الفئة القليلة المتبقية من المزارعين حول الممارسات الأكثر أماناً للحَلْب ولإجراءات إعدادات الجبنة (فيتا) البيضاء.

ABSTRACT Little is known about the risk factors of human brucellosis in Jordan. A case–control study was conducted involving 56 Jordanians who had been treated for brucellosis and at least 3 matched controls for each case (*n* = 247). Matching was for sex, age, locality (the same village) and socioeconomic standard. Univariate and multivariate logistic regression analyses were used. In all, 17 risk factors were examined related to: contact with various livestock, milk and milk product consumption, drinking-water treatment and disease awareness. Most variables were associated with brucellosis in the univariate analysis but the final logistic model included only 4: milking sheep and goats (OR 3.5), consumption of raw feta cheese made from sheep and goat milk (OR 2.8) and consumption of cows' milk (OR 0.4) and boiled feta cheese (OR 0.4). Small ruminant farmers need to be trained in safer milking practices and feta cheese making procedures.

Facteurs de risque de la brucellose humaine dans le nord de la Jordanie

RÉSUMÉ Les données sur les facteurs de risque de la brucellose humaine en Jordanie sont rares. Une étude cas-témoins a été menée auprès de 56 Jordaniens traités pour une brucellose et d'au moins trois témoins appariés pour chaque cas (n = 247). L'appariement concernait le sexe, l'âge, la localité (le même village) et le statut socioéconomique. Des analyses de régression logistique univariées et multivariées ont été utilisées. En tout, 17 facteurs de risque liés aux domaines suivants ont été examinés : contact avec du bétail divers, consommation de lait et de produits laitiers, traitement de l'eau de boisson et connaissance de la maladie. La plupart des variables étaient associées à la brucellose dans l'analyse univariée mais le modèle de régression logistique final n'en a conservé que quatre : la traite des brebis et des chèvres (OR = 3,5), la consommation de fromage feta cru fait à partir de lait de brebis et de chèvre (OR = 2,8), la consommation de lait de vache (OR = 0,4) et de fromage feta bouilli (OR = 0,4). Les éleveurs de petits ruminants doivent être formés à des pratiques de traite et à des méthodes de fabrication du fromage feta plus sûres.

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Received: 24/05/09; accepted: 20/07/09

Introduction

Brucellosis is a zoonosis of importance in most countries of the Middle East and South America and half a million new human cases each year are reported worldwide [1]. The annual incidence of human brucellosis ranges from less than 1 to 78 cases per 100 000 population in the Middle East [2]. Reported numbers greatly underestimate the true incidence of human brucellosis because of variability in the clinical picture [3], misdiagnosis and under reporting to local authorities. *Brucella abortus, B. canis, B. suis* and especially *B. melitensis* are able to cause human infection [2]. The only species isolated in Jordan is *B. melitensis* [4].

In Jordan, a higher seroprevalence of 8% was reported among high-risk people compared to only 0.5% in the control sample [5], and an even higher seroprevalence of 55% was reported among veterinarians [6]. Brucellosis is usually transmitted to man by the ingestion of unpasteurized dairy products or by direct contact with infected animals. The results of previous studies on high-risk people emphasized the importance of contact infection and the occupational nature of the disease in Jordan [5,6].

To date, few community-based studies have been carried out in Jordan and little is known about the risk factors of human brucellosis in the Middle East. We conducted a case–control study to investigate possible risk factors for human brucellosis in northern Jordan, namely contact with livestock, milk and milk products consumption, drinking-water treatment and disease awareness.

Methods

Sample

A matched case–control study was undertaken of indigenous Jordanians who had recently been treated for brucellosis in Irbid, northern Jordan. The laboratory records of 2 teaching hospitals (Princess Basma and King Abdulla) were examined for cases with *Brucella*-positive isolation and/or were seropositive with the Rose Bengal test. A hospital nurse approached each eligible patient, described the purpose of the study and asked for participation in the study. The consent of the patient/patient's legal guardian was sought where appropriate. Out of 65 confirmed brucellosis patients asked to participate, 56 (86%) (42 males and 14 females) agreed to participate and signed a consent form. All cases selected had received treatment during the period January to July 2007. The inclusion criteria for cases were: brucellosis positive with a history of fever, and/or sweating and/or joint pain and *Brucella* spp. isolation (n = 8) or seropositive (n = 56) with Rose Bengal test and confirmed with ELISA; resident in northern Jordan; and agreement to participate in the study.

Each case was matched with at least 3 controls on age, sex, locality (same village) and socioeconomic status. Their consent was also obtained. The inclusion criteria for the controls were: no history of brucellosis and seronegative by the Rose Bengal test, resident in northern Jordan and agreement to participate.

The total sample recruited was 303 people. The study had 80% power at the 5% significance level to detect an odds ratio (OR) ≥ 2 for risk factors present in 50% of controls, and an OR ≥ 3 for those present in 20% of controls.

Data collection

Patients and controls were visited and interviewed at their homes. Data were collected using purposely designed, pretested and validated questionnaire; completion was interviewer-assisted by a trained nurse and it was conducted in Arabic. The interviewer was supervised for the first 10 interviews. Information collected included; name, age, gender, place of residence, medication or health history and telephone number. Potential exposure to livestock was obtained by recording the number of the different livestock animals kept or noted in the immediate area of the residence and whether these animals were ever allowed in the house/yard at the time of the visit. Because of the potential indirect transmission of *Brucella* spp. through drinking-water, contact with ruminants and their manure, consumption of milk and milk products, related questions were asked.

To reduce recall-bias especially on diet, further questions were asked on the source of milk products, i.e. home-made or where the item(s) were usually bought, and children's mothers were asked for confirmation.

Data analysis

Data were stored and analysed using *Epi-Info,* version 6 according to the case–control design and compared in relation to the exposure to the following potential risk factors: livestock near house; contact with sheep; contact with goats; contact with cattle; slaughtering animals; contact with manure; help in animal delivery; milking small ruminants; consumption of sheep's milk, goats' milk, cows' milk, boiled milk, feta cheese and boiled feta cheese; boil water; filter water; and have knowledge of brucellosis.

Uni- and multivariate analyses were performed using the chi-squared test and logistic regression. Seventeen dichotomous variables were studied in the univariate analysis. A multivariate analysis was then conducted separately for each group of factors and the OR and its 95% confidence intervals (CIs) were calculated, starting with the factors that were statistically significant in the univariate analysis ($P \leq 0.05$) or an OR ≤ 0.3 or ≥ 3.0.

A forward stepwise simple logistic regression analysis was done including the significant variables to control for confounding and get a final logistic regression model. Only those factors that remained statistically significant in

the final model are presented. A *P*-value of < 0.05 was considered statistically significant.

Ethical considerations

The study protocol was approved by the concerned committees at Jordan University of Science and Technology. Informed consent was given and all identifying information was kept confidential.

Results

Of the 17 examined factors; 11 were associated with brucellosis in the univariate analysis at $P < 0.05$. Of these, 5 involved contact with animals, namely: keeping livestock near the house, contact with sheep, contact with goats, contact with manure, and milking animals (Table 1). Five factors of milk and milk product consumption, including consumption of sheep and/or cows' milk, boiled milk, feta cheese and boiled feta cheese, were also significantly associated with brucellosis in the univariate analysis (Table 2). On univariate analysis, boiling drinking-water was significantly associated with brucellosis (Table 3). These 11 significant factors were entered in multivariate logistic regression analysis using forward stepwise approach. In the final model after controlling for all the variables only 4 factors remained significantly associated with brucellosis: milking small ruminants and the consumption of raw feta cheese were positively associated with brucellosis, while the consumption of cows' milk and boiled feta cheese decreased the risk of brucellosis (Table 4). The apparent significant association of the other factors found in the univariate analysis disappeared after adjustment for confounding.

Discussion

In our study, although 11 statistically significant risk factors for brucellosis were found in the univariate analysis, only 4 factors remained associated with infection after adjustment for confounding in the logistic regression analysis: milking small ruminants, the consumption of raw

Table 1 Animal contact risk factors for human brucellosis in northern Jordan: univariate analysis

Variable	Controls (*n* = 247)	Cases (*n* = 56)	OR (95% CI)	*P*-value
	No.	No.		
Livestock near house				
No	219	38	3.7 (1.9–7.3)	0.01
Yes	28	18		
Contact with sheep				
No	211	34	3.7 (2.0–7.2)	0.01
Yes	36	22		
Contact with goats				
No	215	34	4.4 (2.3–8.5)	0.01
Yes	32	22		
Contact with cattle				
No	239	52	2.3 (0.7–7.9)	0.30
Yes	8	4		
Slaughtering animals				
No	228	47	2.3 (0.98–5.4)	0.08
Yes	19	9		
Contact with manure				
No	213	38	3.0 (1.5–5.8)	0.01
Yes	34	18		
Help in animal delivery				
No	229	49	1.8 (0.7–4.6)	0.30
Yes	18	7		
Practise milking small ruminants				
No	232	41	5.7 (2.6–12.5)	0.01
Yes	15	15		

OR = odds ratio; CI = confidence interval.

Table 2 Milk and milk product consumption as risk factors for human brucellosis in northern Jordan: univariate analysis

Milk product consumption	Controls (*n* = 247)	Cases (*n* = 56)	OR (95% CI)	*P*-value
	No.	No.		
Sheep's milk				
No	83	9	2.6 (1.2–5.7)	0.01
Yes	164	47		
Goats' milk				
No	161	30	1.6 (0.9–2.9)	0.10
Yes	86	26		
Cows' milk				
No	88	36	0.3 (0.2–0.6)	0.01
Yes	159	20		
Boiled milk				
No	30	17	0.3 (0.2–0.6)	0.01
Yes	217	39		
Feta cheese				
No	127	18	2.2 (1.2–4.1)	0.01
Yes	120	38		
Boiled feta cheese				
No	69	28	0.4 (0.2–0.7)	0.01
Yes	178	28		

OR = odds ratio; CI = confidence interval.

feta cheese, and consumption of cows' milk and boiled feta cheese. The association with small ruminants accords with the findings of Al Sekait [7] from Saudi Arabia, where similar small ruminant husbandry is practised. Infected animals secrete *Brucella* spp. in large numbers in the uterine fluids, as well as in milk [2]. During miscarriage the fetal fluids wet the animal's back and udder. This *Brucella* rich fluid will contaminate the environment and the rear parts of the animal exposing animal handlers, especially milkers, to a high risk of infection.

In northern Jordan, the flock-level prevalence of brucellosis among sheep is 56% [8] and 54% in goats [9] and represents a major source of infection to other animals [10] and humans directly through contact and indirectly through contact with contaminated

Table 3 Univariate analysis of drinking-water treatment and disease awareness as risk factors for human brucellosis in northern Jordan

Variable	Controls (*n* = 247)	Cases (*n* = 56)	OR (95% CI)	*P*-value
	No.	No.		
Water treatment by:				
Boiling				
No	215	55	0.1 (0.02–0.9)	0.02
Yes	32	1		
Filtering				
No	172	45	0.6 (0.3–1.1)	0.20
Yes	75	11		
Knowledge of brucellosis				
No	101	16	1.7 (0.9–3.3)	0.10
Yes	146	40		

OR = odds ratio; CI = confidence interval.

Table 4 Final logistic regression model for brucellosis in humans in northern Jordan

Variable	OR (95% CI)	*P*-value
Contact with animals		
Milking, yes	3.5 (1.5–8.4)	0.02
Consumption of milk and milk products		
Cows' milk, yes	0.4 (0.2–0.8)	0.01
Feta cheese, yes	2.8 (1.4–5.6)	0.01
Boiled feta cheese, yes	0.4 (0.2–0.8)	0.01

OR = odds ratio; CI = confidence interval.

objects and consumption of animal products [4,5].

In Jordan, the only isolated *Brucella* species is *B. melitensis* [4]. Although, *B. melitensis* infects cattle with high morbidity in Jordan (M.N. Abo-Shehada, unpublished observation), the significance of this infection and its role in human infection has never been evaluated. The current results showed brucellosis to have an inverse association (OR = 0.4) with cows' milk consumption. This protective effect of cow's milk can be explained because in northern Jordan cow's milk only is available as a pasteurised or heat-treated product in shops and supermarkets. On the other hand, small ruminant milk is mostly purchased from farmers and consumed directly without heat treatment as most of these farmers are nomads with no milk heat-treatment facilities. Brucellosis had an OR of 2.8 among subjects consuming raw feta cheese of sheep and goat origin, confirming earlier findings in central Greece [11], Khuestan Province in Islamic Republic of Iran [12] and the Gaza Strip [13]. However, this is contrary to the findings of a study in Saudi Arabia which found that greatest risk was associated with the consumption of milk and *laban* (buttermilk), as opposed to cheese [14]. The consumption of homemade milk products has been reported to be associated with *B. melitensis* infection [15], which concurs with our finding of an increased risk associated with consumption of raw feta cheese. Locally, at home and in small dairy businesses, feta cheese is made from heat untreated sheep and goat milk. As the local production of sheep and goat milk is seasonal, the cheese is preserved using boiling and pickling in brine and boiled cheese is used outside the season. The results showed boiled feta cheese consumption to be associated with decreased odds (0.4) of brucellosis contrary to that when consuming the raw feta cheese. This association may explain the seasonality of brucellosis in Jordan and the Middle East, where the peak of infections occurs during the lambing season and small ruminant milking (raw feta cheese production) season and drops afterwards, when the boiled cheese is consumed.

There are some limitations to the study. Case–control studies are prone to bias, especially selection, recall and inter-interviewer bias and measures were taken to minimize this as described. In addition, the use of simple regression analysis in a matched case–control study results in larger odds ratios than conditional regression analysis. However, we kept the stratum sizes large to minimize this effect.

Conclusions

This study showed association with both the practice of manual milking of sheep and goats and the consumption of raw feta cheese, and human brucellosis. Also, the consumption of boiled feta cheese of sheep and goat origin and cows' milk was associated with reduced odds of brucellosis.

Our findings show that contaminated animals and milk products remain sources of brucellosis in Jordanians. Health education efforts should target small ruminant farmers and small ruminant milk product consumers regarding brucellosis symptoms and transmission. Small ruminant farmers need to be trained in safer milking practices and feta cheese making procedures. These may include washing and cleaning the udder before milking and the milkers' hands after milking and heat treating the milk used in making feta cheese.

Acknowledgement

This work received financial support from the Deanship of Research, Jordan University of Science and Technology.

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Research priorities for zoonoses and marginalized infections: Technical Report of the TDR Disease Reference Group on Zoonoses and Marginalized Infectious Diseases of Poverty

The above-mentioned report provides a review and analysis of the research on zoonoses and marginalized infections which affect poor populations, and a list of research priorities to support disease control. The work is the output of the disease reference group on zoonoses and marginalized infectious diseases (DRG6), which is part of an independent "think tank" of international experts, established and funded by the Special Programme for Research and Training in Tropical Diseases (TDR), to identify key research priorities through the review of research evidence and input from stakeholder consultations.

The report covers a diverse range of diseases including zoonotic helminth protozoa, viral and bacterial infections considered to be neglected and associated with poverty. Disease-specific research issues are elaborated under individual disease sections and many common priorities are readily identified among the disease such as need for new and/or improved drugs and regimens, diagnostics and, where appropriate, vaccines. The disease specific priorities are described as micro priorities compared with the macro level priorities which will drive such policies as the need for improved surveillance; the need for intersectoral interaction between health, livestock, agriculture, natural resources and wildlife in tackling the zoonotic diseases; and the need for a true assessment of the burden of the zoonoses.

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