Seroepidemiology of coxiellosis (Q fever) in sheep and goat populations in the northeast of Iran

Keyvani Rad, N.[1]; Azizzadeh, M.[2*]; Taghavi Razavizadeh, A. R.[2]; Mehrzad, J.[3] and Rashtibaf, M.[4]

[1]Graduated from Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [3]Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran, and Veterinary Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran; [4]Department of Survey and Surveillance, Veterinary Head Office of Khorasan-Razavi Province, Iranian Veterinary Organization, Mashhad, Iran

[*]***Correspondence:*** *M. Azizzadeh, Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: m-azizzadeh@ferdowsi.um.ac.ir*

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Summary

Coxiella burnetii is the etiological agent of coxiellosis in animals and Q fever (a zoonotic disease) in humans. Cattle, sheep and goats are the main reservoirs of infection for humans. This serological survey was conducted to determine the seroprevalence of coxiellosis in sheep and goat populations in Khorasan Razavi province, Iran. Four hundred and sixty animals (255 sheep from 29 herds in 12 counties and 205 goats from 28 herds in 11 counties) were selected using multi-stage cluster random sampling. Sera were assayed for antibody against *C. burnetii* using a Q fever ELISA kit. Seroprevalence of *C. burnetii* at animal level was 36.5% (95% CI: 30.6%-42.4%) for sheep and 29.8% (95% CI: 23.8%-36.2%) for goat populations. The proportion of seropositivity for sheep and goats in the studied counties ranged from 4.5%-72.7% and 6.7%-57.1%, respectively. In 26 (89.6%; 95% CI: 78.6%-100%) sheep and 22 (78.5%; 95% CI: 63.5%-93.5%) goat flocks, at least one seropositive case was detected. Logistic regression model showed that age and location correlated with seroprevalence of the antibody against *C. burnetii* at the individual level in both species ($P<0.05$). There was no difference in seroprevalence between sheep and goat populations ($P=0.147$). This study showed that a relatively high proportion of animals are seropositive to *C. burnetii*. Considering the economic and public health importance of *C. burnetii* in animals and humans, measures are to be implemented to prevent its spreading and to reduce the zoonotic risk of *C. burnetii* in the studied region.

Key words: Seroepidemiology, Q fever, Sheep, Goat, Iran

Introduction

Coxiella burnetii is a globally existing small gram negative coccobacillus. It is the etiological agent of coxiellosis in animals and Q fever (a zoonotic disease) in humans (Romich, 2008). A wide variety of animals can be infected with *C. burnetii*, including mammals such as ruminants, dogs, cats, non-human primates and non-mammals such as reptiles, birds, fish and ticks. Ruminants are the main reservoirs. *Coxiella burnetii* infection might be the cause of abortion, stillbirth, lower birth weight and infertility in cattle, sheep and goat (Marrie *et al*., 1996; Marrie, 2007). Decreasing reproductive performance in animals especially livestock can lead to economic loss. Infected animals shed the organisms in urine, faeces, milk and birth products. Transmission of infection to humans is mainly through the inhalation of contaminated aerosols, but can also occur after consumption of raw milk and dairy products. Arricau-Bouvery and Rodolakis (2005) reported 18 outbreaks of Q fever from different countries involving 2 to 289 people from 1999-2004. The most common animals involved in these outbreaks were sheep and goats (McQuiston and Childs, 2002; Arricau-Bouvery and Rodolakis, 2005).

Although 60% of the initial infections are asymptomatic, prolonged fever, pneumonia, hepatitis and cardiac involvement are the main consequence of the acute form of the disease, endocarditis being the most clinical sign of the chronic form in humans (Angelakis and Raoult, 2010).

Considering the zoonotic aspect of *Coxiella burnetii* infection and the recent occurrence of Q fever in humans in Afghanistan and Iran (Hartzell *et al*., 2007; Aronson, 2008; Khalili *et al*., 2010; Bailey *et al*., 2011), the determination of seroprevalence of *C. burnetii* in ruminants, which are the most important reservoir for humans, is necessary. Few studies have investigated the prevalence of coxiellosis in Iran. The prevalence of the antibody against *C. burnetii* in the south of Iran has been reported to be 29.4% and 65.8% for sheep and goats, respectively (Khalili and Sakhaee, 2009; Sakhaee and Khalili, 2010). Another study on commercial Holstein dairy cows showed a seroprevalence of 22.6% in the northeast of Iran (Azizzadeh *et al*., 2011). In fact, the epidemiology of coxiellosis is unclear in Iran. In the present seroepidemiological study, we aimed to determine the seroprevalence of *C. burnetii* in sheep and

goat populations in the northeast of Iran.

Materials and Methods

Study area

The present study was conducted in Khorasan Razavi province, the fourth largest province of Iran, located in the northeast of the country. The province is a 118854 square km region inhabited by 5.6 million people. Half of this population lives in the province capital, Mashhad. This area has arid and semi-arid climate. Average rainfall is about 250 mm per year. The province has shared borders with Afghanistan to the east and Turkmenistan to the north (Fig. 1). There are about 5.8 million sheep and 1.1 million goats in this province (Anonymous, 2012). Small ruminants are raised semi-intensively that graze during daylight hours and are gathered indoors at night.

Fig. 1: Map of the provinces of the Islamic Republic of Iran showing the location of Khorasan Razavi province (gray colour)

Animals

460 animals (255 sheep and 205 goats) were randomly selected. Multi stage cluster random sampling was used to select study samples. From the 19 counties of the province, 12 were randomly selected. From those 12 counties, 29 sheep flocks and from 11 of them 28 goat flocks were randomly selected (1-5 flocks per county). 6-20 animals per flock, depending on herd size, were tested.

Blood samples (10 ml) were collected from the animals' jugular vein. For each animal, flock location, flock size, age (under one year, 1-2 year and more than 2 year) and sex and breed were recorded.

Samples were transported to the laboratory on ice. They were centrifuged at 1800 g at 4°C for 10 min to obtain the serum. Sera were stored in different labeled vials at -20°C until testing.

Serological test

Sera were tested for the presence of antibodies against *C. burnetii* using a CHEKIT Q-fever ELISA kit (IDEXX Laboratories, Switzerland) according to the manufacturer's instructions. Positive and negative control sera were provided by the manufacturer. After the preparation of reagents, serum samples and controls were diluted 1/400 in microtubes using CHEKIT-Wash solution. One hundred µl of the pre-diluted samples and controls were dispensed into appropriate wells of the microtiter plate. The microtiter plate was covered by a lid and incubated for 60 min at 37°C, the plate was then washed 3 times using 300 µl washing solution. One hundred µl of CHEKIT-Q-FEVER-Anti-ruminant-Ig-PO conjugate was dispended into each well. The microtiter plate was incubated for 60 min at 37°C in a humid chamber. Washing of the plate was repeated as described above. One hundred µl of the substrate was dispensed into the wells. The plate was incubated at room temperature for 15 min. Afterwards, one hundred µl of a stop solution were added to all wells and the microplates were read in an ELISA plate reader at 450 nm. Results were finally expressed as a percentage of the optical density of the test sample (%OD) calculated as below:

$$\%OD = 100 \times (S-N)/(P-N)$$

where
S: The OD value of the test sample
N: The OD value of the negative control
P: The OD value of the positive control

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%. Re-analysing suspect samples was performed as recommended by the manufacturer.

A flock was considered positive if at least one of the selected animals from the herd was positive.

Statistical analysis

Herd and animal level seroprevalence and a 95% confidence interval were calculated. Significance testing of each independent variable was performed by running a Chi-Square test. Predictors with $P<0.20$ were placed into a logistic regression model. A backward stepwise approach was used to identify explanatory variables, related to the seropositivity. All statistical analyses were performed using SPSS statistical software version 16 (SPSS Inc., Chicago), and a p-value less than 0.05 was considered as significant.

Results

Description of seropositivity

Seroprevalence of *C. burnetii* at animal level was 36.5% (95% CI: 30.6%-42.4%) for sheep and 29.8% (95% CI: 23.8%-36.2%) for goat populations. In both populations, antibodies were detected in all selected counties. The proportion of seropositivity at animal level in the studied counties for sheep and goats ranged from 4.5%-72.7% and 6.7%-57.1%, respectively. Sero-prevalence in sheep and goat populations was similar in most studied counties. Khvaf, Sabzevar and Torbat-e-

Heydarieh had high seropositivity for both populations (Fig. 2). In 26 (89.6%; 95% CI: 78.6%-100%) sheep herds and 22 (78.5%; 95% CI: 63.5%-93.5%) goat herds, at least one seropositive case was detected. The number and proportion of seropositive animals with respect to sex, age, breed, flock size and location for sheep and goat populations are shown in Tables 1 and 2, respectively.

Risk factors of seropositivity

The final model showed that age and location were correlated with the seroprevalence of the antibody

Table 1: Animal level prevalence of antibody to *Coxiella burnetii* with respect to sex, age, breed, district and flock size for sheep population of Khorasan Razavi province

Variables	Levels	No. of animal tested	Seropositive, N (%)	95% confidence interval	Significant χ^2 test
Sex	Female	207	75 (36.6%)	(36-37%)	0.957
	Male	48	17 (36.2%)	(34-38%)	
Age	<1 yr	33	8 (25%)	(22-28%)	0.081
	1-2 yr	82	25 (30.1%)	(29-31%)	
	>2 yr	125	52 (42.3%)	(41-43%)	
Breed	Afshari	34	17 (50%)	(47-53%)	0.003
	Kordi	66	30 (46.9%)	(45-49%)	
	Baloochi	80	17 (21.3%)	(20-22%)	
	Other	75	28 (37.8%)	(37-39%)	
District	Mashhad	24	4 (20%)	(16-24%)	<0.001
	Fariman	22	8 (33.3%)	(29-37%)	
	Khvaf	30	16 (72.7%)	(69-77%)	
	Sabzevar	19	16 (55.2%)	(52-59%)	
	Torbat-e-Heydarieh	22	10 (55.6%)	(50-61%)	
	Kashmar	18	7 (33.3%)	(29-38%)	
	Sarakhs	22	4 (22.2%)	(18-27%)	
	Kalat	18	1 (4.5%)	(3-6%)	
	Neyshaboor	18	4 (22.2%)	(18-27%)	
	Gonabad	18	8 (44.4%)	(39-50%)	
	Torbat-e-Jam	24	6 (33.3%)	(28-38%)	
	Ghoochan	20	8 (33.3%)	(29-37%)	
Flock size	<300	45	17 (38.6%)	(37-41%)	0.65
	300-1000	150	51 (34.2%)	(33-35%)	
	>1000	60	24 (40.7%)	(39-43%)	

Table 2: Animal level prevalence of antibody to *Coxiella burnetii* with respect to sex, age, breed, district and flock size for goat population of Khorasan Razavi province

Variables	Levels	No. of animal tested	Seropositive, N (%)	95% confidence interval	Significant χ^2 test
Sex	Female	165	46 (28.2%)	(27-29%)	0.251
	Male	40	15 (37.5%)	(36-40%)	
Age	<1 yr	30	5 (16.7%)	(15.19%)	0.041
	1-2 yr	79	18 (23.1%)	(22-24%)	
	>2 yr	81	30 (37.5%)	(37-39%)	
Breed	Boomi	72	18 (25.4%)	(24-29%)	0.237
	Pakistani	28	6 (22.2%)	(19-25%)	
	Other	105	37 (35.2%)	(34-36%)	
District	Mashhad	20	6 (30%)	(26-34%)	<0.001
	Fariman	17	2 (11.8%)	(8-16%)	
	Khvaf	19	8 (42.1%)	(37-47%)	
	Sabzevar	28	14 (51.9%)	(48-56%)	
	Torbat-e-Heydarieh	14	8 (57.1%)	(50-64%)	
	Kashmar	21	2 (10%)	(7-13%)	
	Sarakhs	15	1 (6.7%)	(4-10%)	
	Kalat	15	2 (13.3%)	(9-17%)	
	Gonabad	15	1 (6.7%)	(4-10%)	
	Torbat-e-Jam	20	5 (25%)	(21-29%)	
	Ghoochan	20	12 (57.1%)	(52-62%)	
Flock size	<300	48	17 (31.3%)	(29-33%)	0.173
	300-1000	91	51 (24.2%)	(23-25%)	
	>1000	57	24 (38.6%)	(37-41%)	

Table 3: Multivariate logistic regression model showing factors influencing the risk of seropositivity in sheep and goat populations

Variable	Levels	Sheep					Goat				
		β	SE	OR	%95 CI	P-value	β	SE	OR	%95 CI	P-value
	Constant	-2.45	0.75				-1.98	0.79			
District	Mashhad*	0		1			0		1		
	Fariman	1.02	0.73	2.76	0.66-11.43	0.161	-0.99	0.91	0.37	0.06-2.22	0.278
	Khvaf	2.89	0.77	18.05	3.96-82.27	<0.001	0.72	0.70	2.04	0.51-8.07	0.306
	Sabzevar	2.03	0.70	7.59	1.92-29.94	0.004	1.05	0.64	2.84	0.80-10.03	0.103
	Torbat-e-Heydarieh	2.38	1.29	10.75	0.86-133.42	0.064	-**	-	-	-	-
	Kashmar	1.24	0.76	3.46	0.78-15.34	0.102	-0.95	0.91	0.38	0.06-2.31	0.297
	Sarakhs	0.51	0.82	1.66	0.33-8.31	0.538	-1.06	1.21	0.34	0.03-3.70	0.382
	Kalat	-1.31	1.18	0.27	0.02-2.71	0.226	-0.94	092	0.39	0.06-2.34	0.304
	Neyshaboor	0.2	0.80	1.02	0.21-4.90	0.976	-	-	-	-	-
	Gonabad	1.52	0.76	4.54	1.03-20.07	0.046	-1.78	1.15	0.16	0.01-1.62	0.124
	Torbat-e-Jam	1.07	0.77	2.92	0.64-13.35	0.166	-0.17	0.72	0.84	0.20-3.47	0.081
	Ghoochan	1.32	0.75	3.75	0.86-16.20	0.076	1.72	0.71	5.60	1.38-22.70	0.016
Age	<1*	0		1			0		1		
	1-2	0.23	0.52	1.25	0.45-3.50	0.663	0.57	0.64	1.77	0.50-6.26	0.374
	>2	1.18	0.50	3.23	1.20-8.69	0.020	1.44	0.65	4.23	1.18-15.1	0.026

* Mashhad was considered as reference for district levels, and the age of <1 year considered as reference for age variables. ** Data obtained from this district were omitted from the logistic regression model because the age of selected goats was missing. OR: Odds ratio, and CI: Confidence interval

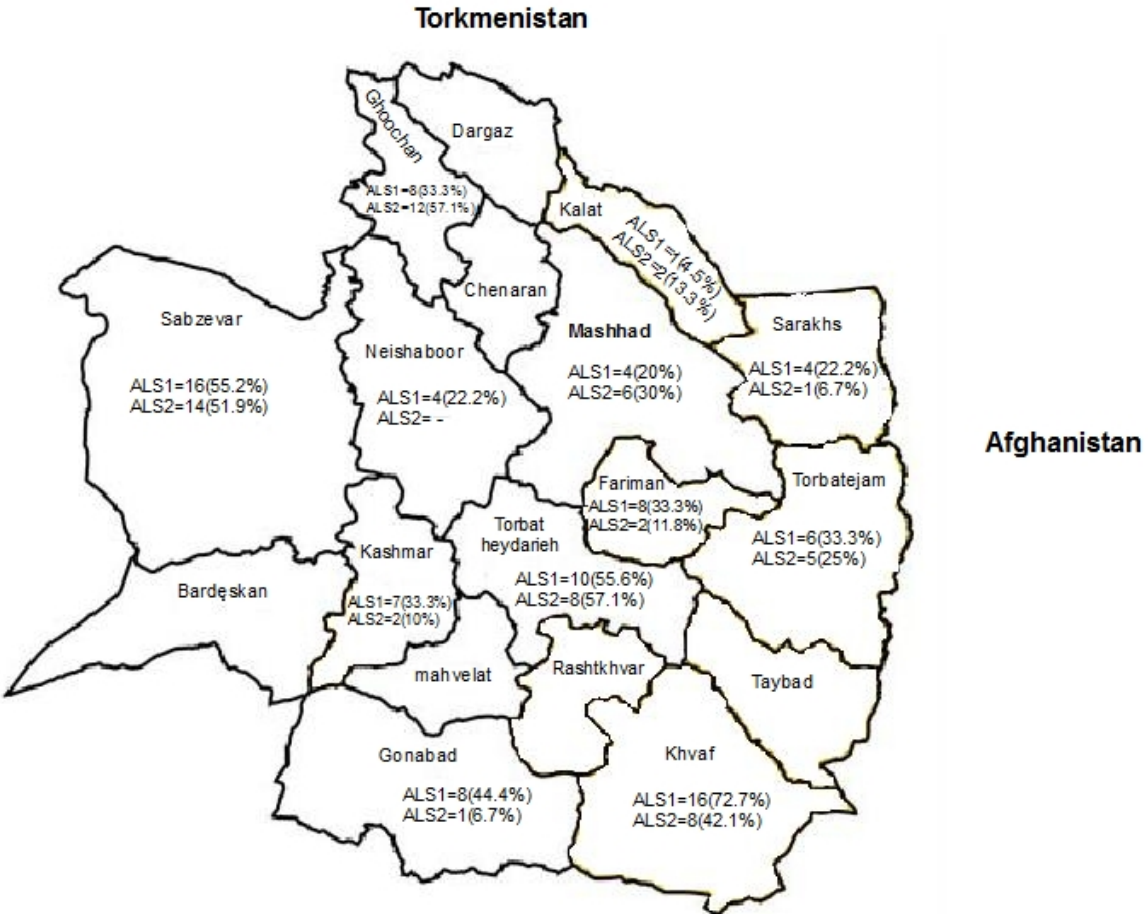

Fig. 2: Animal level seroprevalence (ALS) of coxiellosis in Khorasan Razavi province. Values shown as ALS are number (percentage) of positive animals for sheep (ALS1) and goat (ALS2) population in each district

against *C. burnetii* at the individual level in both species (P<0.05). Sheep located in Khvaf (OR=18.05; 95% CI: 3.96-82.27), Sabzevar (OR=7.59; 95% CI: 1.92-29.94) and Gonabad (OR=4.54; 95% CI: 1.03-20.07) were more likely to be seropositive than those located in the province capital, Mashhad. The probability of seropositivity for goats located in Ghoochan was 5.61 (95% CI: 1.38-22.70) times greater than that of Mashhad goats (Table 3).

Sheep and goats aged over 2 years had a significantly higher chance of seropositivity than those under a year old (P<0.05).

Comparison of seropositivity between sheep and goat populations

There was no difference in the seroprevalence of the antibody against *C. burnetii* between the two species (P=0.147).

Discussion

The present study showed a relatively high proportion of seropositive animals in this area of Iran. In addition, the presence of seropositivity was confirmed in all districts and most of the herds in the study area. Another study performed on commercial dairy cows in this province revealed a seroprevalence of 22.3 and 78.9% at animal and herd level, respectively (Azizzadeh *et al*., 2011). Recent studies in southern Iran have reported a high seropositivity of 29.42% in sheep and 65.78% in goats from flocks with a history of abortion (Khalili and Sakhaee, 2009; Sakhaee and Khalili, 2010). Results of these studies imply that *C. burnetii* exists and is circulating among farm animals that are the most common source of infection for human beings.

Coxiella burnetii seropositivity has also been reported in neighboring countries. In Turkey (western neighbour of Iran) two studies in 2000 and 2010 showed that 10.5 and 20% of the sheep were seropositive and 44.7 and 81% of the flocks revealed at least one seropositive animal (Cetinkaya *et al*., 2000; Kennerman *et al*., 2010). In Oman, located to the south of Iran, 52% of the goats were reported to be infected (Scrimgeour *et al*., 2003). Although there is no information about the seroprevalence of the antibody against *C. burnetii* in domestic animals in Afghanistan (eastern neighbor of Iran), infection with *C. burnetii* among US soldiers deployed to Afghanistan confirmed the presence of *C. burnetii* in this country (Hartzell *et al*., 2007; Aronson, 2008; Bailey *et al*., 2011). The study area has a shared border with Afghanistan and sometimes sheep and goat flocks of both countries cross the border to forage. In addition, the price of meat in Iran is much higher than Afghanistan, encouraging people to import animals to Iran illegally.

Numerous studies have reported the serological prevalence of coxiellosis in farm animals in different parts of the world. In the United State of America and European countries, seroprevalence of coxiellosis was reported at the a lower level for sheep and goat populations than our studied area (Marrie *et al*., 1985; Deforge and Cone, 2006; García-Pérez *et al*., 2009; Ruiz-Fons *et al*., 2010). The higher prevalence of the antibody against *C. burnetii* in the study area might be due to the different climate and type of the sheep and goat flocks' husbandry. Most parts of Khorasan Razavi province are semi arid. The dry atmosphere might enhance the dispersion of aerosols (Nakouné *et al*., 2004). In addition, sheep and goat flocks are housed in a closed space mostly at night and during winter, which can facilitate the transmission of infectious agents.

The prevalence of coxiellosis was different among the studied districts. Different climates and various densities of animals might be related to the dispersion of seropositivity. Torbat-e-Heydarieh and Sabzevar, which showed high seroprevalence of coxiellosis for sheep and goats, are districts with high densities of farm animals and Khvaf (the other district with high seroprevalence at animal and herd level) is a dry-windy area, close to the Afghanistan border.

In both populations, seroprevalence increased by age and the logistic regression model showed that animals with an age more than 2 years are most likely to be seropositive than those less than a year old. This finding is in agreement with those of McCaughey *et al*. (2010) and Ruiz-Fons *et al*. (2010). Large amounts of bacteria are shed during parturition. 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 (Romich, 2008).

The results reported herein showed that *C. burnetii* is endemic in the study area and distributed all over the province. An estimation of the proportion of seropositive animals, the determination of risk factors of seropositivity and its distribution could help authorities prioritize appropriate actions to control coxiellosis.

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