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Original Article

Sero-epidemiological survey of brucellosis in small ruminants in Hamedan province, Iran

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ABSTRACT

Objective: Brucellosis is a highly contagious zoonosis with global distribution. The disease remains endemic in many countries including Iran, while its seroprevalence in endemic area is not well documented. We aimed to determine the seroprevalence of brucellosis in sheep and goats in Hamedan province, west of Iran.

Material and methods: A total of 3,250 blood samples from 2,550 sheep and 700 goats were collected randomly. All samples were analyzed for the presence of *Brucella* antibodies using Rose Bengal, Wright standard tube agglutination and 2-mercaptoethanol agglutination tests.

Results: The seroprevalence rate of brucellosis in animals and flock level were found in 4.6% and 13.6% of goats and 3% and 27.9% of sheep, respectively. No evidence of correlation between gender and *Brucella* infection rate were found in animals ($P>0.05$). Statistical significant differences was seen between age groups and infection rate in goats ($P=0.033$, OR=2.1); unlike to sheep ($P=0.373$). Also, the infection rate in nomads population of sheep was higher than fix location animals ($P=0.003$; OR=1.9); unlike to goats ($P=0.195$). In animals with history of abortion and vaccination against brucellosis, seroprevalence rate was significantly lower than other ($P<0.05$).

Conclusion: This is the first report of brucellosis in sheep and goats in Hamedan province. The design of a comprehensive control program including vaccination, screening, and culling of brucellosis-positive animals is recommended.

KEYWORDS

Brucella, Serology, Small ruminants

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INTRODUCTION

Brucellosis is a zoonotic infectious disease caused by Gram-negative bacteria of genus *Brucella* (Chothe and Sakena, 2011). Though it has been eradicated in few countries such as, Australia, Germany, Sweden, Finland, Norway, United Kingdom, Canada, Israel, Japan and New Zealand. The infection is endemic in sheep and goats in most countries of the Mediterranean basin, some parts of Asia and Middle East including Iran (Gul and Khan, 2007; Samadi et al., 2010; Hasannia et al., 2015).

The bacterium is transmitted by sexually adult animals with predilection of placenta and fetal fluid. Brucellosis is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. In female animals, the bacteria are localized in the udder followed by excretion via milk and in male animals orchitis and epididymitis can lead to infertility (Seyyed-Gholizadeh, 2013).

B. melitensis occurs naturally in sheep and goats. Also this species is the main cause of human brucellosis worldwide (Chothe and Sakena, 2011; Parlak et al., 2013). *B. melitensis* and *B. abortus* are the most important species in Iran (Hasannia et al., 2015). Humans are usually infected through contact with fluid discharges from an infected animals or by ingestion of unsterilized dairy products mainly sheep and goat's milk and fresh soft cheese made out of unpasteurized milk (Gul and Khan, 2007). The disease in human is serious and long lasting and often results in chronic and disabling symptoms (Seyyed-Gholizadeh, 2013). The prevalence of brucellosis can vary according to climatic conditions, geography, species, sex and age. In addition, the applied diagnostic tests might affect the results (Gul and Khan, 2007).

Infection in animals is strongly correlated with abortion and commonly affected animals such as sheep and goats. In male animals, the primary sign of infection is epididymitis and orchitis. Cross-transmission of brucellosis can occur among cattle, sheep, goats, camels and other species (Tegegn et al., 2016). Brucellosis is a major cause of direct economic losses resulting from clinical disease, abortion, neonatal losses, reduced fertility, decreased milk production, emergency slaughtering of the infected animals. Economic losses in small ruminants stem from breeding inefficiency, loss of lambs and kids, reduced wool, meat and milk production (Lone et al., 2013).

The serological tests are the most useful epidemiological tool for diagnosis of brucellosis in animals and humans (Chothe and Sakena, 2011). Currently, rapid, simple and low-cost assays as Rose Bengal tests (RBT), and standard tube agglutination test (STAT) are very useful for detection of brucellosis. RBT and STAT are used as screening and confirmatory assays, respectively. None of these tests differentiate between active and inactive stages of disease; because they do not differentiate between IgG and IgM agglutinins. The IgM titer is due primarily to 2-Mercaptoethanol (2-ME) resistant antibody (IgG) (Sareyyupoglu et al., 2010).

Control measures are based on strict hygiene and vaccination programs. Vaccination is regarded as a measure for reducing the prevalence of the disease to a level where eradication by test and slaughter can be implemented. Also, vaccination may be the most economical means of controlling the brucellosis (Hasannia et al., 2015). Of the vaccines used for immunizing small ruminants against *B. melitensis*, Rev-1 vaccine is generally preferred. The Rev-1 vaccine is used to protect small ruminants against brucellosis and to protect females from abortion in regions where the disease occurs (Samadi et al., 2010).

Little is known of brucellosis prevalence in animals in Iran (Seyyed-Gholizadeh, 2013; Akbarmehr and Ghiyamirad, 2011; Ghobadi and Salehi, 2013; Ebrahimi et al., 2014; Gharib-Mombeini et al., 2014). However, there is no status quo of the disease in sheep and goats in western Iran. The principal objective of current investigation was to determine the seroprevalence of brucellosis in sheep and goats in Hamedan province, West of Iran.

MATERIALS AND METHODS

Study area: Hamedan is a mountainous province with mild climate that is located in West part of Iran (19,546 km²: 34°49'11" N, 48°40'15" E). The mean annual rainfall and temperature is 317.7 mm and 11.3°C, respectively. The economy of this region is mainly based on agriculture and farm animal industry (**Figure 1**).

Sample collection: A cross-sectional seroepidemiologic survey was conducted during April to September 2015. The samples were taken from different regions of Hamedan province using cluster randomly sampling. 90 and 50 flock was selected in different rural area and nomads population, respectively. In the each flock, 20 sheep and 5 goats were selected and sampled randomly. A total of 3250 blood samples (5 mL from the jugular vein) were collected from 2,550 sheep and 700 goats (Thrusfield, 1997). The animals were categorized into two age groups (≤ 2 and > 2 year). Also, the gender, history of abortion in the previous gestation (using the files of

farm) and history of vaccination against brucellosis (documented in local veterinary office) in the last year were recorded (**Table 2**).

Figure 1. Map of Iran and location of Hamedan province (studied area).

Serology: The sera were obtained by centrifugation at 1500×g for 10 min and stored at -20°C until laboratory testing (Reviriego et al., 2000). Anti-*Brucella* antibodies of samples were detected using RBT, STAT and 2-ME tests. Firstly, all of the serum samples were evaluated for anti-*Brucella* antibodies using RBT screening test. The RBT positive samples were then tested by STAT and 2-ME methods. The state of brucellosis (positive or negative) was determined according to the **Table 1** (Bertu et al., 2010).

Rose Bengal plate test: Briefly, equal volumes (30 µL) of RBT antigen (Institute Pourquier, France) and of serum sample were placed on a white ceramic tile, mixed using sterile applicator stick, rocked gently for 4 min, and observed for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive while the absence of agglutination was recorded as negative (Bertu et al., 2010).

Wright standard tube agglutination test: The British method in which five test tubes per sample were required was used. For the 1st tube, 0.8 mL of phenol saline (0.5%, Institute Pourqier, France) was dispensed while 0.5 mL of the compound was applied to the 2nd, 3rd, 4th and 5th tubes. Subsequently, 0.2 mL of the test serum was added to the 1st tube and mixed properly. Serial dilution was then carried out by pipetting 0.5 mL of mixture in the 1st tube to 2nd, then to the 3rd, then to the 4th and then the 5th tubes. The final 0.5 mL from the 5th tube was discarded. Then, 0.5 ml of diluted (1:10 with phenol saline) antigen (Institute Pourqier, France) was added to all the tubes. The tubes were covered, shaken and incubated at 37°C for 20 h. The result was then read and agglutination titres were determined (Bertu et al., 2010).

2-Mercaptoethanol agglutination: This method is similar to STAT. For the 1st tube, 0.3 mL of phenol saline (0.5%, Institute Pourqier, France) was dispensed while 0.5 ml was applied to the 2nd, 3rd, 4th and 5th tubes. Similarly, 0.2 mL of the test serum and 0.5 mL of 2-ME solution (68 µL in 5 mL distilled water, Merck, Germany) was added to the 1st tube and mixed properly. The tube was covered, shaken and incubated at 37°C for 1 h. Then, Serial dilution was carried out by pipetting 0.5 ml of mixture in the 1st tube to 2nd, then to the 3rd, then to the 4th and then the 5th tubes. The final 0.5 ml from the 5th tube was discarded. A volume of 0.5 mL of diluted (1:10 with phenol saline) antigen (Institute Pourqier, France) was added to all the tubes. The tubes were covered, shaken and incubated at 37°C for 20 h. The result was then read and agglutination titres were determined (Bertu et al., 2010).

Data analysis: Statistical analysis was performed by using the software package SPSS version 16.0 for windows. Odds ratios (OR), confidence interval (CI), χ^2 and *P*-value were calculated separately for each variable. *P*-value of less than 0.05 was considered statistically significant.

Table 1. The Manual of serological results interpretation

RBT	STAT	2-ME	Result
Positive	≥4/40	Each value of titer	Positive
	≤3/40	≥1/20	
		≤4/10	Negative

RESULTS

The seropositivity of brucellosis was detected in 3% of sheep and 4.6% of goats. This prevalence in goats was found to be 1.8-fold higher than sheep (*P*=0.035, OR=1.8). The herd seroprevalence was 27.9% (n=39/140) in sheep and 13.6% (n=19/140) in goats. No evidence of correlation between gender and *Brucella* infection rate were found in animals (*P*>0.05, **Table 2**). Statistical significant differences was seen between age groups and infection rate in goats (*P*=0.033, OR=2.1); unlike to sheep (*P*=0.373). Also, the infection rate in nomads population of sheep was higher than fix location animals (*P*=0.003; OR=1.9); unlike to goats (*P*=0.195). In animals with history of abortion and vaccination against

Table 2. Seroprevalence of *brucellosis* in sheep and goats in different variables from Hamedan province, Iran

Variables		Sheep		Goats	
		Examined (%)	Seropositive (%)	Examined (%)	Seropositive (%)
Age groups	≤2	1034(40.5%)	35(3.4%)	310(44.3%)	20(6.5%)
	>2	1516(59.5%)	42(2.8%)	390(55.7%)	12(3.1%)
	SA	*P*=0.373		*P*=0.033, OR=2.1	
Gender	Male	429(16.8%)	16(3.7%)	90(12.9%)	6(6.7%)
	Female	2121(83.2%)	61(2.9%)	610(87.1%)	26(4.3%)
	SA	*P*=0.346		*P*=0.307	
Abortion hisrory	Yes	299(14.1%)	27(9%)	73(12%)	9(12.3%)
	No	1822(85.9)	41(2.3%)	537(88%)	22(4.1%)
	SA	*P*<0.0001, OR=4.3		*P*=0.002, OR=3.3	
Vaccination hisrory	Yes	1962(76.9)	36(1.8%)	516(73.7%)	8(1.6%)
	No	588(23.1)	41(7%)	184(26.3%)	24(13%)
	SA	*P*<0.0001, OR=4		*P*<0.0001, OR=9.5	
Type of herd	Fix location	1800(70.6)	43(2.4%)	450(64.3%)	24(5.3%)
	Nomads	750(29.4)	34(4.5%)	250(35.7%)	8(3.2%)
	SA	*P*=0.003, OR=1.9		*P*=0.195	
Herd		140(100%)	39(27.9%)	140(100%)	19(13.6%)
Total		2550(100%)	77(3%) CI95%=3%±0.6	700(100%)	32(4.6%) CI95%=4.6%±1.5

SA=Statistical analysis

brucellosis, seroprevalence rate was significantly lower than other (*P*<0.05, Table 2).

DISCUSSION

There are only a few reports of *brucellosis* in livestock of Iran. In Sarab (Northwestern of Iran), the seroprevalence rate of brucellosis in cattle, sheep and goats were reported 3.66%, 4.18% and 5%, respectively (Akbarmehr and Ghiyamirad, 2011). Ebrahimi et al. (2014), seroprevalence rate was reported 13.9% and 1.6% of goats in Shahrekord (Southwestern Iran) using Rose Bengal plate test and tube agglutination test, respectively. In Gharib-Mombeini et al. (2014) study from Khuzestan province (Southwestern Iran), this rate was 0.72% in cattle and 3.01% in sheep. In a similar investigations, the rate in sheep and goats was reported 0% and 1.7% in Pakistan, 0.7% and 0.1% in Spain, 1.8% and 12.2% in Bulgaria, 8.7% and 9.4% in Ethiopia, 8.9% and 8.8% in Portugal, 14.5% and 16.1% in Nigeria, 17.7% and 7.3% in India, 21.1% and 24.6% in Jordan, respectively (Reviriego et al., 2000; Bertu et al., 2010; Likov et al., 2010; Mustafa et al., 2011; Hawari, 2012; Negash et al., 2012; Coelho et al., 2013; Suryawanshi et al., 2014).

In Nigatu et al. (2014) study from Ethiopia, 3.1% of goats and 3.6% of sheep were seropositive using Complement Fixation Test (CFT). In Bangladesh, this rate was reported 2.3% and 3.2% in sheep and goats, respectively using Enzyme Linked Immunosurbant Assay (ELISA) (Rahman et al., 2011).

This study is the first report of brucellosis in sheep and goats in Hamedan province. In the present survey, the seroprevalence rate of brucellosis was detected in 3% of sheep and 4.6% of goats; this rate in goats was 1.8-fold higher than sheep. In Nigatu et al. (2014) study from Ethiopia, this rate was 14.4% in goats and 6.7% in sheep (*P*=0.001). Our results are consistent with investigations in Portugal, Bulgaria, Ethiopia and Pakistan (Likov et al., 2010; Coelho et al., 2013; Teklue et al., 2013; Nigatu et al., 2014; Shahzad et al., 2015). Also, in studies from India, Spain and Iraq, this rate in sheep was higher than goats, in opposition to our finding (Karim et al., 1979; Reviriego et al., 2000; Suryawanshi et al., 2014). Hawari (2012), Negash et al. (2012), and Coelho et al. (2013) reported equal levels of brucellosis infection rate in sheep and goats herds. Difference in management of farms, study design, diagnostic methods and sample size are main cause of varied results.

Abortion is the predominant symptom of brucellosis in naturally infected in sheep and goats. The animals usually abort only once, but reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies. Some infected animals carry the pregnancy to term, and shed the organism (Gul and Khan, 2007; Samadi et al., 2010; Lone et al., 2013). It can be concluded that abortion of infected animals is important

for public health (Benkirane et al., 2015). In the present study, 9% of sheep and 12.3% of goats with abortion history were seropositive (Table 2); significant differences was seen (P<0.05). Our result taken together with previous investigations supports the notion that the seropositivity rate is correlated with abortion (Rahman et al., 2011; Benkirane et al., 2015; Tegegn et al., 2016).

Age is probably the most important risk factor in brucellosis because the risk of infection is more closely related to age than other factors. Thus age should always be taken into consideration while describing the disease (Suryawanshi et al., 2014; Yilma et al., 2016). In our work, there was a significant difference in seroprevalence rate between the different age groups of goats (**Table 2**); which was opposed to studies in different regions of Ethiopia (Ashagrie et al., 2011; Teklue et al., 2013). In Negash et al. (2012) research, a slightly higher prevalence was noted in younger animals. The animals at the age of 1-2 yr-old were more seropositive (10.19%) than those of >4 yr-old (7.14%). In Nigatu et al. (2014) study, seroprevalence rate was detected 2.6% in young animals and 2.8% in adult animals (P=0.830). In Tegegn et al. (2016) study from Ethiopia, this rate was detected 2.8% in <2 yr-old, 16.4% in 2-5 yr-old and 20% in >5 yr-old; the statistical significant was seen (P=0.001). However, the variation was statistically non-significant; and could be due to the low number of sampled animals in their study.

In the current survey, the brucellosis in male animals (3.7% in sheep and 6.7% in goats) was higher than females (2.9% in sheep and 4.3% in goats); no statistically significant difference was found (P>0.05; Table 2). In similar research from Ethiopia, seropositive rate in females (13.8%) was 2.1-fold higher than male animals (6.5%, P=0.003) (Tegegn et al., 2016). In contrast to our results, Teshale et al. (2006), Akbarmehr and Ghiyamirad (2011), Bekele et al. (2011), Rahman et al. (2011), Negash et al. (2012), Mohammed et al. (2013), and Shahzad et al. (2015) documented a higher prevalence in both female sheep and goats than males. The higher rate in females might be due to high erythritol content of placenta that facilitates the establishment and multiplication of *brucella* organisms in gravid uterus (Mohammed et al., 2013; Suryawanshi et al., 2014).

Vaccination of animal against brucellosis has been used successfully in most countries. It is one of the most effective measures to reduce the prevalence of disease and has largely contributed to the success of many control programs (Hasannia et al., 2015). In this study, the prevalence of the infection in vaccinated animals was significantly lower than in non-vaccinated (P<0.05; **Table 2**).

CONCLUSION

The results of this research can provide baseline information for the future studies. It should be noted that, evaluation of brucellosis in other hosts such as wild animals is necessary for design of control strategies. The rate of small ruminants brucellosis in Hamedan is lower than other regions; but the flock level prevalence is higher than individual animal level and this characterizes the nature and importance of the disease in the large flock size. Therefore, it warrants a complete overhaul of management in livestock farms. A comprehensive control program includes vaccination, screening, and culling of brucellosis-positive animals is recommended. Further studies focused on the isolation and molecular characterization of the circulating *Brucella* species is imperative.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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