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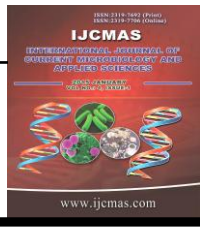

Original Research Article

Q fever in domestic ruminants: A Seroepidemiological survey in Hamedan, Iran

Hossein Edalati-Shokat[1], Ehsan Abbasi-Doulatshahi[1], Hassan Hajian-Bidar[2], Jamal Gharekhani[2]* and Ali-Akbar Rezaei[1]

[1]Department of Microbiology, Central Veterinary Laboratory, Iranian Veterinary Organization, Hamedan, Iran
[2]Department of Parasitology, Central Veterinary Laboratory, Iranian Veterinary Organization, Hamedan, Iran
**Corresponding author*

Keywords

Coxiella burnetii, Q fever, domestic animals, ELISA, Iran

A B S T R A C T

Q fever is a zoonosis caused by an obligate intracellular bacterium, *Coxiella burnetii*. The ruminants are identified as the source in the majority of Q fever outbreaks in humans. The main aim of current survey was to determine the seroprevalence of *C. burnetii* infection in sheep, goats and cattle in Hamedan, western Iran. In a cross-sectional study between May to October 2014, blood samples were collected randomly from 200 sheep, 50 goats and 120 dairy cattle in ten different locations of Hamedan. All samples were evaluated for the presence of antibodies to *C. burnetii* using enzyme-linked immunosorbent assay. Antibodies to *C. burnetii* were found in 27.5% of sheep, 54% in goats and 0.83% in dairy cattle. Herd and within herd level prevalence was 100% and 5-56% for sheep, 100% and 20-100% for goat, and 10% and 0-8.3% for dairy cattle, respectively. No evidence of significant correlation between abortion history, gender, age groups and seroprevalence rates was found in animals. The infection rate in sheep was reported 41.9% and 23.6% in ≤2 and >2 age group, respectively (P=0.017, OR=2.3). In conclusion this is the first report of *C. burnetii* infection in animals in western Iran. The results indicate that coxiellosis may partly be responsible for major economic losses in domestic ruminants husbandry especially in goats in this region. Therefore, it is necessary to take integrated strategies for prevent and control of infection in animals, which could help to reduce human infection. Further comprehensive studies are recommended.

Introduction

Coxiellosis (Q fever) is a highly contagious zoonotic disease caused by an obligate intracellular gram-negative bacterium, *Coxiella burnetii* (Asadi et al., 2013).The Q fever infection has been described in most countries except in New Zealand (Greenslade et al., 2003).A wide variety of animals can be infected with *C. burnetii*.

Ruminants are the most common reservoirs of disease (Keyvanirad et al., 2013).

The infection is mainly asymptomatic in humans and subclinical in animals (Khalili and Sakhaee, 2009; Asadi et al., 2013); but the infection might be the cause of abortion (mostly in sheep and goats), stillbirth, delivery of weak offspring and infertility in sheep, goats and cattle (Keyvanirad et al., 2013). Other symptoms such as mastitis, metritis, pneumonia, conjunctivitis and hepatitis occur rarely in infected animals (Porter et al., 2011); which isresponsible for major economic losses in animals and is considered a public health problem (Cetinkaya et al., 2000).

Infected animals shed the infectious particles in vaginal mucus, urine, feces, milk, birth products and semen (Guatteo et al., 2010; Keyvanirad et al., 2013). Transmission of infection to human is mainly through the inhalation of contaminated aerosols, consumption of raw milk or fresh dairy products (Rahimi, 2010). Ticks are considered to the natural primary reservoir of *C. burnetii* and play a significant role in the transmission of infection to both humans and animals (Norollahifard and Khalili, 2011; Porter et al., 2011).

Q fever is an occupational hazard for veterinarians, abattoir workers, dairy farmers, laboratory personnel and anyone with regular contact with animals or their products (Khalili et al., 2011; Esmaeili et al., 2013).

The determination of coxiellosis prevalence rate in ruminants is very important; because this animals are the most reservoir for humans (Keyvanirad et al., 2013).Q fever in animals can be detected using Immuno-histochemistry, complement fixation test (CFT), enzyme-linked immune sorbent assay (ELISA), indirect immuno-fluorescence assay (IFA)and molecular methods (Roest et al., 2013). The serological methods are recommended to detect of Q fever on herd level; Because, the molecular biology techniques have low sensitivity and are available only in reference laboratories (Sakhaee and Khalili, 2010).

Domestic ruminants such as sheep, goats and cattle are widely used as food animals (meat and milk) in Iran. These infected animals by *C. burnetii* represent source of human infection (Keyvanirad et al., 2013).

Little is known concerning of *C. burnetii* infection in animals in Iran (Rahimi, 2010; Sakhaee and Khalili, 2010; Esmaeili et al., 2013; Keyvanirad et al., 2013; Asadi et al., 2014; Esmaeili et al., 2014).However, there is no published information on Q fever in animals in western Iran.

The principal aim of this study was to determine the seroprevalence of *C. burnetii* infection (Q fever) in sheep, goats and cattle in Hamedan, west part of Iran using ELISA.

Materials and Methods

Study area

Hamedan city by mountainous and mild climate is located in west part of Iran (34.77°N and 48.58°E) (Figure 1). The mean annual rainfall and temperature is 317.7 mm and 11.3°C, respectively. This region is economically impressed by an agricultural and animal husbandry such as cattle, sheep and goat. According to Iranian Veterinary Organization (IVO) report, the population of sheep, goats and cattle in Hamedan is 130,000; 20,000 and 12,000, respectively.

Sampling

A cross-sectional study was performed during May to October 2014. The multi-stage cluster random method was used for sample collection. Three-hundred seventy blood samples (200 sheep and 50 goats samples form 10 flocks in different rural regions of Hamedan; 120 dairy cattle samples from all of 10 dairy cattle farms from hamedan) were taken in animals (Thrusfield, 1997).

Information about age, gender and history of abortion (yes or no) were taken from owners and/or physical examiner (Table 1). In small and large ruminants (cattle), all of animals were native and Holstein breed, respectively. All of animals had no history of vaccination against *C. burnetii* and tick infestation.

Serology

The sera were obtained by centrifugation at 1500×g for 10 min and stored at -20°C until laboratory testing. Anti-*C.burnetii* antibodies of samples were detected using a commercially available *C. burnetii* ELISA kit (ID Screen® Q fever indirect ELISA in ruminants; ID-Vet company, France). The presence of antibodies was detected by calculating of sample to positive (S/P) ratio using the kit manufacturer's protocol(S/P ≥50% = positive).

Statistical analysis

Statistical analysis was performed by using the software package SPSS version 16.0 for windows. Odds ratios (OR), confidence interval (CI), $\chi 2$ and *p*-value were calculated separately for each variable. *P*-value of less than 0.05 was considered statistically significant.

Result and Discussion

Antibodies to *C. burnetii* were found in 27.5% of sheep (CI 95%: 21.3-33.7%), 54% in goats (CI 95%: 14.7-39.3%) and 0.83% in dairy cattle (CI 95%: 0-2.46%)(Table 1).Herd and within herd level prevalence was 100% and 5-56% for sheep, 100% and 20-100% for goat, and 10% and 0-8.3% for dairy cattle, respectively.

The infection rate in goats was reported significantly higher than sheep ($\chi 2$=12.744, *P*=0.0003, *df*=1, OR=3.2) and dairy cattle ($\chi 2$=72.516, *P*<0.0001, *df*=1, OR=139.7). Also, this rate in sheep was higher than dairy cattle ($\chi 2$=36.941, *P*=0.0004, *df*=1, OR=45.1).

There was no statistical significant difference between infection rate and age groups in goats ($\chi 2$=0.061, *P*=0.804, *df*=1) and dairy cattle ($\chi 2$=0.475, *P*=0.788, *df*=1). In sheep, this rate was detected 41.9% in ≤2 and 23.6% in >2 age group; the statistical significant was seen ($\chi 2$=5.665, *P*=0.017, *df*=1, OR=2.3). No evidence of correlation between abortion history, gender and seroprevalence rates was found in animals (Table 1, *P*>0.05).

Q fever is widespread in livestock, and its seroprevalence is thought to have increased in recent years (Porter et al., 2011). In the present manuscript, for the first time, infection by *C. burnetii* was described in sheep, goats and cattle from western Iran.

In past studies, different range of *C. burnetii* infection was reported in ruminant worldwide (Guatteo et al., 2010). This range was Zero (Western Australia) to 40% (Mexico) in sheep (Salinas-Meledez et al., 2002; Banazis et al., 2010), Zero (Switzerland) to 89% (Canada) in goats (Dolce et al., 2003; Fretz et al., 2007), and

Zero (New Zealand) to 82% (USA, California) in cattle (Hilbink et al., 1993; Guatteo et al., 2010). Also, the lowest and highest herd prevalence rate was Zero (Switzerland) and 89% (Canada) in sheep (Dolce et al., 2003; Fretz et al., 2007), Zero (Switzerland) and 100% (Iran) in goats (Fretz et al., 2007; Khalili and Sakhaee, 2009), and 4.4% (Italy) and 100% (USA and Czech republic) in cattle (Guatteo et al., 2010; Martini et al., 1994; Literak and Kroupa, 1998), respectively. The apparent prevalence was slightly higher in cattle (20.0% and 37.7% of mean apparent prevalence at animal and herd level respectively) than in small ruminants (around 15.0% and 25% respectively for animal and herd level in sheep and goat) worldwide(Guatteo et al., 2010).

In the present survey, the overall seroprevalence was determined 27.5% in sheep, 54% in goats and 83% in dairy cattle. On the other hand, our prevalence results were similar to studies in Australia, Japan and Oman (Htwe et al., 1992; Scrimgeour et al., 2003; Banazis et al., 2010).

In total higher percentage of antibodies was detected in goats, sheep than in dairy cattle (0.83%) (P<0.05). Our finding was parallel to investigations in Turkey, Spain and Albania (Cetinkaya et al., 2000; Cekani et al., 2008; Ruiz-Fons et al., 2010). In Keyvanirad et al (2013) study from northeastern Iran, no significant differences was seen in seroprevalence rate in sheep and goats (P=0.147).

In the previous studies from Iran, Asadi et al (2014) was reported 27.2% of seropositivity in Iranian goats. In Rahimi(2010) study, 2% of goat milk samples were positive using molecular techniques. Also, in the other hand, animals and herd-prevalence were 29.8% and 78.5% in northeastern Iran (Keyvanirad et al. 2013), and 65.78% and 100% in southeastern Iran (Khalili and Sakhaee, 2009), respectively. Sakhaee and Khalili (2010) reported the 29.4% of seropositivity in Iranian sheep for the first time. Also, this rate was detected 23.7% in northern Iran (Esmaeili et al., 2013), 33.6% of animals and 87.5% of herd-level in northwestern Iran (Esmaeili et al., 2014), 36.5% of animals and 89.6% of herd-level in northeastern Iran (Keyvanirad et al., 2013).In Khalili and Sakhaee(2009)investigation, 10.75% of cattle and 16.6% of dairy herds were seropositive. Also, 45.4% of dairy cattle farms were positive based on bulk tank milk analysis (Khalili et al., 2011).

Abortion during coxiellosis epizootics have been described in sheep and goats; but abortion in cattle is rare(Masala et al., 2004). Abortion in cattle has been successfully induced experimentally with *C. burnetii* (Cetinkaya et al., 2000).Pregnant ruminants are highly susceptible to infection, and abortions occurred only at the first parturition after infection. The following gestation terminated normally without any reproduction failures. Detection of *C. burnetii* antibodies in fetal fluids or serum is useful in the diagnosis of abortion in sheep and goats(Khalili and Sakhaee, 2009).

In our finding, 50% (3/6) of sheep, 100% (1/1) of goats and 0% (0/11) of dairy cattle with history of abortion were seropositive. No significant difference was seen between seroprevalence rate and animals with history of abortion (P>0.05); unlike to other studies in Iran, Turkey and Greece (P<0.05) (Cetinkaya et al., 2000; Khalili and Sakhaee, 2009; Bisias et al., 2010; Asadi et al., 2013). *C. burnetii* has been suspected as a cause of sheep and goats abortion in Italy, Cyprus, Turkey, Spain and Iran using serological techniques (Cetinkaya et al., 2000; Masala et

al., 2004; Ruiz-Fons et al., 2010; Cantas et al., 2011; Asadi et al., 2013).

Age is the most widely discussed variable in literature. An age-related difference in *C. burnetii* infection is expected because older animals are exposed to tick bite and *C. burnetii* for longer periods (Guatteo et al., 2010).

In the current investigation, seroprevalence rate of sheep in ≤2 age group (41.9%) was higher than >2 age old (23.6%) (Table 1, *P*=0.017, OR=2.3); also no significant differences between age class seroprevalence values were found in goats and dairy cattle (*P*>0.05). Our finding was agreement to studies in northern Iran, Turkey and Spain (Cetinkaya et al., 2000; Ruiz-Fons et al., 2010; Esmaeili et al., 2013). In Keyvanirad et al(2013)work, sheep and goats aged over 2year had significantly higher chance of seropositivity than those under a year (*P*<0.05).Some researchers believe that the animals less than a year old are less likely to experience parturition than older ones. Also, the antibody has been shown to last for months or years after initial infection(Keyvanirad et al., 2013). It seems the disease has affected in all age of animals in this region, equally.

In the presented study, there was no significant relationship between gender and infection rate in sheep and goats (Table 1, *P*>0.05). Sakhaee and Khalili(2010)was reported 18.82% of seropositivity in female and 10.58% in male animals from Iran; the difference was statistically significant (*P*<0.05). Moreover, there are many reports that did not show significant correlation between *C. burnetii*infection and gender, similar to our study (Cetinkaya et al., 2000; Esmaeili et al., 2013).

The hormonal differences between males and females play an important role in determining susceptibility to infection. Estrogen enhances antibody production and androgen suppress both T-cell and B-cell immune responses, but immunity in females can be broken down due to various factors e.g., nutrition, age, pregnancy and environmental factors (Cantas et al., 2011; Porter et al., 2011).

Discrepancies in the rates might be attributed to difference in diagnostic methods used such as serological assays and cut-off value, type of sample (serum and milk), study design, experimental strategies, climatic variations, frequency of tick population and farms. The difference between farms may be partly related to differences in management and hygienic measures (Guatteo et al., 2010; Esmaeili et al., 2013).

The most of management system is traditional in sheep and goat farms in Iran. In this system, the animals are allowed to roam free on pasture during the day. This may increase the chance of tick infestation and *C.burnetii* infection, subsequently. The high seropositivity in goats can be associated with poor sanitary practices in most sheep and goat flocks in Iran such as inappropriate disposal of fetal fluids and membranes, aborted fetuses, and use of placentas to feed dogs. Indeed, in many countries, goats are the most common source of human infection duo to their extensive raising and close contact with humans(Porter et al., 2011).

The control of coxiellosis in animals is difficult owing to the absence of obvious clinical signs. Farmers know little about the disease and are unaware of its economic importance, and they are therefore unwilling to cooperate its control. The widespread distribution of the infection and the persistence of the organism in the

environment also make it difficult to develop effective control strategies. The eradication of the disease from herds is a difficult and costly process owing to the fact that some seronegative animal may also shed *C. burnetii*. Vaccination programs have been reported to be inadequate for the eradication of *C. burnetii*, although they decrease the number of organisms shed by infected animal (Cetinkaya et al., 2000).

Table.1 Seroprevalence of *Coxiella burnetti* infection in animals in different variables in Hamedan, west part of Iran

Animals	Number of tested animals (Sero-positive %)										CI 95%	Within herd level prevalence	Herd level prevalence
	Age groups (year)			Abortion history			Gender			Total			
	≤2	>2	*P*-value	Yes	No	*P*-value	Male	Female	*P*-value				
Sheep	43 (41.9)	157 (23.6)	0.017	6 (50)	171 (26.9)	0.213	23 (26.1)	177 (27.7)	0.871	200 (27.5)	21.3-33.7%	5-65%	100%
Goat	8 (50)	42 (54.8)	0.804	1 (100)	47 (51.1)	0.332	2 (100)	48 (52.1)	0.182	50 (54)	14.7-39.3%	20-100%	100%
	≤4	**>4**									0-2.46%	0-8.3%	10%
Dairy cattle	79 (1.3)	41 (0)	0.469	11 (0)	1 (0.92)	0.749				120 (0.83)			

Figure.1 Map of locations and geographic distribution of the sampled herds in Hamedan, Iran

In conclusion, this work reports basic epidemiologic information of *C. burnetii* infection in domestic ruminants from western Iran. The results indicate that coxiellosis may partly be responsible for major economic losses in domestic ruminants husbandry especially in goats in this region. Therefore, it is necessary to take integrated strategies for prevent and control of infection in animals, which could help to reduce human infection. Further comprehensive studies are recommended.

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