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Seroprevalence Survey of Q Fever among Sheep in Northwestern Iran

Saber Esmaeili,[1] Fahimeh Bagheri Amiri,[1,2] and Ehsan Mostafavi[1]

Abstract

Q fever is a disease caused by *Coxiella burnetii*, which infects both humans and certain animals, and is considered a public health problem. There is a lack of information on its status in most parts of Iran, including the northwestern area, which is one of the main centers of animal husbandry in Iran. This study was carried out to evaluate the seroprevalence of Q fever among sheep in the province of Ardabil in northwestern Iran. In all, 253 sheep samples were collected from different regions (north, central, and south) of Ardabil Province. Sera were tested by a CHEKIT Q fever enzyme-linked immunosorbent assay (ELISA) kit for detection of *C. burneti*i immunoglobulin G (IgG). A total of 33.6% of sheep sera and 87.50% of herds were positive for *C. burnetii* IgG. There was a significant difference between the regions studied ($p < 0.001$) and the highest and the lowest rate of seroprevalence were seen in the south (58.6%) and central (23.3%) regions, respectively. Most of the seropositive cases were observed in 3- to 4-year-old the sheep (46.1%). There was no relationship between gender and the seroprevalence rate. Although this study was the first survey of Q fever in northwestern Iran, the high seroprevalence rate indicates that further attention should be paid to this disease in this region of the country.

Key Words: *Coxiella burnetii*—ELISA—Ardabil—Animal—Iran.

Introduction

Q FEVER IS A WORLDWIDE ZOONOSIS caused by *Coxiella burnetii*, which is an obligate, intracellular, Gram-negative bacterium. (Angelakis and Raoult 2010). The reservoirs of this disease are wide-ranging and include mammals, birds, and arthropods (mainly ticks) (Van den Brom and Vellema 2009). Cattle, sheep, and goats are considered the most common sources of infection for humans (McQuiston and Childs 2002). Although Q fever infection in animals is mostly asymptomatic or subclinical, in some cases *C. burnetii* can cause abortion, stillbirth, infertility, metritis, and endometritis in domestic animals (Arricau-Bouvery and Rodolakis 2005). This pathogen can be shed via milk, urine, feces, vaginal mucus, and especially birth products (placenta and fetal fluids) of infected animals. Birth products of infected animals contain high concentrations of *C. burnetii* and are considered an important source of environmental contamination (Guatteo et al. 2011). Serologic prevalence surveys of Q fever among domestic animals can provide valuable information on the disease status for the health system so appropriate measures to control and prevent the disease can be taken (Arricau-Bouvery and Rodolakis 2005).

Q fever is considered to be an occupational disease, and livestock handlers, farmers, veterinarians, slaughterhouse workers, people in contact with raw milk and its products, and laboratory personnel are at higher risk of infection (Madariaga et al. 2003). In approximately 60% of humans infected with *C. burnetii*, infection is asymptomatic, and many cases of infection are not diagnosed because of the mild and nonspecific clinical symptoms (Raoult et al. 2005). The fatality rate of Q fever in its acute form is reported to be between 1% and 2% (Angelakis and Raoult 2010). Chronic Q fever generally presents as endocarditis or vascular infection (Parker et al. 2006). Risk factors of chronic Q fever include valvulopathy, immunosuppression, and pregnancy (Angelakis and Raoult 2011).

The first report of human Q fever in Iran was in 1952 (Courdurier et al. 1952), and subsequently there have been reports of human cases and serological prevalence of Q fever in the human population in some regions of Iran (Caughey and Harootunian 1976). From 1977 onward, no human cases of the disease were reported in Iran and the disease was more

[1]Department of Epidemiology, Pasteur Institute of Iran, Tehran, Iran.
[2]Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

or less forgotten until 2010 when prevalence of *C. burnetii* antibodies was reported in febrile patients in Kerman Province (southern Iran) (Khalili et al. 2010). The first study of Q fever among animals in Iran was carried out approximately 60 years ago, and serologic evidence of the disease was reported in domestic animals in the provinces of Kermanshah (western Iran) and Tehran (northern Iran) (Giroud and Yassemi 1952). Subsequently several studies conducted in various regions of Iran showed infection of wild and domestic animals (Bashiribod 1989).

Given that there is little epidemiological data on Q fever in most parts of Iran, including northwestern regions, this survey has been carried out in various geographical regions of Ardabil Province to survey the seroprevalence of Q fever among sheep in northwestern Iran, one of the main centers of animal husbandry in Iran.

Materials and Methods

This study was carried out in 2011–2012. Ardabil is a province located in northwestern Iran with an area of approximately 17,953 km^2 and a population of approximately 1.2 million. The province has a cold semiarid climate and the average annual rainfall is 380 mm. It borders the Republic of Azerbaijan (on its northern and northeastern borders) and the provinces of East Azerbaijan (on its western border), Zanjan (on its southern border), and Gilan (on its eastern border) (Fig. 1). On the basis of a report by the Iranian veterinary organization (2009, unpublished data), this province has more than 2,316,990 sheep, 262,190 goats, 109,610 buffalo, and 348,150 head of cattle.

In this study, the blood samples of sheep were collected from the northern (Parsabad and Bilasavar counties), central (Ardabil, Nir, and Meshkin-Shahr counties), and southern (Khalkhal and Kowsar counties) regions of Ardabil Province and from 32 herds using a cluster sampling method. Blood samples were taken from the jugular veins of sheep, and their history, including age, gender, and area of habitat, was recorded. Samples were immediately taken to the laboratory, and their serum was extracted and kept under $-20°C$. Collected sera were transferred to the Department of Epidemiology at the Institute Pasteur of Iran. Sera were analyzed by indirect enzyme-linked immunosrbent assay (ELISA) test using *C. burnetii* antibody (immunoglobulin G [IgG] phases I and II) diagnosis kit (CHEKIT Q fever ELISA kit, IDEXX, USA.). Sera were prepared at a dilution of 1:400. Sera were considered to be ELISA positive if they had an optical density (OD) value of 40% or more, suspect (borderline) if the OD value was between 30% and 40%, and negative if the OD value was $<30\%$ (Sakhaee and Khalili 2010). In this study, 256 serum samples were collected from Ardabil Province from 36 (14.1%) rams and 220 (83.9%) ewes. The average (standard error [SE]) age of the sampled sheep was 4.38 (2.64).

Data were analyzed with SPSS statistical software, v. 16 (SPSS Inc., Chicago, IL). The chi-squared test was used to compare the variables during analysis. p values less than 0.05 were considered statistically significant. ArcGIS version 9.3 software (ESRI) was used for mapping the results.

Results

In total, 33.6% and 3.45% of sheep's sera were positive and borderline for *C. burnetii* IgG, respectively. In addition, 87.50% of herds were seropositive (Fig. 2). There was a statistically significant difference between the regions (north, center, and south) studied ($p<0.001$), and the highest and the lowest rates of seroprevalence were shown in southern (58.6%) and central (23.3%) regions, respectively. Among

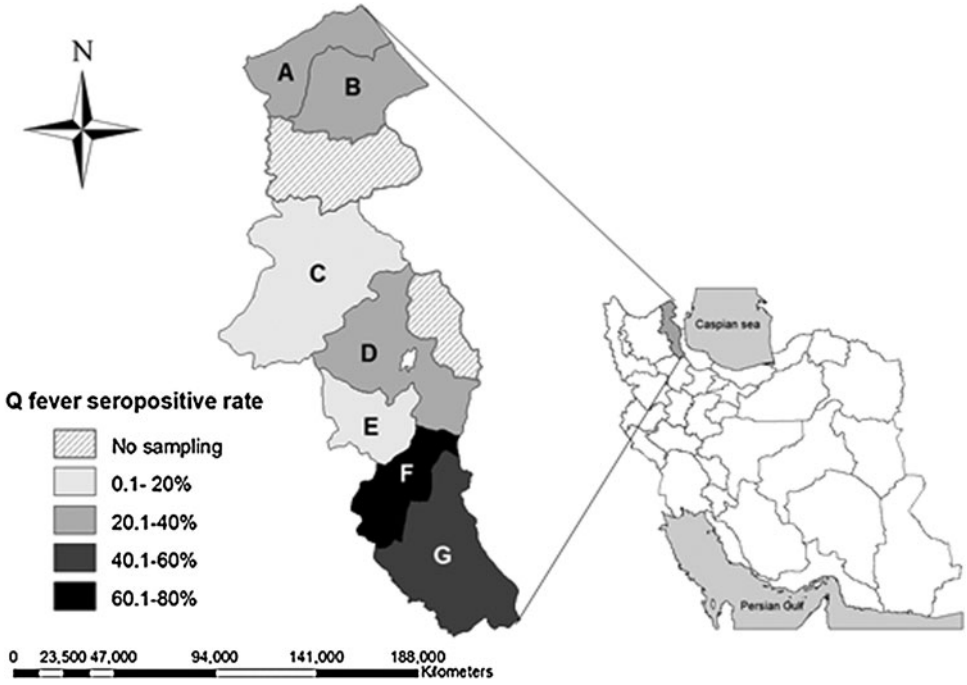

FIG. 1. Geographical distribution of Q fever seropositivity among sheep of Ardabil province. Sampling was conducted in Parsabad (**A**) and Bilasavar (**B**) in the northern region, Meshkin-Shahr (**C**), Ardabil (**D**), and Nir (**E**) in the central region, Kowsar (**F**) and Khalkhal (**G**) in the southern part of the province. Seroprevalence of Q fever is shown with different colors.

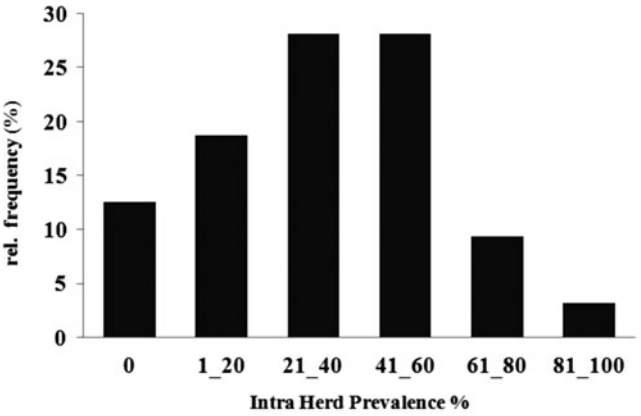

FIG. 2. A total of 256 sera from 32 herds of sheep were analyzed for antibodies to *C. burnetii* (CHEKIT-Q-Fever ELISA; IDEXX). For each herd, the relative intra-herd prevalence percentage of positive samples was calculated.

the counties, the highest seroprevalence rate was seen in Kowsar county (72%), and the lowest seroprevalence rate was seen in Nir county (13.3%) (Table 1).

In this study, animals were divided into three age groups (1–2, 3–4, and over 5 years old). A significant difference in the prevalence of Q fever IgG was found among these three groups ($p=0.01$), and the highest seropositive cases were observed in the age 3- to 4-year-old group (46.1%). Sheep in the 3- to 4-year-old group were 2.52 times (95% confidence interval [CI] 1.26, 5.01; $p=0.01$) and 1.95 times (95% CI 1.06, 3.60; $p=0.03$) more at risk of being seropositive compared to the 1- to 2- and over 5-year-old groups, respectively. Although in this study the seropositive rate in rams (33.5%) was higher than ewes (22.2%), this difference was not statistically significant ($p=0.25$).

Discussion

In this survey, 33.6% of 256 tested sheep of Ardabil Province in northwestern Iran had a history of *C. burnetii* infection, which was higher than most results of other studies in Iran. In a survey conducted on sheep in Mazandaran Province, in northern Iran, the seroprevalence rate of Q fever was 23.7% (Mostafavi et al. 2012). In other recent studies, the seropositive rate of Q fever in sheep in Kerman Province, southern Iran, was 29.42% (Sakhaee and Khalili 2010), and in southeastern Iran, the seropositivity among goats and cattle was 65.78% and 10.75%, respectively (Khalili and Sakhaee 2009). In addition 6.7%, 2%, and 1.1% of goats in Fars Province (southern Iran), Yazd Province (central Iran), and Khuzestan Province (southwestern Iran), respectively, had *C. burnetii* antibodies (Khalili et al. 2011). Using PCR, *C. burnetii* was also detected in the milk of 3.2% of livestock (bovine, ovine, caprine, and camel) in the Isfahan Province (central Iran) (Rahimi et al. 2011) and in 1.1%, 2%, and 6.7% of the milk of goats in the provinces of Khuzestan (southwestern Iran), Yazd (central Iran), and Fars (southern Iran), respectively (Rahimi 2010). The sheep seroprevalence rate in this study is also slightly higher than its average prevalence in other studies from around the world, which have reported it as approximately 30% (Guatteo et al. 2011). Furthermore, in our study, 87.50% of herds were seropositive, and seroprevalence in most (56.25%) herds was between 20% and 40%. The results presented here showed that, 3- to 4-year-old sheep had a history of a significantly higher rate of the disease than younger ones. Other previous studies have also shown that with an increase in age, the chance of having IgG against the bacteria increased (Kennerman et al. 2010, McCaughey et al. 2010), but there have also been studies that have shown no relationship between age and seroprevalence of Q fever (Mostafavi et al. 2012).

There was a significant difference between seropositive rates of different regions of Ardabil province, with the southern area having a higher seroprevalence rate than northern and central areas. Because there were no surveys of neighboring provinces, further studies of surrounding regions and provinces in northwestern Iran are required to explain the reasons for these differences. Studies conducted in a neighboring country, the Republic of Azerbaijan, are also limited. In a report that was published in 2009, there was evidence of human Q fever infection in this country; prior to this report, there was only an older study reporting Q fever in Azerbaijan (Sterkhova and Mirzoeva 1956).

Although there was no significant relationship between gender and seropositive rate, which is the same as the findings of other studies (Mostafavi et al. 2012), it should be mentioned that the low number of ram samples ($n=36$) in comparison with ewe samples ($n=220$) in our study made it difficult to reach a valid conclusion. However, higher exposure of humans with female animals than male ones, especially during milking and parturition, makes female animals more important in transmitting the disease to humans. *C. burnetii* has the ability to localize in the uterus and mammary glands of female animals, and these infected

Table 1. Seropositive Rate of Q Fever among Sheep in Different Geographical Regions in Ardabil Province

County name	*Number tested (% Positive)*	*Region*	*Number tested*	*Borderline (%)*	*Positive (%)*
Parsabad	35 (28.6)	North	66	1.5	25.8
Bilasavar	31 (22.6)				
Ardabil	54 (31.5)	Central	120	5	23.3
Nir	30 (13.3)				
Meshkin-Shahr	36 (19.4)				
Khalkhal	45 (51.1)	South	70	4.3	58.6
Kowsar	25 (72.0)				
		Total	**256**	**3.9**	**33.6**

animals can discharge this pathogen through milk, urine, feces, vaginal mucus, and birth products (placenta and fetal fluids) (Angelakis and Raoult 2010).

Commercially available Q fever ELISA tests, which simultaneously detect phase I and II antibodies in ruminants (such as the test used in this study), may have false-positive and false-negative results (Böttcher et al. 2011). Therefore, in future studies, it is recommended that ELISA tests, which detect each phase separately, should be used to improve the validity of results.

This study was the first survey of Q fever in northwestern Iran. Therefore, it is necessary to employ appropriate strategies for the diagnosis of Q fever patients by establishing local and national reference laboratories and increasing the knowledge of physicians of potential clinical signs of the disease and carrying out essential measures, such as the use of insecticides on domestic animals, to prevent the disease in this province. Furthermore, it is suggested that a seroprevalence study in humans be done to actually characterize the burden of disease. It is recommended that complementary studies be carried out to delineate the status of Q fever disease in this province on other livestock and ticks. It is also recommended that further studies be carried out in neighboring provinces and in the Republic of Azerbaijan to highlight the epidemiological feature of this disease in the northwest of Iran.

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Author Disclosure Statement

The authors declare that they have no competing financial interests.

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Address correspondence to:
Ehsan Mostafavi
Department of Epidemiology
Pasteur Institute of Iran
No. 69, Pasteur Avenue
1316943551, Tehran
Iran

E-mail: mostafavi@pasteur.ac.ir