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SEROLOGICAL DETECTION OF ANTI-COXIELLA BURNETII ANTIBODIES IN ROMANIAN SMALL RUMINANTS

DETECTARE SEROLOGICĂ A ANTICORPILOR ANTI-COXIELLA BURNETII LA RUMEGĂTOARE MICI DIN ROMÂNIA

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ABSTRACT | REZUMAT

Coxiella burnetii is the agent of Q fever, an important zoonosis, maintained in nature in various domestic and wild mammals, birds, reptiles, and ticks. Domestic animals, especially ruminants (cattle, sheep, and goats) are the most important sources of human infections, but the role played in the *C. burnetii* epidemiology by each species is generally unknown. Data concerning the natural reservoir of *C. burnetii* in Romanian counties are limited and more studies are recommended to add knowledge in the role played by each species in specific areas. In this survey, a commercial iELISA kit was used to detect antibodies to *C. burnetii* in 474 small ruminant serum samples from 16 Romanian counties and the municipality of Bucharest. The selection of goats and sheep flocks was based on the recent history (up to 12 month) of reproductive disorders, whose aetiology was not established with certainty and without clinical suspicion of Q fever. To cover as many animals as possible, sample testing was done in pools. A pool included only sera from animals from the same flock. The administrative divisions of the 140 pools included in the survey were as follows: Alba (n=15), Arad (n=4), Argeș (n=10), Brăila (n=30), Bucharest (n=13), Călărași (n=1), Caraș Severin (n=7), Constanța (n=2), Dâmbovița (n=4), Giurgiu (n=5), Maramureș (n=2), Mureș (n=6), Olt (n=4), Sibiu (n=15), Timiș (n=14), Vâlcea (n=4), Vrancea (n=4). Out of 98 sheep tests, anti-*Coxiella burnetii* antibodies were in 24.49 % (n=24), while 2.04% (n=2) were suspect and 73.47% (n=72) negative. Out of 42 goat tests, 14.28% (n=6) were positive and 85.72% (n=36) negative. Out of the total serological tests, 21.43% (n=30) were positive, 1.43% (n=2) suspect and 77.14% (n=108) negative. Out of 17 administrative divisions, in 76.47% (n=13) there were detected seropositive small ruminants, respectively: Sibiu, Brăila, Giurgiu, Arad, Timiș, Caraș Severin, Olt, Alba, Mureș, Maramureș, Vrancea, Călărași, and the municipality of Bucharest. The following counties revealed no seropositive samples: Vâlcea, Argeș, Dâmbovița and Con-

Coxiella burnetii este agentul etiologic al febrei Q, o zoonoză importantă, menținută în natură de diverse mamifere domestice și sălbatice, păsări, reptile și căpușe.Animalele domestice, în special rumegătoare (bovine, ovine și caprine) sunt cele mai importante surse de infecție pentru om, iar rolul jucat în epidemiologia *C. burnetii* de către fiecare specie este în general necunoscut. Datele privind rezervorul natural de *C. burnetii* în județele din România sunt limitate și se recomandă efectuarea mai multor studii în vederea înțelegerii rolul jucat de fiecare specie în anumite regiuni. În cadrul acestui studiu, a fost folosit un kit iELISA comercial pentru detectarea anticorpilor anti-*C. burnetii*, în 474 probe de ser recoltat de la rumegătoare mici de pe raza a 16 județe din România și din municipiul București. Selecția turmelor de capre și ovine s-a bazat pe istoricul recent (de până la 12 luni) al existenței tulburărilor de reproducere în acel efectiv, a căror etiologie nu a fost stabilită cu certitudine și la care suspiciunea clinică de febră Q nu a fost formulată. Pentru a acoperi un efectiv cât mai mare de animale, testarea acestor seruri s-a realizat în pool-uri. Pool-urile au fost constituite doar din seruri provenite de la animale din aceeași turmă. Unitățile administrativ-teritoriale de origine ale celor 140 de pool-uri incluse în studiu au fost următoarele: Alba (n=15), Arad (n=4), Argeș (n=10), Brăila (n=30), București (n=13), Călărași (n=1), Caraș Severin (n=7), Constanța (n=2), Dâmbovița (n=4), Giurgiu (n=5), Maramureș (n=2), Mureș (n=6), Olt (n=4), Sibiu (n=15), Timiș (n=14), Vâlcea (n=4), Vrancea (n=4). Din cele 98 de pool-uri ovine testate serologic, 24,49% (n=24) au fost pozitive, 2,04% (n=2) au fost suspecte, iar 73,47% (n=72) au fost negative. Din cele 42 de pool-uri caprine testate serologic, 14,28% (n=6) au fost pozitive și 85,72% (n=36) au prezentat rezultate negative. Din totalul pool-urilor testate serologic, 21,43% (n=30) au fost pozitive, 1,43% (n=2) au fost suspecte, iar 77,14% (n=108) au fost negative. Din cele 17 unități administrativ-teritoriale cercetate, în 76,47% (n=13) au fost înregistrate rezultate seropozitive, respectiv: Sibiu, Brăila, Giurgiu, Arad, Timiș, Caraș Severin, Olt, Alba, Mureș, Maramureș, Vrancea, Călărași și municipiul București. Rezultate negative s-au înregistrat în județele Vâlcea, Argeș, Dâmbovița și Constanța. În acest studiu a fost demonstrată prezența an-

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stanța. Anti-*Coxiella burnetii* antibodies shown in healthy small ruminants (sheep and goats) suggest a previous exposure to *C. burnetii,* as well as a possible circulation of the bacteria in the area.

Keywords: *Coxiella burnetii*, iELISA, sheep and goat diseases, zoonosis

ticorpilor anti-*C. burnetii* la animale sănătoase, ceea ce sugerează expunerea animalelor la această bacterie, precum și posibila circulație a bacteriei în areal.

Cuvinte cheie: *Coxiella burnetii*, iELISA, bolile ovinelor și caprinelor, zoonoze

Coxiella burnetii is the agent of Q fever, a neglected zoonosis reported in many regions, including Romania (3, 4, 7, 8, 10, 11, 12). Since 1937, when the first report of the Q fever ("query fever") was recorded in Australian slaughterhouse workers (5), knowledge about this disease and its etiological agent has increased radically (6). However, information of the *C. burnetii* epidemiology (e.g., potential reservoir animals in specific areas) and its interactions with the infected host are still limited (9, 12). Domestic ruminants (cattle, sheep, and goats) proved to be the main host reservoirs in various endemic areas (2, 7, 9), but several other species of animals (domestic mammals, marine mammals, reptiles, birds, and ticks) have been reported to shed the *C. burnetii* (1, 6, 13), and their role in transmitting infection to humans should be better documented. *C. burnetii* interactions with the infected host, humans and animals, is expressed through several nonspecific clinical symptoms, making diagnosis challenging (1, 6, 12). Although several PCR-based assays have been developed, serology is still the most common method for testing for *C. burnetii* infection and the first-line diagnostic technique (6). Possibility of *C. burnetii* circulation in Romanian small ruminant herds was recently analysed (2), but the available data does not allow the prevalence and distribution of contaminated flocks to be determined. In order to increase the quantity of information in the *C. burnetii* circulation in small ruminant herds, in this survey serological investigation was performed following a methodology previously described (2).

MATERIALS AND METHODS

Geographic area, target population and samples used in survey

The blood samples included in this study were collected from clinically healthy goats and sheep (Table 1) in 16 different counties and the municipality of Bucharest (Fig. 1). The serological results of samples from Alba, Arad, Argeș, Călărași, Caraș Severin, Dâmbovița, Maramureș Olt, Timiș, Vâlcea and Vrancea counties are for first time presented in this survey. Samples from Brăila, Constanța, Giurgiu and Sibiu counties were investigated and preliminary results were presented in a previous paper (2), and included in this survey for a better analysis of our results.

Table 1

The blood samples included in survey

County	Number of blood samples	
	Sheep	Goats
Alba	15	0
Arad	16	0
Arges	6	4
Brăila	55	95
Bucharest	28	14
Călărași	5	0
Caraș Severin	32	0
Constanța	0	10
Dâmbovița	0	14
Giurgiu	6	15
Maramureș	2	0
Mureș	6	0
Olt	2	2
Sibiu	75	0
Timiș	64	0
Vâlcea	2	2
Vrancea	4	0
Total:	**318**	**156**

Fig. 1. The geographic distribution of goats and sheep herds included in the survey. Yellow colour: counties with tested samples

The selection of goats and sheep herds was based on the recent history of reproductive disorders, whose aetiology was not established with certainty and without clinical suspicion of Q fever. Blood specimen collection was in accordance with the Directive 2010/63/EU of the European Parliament and of the Council.

Serological assay

Serum samples were investigated with an indirect ELISA (Q-fever *Coxiella burnetii* Antibody test kit (IDEXX Laboratories, Switzerland) able to detect antibodies against *C. burnetii*, according to the manufacturer's instructions. A brief description of the assay was presented in our previous paper (2).

RESULTS AND DISCUSSIONS

Because *C. burnetii* infections are generally subclinical or confused with more common diseases, the prevalence of infections in humans and animals is often underestimated (7). A previous study in four Romanian counties reported seropositive small ruminants (9.62%, 5/52 positive pools) in three out of four counties. The previous results indicated that *C. burnetii* may be present in several places and the exposure risk of human and animals could be considered (2). In this study, the results are indicating that infections are widespread among small ruminants in all Macroregions (Table 2), with seropositive results recorded in other nine counties and in the municipality of Bucharest.

The results were grouped and analysed by using the Romanian Macroregions, as follows: Macroregion 1 - Northwest and Center, Macroregion 2 - North-East and South-East, Macroregion 3 - South-Muntenia and Bucharest-Ilfov, Macroregion 4 - Southwest and West (https://en.wikipedia.org/wiki/NUTS_statistical_regions_of_Romania).

The serum samples collected from 318 sheep were distributed in 98 pools (Table 2). The serum samples collected from 156 goats were distributed in 42 pools (Table 2) that were tested using the ELISA method.

The samples of Macroregion 1 provided positive results in all tested counties: Alba, Maramureș, Mureș and Sibiu. The samples from Alba county were from 15 sheep. All samples were individually tested and the results revealed four positive (27%) and 11 negative samples (73%). From Maramureș county we tested only two sheep serum samples and one was seropositive. In Mureș, six sheep serum samples were individually tested, and the serological assay revealed three positive and three negative results. In our previous survey, from Sibiu county 75 samples supplied from sheep farms were tested, grouped in 15 pools of 5 serums each. The serological assay revealed one positive pool and 14 negative pools. These results indicate the presence of anti-*C. burnetii* antibodies among the sheep flocks from this county (2).

The samples of Macroregion 2 were positive in Brăila and Vrancea counties and negative in Constanța. In Brăila, out of 55 sheep samples (11 pools) and 95 goat samples (19 pools), 19.00% (n=2) of the sheep pools were seropositive, while goat serum pools were all negative. A percentage of 7.00% of the total serum pools tested from this county were positive. From Vrancea four sheep samples were collected. Results indicated three positive pools and a negative one. In Constanța all samples were from one goat farm and all results were negative.

The samples of Macroregion 3 provided positive results in Călărași, Giurgiu, and the municipality of Bucharest and negative results in Argeș and Dâmbovița counties. The samples from Călărași were collected from five sheep and were distributed in one pool. The result was positive. In Giurgiu, six sheep serum samples were tested in two pools, and 15 goat samples were tested in three pools. Both sheep pools were negative, while two out of the three goat pools were positive. From the municipality of Bucharest we tested 28 sheep and 14 goat samples collected from 3 different administrative units (Sector 1, Sector 3 and Sector 6). Samples were distributed in 13 pools (six goat sample pools and seven sheep samples pools). Two goat pools and three sheep pools were positive, and eight pools were negative. In Argeș six sheep samples were tested (provided by different sheep farms) and four goat samples, and all results were negative. In Dâmbovița, 14 goat samples were distributed in four pools. All samples coming from this county revealed no trace of anti-*Coxiella* antibodies.

The samples of Macroregion 4 provided positive results in Arad, Caraș Severin, Olt and Timiș counties, and negative results in Vâlcea. From Arad county, 16 sheep samples were distributed in four pools of 3-5 serums each. Out of the four pools, one was positive, two pools were negative, and one was suspect. In Caraș Severin, 32 sheep samples were tested in seven pools and two were positive. In Olt, we tested two sheep samples and two goat samples. The serological assay revealed seropositive results in all samples. In Timiș, 64 sheep samples were tested in 14 pools. One pool was seropositive, one was suspect and 12 nega-

Table 2

Results of serological investigation with iELISA Q-fever *Coxiella burnetii* Antibody test kit in Romanian sheep and goat herds from 16 Romanian counties and the municipality of Bucharest

County	Species	Total pools	Results					
			Positive		Negative		Suspect	
			#	%	#	%	#	%
Macroregion 1- Northwest and Center								
Alba	sheep	15	4	26,67	11	73,33	0	0,00
Maramureș	sheep	2	1	50,00	1	50,00	0	0,00
Mureș	sheep	6	3	50,00	3	50,00	0	0,00
Sibiu*	sheep	15	1	6,67	14	93,33	0	0,00
Macroregion 2- North-East and South-East								
Brăila*	sheep	11	2	18,18	9	81,82	0	0,00
	goats	19	0	0,00	19	100	0	0,00
Vrancea	sheep	4	3	75,00	1	25,00	0	0,00
Constanța*	goats	2	0	0,00	2	100	0	0,00
Macroregion 3- South-Muntenia and Bucharest-Ilfov								
Argeș	sheep	6	0	0,00	6	100	0	0,00
	goats	4	0	0,00	4	100	0	0,00
Bucharest	sheep	7	3	42,86	4	57,14	0	0,00
	goats	6	2	33,33	4	66,67	0	0,00
Călărași	sheep	1	1	100	0	0,00	0	0,00
Dâmbovița	goats	4	0	0,00	4	100	0	0,00
Giurgiu*	sheep	2	0	0,00	2	100	0	0,00
	goats	3	2	66,67	1	33,33	0	0,00
Macroregion 4- Southwest and West								
Arad	sheep	4	1	25,00	2	50,00	1	25,00
Caraș Severin	sheep	7	2	28,57	5	71,43	0	0,00
Olt	sheep	2	2	100	0	0,00	0	0,00
	goats	2	2	100	0	0,00	0	0,00
Timiș	sheep	14	1	7,14	12	85,71	1	7,14
Vâlcea	sheep	2	0	0,00	2	100	0	0,00
	goats	2	0	0,00	2	100	0	0,00
Total		**140**	**30**	**21.43**	**108**	**77.14**	**2**	**1.43**

*Previously published data (Baraitareanu et al., 2018)

tive. From Vâlcea, two sheep samples and two goat samples were collected and individually tested. The serological assay revealed only negative results.

Out of the 16 counties, in 12 counties were found seropositive reactors: Sibiu, Brăila, Giurgiu, Arad, Timiș, Caraș Severin, Olt, Alba, Mureș, Maramureș, Vrancea and Călărași. Also, seropositive results were found in Bucharest as well.No anti-*C. burnetii* antibodies were found in Vâlcea, Argeș, Dâmbovița and Constanța (Fig. 2). The serological assay revealed the presence of anti-*Coxiella burnetii* antibodies in 24.49% of the sheep pools (24/98), suspect results were found in 2.04% (2/98), while 73.47% of the pools were negative (72/98) (Fig. 3).

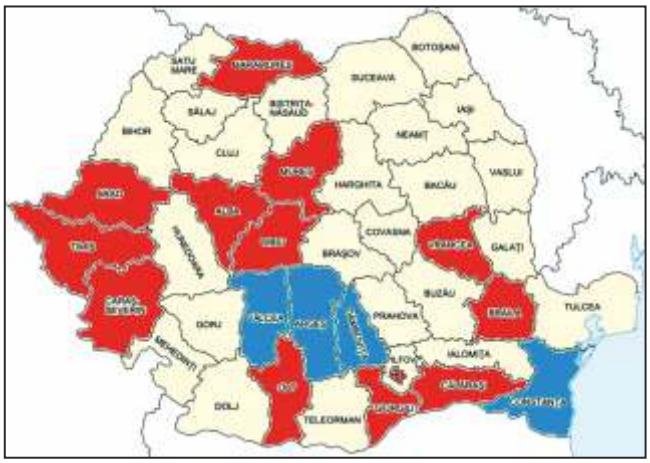

Fig. 2. Geographic distribution of seropositive indirect ELISA tests for anti-*Coxiella burnetii* antibodies. Red colour: counties with positive results; Blue colour: counties with negative results

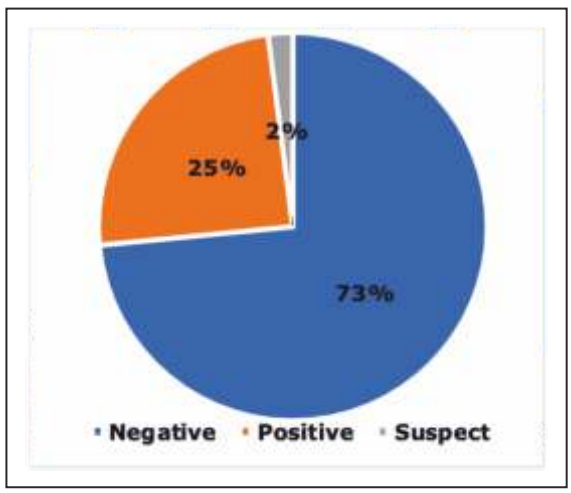

Fig. 3. ELISA results for anti-*C. burnetii* antibodies found in sheep, in percentages

The results revealed the presence of anti-*C. burnetii* antibodies in 14.28% of the goat pools (6/42).

No specific antibodies were found in 82.72% of the pools (36/42) (Fig. 4). Out of the total pools included in this study, 21.42 % were seropositive (30/140), 1.44% were suspect (2/140), and 77.14% were negative (108/140) (Fig. 5).

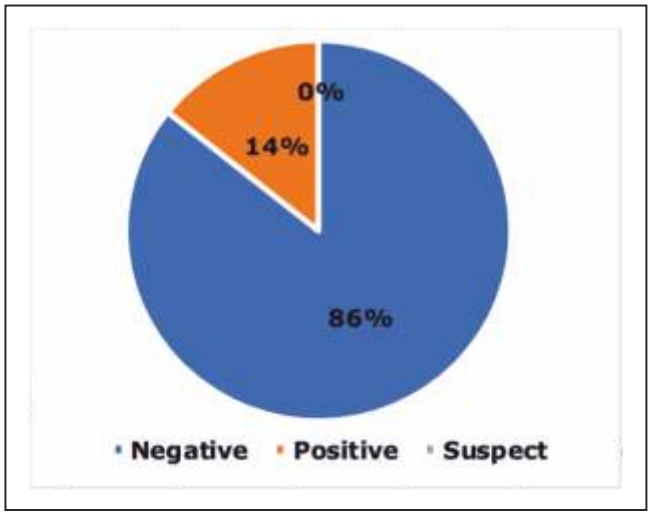

Fig. 4. ELISA results for anti-*C. burnetii* antibodies found in goats, in percentages

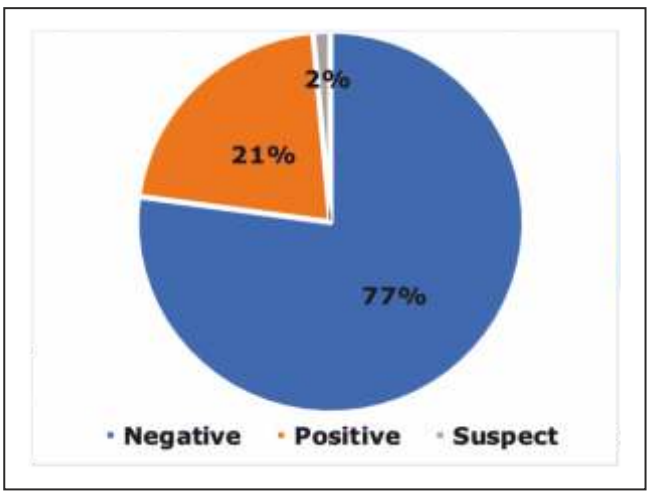

Fig. 5. ELISA results for anti-*C. burnetii* antibodies in small ruminants, in percentages

This study indicated the presence of anti-*C. burnetii* antibodies in various small ruminant flocks in Romania. Although animals included in the survey showed no clinical signs of Q fever, and the prevalence is under 50%, the potential zoonotic risk should be considered, especially due to the persistent shedding of the bacteria by infected animals.

Considering the zoonotic risk, active surveillance of *C. burnetii* infections should be performed among ruminant flocks in Romania. The bacteria are usually excreted by infected animals through milk, urine, fae-

ces, genital fluids and placenta – up to one billion bacteria per gram (12), and animals handlers, as well as slaughter house workers, should be aware of the risk and control measures need to be set up.

For a more accurate prevalence of *C. burnetii* further studies should be performed on a large number of biological samples collected from uniformly distributed flocks, as well as individual animal studies for the seropositive ones.

People in an occupation that deals with livestock, including veterinarians, meat-processing technicians, sheep and dairy workers, livestock farmers and sheep and livestock researchers, should be tested as well.

CONCLUSIONS

Out of 98 sheep tests, anti-*Coxiella burnetii* antibodies were found in 24.49 % (n=24), while 2.04% (n=2) were suspect and 73.47% (n=72) negative. Out of 42 goat tests, 14.28% (n=6) were positive and 85.72% (n=36) negative. Out of the total serological tests, 21.43% (n=30) were positive, 1.43% (n=2) suspect and 77.14% (n=108) negative.

Out of the 17 administrative divisions, in 76.47% (n=13) we detected seropositive small ruminants, respectively: Sibiu, Brăila, Giurgiu, Arad, Timiș, Caraș Severin, Olt, Alba, Mureș, Maramureș, Vrancea, Călărași, and the municipality of Bucharest. The following counties revealed no seropositive samples: Vâlcea, Argeș, Dâmbovița and Constanța.

Anti-*Coxiella burnetii* antibodies shown in healthy small ruminants (sheep and goats) suggests a previous exposure to *C. burnetii,* as well as a possible circulation of the bacteria in the area.

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