A cross-sectional study of the seroprevalence and flock-level factors associated with ovine and caprine brucellosis in southeastern Iran

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Summary

This cross-sectional study was conducted to estimate seroprevalence and to identify flock-level factors associated with seropositivity to brucellosis in small ruminants in Kerman province, southeastern Iran. In October-November 2011, serum samples were randomly collected from 1767 sheep and 1233 goats, older than 18 months, from 300 flocks. The sera were initially screened for the presence of anti-*Brucella* antibodies using the Rose-Bengal test; those found to be positive were then examined by Wright and 2-mercaptoethanol *Brucella* agglutination tests. A questionnaire was used to collect data on flock-level factors likely associated with the within flock seroprevalence of brucellosis. The associations were statistically evaluated for significance in multivariable logistic models. Sixty three flocks (21.00%; 95% CI: 16.80-26.60) had at least one seropositive animal. The mean within-flock seroprevalence was 3.10% (95% CI: 2.60-3.90). The presence of newly purchased animals (OR=3.42; 95% CI: 1.35-8.65) was significantly associated with seropositivity. Our findings highlight the role of animal movement among flocks in the epidemiology of brucellosis in this region. Thus, a control program for brucellosis in the region is suggested to impose appropriate restrictions on animal trade and improve knowledge of livestock owners about quarantine principles for newly purchased animals.

Key words: Brucellosis seroprevalence, Flock-level risk factors, Small ruminant, Iran

Introduction

Brucellosis, a bacterial disease caused by *Brucella* spp., is a public health concern and an economically important disease with a worldwide distribution (Refai, 2002; Coelho *et al*., 2007). Consumption of infected unpasteurized dairy products as well as contact with tissues and secretions of infected animals can lead to human infection. In this respect, sheep and goats have a more important role in the zoonosis of brucellosis than cattle and camels (Corbel, 1997; Pepin *et al*., 1997; Refai, 2002; OIE, 2012). The economic losses caused by ovine and caprine brucellosis are mainly attributed to abortions and to a lesser extent, to orchitis and epididymitis (Hirsh and Zee, 1999).

Despite its eradication in some countries, brucellosis is still present in the Middle East, Africa, Central Asia and Latin America (Refai, 2002; Coelho *et al*., 2007). In Iran, which has one of the largest populations of sheep and goats in the Middle East, brucellosis is under a national control program through vaccination (Iran Veterinary Organization, 2011). Regardless of the economic losses, the significance of animal brucellosis is in its human impact; 34 new cases of human brucellosis occur annually in Iran per 100,000 inhabitants (Zeinali, 2007).

Valid estimations of animal-level seroprevalence and identification of flock-level managerial factors associated with the risk of seropositivity may improve control efforts by highlighting weak points and accelerating future eradication efforts. However, to the best of our knowledge, there has been no study aiming at estimating seroprevalence and identifying factors associated with the risk of seropositivity to *Brucella* spp. of Iranian small ruminants. Therefore, this cross-sectional study was conducted to investigate the seroprevalence of ovine and caprine brucellosis and to identify flock-level factors associated with seropositivity of brucellosis in Kerman province, Iran.

Materials and Methods

Study area

Kerman province, with an area of 181,714 km^2 is located in the southeast of Iran. Animal husbandry and agriculture are among the most economically and socially important industries in the region. More than five million sheep and goats are reared in this area.

Study design and sample size

We conducted a cross-sectional study with a two-stage random sampling design. The required minimum within-flock sample size was calculated based on the formula for simple random sampling multiplied by the design effect (Dohoo *et al*., 2010). The assumptions for this calculation were:

Estimated prevalence of anti-*Brucella* antibodies in sheep and goat population in the area (p) = 0.05
Maximum acceptable deviation (precision of the estimate) (d) = 0.2 p = 0.01
Acceptable confidence interval for p = 95%
Intra herd correlation coefficient (rho) = 0.07
Number of samples in each herd (m) = 10
Design effect of the sampling (DE) = 1 + rho * (m-1) = 1.65

According to the mentioned assumptions, 3000 serum samples were to be taken from 300 different epidemiologic units.

Sampling procedure

Three thousand blood samples were taken from sheep and goat flocks throughout all counties, each county was considered as a stratum. To exclude healthy animals with interfering residual antibodies due to vaccination, in addition to using appropriate cut off values for serological tests, only animals older than 18 months of age were selected (Corbel, 2006). The number of sampled animals from each county was adjusted to the population size of each region. Each county was then divided into sample districts which corresponded to the epidemiological units (clusters) recorded in GIS of Iranian veterinary organization; each epidemiological unit was an industrialized farm or a village. The number of districts to be sampled in each county was then calculated and ten animals were sampled from each flock in the randomly selected districts.

Five ml of blood were collected from the jugular vein of each animal. After centrifuging the blood samples, the collected sera were transferred to the provincial veterinary laboratory and stored at -20 degrees centigrade until testing. Hemolyzed samples were replaced by other random samples from the same herds.

Collection of epidemiological information

To determine the potential factors associated with the flock-level risk of *Brucella* seropositivity in sheep and goats, a questionnaire was designed. The data were collected on the following factors from the herd owner at the time of sampling: herd size, presence of newly purchased animals, presence of non-indigenous animals, presence of dogs in the herd, the procedure of elimination of aborted fetus and other related materials, herd owner's knowledge of brucellosis, herd owner's education level, occurrence of *Brucella* infection in the herd owner's family members. Sheep and goat population in each epidemiological unit was also considered as a potential risk factor and included in the study.

Laboratory examination procedure

The sera were first screened for the presence of anti-*Brucella* antibodies using the Rose-Bengal test. Positive samples were then examined by 2-mercaptoethanol (2-ME) and Wright tests (Alton *et al*., 1975). Results were serially interpreted according to the guidelines of the Iranian Veterinary Organization for control and eradication of brucellosis. Animals with a titer of ≥40 (only 4+ readings) in the Wright test were considered positive. Animals with a titer less than 40 (4+ readings) in the Wright test and a titer of ≥20 in the 2-ME *Brucella* agglutination test were also considered positive (Iran Veterinary Organization, 2011).

Statistical analysis

Estimates were weighted according to the sampling fraction. To this end, we set the software based on sampling fraction with the *svyset* command and carried out all the analyses. Descriptive statistics and 95% confidence intervals were used to calculate the prevalence of the disease. A multivariable logistic model was built (by svy: logit command) to evaluate the association between the potential herd-level risk indicators (Table 1) and the herd infection status (Dohoo *et al*., 2010).

Variable selection

A four-stage model building approach was used to assess herd level risk factors of brucellosis in sheep and goat herds. In the first stage, a correlation analysis of the independent variables was conducted to identify the pairs of variables that essentially contained the same information and had to be removed due to collinearity. To this end, cross-tabulation with a two sided Chi-square test was applied. In the second stage, univariable analysis was carried out to identify variables that were unconditionally associated with brucellosis. During this screening phase, a significance level of 0.20 was set. In the third stage, all variables with $P<0.20$ were simultaneously plugged in a multiple logistic model which was subsequently reduced by a backward elimination strategy. Wald's tests were used to choose the final predictors of the model. The procedure was repeated until all remaining variables were significant at a 0.05 level. In the final stage, we evaluated two-way interactions between important predictors by constructing effect modifier terms for the significant main effect variables in the final model which were retained if significant (Muma *et al*., 2007; Dohoo *et al*., 2010).

Table 1: Epidemiological data collected from 300 sheep and goat flocks in Kerman province

Variable	Number of brucellosis-positive herds	Number of brucellosis-negative herds
Herd size		
≤200	30	113
>200	33	124
Population of sheep and goats in epidemiological unit		
≤1000	29	112
>1000	34	125
Presence of newly purchased animal		
Yes	9	11
No	54	226
Presence of dogs		
Yes	7	25
No	56	212
Disposal of aborted material by burial or incineration		
Yes	27	111
No	36	126
Herd owner's knowledge of brucellosis		
Yes	36	135
No	27	102
Herd owner's education		
Academic	3	11
High school diploma	10	41
Below high school diploma	50	185
Occurrence of brucellosis infection in herd owner's family		
Yes	11	37
No	52	200

Table 2: Flock-level risk factor associated with ovine and caprine brucellosis in Kerman province, based on final multivariable logistic model

Independent variable	B	Odds ratio	SE*	Z	Wald	95% CI**	P-value
Presence of newly purchased animals	1.23	3.42	1.62	2.60	6.76	1.35-8.65	0.009

* Standard error for odds ratio, ** 95% Confidence interval

Statistical software

Data management and analyses were performed using Stata Statistical software (StataCorp 2007, Stata Statistical Software: Release 10.1 College Station, TX: *StataCorp LP*.).

Results

From October to November 2011, 3000 small ruminants, 1767 sheep and 1233 goats, were examined in 300 epidemiological units in 10 counties of Kerman province. Sixty three epidemiological units out of 300 units under study, 21.00% (95% CI: 16.80-26.60), had at least one *Brucella* infected animal. It was found that 3.10% (95% CI: 2.60-3.90), 93 out of 3000, of the studied animals were infected with *Brucella*. The prevalence of *Brucella* infection in sheep and goats was 2.70% (49 out of 1767; 95% CI: 2.00-3.50) and 3.50% (44 out of 1233; 95% CI: 2.50-4.60), respectively. The spatial pattern of *Brucella* infection significantly varied among the epidemiological units as well as the studied counties (P<0.0001).

The results of multiple regression analyses revealed that the presence of newly purchased animals and non-indigenous breeds in the flock was significantly associated with the risk of brucellosis (Table 2).

Discussion

In the current study, we tried to provide more data on the prevalence of brucellosis in Iranian sheep and goats and to identify the potential risk factors of small ruminant brucellosis in Iran. We found that 3.10% of all the tested sheep and goats were *Brucella*-seropositive. Compared to the findings of earlier studies in Iran, it can be noted that the prevalence of brucellosis in sheep and goats has declined in recent years. This may be due to vaccination, implementation of a test and slaughter program, and the movement toward industrial livestock production (Zowghi and Ebadi, 1985). Previous epidemiological studies in other parts of the world have shown that the seroprevalence of brucellosis in small ruminants ranges from 0.5% to 5.8% (Mainar-Jaime and Vazquez-Boland, 1999; Kabagambe *et al*., 2001; Jackson

et al., 2007; Lilenbaum, 2007). However, a higher prevalence of ovine and caprine brucellosis (24-60%) has been reported in some countries (Al-Majali, 2005; Ahmed *et al*., 2010; Al-Mariri *et al*., 2011).

In our study, introduction of new animals to the herds was found to be associated with a higher risk of *Brucella* seropositivity, which was in accordance with the findings in Mexico (Mikolon *et al*., 1998). The majority of these newly purchased animals belonged to non-indigenous breeds with an unknown history of brucellosis imported from neighboring countries, mostly Pakistan. As indicated by several researchers (Kabagambe *et al*., 2001; Refai, 2002; Coelho *et al*., 2007), introduction of imported animals to the herds increases the risk of *Brucella* seropositivity. Hence, they can be considered an important source of *Brucella* infection for domestic herds in Iran. Based on brucellosis control programs, suspicious animals should not enter the herd, and unauthorized entrance of animals from an infected herd to other herds is forbidden (Corbel, 2006). Despite its low individual prevalence, the high flock-level prevalence of brucellosis could be due to animal movement as well as inappropriate quarantine measures. Studies suggest that the introduction of infected animals can lead to an increase in the individual level prevalence due to the fact that the longer they are in contact with rest of the flock, the higher the risk of spread would be (Corbel, 2006; Rahman *et al*., 2013).

The major concern in this study was the possible confusion between post-vaccinal and infection antibodies. In order to minimize this confusion, we only chose animals ≥18 months to reduce FD-Rev1 vaccine antibodies since these antibodies do not remain a year after injection (Corbel, 2006). Moreover, we delayed our sampling at least three months after Rd-Rev1 vaccination in this region. Furthermore, the positive samples were detected based on instructions provided by the Iranian Veterinary Organization to use tests serially. Another limitation of this study was its design; being a cross-sectional study, we could not confirm causality between brucellosis and risk factors (Dohoo *et al*., 2010); thus, the study was confined to flock-level risk factors.

Although brucellosis had a low prevalence at the animal level, it was present in approximately a quarter of the herds studied, which is sufficient to be considered as a public health concern. Furthermore, the identified risk factor highlights the role of animal movement in the epidemiology of brucellosis. Thus, a regional control program for brucellosis must impose appropriate and strict measures on animal transportation, particularly in the eastern borders of Iran and improve knowledge of livestock owners regarding quarantine principles for newly purchased animals.

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Conflict of interest

The authors declare no conflict of interest.

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