Seroepidemiological study of Q fever in small ruminants from Southeast Iran

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Summary   The aim of the present study was to determine the prevalence of *Coxiella burnetii* antibodies in small ruminants in Southeast Iran. A total of 368 small ruminant blood samples (241 caprine blood samples and 127 ovine blood samples) were collected from January to May of 2011 in Southeast Iran. A commercial ELISA test kit was employed to identify specific antibodies against *C. burnetii* in the sheep and goats. Seropositivity in the examined counties ranged from 17.1% to 39.2%. Of the animals tested, 97 animals (26.4%), including 43 sheep (33.9%) and 54 goats (22.4%), had antibodies to *C. burnetii*. The results of the current study reveal the high prevalence of antibody positivity in small ruminants in Southeast Iran. Thus, sheep and goats are important reservoirs in this area. Additionally, we performed a logistic regression to the identify risk factors for positivity and concluded that age was an important risk factor ($P < 0.001$).

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Introduction

Query fever (Q fever) is a widely disseminated illness with a broad range of susceptible hosts and is a public health concern throughout the world [1]. *Coxiella burnetii* is the causative agent of Q fever and belongs to the order *Legionellales*, the class gamma *Proteobacteria*, the family *Coxiellaceae* and the genus *Coxiella*. Q fever is caused by a short (0.3—1.0 μm) obligate intracellular parasitic, pleomorphic, gram-negative bacterium [2].

C. burnetii infects a very wide range of animals, including domesticated animals, wild animals, pets, birds, arthropods and humans [1,3]. In some areas, cattle seem to be the main domestic reservoir, whereas in other areas, sheep or goats are the main reservoir. These reservoirs are the primary source of human infections, and clinical Q fever in man is caused by transmission from these animals [4—7]. Sheep are primarily asymptomatic carriers, but they can shed massive numbers of bacteria at parturition and intermittently in various secretions [8].

Vertical and sexual transmissions of *C. burnetii* have been reported [9]. In ruminants, Q fever has been particularly associated with reproductive disorders, such as late abortions, stillbirths, weak offspring, metritis and infertility [10—12]. Although abortions during disease epizootics have been reported in sheep and goats [13], this bacterium rarely causes abortions in sheep [14].

Serological surveys have been performed in many countries to evaluate the distribution of *C. burnetii* in domestic ruminants. Routine diagnoses of Q fever are established by serological tests that include immunofluorescence assays (IFAs), complement fixation and enzyme-linked immunosorbent assays (ELISAs) [15]. ELISA is preferable to IFA because it is more sensitive [16].

Q fever is endemic in Iran, and serological evidence indicates that Iranian people and animals are exposed to *C. burnetii* [14,17—19]; however, very limited data about the geographic distribution of the disease in Southeast Iran are available [17,18]. In the current study, we determined the seroprevalence of *C. burnetii* in the two main domestic ruminant species (sheep and goats) of Southeast Iran. Additionally, we investigated whether age, gender, species and climatic conditions are possible risk factors for the presence of *C. burnetii* in goats and sheep in this area.

Materials and methods

Samples

Herd information was retrieved from *the Iran Veterinary Organization*. We randomly selected 50 herds from five counties of Southeast Iran (Fig. 1). In a cross-sectional study, random cluster sampling was performed to acquire samples from five percent of the animals per herds, and a total of 368 small ruminant blood samples (241 goat samples and 127 sheep samples) were collected from January to May 2011. The blood samples were collected by jugular vein puncture with Vacutainer tubes without EDTA and were transported on ice to the microbiology laboratory of the *Razi Vaccine and Serum Research Institute-Kerman branch*. Upon arrival at the laboratory, the samples were centrifuged at 3000 rpm for 10 min at room temperature, and the sera were gathered. All serum samples were transported on ice and stored at −20 °C until they were tested
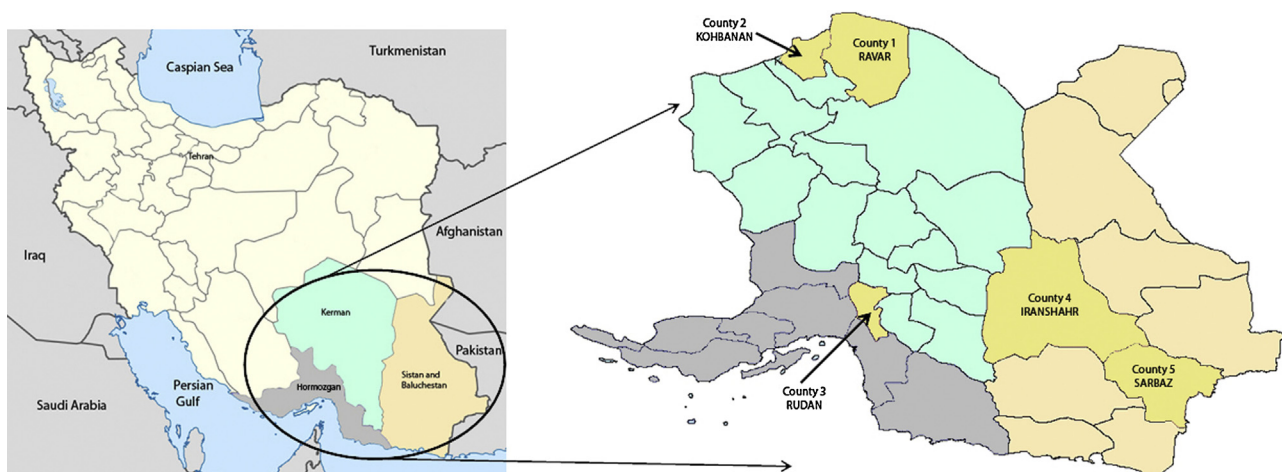
Figure 1 The counties in Southeast Iran that were sampled (the sampled counties are indicated in yellow).

for antibodies at a later time. Moreover, meteorological information was retrieved from the *Iran Meteorological Organization* for the logistic regression analysis.

ELISA

The commercial CHEKIT* Q fever (*C. burnetii*) antibody ELISA test kit (CHEKIT®; Idexx Switzerland, Switzerland) was used. The plates were read at 450 nm with an ELISA reader (Anthos 2020, Wals, Austria) according to the manufacturer's recommendation. To calculate the results, the optical densities (ODs) of samples were analyzed in relation to the negative and the positive controls with the following formula:

$$Value (\%) = \frac{OD_{sample} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100$$

The sera were considered to be ELISA-positive if they had a value of 40% or more, were suspected to be positive if the value was between 30% and 40% and were considered negative if the value was <30%.

Statistical analyses

To calculate the prevalence, we used descriptive statistics with 95% confidence intervals (CIs).

Moreover, a logistic regression model was created to determine the associations of age, species, animal gender and the climatic conditions of the counties with the prevalence of *C. burnetii* antibodies. For this purpose, chi-square tests were used to identify the variables that were associated with the prevalence of *C. burnetii* antibodies, and a significance level of 0.20 was considered. In the third stage, all variables with $P < 0.20$ were simultaneously included in a full model that was subsequently reduced via backwards elimination. At each step, the reduced model was compared to the full model via a likelihood ratio test. The procedure was repeated until all remaining variables were significant at the level of $P < 0.05$ [20]. The statistical analyses were performed using Stata software version 10.1.

Results

The mean annual rainfalls (averages of five years) in the different counties ranged from 18 mm in Rudan to 229 mm in Sarbaz. Other climatic data for the counties are shown in Table 1. The rates of seropositivity of the counties ranged from 17.1% to 39.2%. The distributions of samples and antibody prevalence are shown in Tables 2 and 3. Ninety-seven animals (26.4%; 95% CI: 21.9−31.2) had antibodies to *C. burnetii*, including 43 sheep (33.9%; 95% CI:

Table 1 The mean annual rainfall, minimum and maximum temperature and Altitude from sea.

	County	The mean annual rainfall (5 years average) (mm)	The minimum temperature (5 years average) (°C)	The maximum temperature (5 years average) (°C)	Altitude from sea (m)
1	Ravar	41	6.0	43.6	2296
2	Kohbanan	60	12.3	36.6	2184
3	Rudan	18	9.2	46.7	200
4	Iranshahr	155	10.9	43.4	369
5	Sarbaz	229	16.9	32.4	1078

Table 2 Prevalence of positive specific antibodies to *C. burnetii* in sheep and goat sera in Southeast Iran.

County	Sheep			Goat			Summation		
	Total	Positive	95% CI	Total	Positive	95% CI	Total	Positive	95% CI
Ravar	37	7 (18.9%)[a]	7.9−35.2	42	10 (23.8%)	12.1−39.5	79	17 (21.5%)	13.1−32.2
Kohbanan	0	0	0	94	16 (17.1%)	10.1−26.2	94	16 (17.1%)	10.1−26.2
Rudan	12	2 (16.7%)	2.1−48.4	39	18 (46.2%)	30.1−62.8	51	20 (39.2%)	25.9−53.9
Iranshahr	78	34 (43.6%)	32.4−55.3	52	5 (9.6%)	3.2−21.1	130	39 (30.0%)	22.3−38.7
Sarbaz	0	0	0	14	5 (35.7%)	12.8−64.9	14	5 (35.7%)	12.8−64.9
Total	127	43 (33.9%)	25.7−42.8	241	54 (22.4%)	17.3−28.2	368	97 (26.4%)	21.9−31.2

[a] The number in parentheses shows percentage of frequency.

Table 3 Sex distribution of seropositivity against *C. burnetii* in sheep and goats in Southeast Iran.

Species	Male			Female			Summation		
	Total	Positive	95% CI	Total	Positive	95% CI	Total	Positive	95% CI
Sheep	28	5 (17.9%)[a]	6.1—36.9	99	38 (38.4%)	28.8—48.78	127	43 (33.9%)	25.7—42.9
Goat	112	18 (16.1%)	9.8—24.2	129	36 (27.9%)	20.4—36.5	241	54 (22.4%)	17.3—28.2
Total	140	23 (16.4%)	10.7—23.6	228	74 (32.5%)	15.6—36.5	368	97 (26.4%)	21.9—31.2

[a] The number in parentheses shows percentage of frequency.

Table 4 Age distribution of seropositive samples in sheep and goats in Southeast Iran.

Age	Sheep			Goat			Summation		
	Total	Positive	95% CI	Total	Positive	95% CI	Total	Positive	95% CI
≤6 months	15	2 (13.3%)[a]	1.7—40.5	30	2 (6.7%)	0.8—22.1	45	4 (8.9%)	2.4—21.2
6—24 months	33	10 (30.3%)	15.6—48.7	98	17 (17.4%)	10.4—26.3	131	27 (20.6%)	14.1—28.6
≥24 months	79	31 (39.2%)	28.6—50.9	113	35 (31%)	22.6—40.4	192	66 (34.4%)	27.7—41.6
Total	127	43 (33.9%)	25.7—42.8	241	54 (22.4%)	17.3—28.2	368	97 (26.4%)	21.9—31.2

[a] The number in parentheses shows percentage of frequency.

25.7—42.8), 54 goats (22.4%; 95% CI: 17.3—28.2), 74 females (32.5%; 95% CI: 15.6—36.5) and 23 males (16.4%) (95% CI: 10.7—23.6).

The age distribution of the seropositive samples is shown in Table 4. The results of the logistic regression revealed that age was an important risk factor for the prevalence *C. burnetii* ($P < 0.001$). The findings also revealed that the odds of seropositivity increased with increasing age (Table 5).

Table 5 Multivariable logistic regression analysis for likelihood for odds of counties, species, age and sex in Southeast Iran.

	Odds ratio	95% CI	*P* value
County			
Iranshahr	1	—	—
Ravar	0.67	0.33—1.35	0.26
Kohbanan	0.51	0.23—1.13	0.10
Rudan	2.24	0.98—4.87	0.06
Sarbaz	1.86	0.52—6.74	0.34
Species			
Sheep	1	—	—
Goat	0.67	0.36—1.24	0.20
Age			
≤6 Months	1	—	—
6—24 months	4.47	1.34—13.5	0.01
≥24 Months	8.02	2.65—24.2	<0.001
Sex			
Male	1	—	—
Female	1.43	0.79—2.60	0.24

Discussion

The study results revealed that 26.4% (95% CI: 21.9—31.2) of the tested animals had antibodies to *C. burnetii*, and the seropositivities of the studied counties ranged from 17.1% to 39.2%. We found that sheep and goats are important reservoirs. Moreover, the logistic regression analysis revealed that only age was an important risk factor for the prevalence of *C. burnetii* in Southeast Iran.

In the present study, 43 sheep (33.9%; 95% CI: 25.7—42.8) and 54 goats (22.4%; 95% CI: 17.3—28.2) had antibodies to *C. burnetii* (Table 2), and there was no significant difference between the seropositivities of the sheep and goats. Two previous studies that were conducted in Southeast Iran by Khalili and Sakhaee demonstrated that 29.42% of sheep and 65.78% of goats have anti-*C. burnetii* antibodies [14,17]. In those studies, the numbers of animals were limited, and the sampling was performed in herds with histories of abortion that affected the results. In a study of four counties of Iran, the overall seroprevalences of *C. burnetii* in sheep and goats were 19.5% and 27.2%, respectively. The result regarding seropositivity among goats from this study [19] was higher than that of the present study, but this difference was not significant.

It can be suggested that the seroprevalence observed in current study exceeded the ranges of herd prevalence that have been described for other countries. Q fever has been reported in more than 50 countries in different parts of the world,

and the epidemiology of this disease is remarkably diversity across different geographical regions [21]. This disease has recently been reported in countries neighboring Iran, including Oman, Iraq, Afghanistan, the United Arab Emirates, Turkey and Saudi Arabia [22,23]. Previous studies of the seroprevalence of *C. burnetii* in sheep populations have reported estimates of 20% and 13.5% in two region of Turkey [5,24], 10% in the USA [25], 21% in Spain [26], 1.3% in Germany [27] and 18.9% In Cyprus [28]. Moreover, the seroprevalence of *C. burnetii* has been studied in goat across the world. Goat seroprevalences have been reported to be 8.8% in Albania [29], 6.5% in Northern Greece [30], 8.7% in Spain [26] and 13% in Italy [31]. The study by McQuiston and Childs also reported differences the seroprevalences among sheep and goats in the USA; in the USA goats have been shown to have the highest species-specific prevalence of antibodies to *C. burnetii* (41.6%) followed by sheep (16.5%) and cattle (3.4%) [25].

Due to the high concentrations of the bacteria in the placentas of infected animals [32] and the occurrence of outbreaks of the disease after lambing [33], female animals are at a higher disease risk than males. Regarding to the results presented in Table 3, the univariate analyses revealed a significant difference in the seropositivities of the male and female groups ($P < 0.05$). This difference is likely due to the greater susceptibility of pregnant ruminants and the shedding of bacteria into the environment during normal parturition and abortion [1,34]. However, the logistic regression analysis did not identify gender as an important risk factor for the prevalence of *C. burnetii* (Table 5), and this finding indicates that the effect of sex was confounded by age.

The logistic regression results showed that age was an important risk factor for the prevalence of *C. burnetii* ($P < 0.001$) because increasing age was associated with an increasing likelihood of seropositive results (Table 5) [35]. In present study, the small ruminants that were 24 months of age or older exhibited a greater rate of seropositivity for *C. burnetii* (34.4%) than did the two other age groups (8.9% for animals ≤6 months old and 20.6% for the 6−24-month-old animals; Table 4). Based on these findings, the odds of seropositivity increased with age (Table 5). This finding is consistent those of a previous report from the Netherlands [36]. These results are suggestive of the occurrence of horizontal transmission among animals and the maintenance of infections within adult populations [35].

We found no differences in *C. burnetii* seropositivity due to the mean annual rainfalls of the studied counties. Unusually dry periods have been proposed to encourage the formation and propagation of infectious dusts and aerosols [37]. Dry and windy conditions have been suggested to play roles in the aerosol transmission of *C. burnetii* in several outbreaks [38,39]. In recent years, Southeast Iran has experienced a drought and the associated reduced rainfalls and drier lambing seasons, which are predisposing conditions for the aerosol transmission of spore-like *C. burnetii* [19]. The highest prevalences in goats were observed in Roodan and Sarbaz (46.2 and 35.7%, respectively), whereas the highest prevalence in sheep was observed in Iranshahr (43.6%). As seen in our results, there were no relation between low annual rainfalls and increases in *C. burnetii* seropositivity. The minimum and maximum temperatures (based on five-year averages) were positively associated with the Q fever seropositivity in these counties over the study period; i.e., the univariate analyses indicated that decreases in the minimum and maximum temperatures (in county 2, Kohbanan) were associated with decreases in seropositivity. The logistic regression analysis confirmed that the annual rainfall and meteorological conditions were not important risk factors for the prevalence of this pathogen.

In conclusion, the present study confirmed the presence of specific *C. burnetii* antibodies in small ruminants in Southeast Iran, which demonstrated the presence of these bacteria in the herds. Moreover, we confirmed that sheep and goats are important reservoirs of *C. burnetii* in this area. Furthermore, the logistic regression analysis revealed that only age was an important risk factor for the prevalence of *C. burnetii* bacteria. Further studies of the extent of *C. burnetii* infection in humans are needed. Although the information provided by this study might be geographically limited, it is important for the understanding and control of this disease in Iran.

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Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

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