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Original Article

Risk Factors of Human Brucellosis in the Northeast of Iran

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Introduction: Brucellosis is a major health problem in northeast of Iran. There is not much data on the association of nutrition and lifestyle factors with the risk of brucellosis in this area. We conducted the present study to determine the risk factors of brucellosis in Khorasan-e Razavi Province, northeastern Iran. **Methods**: we conducted a case-control study from July 2015 to March 2016 in three cities of Khorasan-e Razavi Province. Cases and controls were recruited from individuals attending primary care and were matched together based on their age (± 2 years) and gender. Clinical and epidemiological data were collected with a valid questionnaire. Conditional logistic regression was used in Stata software V13. **Results**: We recruited 180 incident cases and 360 controls, of which 53.9% were male, and 90.0% were living in rural areas. Consumption of unpasteurized yogurt (OR): 5.4; 95% CI: 2.5-11.5), milk (OR: 6.0; 95% CI: 3.0-11.9), and cheese (OR = 3.7; 95% CI: 1.7-8.1), as well as engagement in livestock-related occupations (OR: 2.6; 95% CI: 1.2-5.2) significantly increased the adjusted risk of brucellosis. Conversely, having academic education (OR: 0.1; 95% CI: 0.01-0.5), consuming unpasteurized butter (OR: 0.4; 95% CI: 0.2-0.8), and timely animal vaccination (OR: 0.3; 95% CI: 0.1-0.5) had a protective effect on brucellosis. **Conclusion**: The risk factors identified in this study are lifestyle- and occupation- related modifiable factors. So, the disease incidence is expected to decrease in this region with modification of these risk factors, such as animal vaccination, personal protection at work, and public health education. *J Med Microbiol Infec Dis, 2016, 4 (1-2): 20-24.*

Keywords: Brucelloses, Case Control, Risk Factors, Iran.

INTRODUCTION

Brucellosis is the most common zoonotic infection caused by the bacterial genus *Brucella*. The disease remains a major public health concern worldwide, causing enormous global burden [1, 2]. Brucellosis causes a severely debilitating and disabling illness, accompanied by fever, diaphoresis, chills, fatigue, weight loss, headache, and joint pain persisting for weeks to months. Long term sign and symptoms of this bacterial disease include arthritis, anemia, leucopenia, thrombocytopenia, granulomatous hepatitis, meningitis, and endocarditis [3-7]. Iran is an endemic region for brucellosis with about 500,000 new cases annually [8]. Khorasan-e Razavi Province is located in the northeast of Iran, sharing long common borders with Afghanistan, which is a country with high prevalence of the disease. Due to the high rate of human mobility and animal trafficking between Afghanistan and Iran, which partially happens through Khorasan-e Razavi Province, this region is at a high risk for brucellosis. In Torbat-e Heydarieh, a city in the south of Khorasan-e Razavi Province, the disease incidence has shown an increasing trend since 2011, which is much greater (21 cases per 100,000 individuals) than the average estimate of the country.

Despite debilitating and disabling effects of the infection, no human vaccine is currently available to prevent the disease. Therefore, the transmission of the infection should be intercepted by eliminating human-level risk factors for disease. Previously, some studies investigated the risk factors associated with brucellosis, among which, keeping livestock, consuming unpasteurized milk, and infection of family members were identified as the critical risk factors [4, 5, 9]. However, specific types of dairy products are traditionally produced in Khorasan-e Razavi Province, whose risk for brucellosis infection has remained unstudied.

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Given the high incidence of brucellosis in Khorasan-e Razavi and lack of knowledge on the role of nutrition and lifestyle factors in the risk of brucellosis contraction, we conducted this study to determine risk factors associated with brucellosis infection in this area. The data about such factors would help policy makers to develop more effective preventive strategies [10, 11].

MATERIAL AND METHODS

We performed an age- and gender-matched retrospective case control study with 1:2 matching ratio. The study was conducted in three cities of Khorasan-e Razavi Province, Torbat-e Heydarieh, Zaveh, and Mahvelat. The study ran from July 2015 to March 2016, during which 180 patients were recruited.

Inclusion criteria for enrollment of cases included: 1) individuals with clinical signs and symptoms of brucellosis attending the health centers of Torbat-e Heydarieh, Zaveh, or Mahvelat cities of Khorasan-e Razavi Province, Iran; 2) incident cases diagnosed as brucellosis with both Coombs Wright serology test and physician's diagnosis [12]; 3) incident cases who were also confirmed as brucellosis case by the national portal for zoonotic diseases management [13]; and 4) individuals who consented to participate in the study. The control group was selected from non-brucellosis individuals attending the same health centers. Control individuals were matched to the case patients based on their age (± 2 years) and sex. For each case, two eligible controls were selected. We excluded the recurrent or treatment failure brucellosis cases based on the records of the national portal for zoonotic disease management [13]. We would exclude controls if they were diagnosed with brucellosis during the study period, or were unwilling to participate in the study for any reason.

Sample size. Convenience sampling method was used to select study samples from individuals referring to the health centers. Based on previous estimates, we considered a probability of 0.80 and 0.58 for bovine exposure in cases and controls, respectively. Considering a type I error of 0.05 and a power of 0.9, the initial sample size was calculated as 44 and 88 for case patients and controls, respectively. We also intended to evaluate the effect of some potential confounders on the risk of brucellosis. Therefore, considering 10 additional samples per confounding variable, the final sample size increased to 180 cases and 360 controls.

Data collection. Data collection was performed during July 2015 to March 2016. Information about brucellosis risk factors was collected through a questionnaire. The questionnaire itself was a combination of two relevant, valid, and reliable questionnaires. The first one was a 17-item multiple-choice questionnaire assessing the risk factors of brucellosis in high-risk occupations. Validity and reliability of this questionnaire have been confirmed previously [14]. The other questionnaire was the standard data collection form of brucellosis surveillance in Iran [12]. Ten experts evaluated the content and face validity of the final questionnaire, and their recommendations were applied based on the research team's consensus.

Final questionnaire had eight domains, including 1) geographical characteristics of area; 2) the participants' demographic characteristics, including age, sex, educational level, and occupation; 3) laboratory findings for brucellosis based on Wright, Coombs Wright, and 2ME tests; 4) disease stage (incident case, treatment failure, and recurrence); 5) clinical signs and symptoms of brucellosis; 6) lifestyle factors, in the past year, including consumption and type of non-pasteurized dairy products, contact history with livestock, vaccination history of livestock, and family history of infection; 7) type and duration of brucellosis treatment in the past year; and 8) occupational condition in the past year, including type of occupation, working experience, use of personal protection at work, type of personal protection used, and existence of ventilation in the workplace. After adequate training and examination, the staff of the health centers collected the data fo this study. The senior staff of the health centers supervised data collection procedure. We placed health center staffs for data collection because they were familiar with the participants' culture.

Statistical analysis. Continuous and categorical data were described using mean ± SD and number (percentage), respectively. Univariable conditional logistic regression was used to test each independent variable against study group (case and control). Variables with a significance level less than 0.2 in this model were included into the multivariable conditional logistic regression model. Also, a backward method using Likelihood Ratio Tests (LRT) was used to make the model as simple as possible. Analyses were performed in the Stata software (Version 13).

Ethical Consideration. Informed consent was obtained from all participants. The study protocol was reviewed and approved by the ethics committee of Kerman University of Medical Sciences (IR.KMU.REC.1394.438.)

RESULTS

Totally, 180 brucellosis patients and 360 controls were included in this study. The controls were matched with the patients based on their sex and age. About 90% of both patients and controls were residents of rural areas, amongst which 65% of patients and 73% of controls were keeping livestock in their backyard. Other characteristics of study participants are presented in table 1.

There was no significant difference between cases and controls regarding the participants' age, gender, educational level, pregnancy, place of residence, and history of hospitalization. However, the patients had more frequently consumed raw milk (OR: 7.2; 95% CI: 4.4-11.8), unpasteurized fresh cheese (OR: 8.9; 95% CI: 4.1-16.0), and unpasteurized yogurt (OR: 6.9; 95% CI: 3.9-12.2) than controls. They had also been in contact with livestock (OR: 2.9; 95% CI: 1.6-5.5) and engaged in stockbreeding and animal husbandry (OR: 4.1; 95% CI: 2.5-6.7) more frequently than controls. In the multivariable model, however, consumption of raw milk (adjusted OR: 6.0; 95% CI: 3.0-11.9), unpasteurized fresh cheese (adjusted OR: 3.7; 95% CI: 1.7-8.1), and unpasteurized yogurt (adjusted OR:

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5.4; 95% CI: 2.5-11.5) as well as being involved in livestock-dependent occupations (adjusted OR: 2.6; 95% CI: 1.2-5.2) remained significant (Table 2).

On the other hand, in the univariable model, livestock vaccination (OR: 0.5; 95% CI: 0.3-0.8) and keeping livestock in the backyard (OR: 0.5; 95% CI: 0.3-1.0) conversely related to contraction of brucellosis (Table 1). In the multivariable model, however, only livestock vaccination remained as a significant protective factor (adjusted OR: 0.3; 95% CI: 0.1-0.5). In this model, having an academic education (adjusted OR: 0.01; 95% CI: 0.01-0.5) and consuming butter (adjusted OR; 0.4; 95% CI: 0.2-0.8) also turned to be significant protective factors, decreasing the risk of brucellosis. Table 2 shows the results of the multivariable regression model.

Table 1. Risk factors of human brucellosis in the univariable conditional logistic regression

Variables		Case	Control	Crude OR	*P-value*
		n (%)	n (%)	95% CI	
Occupation	Unemployed	27 (15)	76 (21)	1	
	Livestock-dependent profession	92 (51)	88 (24)	8.5 (2.8-25.5)	<0.001
	Livestock-independent profession	61 (34)	196 (55)	2.1 (0.7-6.1)	0.19
Education level	Illiterate	57 (32)	96 (27)	1	
	Elementary	57 (32)	133 (37)	0.6 (0.3-1.0)	0.08
	Secondary	39 (21)	67 (18)	0.7 (0.3-1.5)	0.4
	Diploma	32 (13)	47 (13)	0.6 (0.3-1.4)	0.2
	University	4 (2)	17 (5)	0.3 (0.1-1.0)	0.05
Contact with livestock*	Yes	111 (73)	100 (50)	2.9 (1.6-5.5)	<0.001
	No	42 (27)	92 (48)	1	
Keeping animals at home	Yes	99 (65)	141 (73)	0.5 (0.3-0.9)	0.04
	No	54 (35)	51 (27)	1	
Livestock Vaccination*	Yes	34 (68)	111 (67)	0.5 (0.3-0.8)	<0.001
	No	16 (32)	54 (33)	1	
Consumption of unpasteurized milk*	Yes	142 (79)	140 (39)	7.2 (4.4-11.8)	<0.001
	No	38 (21)	220 (61)	1	
Consumption of unpasteurized cream*	Yes	26 (15)	43 (12)	1.3 (0.7-2.2)	0.4
	No	153 (85)	317 (88)	1	
Consumption of unpasteurized cheese*	Yes	70 (39)	33 (9)	8.9 (4.1-16.0)	<0.001
	No	110 (61)	327 (91)	1	
Consumption of unpasteurized butter*	Yes	28 (16)	63 (17)	0.8 (0.5-1.4)	0.5
	No	151 (84)	297 (83)	1	
Consumption of heated milk fat*	Yes	30 (17)	53 (15)	1.2 (0.7-1.9)	0.5
	No	149 (83)	307 (85)	1	
Consumption of unpasteurized yogurt*	Yes	62 (35)	29 (8)	6.9 (3.9-12.1)	<0.001
	No	117 (65)	331 (92)	1	

* These items show the consumption/contact history in the past year (at the time of study)

Table 2. Risk factors of human brucellosis in multivariable conditional logistic regression

Variables		Multivariable model	*P-value*	Final model	*P-value*
		AOR (95% CI)§		95% CI	
Occupation	Unemployed	1			
	Livestock-dependent profession	2.6 (1.2-5.2)	0.008	2.16 (1.2-4.0)	<0.015
	Livestock-independent profession	0.6 (0.1-2.8)	0.5	0.46 (0.1-1.9)	0.28
Education level	Illiterate	1			
	Elementary	0.5 (0.2-1.1)	0.09		
	Secondary	0.6 (0.2-1.8)	0.4	-	-
	Diploma	0.7 (0.2-2.1)	0.4		
	University	0.1 (0.01-0.5)	0.01		
Contact with animals*	Yes vs. No	2.6 (0.8-8.9)	0.1	-	-
Consumption of unpasteurized milk*	Yes vs. No	6.0 (3.0-11.9)	<0.001	4.59 (2.5-8.3)	<0.0001
History of Cream in the past year	Yes vs. No	2.3 (0.4-13)	0.3	-	-
Consumption of unpasteurized cheese*	Yes vs. No	3.7 (1.7-8.1)	0.001	3.58 (1.9-7.7)	<0.0001
Consumption of unpasteurized butter*	Yes vs. No	0.4 (0.2-0.8)	0.01	0.31 (0.2-0.6)	<0.002
Consumption of heated milk fat*	Yes vs. No	0.6 (0.3-1.3)	0.2	-	-
Consumption of unpasteurized yogurt*	Yes vs. No	5.4 (2.5-11.5)	0<0001	4.42 (2.3-8.6)	<0.0001
Livestock Vaccination*	Yes vs. No	0.3 (0.1-0.5)	<0.001	-	-
Keeping animals at home	Yes vs. No	0.6 (0.2-1.9)	0.3	-	-

* These items show the consumption/contact history in the past year (at the time of study)

§ In the multivariable model, AOR for each variable is estimated after adjustment for the rest of variables presented in this table

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DISCUSSION

In this matched case control study, people who consumed raw milk, unpasteurized cheese, and yogurt, as well as being involved in livestock-dependent occupations in the past year, had an increased risk of brucellosis. On the other hand, livestock vaccination, having an academic education, and butter consumption showed a protective effect on brucellosis.

The association between participants' occupation and risk of brucellosis has been reported in previous studies [15-17]. This finding suggests that direct contact with livestock, which is common among poulterers, stockbreeders, shepherds, butchers, and slaughterhouse workers, provides exposure to *Brucella* spp., and hence, increases the risk of human infection. The pathogen probably transmits to human through contact with infected animal's skin, wool, blood, tissue, and body secretions.

The association between raw milk consumption and risk of brucellosis is also consistent with previous findings [4, 18-20]. Likewise, unpasteurized cheese consumption increased the risk of brucellosis, which is in line with previous studies [21-23]. These findings highlight the preventive role of milk pasteurization/boiling in brucellosis infection.

Unlike Meky *et al.* (2007), who observed an increased risk of brucellosis contraction in individuals consuming butter in Alexandria, northern Egypt [24], we observed a decreased risk of disease in such persons. This discrepancy might be attributable to different butter production methods applied in northeastern Iran versus northern Egypt. In northeastern Iran, butter is produced from boiled milk through the fermentation process, which is known to be fatal for *Brucella* bacteria, and hence, can reduce the risk of brucellosis.

The association observed between unpasteurized yogurt consumption and brucellosis might also be attributable to the process of yogurt production in northeastern Iran. In this region, yogurt is produced by mixing fresh milk of different livestock with a particular type of stored milk, which has a sour taste and acidic PH. Since milk is obtained from various animal sources, this method of yogurt production increases the risk of milk contamination. Currently, little is known about yogurt production methods in other regions of Iran. The effect of each method on product's contamination is also unclear.

We also observed an association between participants' educational level and brucellosis infection, in a way that brucellosis patients tended to have lower educational levels. This finding is in contrast to the results of Rubach *et al.* (2013) and Meky *et al.* (2007) [24, 25], but is consistent with that of Sofian *et al.* (2008) [4]. Our finding highlights the role of education on individuals' lifestyle and their awareness of transmission routes of the diseases, which ultimately preserves them from acquiring infections.

Finally, timely vaccination of livestock during the last year showed a protective effect on the risk of human brucellosis. This result is consistent with those previous studies [18, 26-28], and highlights the need for livestock vaccination as one of the most efficient ways to prevent livestock and human brucellosis.

This is the first study reporting socio-behavioral and lifestyle factors associated with human brucellosis in northeastern Iran. Recall bias may have influenced our results. We aimed to reduce recall bias by selecting cases from incident brucellosis patients and controls from individuals referred to health centers as proposed by [29].

Identified risk factors for brucellosis in northeastern Iran include consumption of unpasteurized milk, cheese, and yogurt, as well as engagement in livestock-related occupations. It is expected that by controlling these modifiable factors, the incidence of brucellosis would decrease in this endemic region. In this regard, timely vaccination of livestock is one of the priorities. These efforts should be completed with livestock follow-ups, to ensure elimination of the infection. Moreover, the corpses of infected animals should be actively identified and immediately isolated and eliminated by veterinarians and veterinary laboratory staff. Use of personal protection while working with animals is also recommended. Health education to the general population as well as people who are engaged in the dairy production and livestock-related occupations also can decrease the risk of infection. Further studies are needed to detect pathogen survival during the manufacture and storage of dairies.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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