

Risk facts of small ruminant brucellosis: a cross-sectional study in Southeast Iran 2012

^{1,2}Hamid Sharifi, ³Khobayar Mashayekhi, ⁴Mohaddeseh Manzari Tavakoli

¹ Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran; ² Research Center for Modeling in Health, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran; ³ South of Kerman Province Veterinary Office, Iranian Veterinary Organization, Iran; ⁴ Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract. Objective: Brucellosis is an important zoonosis around the world being transmitted from animals to humans. To control the infection, understanding the risk factors is very important. This cross-sectional study was carried out to detect the risk factors of brucellosis in small ruminants in the southeast of Iran. Materials and Methods: Between March and April 2012, blood samples were randomly collected from 3000 sheep and goats from 300 flocks across all counties in the South part of Kerman province. The sera of 2952 animals that did not have hemolysis were first screened using Rose-BengalTM test for the presence of anti-*Brucella* antibodies and the positive samples were then tested by Wright and 2-ME tests. All tests were produced by Razi Vaccine and Serum Research center in Iran. A carefully structured questionnaire was used to collect epidemiological data from each herd. After adjustment for the sampling fraction, a multivariable multilevel logistic model was used to detect the potential risk factors of the infection. Result: The final model identified three predictor variables as the risk factors in herd and animal level: presence of purchased animals (OR = 8.39; 95% CI: 1.10-64.90), *Brucella* infection in a family member of farmer (OR = 0.26; 95% CI: 0.69-0.98 for infected family), species (OR = 2.12; 95% CI: 1.10-4.11 for sheep). Conclusion: Our findings suggest that the movement of animals between herds, insufficient knowledge, and negative attitude of farmers towards infection can increase the risk of infection.

Key Words: risk factors, brucellosis, cross-sectional, Iran.

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Corresponding Authors: H. Sharifi, email: hamidsharifi@uk.ac.ir

Introduction

Brucellosis is a zoonosis disease with a worldwide distribution that is important in public health and economically (Coelho et al 2007). The contact with infected animals and the consumption of unpasteurized infected dairy products may transmit the infection to humans. (Pepin et al 1997; OIE 2012; Corbell 1997). Ovine and caprine brucellosis due to *B. melitensis* is more important than other species in human. Abortion and retained placenta are the most important economic losses due to ovine and caprine brucellosis. The infection in animals is very important in some area like Iran that animal husbandry is an important component of people life (Zeinalian Dastjerdi 2012; Karaca 2007).

Brucellosis is endemic in the Middle East, Africa, Central Asia and Latin America (Coelho et al 2007; Refai 2002). Brucellosis in Iran is an endemic infection from many years ago and is under a national control program via vaccination in sheep and goats and vaccination, test and slaughter in cattle population (Iran Veterinary Organization 2011).

To control and eradicate the disease, understanding the risk factors is very important. Different factors, such as herd management, animal movement, ecological conditions, socio-economic factors are important in the prevalence of brucellosis in sheep and goats in some countries (Corbell 1997; Dohoo et

al 2010; Reviriego et al 2000). To the best of our knowledge, there are no relevant studies aiming to detect the potential risk factors, herd-level and animal-level, of the infection in Iranian small ruminants. Knowing these risk factors are essential for the development of cost-effective and efficient brucellosis control program. Therefore, we designed this cross-sectional study to investigate the associated risk factors in the south part of Kerman province of Iran.

Materials and Methods

Study Area

The study was conducted in the South part of Kerman province, located in the Southeast of Iran. The main activity of people in this area is agriculture followed by livestock production. More than two million sheep and goats are reared in this area with semi-intensive type system and poor technology, infrastructure and equipment.

Study Design and Sampling Procedure

This cross-sectional study, with a two-stage random sampling design, was conducted between March and April 2012. The number of sampled animals from each county was adjusted for the population size of different regions. Each county was then

divided into sample districts which corresponded to the epidemiological units (clusters) recorded in GIS of the Iran governmental veterinary organization. We calculated the sample size based on the formula for simple random sampling and then adjusted for cluster sampling and multiplied by the design effect (Dohoo et al 2010). Our assumptions for sampling were: Estimated prevalence of anti-*Brucella* antibodies in the area (p)=0.05; the maximum acceptable deviation (precision of the estimate) (d)=0.2 p =0.01; the acceptable confidence interval for p =95%; the intra herd correlation coefficient (ρ)=0.07; the number of samples in each herd (m)=10; The design effect of the sampling (DE)= $1 + \rho * (m - 1) = 1.65$. The number of epidemiologic unit to be sampled in each county was calculated and 10 animals were sampled from randomly selected districts. Totally, 3000 blood samples were taken from 300 epidemiologic units were selected randomly. In this study, only animals older than 18 months were recruited and sampled to exclude healthy animals with interfering residual antibodies due to the vaccination. We designed a structured questionnaire to determine the potential risk factors of *Brucella* seropositivity. Five milliliters of blood was collected from the jugular vein of each sampled animal. After centrifuging the blood samples, the collected serum were transferred to the provincial veterinary laboratory and stored at -20°C until testing. Samples with hemolysis were removed.

Independent variable

Animal-level studied variables were: age (18 months to 36 month, more than 36 months), species, sex and race (indigenous and exotic).

Herd-level studied variables were: herd size, population of sheep and goat in the epidemiologic unit, presence of purchased animal, presence of dug in the herd, farmer education, disposal aborted material by burial or incineration technique, occurrence of brucellosis infection in a family member of the farmer and keep cattle in addition to small animals

Laboratory Examination Procedure

Rose-BengalTM test was used to screen samples and positive samples were examined by Wright and 2-ME tests. The results were serially interpreted according to the guidelines of Iran Veterinary Organization for control and eradication of brucellosis (Iran Veterinary Organization 2011). All tests were produced by Razi Vaccine and Serum Research center in Iran.

Statistical Analysis

A multivariable multilevel logistic regression model was used (by svy: logit command) to evaluate the association between the potential animal- and herd-level risk indicators. Before analysis we weighted the estimates according to the sampling fraction. For this, we set Stata software based on sampling fraction with svyset command and carried out all the analyses (Dohoo et al 2010).

Variable selection

For modeling the risk factors of brucellosis in herd- and animal-level, we firstly conducted correlation analysis of independent variables to identify the pairs of variables that contained the same information to control collinearity. For this, two sided

Chi-square test was used. Then, to identify variables which were unconditionally associated with *Brucella* infection we conduct a univariable analysis and during this screening phase, a significance level of 0.20 was set. After that, all variables with $p < 0.20$ were simultaneously put in a multiple logistic model which was subsequently reduced by backward elimination strategy. Wald's tests was used to choose the final predictors of the model. The procedure was repeated until all of the remaining variables were significant at a significance level of 0.05 (Dohoo et al 2010; Muma et al 2007).

Statistical Software

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

Results

In total, 2952 samples without hemolysis were examined. The prevalence of the infection at animal level was 6.4% (95% CI: 4.1-7.8) and 26.1% (95% CI: 21.1-31.5) of herds had at least one *Brucella* infected animal. The spatial pattern of infection varied among the different counties ($P < 0.001$). Table 1 shows the odds ratio (OR), 95% confidence interval (CI) and p value of significant risk factors of infection in the study. Based on the results of this study, the risk of infection was higher in goats comparing with sheep at animal level and at herd-level presence of purchased animals increased the risk of being infected, however the history of infection in a family member of the owner of herds decreased this risk.

Table 1: Risk factors associated with Brucellosis in small ruminants in the Southeast of Iran

Variable	Odds Ratio	95% CI*	p
Animal level			
Species			
Goat	1	-	-
Sheep	2.12	1.10-4.11	0.026
Herd level			
Presence of purchased animal			
No	1	-	-
Yes	8.39	1.10- 64.90	0.03
Infection in a family member			
No	1	-	-
Yes	0.26	0.69-0.98	0.007

Discussion

In the current study, we found a considerable prevalence of infection in animal and especially in herd level in small ruminants in the Southeast of Iran. It can be noted that, compared to the findings of the earlier studies in Iran, the prevalence of brucellosis in sheep and goats has declined in recent years. This can be due to vaccination, implementation of a test and slaughter program, and movement toward industrial livestock production (Zowghi & 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% (Jackon *et al* 2007; Kabagambe *et al* 2001; Mainar-Jamie *et al* 1999). However, a higher prevalence of ovine and caprine brucellosis (24.0-60.0%) has been reported in some countries (Ahmed 2010; Al-Majali *et al* 2007; Al-Mariri *et al* 2011). There are few modern animal farms in this area and raising of sheep and especially goats is in rural area with traditional approach so that this can increase the risk of infection especially in goats.

In the current study, the risk of infection in sheep was higher than that of goats. In a study in Spain the prevalence of infection was 0.7% in sheep and 0.1% in goats (Reviriego *et al* 2000). The prevalence of infection in Egypt was 21.2% in sheep and 14.5% in goats (Kaoud *et al* 2010). The prevalence of infection in goats was higher than that of sheep in Portugal and Bangladesh (Coelho *et al* 2013; Rahman *et al* 2011). In this area, sheep are kept in the corral and goat in the desert and this leads to more contact between sheep and increased the risk of infection.

In our study, the presence of purchased animals in the herd was a risk factor of *Brucella* seropositivity which is similar to findings in Mexico (Mikolon *et al* 1998) and Malaysia (Bamaiyi *et al* 2014). However, in Jordan researches failed to find a similar association in Jordan (Al-Talafhah *et al* 2003; Al-Majali *et al* 2005). This correlation can be due to the importance of small ruminants with an unknown history of brucellosis from other herds especially in neighboring countries, mostly Pakistan. As it has been indicated by several researchers (Coelho *et al* 2007; Refai 2002; Kabagambe *et al* 2001) the introduction of imported animals to the herds increases the risk of *Brucella* seropositivity; consequently, they can be considered an important source of *Brucella* infection for domestic herds in Iran. In this context, the result of a study found a close genetic relationship between Iranian and Pakistani isolates of Foot and Mouth disease virus, which also accentuates the role of animal transportation in the eastern borders of Iran in transmission of infectious diseases to domestic livestock (Rashtibaf *et al* 2012). Another reason of this finding is sharing of male animal in breeding season in this area. The history of infection in a family member of the owner was a protective factor of brucellosis infection in this area. One of the most important reasons of this finding may be that these growers participate better on control strategies like vaccination and culling of infected animals.

Conclusion

The prevalence of brucellosis is considerable in this area especially at herd level which should be considered a public health concern. Necessary measures should therefore be taken to prevent the transmission of brucellosis to the human population. Furthermore, the identified risk factors indicate that animals that originate from outside the herds are an important source of *Brucella* infection and highlight the role of animal movement in the epidemiology of brucellosis. Thus, a control program for brucellosis in the region must impose appropriate strict measures on animal transportation, particularly in the eastern borders of Iran. This study revealed that the changing of farmer attitudes can help us control the infection.

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- of Kerman, 22 Bahman St., Pazhohesh Square, Kerman, IR Iran, AND Research Center for Modeling in Health, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran; email: hamidsharifi@uk.ac.ir
- Khobayar Mahayekhi: Veterinary Medicine Office, Veterinary Medicine Junction, Modarres Street, Jiroft, IR Iran, email: khobayarm@yahoo.com
- Mohaddeseh Manzari Tavakoli: Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, 22 Bahman St., Pazhohesh Square, Kerman, IR Iran, email: m.tavakoli90@yahoo.com.sg

Authors

- Hamid Sharifi, Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahid Bahonar University

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