Seroprevalence of Q fever in small ruminants in the northeast Anatolian region in Turkey

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Received 22.12.2020

Accepted 25.01.2021

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Summary

The aim of our study was to determine the seroepidemiological profile of Q fever in small ruminants in Turkey and to examine its prevalence changes over the years. The study included 573 serum samples taken in 2013 and 472 samples taken in 2017 from animals in mixed herds of sheep and goats from 84 farms in Northeast Anatolia. Phase I and phase II IgG antibodies against *Coxiella burnetii* in serum samples were investigated by IDEXX ELISA (Q fever Ab Test IDEXX Laboratories, USA) indirect ELISA kits. Seroprevalence of *Coxiella burnetii* IgG in Artvin, Gümüşhane and Iğdır provinces was 5.6% in sheep, 1.8% in goats and 4.5% in total in 2013. In contrast, it was 24.4% in sheep, 1.1% in goats and 20.1% in total in 2017. According to the total seroprevalence rates calculated by including both sheep and goat population, it was seen that the province with the highest seroprevalence change in these animals was Iğdır with a 7.3-fold increase. Herd-level seroprevalence was 29.4% in 2013 and 57.6% in 2017. According to these results, the *C. burnetii* IgG seroprevalence nearly doubled after four years. This increase has been evaluated as a major risk for animal and human health as well as for the livestock economy in Northeastern Anatolia, where animal husbandry is intense.

Keywords: *Coxiella burnetii*, Q fever, small ruminants, Turkey

Q fever first has been diagnosed in abattoir workers in Australia in 1935, and was soon considered as a zoonotic disease and a global problem for human and animal health (15, 19). The causative organism is *Coxiella burnetii*, a slow-growing, intracellular bacterium, but the disease is difficult to diagnose, prevent and treat in humans and in animals (7). Payzın and Golem reported the occurrence of Q fever for the first time in Turkey in 1948 and identified *C. burnetii* infection in humans with atypical pneumonia (22). They then investigated the relationship between Q fever in humans and domestic animals.

Domestic ruminants which give birth or abort are the main sources of Q fever infection for humans. However, the reservoir of the infection is large enough to cover free-living mammals and birds (25). Among farm animals, the most susceptible ones to this disease are sheep and goats. *C. burnetii* infection causes significant economic losses for the livestock industry due to abortions and still births and to infertility in adult animals. The disease is mainly transmitted to humans by inhalation of contaminated aerosol particles from parturient or slaughtered ruminants (21). It is stated

that ticks also play a role in the transmission of Q fever among animals. In the experimental studies, some tick species belonging to the *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Ornithodoros* genera have been shown to function as vectors of *C. burnetii* (7).

C. burnetii is a highly infectious pathogen that with difficulty grows in artificial media, so diagnosis of infection must rely on molecular techniques and serological methods, such as indirect fluorescent antibody tests, complement fixation tests and enzyme-linked immunosorbent assay (ELISA). ELISA methods are deemed the most sensitive, easiest to apply, suitable for epidemiological research and valuable for the diagnosis of acute and chronic Q fever (26).

In Turkey, seroepidemiological studies of Q fever have continued and have increased its topicality since the early 1950s. Studies of the small ruminant population in the eastern Anatolia region revealed a seroprevalence in sheep of 22.1% in Erzurum, Kars and Ağrı; 10.5% in Elazığ; 23.3% in Elazığ, Malatya, Bingöl and Muş; 20.0% in the southern Marmara region including Bursa, Balıkesir and Çanakkale and 13.3% in the Marmara region covering 11 different provinces

(4, 12-14, 17). Seroprevalence in goats was 7.6% in Kilis, located in the southeastern Anatolia region (8). These studies, conducted in different years in Turkey, indicated a prevalence of Q fever in small ruminants ranging from 7.6% to 23.3%.

Studies from European countries have revealed seroprevalence in sheep/goats of 1.8/3.4% in Switzerland, 6.0/N% in Hungary and 2.4/7.8% in the Netherlands (3, 9, 18). The total seroprevalence in sheep and goats population was 15.9% in northwestern Italy and 11.4% in the central region of Portugal (1, 24). The rates in countries outside Europe show wide differences; for example, in the sheep/goat population it was 19.5/27.2% in Iran, an Asian country, 10.0/17.2% in Lebanon, one of the Middle Eastern countries and 9.5/3.3% in Bangladesh (2, 5, 23).

Although prevalence rates have been reported from many regions of Turkey, information about the extent of Q fever infection is lacking in small ruminants in the northeastern Anatolian region. The aim of the present study was therefore to investigate the *C. burnetii* seroprevalence on animal-level and herd-level in small ruminants in Artvin, Gümüşhane and Iğdır provinces in 2013 and 2017. The four-year interval was chosen provide the first information about any changes in the prevalence of this disease in this region.

Material and methods

Ethics committee approval and publication permission. In our study, institutional ethics committee approval was not required since blood samples from sheep and goats were taken for diagnostic evaluation. The General Directorate of Food and Control, The Ministry of Forestry and Water Affairs in Turkey has permitted the publication of this study in national or international journals with an official letter dated 04.12.2019 and numbered E.3746139.

Calculation of the sample size. The study material consisted of blood samples taken from sheep and goats in the mixed herds in villages of the Artvin, Gümüşhane and Iğdır provinces in March-April, at a 4 year interval in 2013 and 2017. Since the animals in each village graze on the common pasture, the animal population in each village was evaluated as a single herd. Data on the number of animals and villages belonging to the cities was obtained from the official website of the Turkey Statistical Institute (TUIK) (<https://www.tuik.gov.tr/>). According to the TUIK data, the number of sheep/goats in Artvin, Gümüşhane and Iğdır provinces in 2013 was approximately 640,000, 110,000 and 37,000, the number of villages in these provinces are 320, 318 and 161, respectively. In the study, it was planned to take samples from at least 4% of the herds in each province. A free online sample size calculator (provided by "Maple Tech International LLC", <https://www.calculator.net/sample-size-calculator.html>) was used to determine the sample size. The sample size was determined with a 95% confidence interval (95% CI) and 5% margin of error, taking into account the 8.0% estimated prevalence of Q fever (based on data from Turkey). The minimum blood sample number capable of representing our research was found as

114. For both Artvin, Gümüşhane and Iğdır provinces, blood samples were collected from 573 animals (409 sheep and 164 goats) from 51 herds in 2013 and from 472 animals (384 sheep and 88 goats) from 33 herds in 2017. Since we could not go to some farms due to the insufficient budget allocated for our research for 2017, the number of herds examined in 2017 was lower than in 2013.

Collection and preservation of samples. Blood samples were collected with two-stage cluster sampling from small ruminant animals older than 12 months. All blood was sampled into sterile 5 mL vacuum tubes (without anticoagulation) from the vena jugular of animals. The samples were brought to Erzurum Veterinary Control Institute under cold chain conditions at an average temperature of +4 degrees in vehicle refrigerators and centrifuged there for 10 minutes at 1500 rpm. The separated serum samples were transferred to sterile Eppendorf tubes and stored at -20°C until serological analysis was performed.

Serological analysis. The serum samples were analyzed using a commercial IDEXX ELISA kit (Q fever Ab Test IDEXX Laboratories, United States of America) for the detection of phase I and phase II antibodies specific to *C. burnetii*. Serum samples were analyzed in accordance with the kit protocol. The test results were evaluated as positive if the optical density (OD) values were 40% and above, as doubtful for OD of 30-40%, and negative for OD of 30% and below. Even if there was only one positive animal in any of the herds, that herd was rated positively.

Statistical analysis. A free online sample size calculator (provided by "Maple Tech International LLC" was used to determine the sample size. The Yates' Chi-square (χ^2) test was used to compare the data. A p value < 0.05 was considered statistically significant. Confidence intervals (95% CI) were determined based on percentages.

Results and discussion

The sheep and goats bred in the provinces of Artvin, Gümüşhane and Iğdır in the Northeastern Anatolia region were tested for the seroprevalence of Q fever with the same methodology at a 4-year interval; the first testing was conducted in 2013 and the second in 2017. Table 1 shows that tests were positive for *C. burnetii* IgG antibodies in 5.6% of the sheep and 1.8% of the goats in 2013, and in 24.4% of the sheep and 1.1% of goats in 2017. The total (sheep + goat) prevalence was 4.5% for 2013 (CI: 2.8-6.2%), and this increased about 4.5-fold in 2017 to reach 20.1% (CI: 16.5-23.7%). When the *Coxiella burnetii* IgG seroprevalences of sheep and goats were compared, it was found to be statistically significantly higher in sheep both in 2013 and 2017 (χ^2 : 3.890, p: 0.049 in 2013; χ^2 : 34.782; p: < 0.00001 in 2017). Total *Coxiella burnetii* IgG positivity obtained without discrimination between sheep and goats was found to be significantly higher in 2017 compared to 2013 (χ^2 : 61.436, p: < 0.00001).

Table 2 shows that the seroprevalence rate of *Coxiella burnetii* IgG in small ruminants varied across the three selected provinces in the northeast Anatolian regions. The highest *C. burnetii* IgG seroprevalence

in 2013 occurred in Gümüşhane province (6.8%), followed by Iğdır (6.3%) and Artvin. It was observed that small ruminants in Artvin were more fortunate in terms of the risk of Q fever infection compared to other provinces. *C. burnetii* IgG seroprevalence was found to be significantly lower in animals in Artvin in 2013 compared to animals in Gümüşhane and Iğdır (χ^2 : 7.15; p: 0.028). Four years later, Iğdır was first in seroprevalence, with an increased seroprevalence in all provinces, followed by Gümüşhane and Artvin. IgG antibody seroprevalence was found significantly higher in the animals in Iğdır in 2017 compared to other provinces (χ^2 : 76.59; p: < 0.00001). The province with the highest prevalence change in sheep and goats in 2013 compared to 2017 was Iğdır, with an increase of 7.3-fold, followed by Artvin at 3.8-fold and Gümüşhane at 2.5-fold.

The decrease observed in the number of herds in all provinces in 2017 compared to 2013 occurred as a result of many families combining their herds. Accordingly, the total herd seroprevalence was 29.4% (15/51) in 2013 and 57.6% (19/33) in 2017. The prevalence rates of Q fever in the herds is shown in Table 3.

The seroprevalence on animal-level of Q fever in small ruminants from Artvin, Iğdır and Gümüşhane, was 4.5% in 2013, but this increased to 20.1% in 2017, and this increase was statistically significant. Dorko et al. in Slovakia and Hatchette et al. in Canada also determined that Q fever seroprevalence in sheep is increasing (6, 11). We think that this increase in the

prevalence of IgG antibodies in our case is due to the infected herds in the region grazing on the same pastures with healthy herds and the animals staying together for a long time in the same indoor environment. Some animals were removed from the herd or new individuals were included in the herd before the blood samples were taken; therefore, infected animals that remained in the herd or were included in the herd continued to spread the infection.

A comparison of the total seroprevalence rate (20.1%) determined in 2017 in northeastern Anatolia with other studies conducted in Turkey reveals a relatively lower rate than that reported from Kars and Ağrı, Elazığ, Malatya, Bingöl and Muş. However, our results are higher than the results obtained in the Marmara region and in the southeastern Anatolia region (12, 13, 17). In studies conducted in Turkey, the prevalence of Q fever in small ruminants varies by 7.6% to 23.3%. The seroprevalence in 2017 in our cases representing the northeastern Anatolia region of Turkey fell within the average range for Turkey. Evaluation of our results on a provincial basis showed that Artvin was the least affected province in terms of Q fever, with a low prevalence rate (5.0%), whereas Gümüşhane had close to the average rate for Turkey (16.9%). By contrast, Iğdır had an extremely high rate (46.0%).

Comparison of the seroprevalence rates of Q fever reported from other countries with the results of our study revealed that the total *C. burnetii* seroprevalence we detected in three provinces in 2017 was higher

than the rates reported from European countries, such as Switzerland, Hungary, Netherlands, Northwest Italy and Portugal (1, 3, 9, 18, 24). Between 2013 and 2017, no significant change was observed in the seroprevalence in the goat population, but it increased substantially in sheep, even though the goats and sheep were present in the same herds. Indeed, the seroprevalence in the goat population was very low compared to sheep. By contrast, in Europe, the animal-level prevalence was close for both goats and sheep in Italy and England, whereas it was higher in goats in Portugal, Switzerland and the Netherlands (1, 3, 16, 18, 24).

Herd prevalence is considered to serve more as an indicator of disease when

Tab. 1. *Coxiella burnetii* IgG seroprevalence changes in the sheep and goats over the years*

Years	Sheep		Goat		Total		χ^2	P value
	n	Pos. n (%)	n	Pos. n (%)	n	Pos. n (%)		
2013	409	23 (5.6)	164	3 (1.8)	573	26 (4.5)	3.890	0.049
2017	292	94 (24.4)	88	1 (1.1)	472	95 (20.1)	34.782	< 0.00001

Explanations: * the total number of IgG positive sheep and goats in 2013 and 2017 were compared

Tab. 2. *Coxiella burnetii* IgG seroprevalence changes in the animals according to the provinces and years

Provinces	2013			2017		
	n	Positive n (%)	95% CI	n	Positive n (%)	95% CI
Artvin	224	3 (1.3)	0.0-2.8	180	9 (5.0)	1.8-8.2
Gümüşhane	207	14 (6.8)	3.4-10.2	166	28 (16.9)	11.2-22.6
Iğdır	142	9 (6.3)	2.3-10.3	126	58 (46.0)	37.3-54.7
Yates' χ^2 test results	χ^2 : 7.15; p: 0.028			χ^2 : 76.59; p: < 0.00001		

Tab. 3. *Coxiella burnetii* IgG seroprevalence in the herds according to provinces and years

Province	Artvin		Gümüşhane		Iğdır		Total	
Years	2013	2017	2013	2017	2013	2017	2013	2017
Herd number	18	11	18	15	15	7	51	33
Positive herd number*	3	5	5	8	7	6	15	19
Herd prevalence (%)	16.7	45.5	27.8	53.3	46.7	85.7	29.4	57.6

Explanations: * a single positive animal was considered sufficient to deem a herd positive for infection

compared to animal-level prevalence. An increase in herd-level prevalence is also viewed as a real indicator of control and eradication of the disease. We have seen that herd-level prevalence has increased nearly two-fold in 4 years to reach 57.6%. The seroprevalence in the sheep/goat population was 40.5/24.8% in Italy, 9.7/2.8% in England, 37.5/28.8% in Portugal, 5.0/11.1% in Switzerland and 14.5/17.9% in the Netherlands, indicating a wide variability in herd-level prevalence in terms of countries (1, 3, 16, 18, 24). Factors such as herd management, geographical differences, animal races and methodology differences in diagnosis are the first reasons that come to mind to explain these variations in results. The *C. burnetii* infective agent is known to be carried within inhalable aerosol, so its prevalence is higher in areas open to wind, in herds with high animal density and in areas with high temperatures. People living in these regions will also be at risk for Q fever (20).

Our results show the existence of an important health threat not only for animals but also for the people in these study areas. Ruminants, and especially goats and sheep, are considered primary reservoirs for human infection. For example, Hanssen et al. described human cases in people who live close to dairy goat farms affected by Q fever (10). They also stressed that pregnant animals should be culled to prevent outbreaks in these places. The animals must be closely followed before they become infected, and those that are infected should be immediately treated. However, difficulties in clinical diagnosis may arise because the clinical symptoms of the disease vary or the animals are asymptomatic. We conducted our study on sheep/goat herds in rural areas of Artvin, Gümüşhane and Iğdır provinces. In these regions, people keep their sheep and goats in the pens near their homes and usually express their milk by hand. Close contact of local people with animals poses a serious threat to the health of these people. To eliminate or minimize this threat, sick and symptomatic animals must first be identified and treated.

In conclusion, the results of the present study demonstrate that Q fever seroprevalence has increased in small ruminants in the northeastern Anatolia region of Turkey in terms of both animal-level and herd-level. This study provides the first information about the seroprevalence of Q fever in small ruminant animals from Artvin, Iğdır and Gümüşhane, which are important centres of sheep and goat breeding in Turkey.

References

- Anastácio S., Tavares N., Carolino N., Sidi-Boumedine K., Da Silva G. J.: Serological evidence of exposure to *Coxiella burnetii* in sheep and goats in central Portugal. *Vet. Microbiol.* 2013, 167, 500-505.
- Asadi J., Kafi M., Khalili M.: Seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012. *Vet. Ital.* 2013, 49, 163-168.
- Brom R., Van den, Moll L., Schaik van G., Vellema P.: Demography of Q fever seroprevalence in sheep and goats in The Netherlands in 2008. *Prev. Vet. Med.* 2013, 109, 76-82.
- Cetinkaya B., Kalender H., Ertas H. B., Muz A., Arslan N., Ongor H., Gurçay M.: Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. *Vet. Rec.* 2000, 146, 131-136.
- Dabaja M. F., Greco G., Villari S., Vesco G., Bayan A., Bazzal B. El., Ibrahim E., Gargano V., Sciacca C., Lelli R., Ezzedine M., Mortada H., Tempesta M., Mortada M.: Occurrence and risk factors of *Coxiella burnetii* in domestic ruminants in Lebanon. *Comp. Immunol. Microbiol. Infect. Dis.* 2019, 64, 109-116.
- Dorko E., Pilipčinec E., Rimárová K., Kostovčíková J.: Serological study of Q fever in sheep in the territory of Eastern Slovakia. *Ann. Agric. Environ. Med.* 2010, 17, 323-325.
- Duron O., Sidi-Boumedine K., Rousset E., Moutailler S., Jourdain E.: The importance of ticks in Q fever transmission: What has (and has not) been demonstrated? *Trends Parasitol.* 2015, 31, 536-552.
- Günaydin E., Pekaya S., Müştak H. K., Dalkılıç B.: Investigation of Q fever in Kilis and Şanlıurfa goats by ELISA and Touchdown-PCR. *Ankara Üniv. Vet. Fak. Derg.* 2014, 61, 161-165.
- Gyuranecz M., Dénes B., Hornok S., Kovács P., Horváth G., Jurkovich V., Varga T., Hajtós I., Szabó R., Magyar T., Vass N., Hofmann-Lehmann R., Erdélyi K., Bhidé M., Dán A.: Prevalence of *Coxiella burnetii* in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. *Vector Borne Zoonotic Dis.* 2012, 12, 650-653.
- Hanssen D. A. T., Morroy G., Lange de M. M. A., Wiolders C. C. H., Hoek W. van der., Dijkstra F., Schneeberger P. M.: Notification data and criteria during a large Q-fever epidemic reassessed. *Epidemiol. Infect.* 2019, 147, e191.
- Hatchette T., Campbell N., Whitney H., Hudson R., Marrie T. J.: Seroprevalence of *Coxiella burnetii* in selected populations of domestic ruminants in Newfoundland. *Can. Vet. J.* 2002, 43, 363-364.
- Kalender H.: Elazığ ve komşu illerdeki koyunlarda *Coxiella burnetii* enfeksiyonunun yaygınlığı. *Türk. J. Vet. Anim. Sci.* 2001, 25, 51-55.
- Karagül M. S., Malal M. E., Akar K.: Seroprevalence of Q fever in sheep and goats from the Marmara Region, Turkey. *J. Vet. Res.* 2019, 63, 527-532.
- Kennerman E., Rousset E., Gölcü E., Dufour P.: Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey. *Comp. Immunol. Microbiol. Infect. Dis.* 2010, 33, 37-45.
- Kılıç A., Kalender H., Koç O., Kılınc Ü., İrehan B., Berri M.: Molecular investigation of *Coxiella burnetii* infections in aborted sheep in eastern Turkey. *Iran. J. Vet. Res.* 2016, 17, 41-44.
- Lambert S. L., Smith R. P., Gillard K., Horigan M., Farren C., Pritchard G. C.: Serological survey using ELISA to determine the prevalence of *Coxiella burnetii* infection (Q fever) in sheep and goats in Great Britain. *Epidemiol. Infect.* 2016, 144, 19-24.
- Leloğlu N.: Erzurum Kars ve Ağrı illerinde Q humması üzerinde çalışmalar. *Atatürk Üniv. Ziraat Fak. Derg.* 1977, 8, 113-131.
- Magouras I., Hunninghaus J., Scherrer S., Wittenbrink M. M., Hamburger A., Stärk K. D., Schüpbach-Regula G.: *Coxiella burnetii* infections in small ruminants and humans in Switzerland. *Transbound. Emerg. Dis.* 2017, 64, 204-212.
- Maurin M., Raoult D.: Q fever. *Clin. Microbiol. Rev.* 1999, 12, 518-553.
- Nusinovi S., Frössling J., Widgren S., Beaudeau F., Lindberg A.: Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation. *Epidemiol. Infect.* 2015, 143, 3316-3326.
- Parker N. R., Barralet J. H., Bell A. M.: Q fever. *Lancet* 2006, 9511, 679-688.
- Payzin S., Golem S. B.: 1948. Türkiye'de Q humması (Rapor 1). *Türk. Hij. Den. Biyol. Derg.* 2006, 8, 94-113.
- Rahman M. A., Alam M. M., Islam M. A., Bhuiyan A. K., Rahman A. K.: Serological and Molecular Evidence of Q Fever in Domestic Ruminants in Bangladesh. *Vet. Med. Int.* 2016, 2016, 9098416.
- Rizzo F., Vitale N., Ballardini M., Borromeo V., Luzzago C., Laura Chiavacci L., Mandola M. L.: Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. *Prev. Vet. Med.* 2016, 130, 10-17.
- Woldehiwet Z.: Q fever (coxiellosis): epidemiology and pathogenesis. *Res. Vet. Sci.* 2004, 77, 93-100.
- Wood C., Muleme M., Tan T., Bosward K., Gibson J., Alawneh J., McGowan M., Barnes T. S., Stenos J., Perkins N., Firestone S. M., Tozer S.: Validation of an indirect immunofluorescence assay (IFA) for the detection of IgG antibodies against *Coxiella burnetii* in bovine serum. *Prev. Vet. Med.* 2019, 169, 104698.

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