

Seroprevalence of *Coxiella burnetii* in sheep and goats in the Istrian Region, Croatia

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ABSTRACT

A seroepidemiological study was conducted on the presence of *Coxiella burnetii* (*C. burnetii*) antibodies in sheep and goats in Istria, the largest peninsula in Croatia. Random blood samples were taken from 634 sheep and goats at different localities throughout the region. The aim of the study was to assess the prevalence of *C. burnetii* infection in sheep and goats, which represent the most important reservoir of infection in humans. *C. burnetii* antibody detection was performed by ELISA (LSIVet Ruminant Q Fever-Serum/Milk). Seroprevalence of *C. burnetii* was proven in 6.2% of sheep and 3.5% of goats. Larger herds, poor hygienic conditions on farms, a higher presence of goats in a restricted area, and the northern part of Istria proved to be significant risk factors for the seropositivity of animals. The southern part of Istria is known to have endemic Q fever, but no studies have been carried out so far to explore this issue. To gain a more complete epidemiological picture of Q fever in Istria, studies in humans, especially those professionally exposed to *C. burnetii* infection, should be performed.

Key words: *Coxiella burnetii*; sheep (*Ovis aries aries*); goats (*Capra aegagrus hircus*); Q fever; seroprevalence; Istria, Croatia

Introduction

Q fever is one of the most common zoonoses, presenting as an endemic disease in different geographic regions and climate zones worldwide, except in New Zealand (HILBNIK et al., 1993). It was described for the first time by Derrick in 1937, as a febrile disease of unknown origin in a group of workers in a slaughterhouse in Brisbane, Australia (DERRICK, 1937). The causative agent of Q fever, *Coxiella burnetii*, is an obligate intracellular

bacterium widely present in nature, causing infections in mammals (including humans), as well as in birds, reptiles and fish (NORLANDER, 2000). In humans, *C. burnetii* can cause subclinical infection, as well as acute and chronic forms of disease.

Q fever is usually present in endemic foci, especially in intensive breeding areas. The risk for human infection in endemic areas is higher in

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subjects exposed to frequent close contact with domestic animals or animal products – farmers, abattoir workers, veterinarians, laboratory personnel (MAURIN and RAOULT, 1999). Cattle, goats and sheep represent the most important and frequent source of human *C. burnetii* infection (WOLDEHIWET 2004; MAURIN et al., 1999), although pets, including cats, rabbits, and dogs, can also be potential sources of urban outbreaks (ANGELAKIS and RAOULT, 2010).

Infected animals mostly shed *C. burnetii* during parturition or after abortion, through the placenta and birth fluids. They also shed *C. burnetii* in their faeces, urine, vaginal secretion and milk, from which humans may be contaminated (MAURIN and RAOULT, 1999).

The most important route of Q fever transmission to humans is by inhalation of infected aerosol, followed by direct contact with an infected animal, its placenta and bodily secretions, and indirect contact via animal products such as wool (MAURIN and RAOULT, 1999; ANGELAKIS and RAOULT, 2010). Drinking unpasteurized contaminated milk has induced seroconversion in human volunteers, but without clinical signs of the disease (ARRICAU-BOUVERY and RODOLAKIS, 2005).

Q fever in domestic animals is a chronic, usually asymptomatic disease that can cause reproductive complications. Clinical consequences of *Coxiella* infection in domestic animals are abortion and stillbirth, mostly in sheep and goats, and mastitis and infertility in cattle (ARRICAU-BOUVERY and RODOLAKIS, 2005; AITKEN 1989). In most cases, abortion occurs at the end of the pregnancy, without specific indicative clinical signs. In animals, infection frequently lasts for the entire life, in a more or less dormant state, with periodic increases in *Coxiella* replication during periods of immunosuppression, such as parturition (BYRNE, 1997).

Symptoms of *Coxiella* infection in sheep tend to be transient, followed by a spontaneous remission. Infected sheep usually cease shedding *Coxiella* after a few months, and they are not infectious for other animals in the flock, except during parturition. Epidemiological data indicate that dairy cows and

dairy goats are more frequently chronically infected than sheep, and they represent a more important source of human *C. burnetii* infection (MAURIN and RAOULT, 1999). In cattle and goats, chronic shedding of *C. burnetii* can be expected over months or years (BYRNE, 1997). The seroprevalence of Q fever in humans and animals is not yet known, probably being underestimated due to the different and frequently unrecognized forms of the disease. There are no specific clinical signs of Q fever in animals and humans, so that laboratory diagnostics are necessary to confirm the disease (KOVÁCOVÁ et al., 2000). Van den Brom has shown that the seroprevalence of *C. burnetii* is significantly higher in goats than in sheep. This could mean that goats are perhaps more susceptible to *Coxiella* infection than sheep (VAN DEN BROM et al., 2015). Long-distance wind flow *Coxiella* spread has also been described (MAURIN and RAOULT, 1999; ANGELAKIS and RAOULT, 2010; ELDIN et al., 2017).). *C. burnetii* can survive and remain infectious outside a host in an environment for years, and may be carried by the wind several kilometres from its original source. Thus, Q fever may occur in persons without any known contact with animals. This may explain the appearance of Q fever cases in urban areas, mainly sporadically (MAURIN and RAOULT, 1999; ARRICAU-BOUVERY and RODOLAKIS, 2005).

The first human case of Q fever in Croatia was registered in 1948, while in Istria the first serologically confirmed infection was detected in 1955 (MARETIĆ et al., 1971). Q fever has been a notifiable disease in Croatia since 1954 (Croatian Institute of Public Health), and human and veterinarian health services are obliged to share information, as this zoonosis affects both humans and animals.

The aim of this study was to assess the prevalence of *C. burnetii* antibodies present in sheep and goats in the Istrian Region, and thus gain insight into the naturally acquired immunity to *C. burnetii* in those animal populations.

Materials and methods

Study area. Istria is the largest peninsula in Croatia and on the entire Adriatic coast. More than

88% of its territory belongs to Croatia, which much smaller parts being shared by Slovenia and Italy. The Istrian Region alone covers 2813 km², which is roughly 80% of the territory of Istria. This was the area on which our study was carried out. According to official data from 2014 (Croatian Agricultural Agency), the population of sheep and goats in this area was 16648 sheep and 2 688 goats.

Study design. A two-stage cross-sectional study for *C. burnetii* antibodies in sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*) was performed in the study area. The first stage of the study began from May 2013 until April 2014, and the second one took place between January and December 2015. The localities of the investigated sheep and goat farms were grouped into seven epidemiological areas around the urban centres of Pula, Rovinj, Poreč, Umag, Labin, Pazin and Buzet (Fig. 1). In the first stage of the study, sera samples were collected from 46 sheep flocks and 20 goat flocks. Approximately every tenth animal in each flock was selected for blood collection. The samples were taken at random, but the inclusion criterion was that the animals were at least one year old. In the second stage, only those animals that proved seropositive at the first testing were tested again. We expected this to give us an insight into the dynamics of naturally acquired immunity to *C. burnetii*. Concomitantly with blood sampling in animals, the sheep and goat breeders were asked to fill out a questionnaire about possible risk factors for *C. burnetii* infection. The information collected included the breed of the animals, age (1-2, 3-6 and >6 years), health status, reproductive disorders (occurrence of spontaneous abortion, malformed offspring), the origin of the animals, ear tag number, information on the owners, and hygiene conditions on the farm (poor, good, very good), depending on the hygienic measures implemented on the farm. With regard to the size of the flocks, sheep and goats were divided into three groups: small flock (less than 100 animals), middle-sized flock (101-200 animals), and large flock (more than 200 animals).

Sampling procedures. In both sheep and goats, blood was taken from the jugular vein and centrifuged at 3000 rpm for 5 minutes. The serum

was separated, poured into vials and frozen at -20°C until it was shipped to the Veterinary Institute in Udine, Italy, for serological testing.

Detection of antibodies. The presence of specific phase I and phase II antibodies against *C. burnetii* was determined using Immunoenzyme Indirect ELISA test, according to manufacturer's instructions (LSIVet Ruminant Q Fever Serum/Milk), Life Technologies, Carlsbad, CA, USA). The results were expressed as the ratio between the blood sample and the positive control (S/P) for each blood sample. Titre values > 40 were considered positive. The flocks with at least one seropositive animal were declared positive. An ELISA titre of 40-100 was graded as positive +, a titre of 101-200 as positive ++, a titre of 201-300 as positive +++ and a titre of > 300 as positive ++++.

Statistical analysis. Due to the results obtained by normality testing using the Kolmogorov-Smirnov test, non-parametric tests were used in the further course of analyses. Categorical variables were presented as frequencies and the corresponding percentages, and analysed using the Chi-square test or Fisher's exact test. Fisher's exact test was used to analyse differences in categorical clinical parameters between animal species and regions where there were less than 10 samples per cell. The binary logistic regression model was used to analyse the prediction of seropositive findings among the animals. All p values below 0.05 were considered significant. The software used for the analysis was IBM SPSS Statistics, version 25.0 (<https://www.ibm.com/analytics/spss-statistics-software>).

During blood sampling, the provisions of the Animal Welfare Act (Official Gazette 19/99) were observed, so that the pain inflicted on the animals was minimal. The study of naturally acquired immunity to *C. burnetii* infection in animals was approved by the School of Medicine of the University of Zagreb on September 17th, 2013.

Results

Stage I of the study-initial screening of the animals included 548 sheep and 86 goats, of a total of 6039 sheep and 953 goats on the investigated farms. Sixty-six flocks were investigated, 46

of which were sheep flocks and 20 goat flocks. Seropositivity was found in 37 (5.84%) of the tested animals, 34 sheep (6.2%) and 3 (3.5%) goats (Table 1). The animals were classified by breed, age of animals, size of flock, hygiene conditions on the farms and serology findings (Tables 1, 2 and 3).

In all, there were 6 seropositive flocks of sheep (13%) and one positive flock of goats (5%). The seroprevalence of *C. burnetii* within sheep flocks was 0.5%, 0.6%, 1.0%, 1.3%, 2.3% and 3.2%, respectively. All animals were healthy on the first and second blood samplings.

Table 1. Descriptive and serology indicators on samples of sheep and goats included in the study

Variables	Category	Animals			
		Sheep		Goats	
		N	%	N	%
Region	South	236	43.1	24	27.9
	North	312	56.9	62	72.1
Hygiene conditions on farms	Poor	84	15.3	0	0.0
	Good	445	81.2	86	100.0
	Very good	19	3.5	0	0.0
Size of flock*	Small	162	29.6	55	64.0
	Medium	110	20.1	15	17.4
	Large	276	50.4	16	18.6
Age (years old)	1-2	144	26.3	13	15.1
	3-6	266	48.5	57	66.3
	>6	138	25.2	16	18.6
ELISA	Positive	34	6.2	3	3.5
	Negative	514	93.8	83	96.5

*Size of flock (small 0-100; medium 101-200 and large >200 animals)

Table 2. Differences between the northern and southern parts of the Istrian region with respect to breed of sheep, hygiene conditions on farms, size of flocks and serological findings in sheep: Chi-square test

SHEEP Variables	Category	Region				P
		South		North		
		N	%	N	%	
Breed	Ruda	3	1.3	0	0.0	< 0.001
	East Friesian	0	0.0	58	18.6	
	Istrian	62	26.3	142	45.5	
	Istrian pramenka	78	33.1	14	4.5	
	Romanov	0	0.0	3	1.0	
	Sardinian	0	0.0	42	13.5	
	Jezersko-solcava	79	33.5	53	17.0	
	Pramenka	14	5.9	0	0.0	

Table 2. Differences between the northern and southern parts of the Istrian region with respect to breed of sheep, hygiene conditions on farms, size of flocks and serological findings in sheep: Chi-square test (continued)

Hygiene conditions on farms	Poor	66	28.0	18	5.8	< 0.001
	Good	170	72.0	275	88.1	
	Very good	0	0.0	19	6.1	
Size of flock*	Small	70	29.7	92	29.5	< 0.001
	Medium	10	4.2	100	32.1	
	Large	156	66.1	120	38.5	
ELISA	Positive	2	0.8	32	10.3	< 0.001
	Negative	234	99.2	280	89.7	
Degree of reactions**	1 (+)	2	0.8	12	3.8	< 0.001
	2 (++)	0	0.0	18	5.8	
	3 (+++)	0	0.0	2	0.6	

*Size of flock (small 0-100; medium 101-200 and large >200 animals)

**Degree of reactions (ELISA)

Table 3. Differences between the northern and southern parts of the Istrian region with respect to breed of goats, hygiene conditions on farms, size of flocks and serological findings in goats: Chi-square test

Variables	GOATS Category	Region				P
		South		North		
		N	%	N	%	
Breed	Boer	0	0.0	3	4.8	0.024
	French-alpine	24	100.0	34	54.8	
	Croatian white	0	0.0	5	8.1	
	Croatian coloured	0	0.0	2	3.2	
	Istrian	0	0.0	6	9.7	
	Saanen	0	0.0	1	1.6	
	German improved fawn	0	0.0	11	17.7	
Hygiene conditions on farms	Poor	0	0.0	0	0.0	NA
	Good	24	100.0	62	100.0	
	Very good	0	0.0	0	0.0	

Table 3. Differences between the northern and southern parts of the Istrian region with respect to breed of goats, hygiene conditions on farms, size of flocks and serological findings in goats: Chi-square test (continued)

Size of flock*	Small	8	33.3	47	75.8	< 0.001
	Medium	0	0.0	15	24.2	
	Large	16	66.7	0	0.0	
ELISA	Negative	24	100.0	59	95.2	0.273
	Positive	0	0.0	3	4.8	
Degree of reaction**	1 (+)	0	0.0	1	1.6	0.548
	2 (++)	0	0.0	2	3.2	

*Size of flock (small 0-100; medium 101-200 and large >200 animals)

**Degree of reactions (ELISA)

NA – not applicable

With regard to the age of seropositive animals, young sheep (1-2 years) proved to be more susceptible (22/144) than older age groups (3-6 years; 7/266, >6 years; 5/138) but no statistical significance was found (Table 4). The number of goats was too small for analysis.

As to the size of flocks, this was also found to be relevant. Large flocks with more than 200 animals were seropositive in a significantly higher degree than small- (0-100) and medium-sized (101-200) flocks (Table 4).

Table 4. Binary logistical model of prediction of seropositive findings in animals

	OR	95% CI		P
		Lower	Upper	
Goats vs. sheep	11.33	1.76	73.10	0.011
North vs. south	105.71	13.53	826.07	< 0.001
Poor hygiene conditions on farms	2.63	1.19	5.88	0.017
Larger flock	21.60	5.45	85.59	< 0.001
Age of animal	0.92	0.78	1.09	0.350

Hygiene conditions in which sheep were kept were considered good in 81.2% of flocks, while in 15.3% they were poor. A significant correlation was found between the seropositivity of sheep and poor hygiene conditions (Table 4).

We decided to explore the relationship between the northern and southern endemic parts of the Istrian Region, regarding breed of animals, hygiene

conditions on farms, the size of flocks and serology findings. The dividing line between the northern and southern part of the Istrian Region is marked on Fig. 1. The chi-square test was used for this comparison.

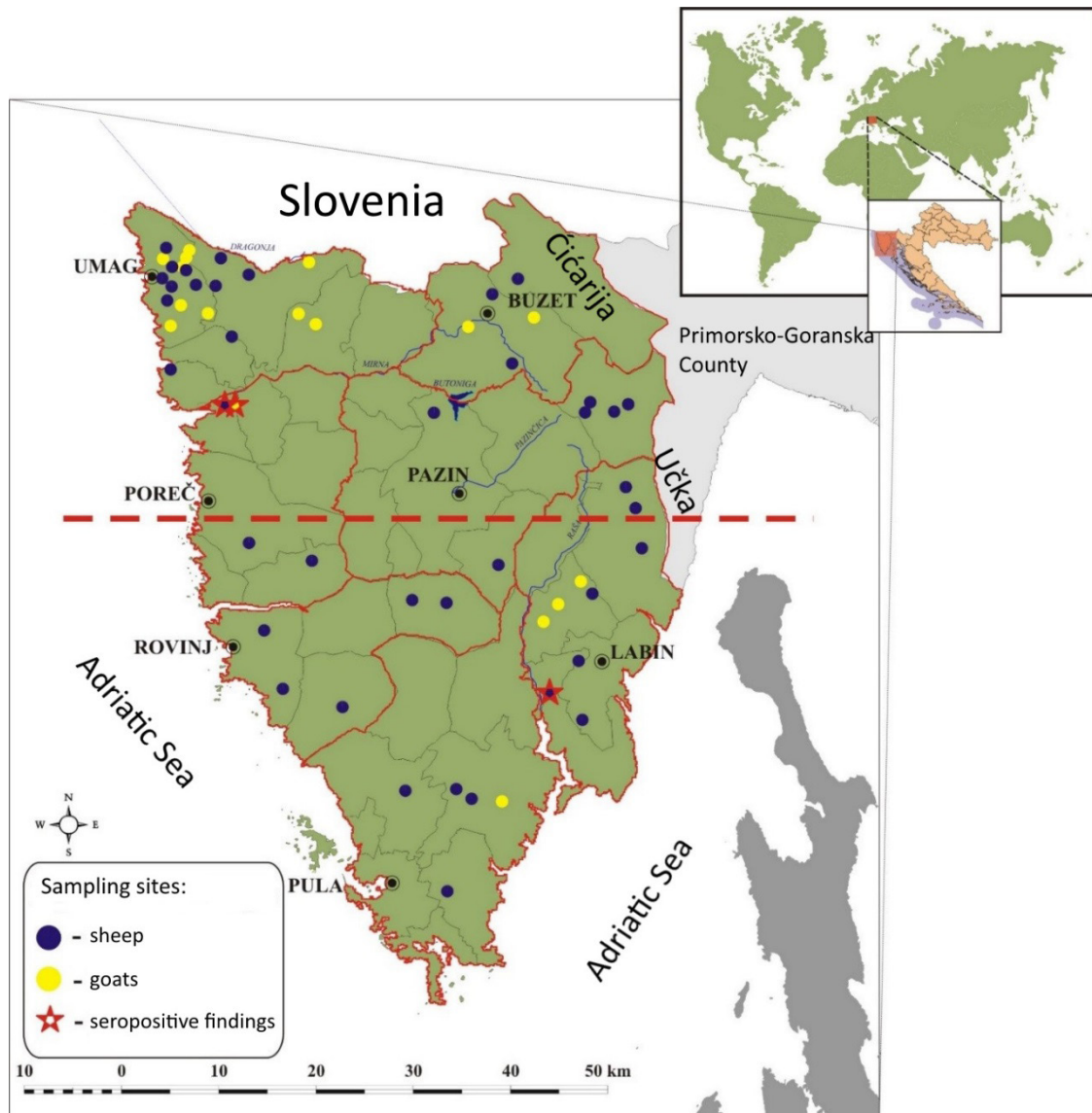


Fig. 1. Map of the Istrian Region showing locations of blood sampling of sheep and goats and seropositive findings

In sheep, significant differences were found with respect to breed (the indigenous Istrian sheep breed prevailed in the northern part), hygiene conditions on farms, which were poorer in the south ($P < 0.001$), the size of flocks which were also larger in the south, ($P < 0.001$), the degree of reaction obtained by ELISA which was higher in the north ($P < 0.001$), and the frequency of positive findings which was also higher in the north (Table 2).

In goats, significant differences were found with respect to the size of flock ($P < 0.001$). It was apparent that larger goat flocks of over 200 animals prevailed in the endemic southern part of Istria, whereas smaller flocks of approximately a dozen animals were found in the north.

Concerning the item in the questionnaire related to reproductive disorders in animals, such as the occurrence of spontaneous abortion, malformed offspring etc., all the owners of the investigated farms denied such occurrences.

The binary logistical model of prediction of seropositive findings in animals showed that poorer hygiene conditions on the farms increased the probability of seropositivity by a factor of 2.63 (OR 2.63; 95% CI 1.19-5.88; P=0.017), followed by larger flock (OR 21.60; 95% CI 5.4-85.59; P<0.001), northern region (OR 105.71; 95% CI 13.53-826.07; P<0.001) and goats, which are probably more susceptible to *Coxiella* infection than sheep (OR 11.3; 95% CI 1.76-73.10; P=0.011) (Table 4).

In stage II of the study, the intention was to retest animals that were seropositive in stage I. However, of 37 such animals, we were able to find only 11 for retesting, 10 sheep and 1 goat, although the owners were warned to pay special attention to seropositive animals from stage I.

Serological testing using the same method showed a significant increase in the concentration of antibodies in four sheep, which might indicate repeated contact with *C. burnetii*. Two sheep and one goat showed a marked decrease in the quantity of antibodies, while in four sheep the concentration of antibodies remained on the same level as in stage I. Considering the small number of animals that were retested, we cannot draw any reliable conclusions regarding the dynamics of naturally acquired immunity.

Discussion

The seroprevalence of *C. burnetii* in sheep in stage I of the study was 6.2%. Some other studies assessing the seroprevalence of *C. burnetii* in sheep found rates ranging from 3.9% in Switzerland (METZLER et al., 1983), 5.03% in Montenegro (LAUŠEVIĆ, 2001), 10.5% in eastern Turkey (ÇETINKAYA et al., 2000), to 20.44% in northern Greece (PAPE et al., 2009). By breed, the highest number of seropositive sheep was found in the indigenous Istrian sheep, with 19/204 positive animals, followed by the East Friesian breed (13/58). Only one seropositive sheep was found in Jezersko-solcava (1/132) and Pramenka (1/92) breeds (Table 2). However, the statistical significance of these breeds was not proven, so that the susceptibility of individual breeds of sheep to *Coxiella* infection remains an open issue.

Our study revealed that 3.5% of the tested goats were serologically positive. Seroprevalence of *C. burnetii* in goats has been investigated in several other countries as well, with the following results: in northern Greece, the presence of antibodies to *C. burnetii* in goats was 6.5%, in Spain 8.7%, in Albania 8.8%, in Northern Ireland 9.3%, on Sardinia 13% and in The Netherlands 21.4% (GUATTEO et al., 2011).

Some authors claim that goats are more susceptible to *Coxiella* infection than sheep (VAN DEN BROM et al., 2015; HACHETTE et al., 2003). This is difficult to corroborate from our data, as we only had 3/86 seropositive goats (Table 3).

A significant correlation was found between hygiene conditions on farms and seropositivity in sheep. Poor hygiene conditions on farms, where sheep were kept in inadequate living conditions, favoured a higher frequency of acquired immunity to *Coxiella* infection. This finding has been corroborated by other authors (SCHIMMER et al., 2011).

All the owners of investigated farms denied any reproductive disorder in their animals, but we believe these responses to be untrue, or at least inconsistent with the literature (ÇETINKAYA et al., 2000; VAIDYA et al., 2010). Çetinkaya et al. demonstrated a statistically significant link between seropositive sheep and abortion data (ÇETINKAYA et al., 2000).

The size of the flock proved to be another significant variable for seropositivity in animals. Larger flocks of sheep had a higher incidence of seropositivity, and the same was observed in goats.

In our study, younger animals showed a higher percentage of seropositivity to *C. burnetii*, unlike in other studies that reported the significantly higher seropositivity of older sheep and goats, due to the greater probability of repeated contacts with this pathogen (ANASTÁCIO et al., 2013).

Of the seven initially defined epidemiological areas (Fig. 1), seropositivity in sheep was found in only two, namely Poreč and Labin. The largest Istrian sheep and goat farm is situated in the Poreč area, with 5 flocks of sheep. In all five, seropositive sheep were detected. The 3 seropositive goats were also on farms in the Poreč area. The other

five epidemiological areas, namely Pula, Rovinj, Umag, Pazin and Buzet, were free of seropositive animals. It is important to note, however, that the two areas in which seropositive animals were found were within 5 km from human settlements, which implies a risk of transmission of the disease to humans. That this risk is real was seen in a major Q fever outbreak in The Netherlands, where intensive breeding of goats took place within 5-8 km from human settlements (VAN DEN BROM et al., 2015; ROEST et al., 2011; SCHIMMER et al., 2011).

Schimmer et al. proved a higher risk of seroconversion in goats raised on farms with more than 800 animals, suggesting that the agent of Q fever is easily transmitted under conditions where the animals are crowded in a limited area, often under inadequate living conditions (SCHIMMER et al., 2011). In our study, we also witnessed the grossly inadequate conditions in which the animals are kept on certain farms. Overcrowded environments, along with poor hygiene and microclimatic conditions represent a significant risk to the health of these animals. Such animals live under stress, become nervous, are prone to injuries, as well as diseases in general, and the mortality rate is higher.

In Croatia, several studies have previously been carried out on domestic ruminants seropositive to Q fever. From 1984 to 1986, Kovačić and Borčić took blood samples from 1001 sheep and 1632 goats from different parts of Croatia, and found the presence of antibodies to *C. burnetii* in 19.2% of sheep and 21.9% of goats (KOVAČIĆ and BORČIĆ, 1988). Istria was not included in this study. In 2008, the CFT method was used in the Istrian Region which proved a 9.16% seropositivity rate to *C. burnetii* in sheep and goats (NEMARIĆ, 2009).

Serological testing of individual animals within a flock has certain drawbacks. Namely, a negative finding does not necessarily mean that the animal was not in contact with *C. burnetii*, and vice versa, a positive finding is not a definitive indicator of disease in the animal. Some animals remain seropositive many years after acute infection, which is proof of a former infection, while others do not produce

antibodies at all. Moreover, *C. burnetii* can also be secreted into the environment by seronegative animals (BERRI et al., 2001). The same authors cite a weakened humoral immunity against *C. burnetii* as the reason for this phenomenon. In such animals, *C. burnetii* may remain localized in the placenta or the uterus without inducing serum antibodies. Also, infected sheep secrete *C. burnetii* in large numbers during normal parturition, and thus contaminate the environment (BERRI et al., 2001). In order to prove the disease in a flock, alongside serological diagnostics, the agent must be proven by molecular diagnostics (ROEST et al., 2013). Serological methods are appropriate for infection diagnostics on the level of a flock.

For the purpose of animal and human safety, it is of outmost importance to regularly apply hygienic measures on farms, and to use protective equipment during contact with animals.

Finally, persons at occupational risk must be properly educated about the enforcement of biosafety measures (ROEST et al., 2011).

Since the study included approximately 10% of sheep and goats from the selected flocks, it can be assumed that the real number of seropositive sheep and goats in the flocks is much larger. In view of the fact that all the tested animals represent only 3.2% of the total number of sheep and goats in the entire region, and that other species, such as cattle, dogs, cats, ticks and others are also susceptible to *Coxiella* infection, we believe that the number of seropositive animals is in reality substantially greater. Therefore, we can assume that the epizootiological situation regarding Q fever in Istria represents a major health challenge.

A zoonosis such as Q fever represents a public health hazard for the urban population as well. It is well known that humans can contract a *Coxiella* infection even if they are not in close contact with animals, nor professionally exposed to infection. For the occurrence of infection in humans it is sufficient to hike along roads on which sheep or goats have stayed for a period of time, or to be exposed to strong winds carrying the agent (RYAN, 2016; ELDIN et al., 2017).

Epidemiological studies of infections in animals are important, because in this way we broaden our

knowledge of potential infection of humans, and are in a better position to devise means of prevention (ÇETINKAYA et al., 2000).

More in-depth knowledge of clinical manifestations of Q fever, its pathogenesis, and the immune response of the infected host, as well as achieving close cooperation between human and veterinary medicine, are required to manage this still mysterious disease more effectively in endemic areas.

Conclusion

The results of this study confirmed the presence of antibodies against *C. burnetii* in sheep and goats in the entire Istrian Region, not only in the previously known endemic southern part. The risk factors which significantly increase the likelihood of seropositivity in animals are poor hygiene conditions on farms, larger flocks, a greater presence of goats in a confined area, and the northern part of Istria. As the study was only conducted on 3.27% of the total sheep and goat population, we assume that the proportion of this zoonosis in the Istrian Region is substantially greater. Although *C. burnetii* cannot be eradicated, prevention of Q fever in humans can largely be achieved by gaining in-depth knowledge of the nature of this zoonosis and implementing preventive measures to suppress the appearance of this infection in domestic animals.

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References

- AITKEN, I. D. (1989): Clinical aspects and prevention of Q fever in animals. *Eur J Epidemiol.* 5, 420-424.
- ANASTÁCIO S., N. TAVARES, N. CAROLINO, K. SIDI-BOUMEDINE (2013): Serological evidence of exposure to *Coxiella burnetii* in sheep and goats in central Portugal. *Vet Microbiol.* 167, 500-505.
DOI: org/10.1016/j.vetmic. 2013.08.004.
- ANGELAKIS, E., D. RAOULT (2010): Q fever. *Vet Microbiol.* 140, 297-309.
DOI: 10.1016/j.vetmic. 2009.07.016.
- ARRICAU-BOUVERY, N., A. RODOLAKIS (2005): Is Q fever an emerging or re-emerging zoonosis? *Vet Res.* 36, 327-349.
DOI: 10.1051/vetres: 2005010.
- BERRI, M., A. SOURIAU, M. CROSBY, D. CROCHET, P. LECHOPIER, A. RODOLAKIS (2001): Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *Vet Rec.* 48, 502-505.
DOI: 10.1136/vr.148.16.502.
- BYRNE, W. R (1997): Q fever. In: Medical aspects of chemical and biological warfare. (Sidell, F. R., E.T. Takafugi, D.R. Franz, Eds.), TMM Publications, Washington, pp. 523-537.
- ÇETINKAYA, B., H. KALENDER, H. B. ERTAS, A. MUZ, N. ARSIAN, H. ONGOR (2000): Seroprevalence of Coxiellosis in cattle, sheep and people in the east of Turkey. *Vet Rec.* 146, 131-136.
DOI: 10.1136/vr 1465.131.
- DERRICK, EH. (1937): Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Med J Aus.* 2, 281-299.
- ELDIN, C., C. MELENOTTE, O. MEDIANNIKOV, E. GHIGO, M. MILLION, S. EDOUARD (2017): From Q fever to *Coxiella burnetii* infection: a paradigm change. *Clin Microbiol Rev.* 30, 115-190.
- GUATTEO, R., H. SEEGER, A. F. TAUREL, A. JOLY, F. BEANDEAU (2011): Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. *Vet Microbiol.* 149, 1-16.
DOI: 10.1016/j.vetmic. 2010.10.1007.
- HATCCHETTE T., N. CAMPBELL, R. HUDSON, D. RAOULT, T. J. MARRIE (2003): Natural history of Q fever in goats. *Vector Borne Zoonotic Dis.* 3, 11-15.
DOI: 10.1089/153036603765627415.
- HILBINK, F., M. PENROSE, E. KOVACOVA, J. KAZAR (1993): Q fever is absent from New Zealand. *Int J Epidemiol.* 22, 945-949.
DOI: 10.1093/ije/22.5.945.
- CROATIAN AGRICULTURAL AGENCY (2014): Sheep farming, goat farming, and small animals. Annual report. 1-112.
- KOVÁCOVÁ, E., J. KAZÁR (2000): Rickettsial diseases and their serological diagnosis. *Clin Lab.* 46, 239-245.
- KOVAČIĆ, H., B. BORČIĆ (1988): Q fever in domestic animals and people. *Vet. stanica.* 19, 209-216. (In Croatian)
- LAUŠEVIĆ, D. (2001): Prevalence of *Coxiella burnetii* antibodies in sheep in the territory of Montenegro. *Acta Veterinaria.* 51, 49-56.
- MARETIĆ, Z., I. BOROVEČKI, M. OGRIZEK (1971): Q fever epidemic in Pula and surroundings in 1969. *G Mal Infett Parassit.* (In Italian)

- MAURIN, M., D. RAOULT (1999): Q fever. Clin Microbiol Rev 12, 518-553.
- METZLER, A. E., J. NICOLET, H. U. BERTSCHINGER, R. BRUPPACHER, J. GELZER (1983): Distribution of *Coxiella burnetii*: a seroepidemiological study of domestic animals and veterinarians. Schweiz Arch Tierheilkd. 125, 507-517.
- NEMARIĆ, N. (2009): Prevalence of Q fever in North Croatian Littoral and Mountain Croatia. Thesis. University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia. (In Croatian)
- NORLANDER, L (2000): Q fever: epidemiology and pathogenesis. Microbes Infect. 2, 417-424.
DOI: 10.1016/S1286-4579(00)00325-7.
- PAPE, M., E. G. BOUZALAS, G. S. KOPTOPOULOS, K. MANDRAVELI, M. ARVANITIDOU-VAGIONA, P. NIKOLAIDIS, S. ALEXIOU-DANIEL (2009): The serological prevalence of *Coxiella burnetii* antibodies in sheep and goats in northern Greece. Clin Microbiol Infect. 15, 146-147.
DOI: 10.1111/j.1469-0691.2008.02159.x.
- ROEST, H. I. J., J. J. TILBURG, W. VAN DER HOEK, P. WELLEMA, F. G. VAN ZIJDERVELD, C. H. KLAASSEN, D. RAOULT (2011): The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol Infect. 139, 1-12.
DOI: 10.1017/S0950268810002268.
- ROEST, H. I. J., A. BOSSERS, F. G. VAN ZIJDERVELT, M. L. REBEL (2013): Clinical microbiology of *Coxiella burnetii* and relevant aspects for the diagnosis and control of the zoonotic disease Q fever. Vet Quart. 33, 148-160.
DOI: 10.1080/01652176.2013.843809
- RYAN, J. R. (2016): Biosecurity and bioterrorism. Containing and preventing biological threats. Butterworth-Heinemann, Oxford, pp 109.
- SCHIMMER, B., S. LUTTIKHOLT, J. L. HAUTVAST, E. A. GRAAT, P. VELLEMA, Y. T. DUYNHOVEN (2011): Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010. BMC Vet Res. 7, 81-94.
DOI: 10.1186/1746-6148-7-81.
- VAIDYA, V. M., S. V. S. MALIK, K. N. BHILEGAONKAR, R. S. RATHORE, SIMRANPREET KAUR, S. B. BARBUDDHE (2010): Prevalence of Q fever in domestic animals with reproductive disorders. Comp. Immun. Microbiol. Infect. Dis. 33, 307-321.
DOI: 10.1016/j.cimid.2008.10.006.
- VAN DEN BROM, R., E. VAN ENGELEN, H. I. J. ROEST, P. VELLEMA (2015): *Coxiella burnetii* infections in sheep and goats: an opinionated review. Veterinary Microbiology. 181, 119-129.
DOI: 10.1016/j.vetmic.2015.07.011.
- WOLDEHIWET, Z. (2004): Q fever (coxiellosis): epidemiology and pathogenesis. Res Vet Sci. 77, 93-100.
DOI: 10.1016/j.rvsc.2003.09.001.

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SAŽETAK

U radu prikazani su rezultati seroepidemiološkog istraživanja prisutnosti protutijela protiv bakterije *Coxiella burnetii* (*C. burnetii*) kod ovaca i koza. Uzeto je slučajnim odabirom 634 uzoraka krvi ovaca i koza s različitih područja Istarske županije, najzapadnije regije u Hrvatskoj. Cilj istraživanja bio je procijeniti proširenost infekcije koksijelom kod tih životinja koje predstavljaju glavni rezervoar infekcije za ljude. Prisutnost protutijela protiv *C. burnetii* utvrđena je serološkom metodom ELISA (LSIVet Ruminant Q Fever-Serum/Milk). Prevalencija *C. burnetii* bila je 6,2% u ovaca i 3,5% u koza. Veća stada, lošiji higijenski uvjeti na farmi, veća prisutnost koza na jednom ograničenom području te sjeverna regija Istre dokazani su kao statistički znakoviti čimbenici rizika za seropozitivnost životinja. Poznato je da je južni dio Istre endemski za Q-groznicu, ali do sada nije provedeno istraživanje koje bi obuhvatilo cijelo područje Istarske županije. Da bi se dobila još kompletnija epidemiološka slika Q-groznice na ovom području, trebalo bi uključiti u istraživanje i ljude, osobito one koji su profesionalno eksponirani ovoj bolesti.

Ključne riječi: *Coxiella burnetii*; ovce (*Ovis aries aries*); koze (*Capra aegagrus hircus*); seroprevalencija; Istra; Hrvatska
