Seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012

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Summary
The purpose of this study was to estimate the seroprevalence of *Coxiella burnetii* infection in sheep and goat flocks with a history of abortion in different areas of Iran. One thousand and one hundred ovine and 180 caprine samples from 43 sheep and goat flocks in four counties located in the Northeast (Mashhad), Central (Isfahan), Western (Arak), and Southwest (Shiraz) Iran were collected randomly between March 2011 and April 2012. The CHEKIT Q fever ELISA kit was used to identify specific antibodies against *C. burnetii* in sheep and goats. The results showed that the overall seroprevalence of *C. burnetii* in sheep and goats was 19.5% and 27.2%, respectively. There was a significant difference in seropositivity between sheep and goats (P<0.05). Central Iran significantly had the highest prevalence among the studied areas, especially in goat coxiellosis (23.8% and 40.8% in sheep and goats, respectively). The lowest prevalence in sheep was 12.8% in Northeast Iran while in Western Iran *C. burnetii* antibodies were absent in goats. The higher prevalence of Q fever in Central Iran may be partly due to persistent favourable conditions to spread *C. burnetii* in this area including drought and dust storms that originated from neighbouring Iraq and Kuwait. In conclusion, the present study demonstrated the relatively high prevalence of Q fever in sheep and goat flocks with a history of abortion. Therefore, Q fever could be responsible for considerable numbers of ovine and caprine abortions in Iran.

Sieroprevalenza di Febbre Q in greggi di pecore e capre con anamnesi di aborto in Iran tra il 2011 e il 2012

L’articolo descrive i risultati di uno studio condotto in Iran tra Marzo 2011 e Aprile 2012 per valutare la sieroprevalenza di infezioni da *Coxiella burnetii* in pecore e capre con anamnesi di aborto. Lo studio si è basato su un campionamento casuale effettuato su 43 greggi di pecore e capre in quattro province dell’Iran: Mashhad (zona nordest), Isfahan (zona centrale), Arak (zona occidentale) e Shiraz (zona sudovest). Sono stati utilizzati 1.100 campioni prelevati da pecore e 180 prelevati da capre. Per identificare gli anticorpi specifici per *C. burnetii* è stato impiegato il test CHEKIT Q fever ELISA. I risultati hanno mostrato la sieroprevalenza totale del microrganismo nel 19,5% delle pecore e nel 27,2% delle capre, differenza che è risultata statisticamente significativa (P<0,05). Nella zona centrale dell’Iran sono stati rilevati valori di sieroprevalenza più elevati nelle capre (40,8%), nella zona nordest e nella zona sudovest, in confronto alle pecore (12,8%) e nella zona occidentale non sono stati evidenziati anticorpi *Coxiella burnetii* nelle capre. La presenza di un maggiore numero di animali affetti da febbre Q nella zona centrale dell’Iran può essere ricondotta al persistere nell’area di condizioni favorevoli alla diffusione del microrganismo come siccità e tempeste di polvere, queste ultime provenienti da Iraq e Kuwait (paesi confinanti). I risultati dello studio hanno mostrato che in greggi di pecore e capre con anamnesi di aborto esiste una prevalenza relativamente alta di febbre Q, il cui agente etiologico potrebbe essere responsabile del considerevole numero di aborti riscontrati.
Introduction

Q fever is a zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacterium (23). The infection has a worldwide distribution with the exception of New Zealand (10). A wide range of hosts is susceptible to infection with *C. burnetii* including farm animals, pets, wild mammals, arthropods (mainly ticks), birds, as well as humans. *C. burnetii* could be maintained in nature following two different cycles: the wild cycle, in which ticks and wild animals are involved, and the domestic cycle, where ruminant and other animal species such as dogs and cats are the main reservoirs (3, 23). However, the link between both proposed cycles is currently poorly understood, especially because the domestic cycle has been considered the main source for human infection (18). The main reservoirs of *C. burnetii* are domesticated ruminants such as cattle, goats and sheep which are also considered the major source of infection for human beings (2). Transmission of infection to humans occurs mainly through inhalation of contaminated aerosols and, less commonly, ingestion of infected milk and/or raw dairy products (3, 30).

The bacterium has a small gram-negative pleomorphic coccobacilli shape and produces two morphologically distinct cell types that comprise a bi-phasic developmental cycle. A small cell variant (SCV), with its characteristic condensed chromatin, is thought to be an extracellular survival form with enhanced resistance to environmental stressors such as desiccation and heat. When the small cell variant invades the host, it develops into a large cell variant (LCV) that is metabolically and divisionally active. Differentiation of LCV to SCV occurs during the stationary phase of the organism’s growth cycle. It involves changes in surface proteins, but changes in lipopolysaccharide (LPS) have not been studied so far. Originally LCV was thought to produce an endospore that served as a progenitor of the SCV, but this developmental form has since been discounted (23).

*C. burnetii* is very resistant to heat, drought and disinfectants. Therefore, the organism can survive for long periods in the environment and be transmitted through contaminated aerosols (2). It has been shown that dry and windy conditions play a role in *C. burnetii* transmission and are epidemiologic factors in Q fever outbreaks occurring near sheep-rearing areas (6, 33, 40).

The infection is mainly asymptomatic in humans. However, acute forms of the disease can cause clinical signs including a flu-like illness, pneumonia, and hepatitis. Endocarditis is the major clinical presentation of chronic Q fever (23, 32). Q fever is often subclinical in animals, however it can lead to abortion, fetal death, delivery of weak newborn animals, and reproductive disorders (1, 18, 39, 41). *C. burnetii* is commonly shed in the amniotic fluid and placenta of infected animals during parturition, but shedding of the bacterium can also occur via milk, urine, and feces. The only clinical finding in sheep and goats is abortion, particularly in late gestation (11, 31). Abortions during coxiellosis epizootics have been described in goats and sheep (4, 22, 28).

In Iran, the incidence of ovine and caprine abortion is very high, mainly in late gestation without specific clinical signs. Although *Brucella* species and other causal agents are responsible for a considerable percentage of abortions in Iran, there have also been a significant number of abortions each year with unknown etiology. So far, *C. burnetii* has not been investigated as a plausible agent of these cases in Iran, in addition, recent serological studies have reported the evidence of *C. burnetii* infection in animals and humans in Kerman, Southeast Iran (16, 17, 36). However, these studies have been conducted with low sample size and limited to this area of the country.

Serological tests are usually used for detection of antibodies against *C. burnetii*. The most commonly used techniques include ELISA and immunofluorescence assay (IFA). ELISA is preferable to IFA because it is more sensitive (23, 34).

Due to lack of information on the epidemiology and biology of *C. burnetii* infection, outbreaks of Q fever may occur unnoticeably in humans and animals. Despite the prevalence of Q fever in Iran, only few studies have been conducted on abortions in relation to this disease. Therefore, the present study was undertaken to estimate the prevalence of Q fever in sheep and goat sera of flocks with a history of abortion in different regions of Iran.

Materials and methods

Sample collection

A total of 1,280 serum samples (1,100 ovine and 180 caprine) was collected from 43 sheep and goat flocks in four counties located in the Northeast (Mashhad), Central (Isfahan), Western (Arak), and Southwest (Shiraz) Iran from April 2011 to March 2012. These four counties were selected on the basis of geographical distribution and local reports of a high occurrence of abortion in both sheep and goats. For each area, information on flock reproductive status (including abortion, fetal death, and newborn animals’ viability) was obtained from the Province Veterinary Department. In all counties except Mashhad, farms with a history of high abortion rate (≥10%) were selected. In the selected farms, about 10–12% of the population of each flock...
was randomly tested. The number of sampled flocks was 15, 15, and 13 farms in Southwest, Central, and Western Iran, respectively. The flock size ranged from 30 to 500 in Southwest, 50 to 1,120 in Central, and 23 to 400 animals in Western Iran.

In Mashhad, blood samples were randomly obtained immediately before slaughter of sheep and goats at the abattoir of the county. The catchment area of this abattoir includes all counties of Razavi Khorasan province, Northeast Iran.

Animals older than 6 months were included in this study. Blood samples were taken aseptically from the jugular vein using a sterile 10-ml anticoagulant-free vacutainer. Samples were transported to the laboratory in containers with ice and sera were separated immediately by centrifugation of blood at 1,500×g for 10 min at room temperature and were stored at −20 °C until the day of analysis.

**ELISA assay**

Serum samples were tested for Q fever antibodies using the indirect ELISA kit (CHEKIT, IDEXX Laboratories, Switzerland), which was carried out following the protocol recommended by the manufacturer using phase I + II purified antigens of *C. burnetii*. Sera were prepared at 1:400 dilution, and specific antibodies were measured using a peroxidase-labeled antiruminant immunoglobulin G conjugate. Results were expressed as a percentage of the optical density reading of the test sample (value), calculated as value = 100×(S−N)/(P−N), where S, N, and P are the OD of the test sample, the negative control, and the positive control, respectively. Sera were considered to be ELISA positive if they had a value of 40% or more, suspect if the value was between 30% and 40%, and negative if the value was <30%. A farm was considered positive if at least one animal on the farm was classified as positive.

**Statistical analysis**

Statistical analyses were performed using SPSS software version 16 (SPSS Inc., Chicago, Ill, USA). A Chi-square test was used to determine the association between prevalence of Q fever antibodies and species of animals. P value < 0.05 was considered statistically significant.

**Results**

A total of 1,280 animals from 43 flocks in four different areas in Iran were sampled and tested for the presence of antibodies against *C. burnetii*. A total of 215 sheep (19.5%; 95% CI: 17-22%) and 49 goats (27.2%; 95% CI: 21-34%) had antibodies specific to *C. burnetii*. There was a significant difference in seropositivity between sheep and goats (p=0.02). The highest prevalence in sheep and goats was 23.8% and 40.8% in Central Iran, respectively. The lowest prevalence in sheep was 12.8% in Northeast Iran while in Western Iran, *C. burnetii* antibodies were absent in goats (Table I).

All flocks had at least one positive animal. Therefore, the flock-level prevalence of Q fever was 100%. Within-herd seroprevalence ranged from 6.1% to 55.30% in the studied flocks.

**Discussion**

The present study demonstrated that the overall seroprevalence of *C. burnetii* in sheep and goats was 19.5% and 27.2%, respectively. With the exception of Northeast Iran - where farm data were not available, all farms had at least one seropositive animal (farm prevalence 100%). This study demonstrated substantial transmission of *C. burnetii* within and between sheep and goat flocks in the studied regions.

The prevalence of seropositive animals (sheep or goats) per farm varied between 6.1 and 55.3%. The difference between farms may be partly related to differences in management and hygienic measures. All studies performed in rural areas have shown that poor hygiene could be an exacerbating factor in the spread of *C. burnetii* (20, 21). The overall high prevalence of *C. burnetii* infection detected in this study can also be associated with poor sanitary practices in most flocks in Iran including inappropriate disposal of fetal fluids and membranes, aborted fetuses, and use of placetas to feed dogs. The seroprevalence estimates, however, are lower than those found in sheep (29.42%) and goats (65.78%) in Southeast Iran (16, 36).

In this study the Q fever seroprevalence in sheep is comparable to those found in neighbouring countries of Iran. In Turkey serosurveys have demonstrated that seropositivity of *C. burnetii* infection is 20% in the southern Marmara region (15) and 13.5% in the West Black Sea region (9).

**Table I. Seroprevalence of *C. burnetii* infection in sheep and goats in Southwest, Central, West and Northeast Iran.**

<table>
<thead>
<tr>
<th>Area</th>
<th>Sheep Positive</th>
<th>Sheep Negative</th>
<th>Goat Positive</th>
<th>Goat Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwest</td>
<td>48 (20.4)</td>
<td>187 (79.6)</td>
<td>16 (21.1)</td>
<td>60 (78.9)</td>
</tr>
<tr>
<td>Central</td>
<td>80 (23.8)</td>
<td>256 (76.2)</td>
<td>31 (40.8)</td>
<td>45 (59.2)</td>
</tr>
<tr>
<td>West</td>
<td>49 (21.1)</td>
<td>183 (78.9)</td>
<td>0 (0)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Northeast</td>
<td>38 (12.8)</td>
<td>259 (87.2)</td>
<td>2 (15.4)</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>Total</td>
<td>215 (19.5)</td>
<td>885 (80.5)</td>
<td>49 (27.2)</td>
<td>131 (72.8)</td>
</tr>
</tbody>
</table>

Numbers in parentheses show the percentage of prevalence in sheep and goat in each area.
The seroprevalence of *C. burnetii* infection in sheep populations has been estimated in several other countries such as USA (10%), Spain (21%), Cyprus (18.9%) and Germany (1.3%) (13, 25, 29, 35).

Seroprevalence of *C. burnetii* has been studied worldwide also in goats. The goat seroprevalence has been reported to be 8.8% in Albania (5) and 6.5% in Northern Greece (27). In Spain, the goat and farm prevalence was 8.7% and 45%, respectively (35), similar to the results for animals tested in Northern Ireland (24). Goat and farm prevalence has been determined to be respectively 21.4% and 43.1% in the Netherland(37) and 13% and 47% in Sardinia (22). A highly different goat prevalence has been observed in Poland, where no *C. burnetii* IgG phase 2 antibodies were found in 918 goats from 48 herds (7). The results of the present study show a 27.2% overall goat prevalence of *C. burnetii*, which is relatively high compared to most seroprevalence studies performed in Europe, while it is low compared to the seroprevalence in Southeast Iran (65.7%). However, the data collected show the highest prevalence in goats (40.8%) in Central Iran, which is more comparable to the one found in Southeast Iran. Absence of *C. burnetii* antibodies in Western Iran could be ascribed to the presence of fewer populations of goats in this area.

The results of the study, as the ones of studies performed in Southeast Iran (16, 36) and the USA (25), show a significantly higher seroprevalence in goats than in sheep. Thus, goats represent a higher risk for environmental contamination and, consequently, for transmission within flocks and between different areas. Goats share a predisposition with dairy cows to be chronically infected (18). Therefore, transmission of *C. burnetii* to humans from infected goats may be significant in areas where they replace cows as a source of milk.

Central Iran had the highest prevalence (P<0.05) among the studied areas especially in goats (40.8%) followed by Southwest, Western, and Northeast Iran. In recent years, the Central and Southwest regions of Iran have experienced drought associated with lower rainfall and drier lambing season, which predisposes the aerosol transmission of *C. burnetii*. Concurrently, and especially since 2004, these regions have been experiencing major dust storm events originating from neighbouring Iraq and Kuwait. This event, which is termed ‘the Middle Eastern Dust (MED) event’, can potentially carry different infective agents and transport them over long distances (38). Interestingly Leski et al. (19) have indicated a high prevalence of *C. burnetii* in breathable dusts from arid regions of Iraq and Kuwait. The role of dry and windy conditions in the aerosol transmission of *C. burnetii* has been suggested in several outbreaks (6, 12, 14, 33, 40, 42). Furthermore, an outbreak of Q fever occurred in US soldiers deployed to Iraq in 2005 (8). The disease has recently been reported in countries neighbouring Iran, including Oman (2000), Iraq (2003), Afghanistan (2006), United Arab Emirates (2008), Turkey (2010), and Saudi Arabia (2011) (26).

The higher prevalence of Q fever in Central Iran can be due to climatic conditions favourable to the transmission of the bacterium in this area. In contrast, the lower prevalence in Northeast Iran can be attributed, at least partly, to different conditions including the higher rainfall and lack of the dusts mentioned above. Besides, the most likely way in which animals acquire infection seems to be by inhalation of organisms from infected dusts especially in Central and Southwest Iran.

The samples of this study were taken from flocks with a history of fetal death, delivery of weak offspring and high abortion rate of unknown etiology. The high prevalence of Q fever detected in the samples collected from different regions of Iran suggests a possible important role of *C. burnetii* as a causal agent of the reproductive failures reported in the flocks of this country. However, further studies, including molecular investigations, are required to determine the precise etiological factor of the abortions.

According to the present status of *C. burnetii* infection in Iran and persistent favourable conditions to the spreading of the bacterium, more attention is needed to control and prevent Q fever in terms of public health and ovine and caprine abortions.

**Conclusions**

The present study demonstrated the relatively high prevalence of Q fever in sheep and goat flocks with a history of abortion in Iran. The results show that goats might play an important role in contaminating the environment and spreading the infection within and between the flocks. Furthermore, the prevalence values found in this study were influenced by geographical location and, possibly, climatic conditions.

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References


Q fever in sheep and goats in Iran