Q-Fever Antibodies in Romanian Small Ruminants

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Abstract

Small ruminants serum samples from Romanian Counties were investigated by iELISA Q-fever *Coxiella burnetii* Antibody test kit (IDEXX Laboratories, Liebefeld-Bern, Switzerland), according to the manufacturer's instructions. In Giurgiu County 10/15 goats serum samples were positive and all sheep samples (6/6) were negative. In Sibiu County 5/75 sheep samples were positive. In Constanta County all goat samples (10/10) were negative. In Braila County all goat samples (95/95) were negative and 10/55 sheep samples were positive. Prevalence of sero-positive results and their distribution does not provide enough data to design a surveillance program, but support the hypothesis of the presence of the pathogen in Braila, Giurgiu and Sibiu Counties. Further studies must be carry on statistically relevant sampling system.

Keywords: *Coxiella burnetii*, iELISA, sheep and goats diseases

Introduction

Despite long history of Q fever existence, several epidemiologic aspects of this infection have not been completely revealed (Parker *et al.*, 2006; Paul *et al.*, 2016). This may be due to the fact that the animals are usually asymptomatic or with latent infections, but able of persistent shedding of bacteria into the environment (Maurin and Raoult, 1999; Angelakis and Raoult, 2010).

The etiological agent of Q fever is *Coxiella burnetii*, an obligate intracellular, Gram-negative bacteria (Maurin and Raoult, 1999), able to infect a large host spectrum of vertebrates (e.g. humans, cattle, sheep, goats, dogs, cats, reptiles, birds) and invertebrates (e.g. arthropod - ticks) (Hartzell *et al.*, 2008; Angelakis and Raoult, 2010).

Epidemiologic studies usually associate Q fever with ruminants because the infected cattle, sheep and goats spread large amounts of bacteria, mainly through parturient products, and contaminates the environment for months, consequently (Parker *et al.*, 2006). Even more,

molecular studies, performed in the Netherlands, incriminate sheep as main reservoir of bacteria for humans (Roest *et al.*, 2013).

In Romania, the first case of Q-fever has been reported in 1947 (Cracea, 1987), since then, there were reported sporadic cases and three notable outbreaks (1978, 1980, 1982), involving 240 human cases (Berger, 2017). The latest studies looking for antibodies against *C. burnetii* in Romanian patients indicated both recent and latent or past infections, and consequently scientists recommended special attention for certain risk groups of patients (e.g. immunosuppression, endocardial impairment, association fever-hepatitis-interstitial pneumonia) exposed to livestock and/or companion animals, even if they live in urban areas (Negru *et al.*, 2014; Popescu *et al.*, 2014; Messinger *et al.*, 2017). In Romania, the disease notification is compulsory for human cases, and in animals is notifiable according to OIE international rules.

In order to investigate on the circulation of Q-fever in farm animals, a serological screening using indirect enzyme-linked immunosorbent assay (iELISA) has been undertaken in different Romanian counties.

Materials and methods

Samples collection

The research has been carried out in four Romanian Counties with long tradition in sheep and goats breeding: Braila, Constanta, Giurgiu, and Sibiu. The selection of herds was based on the history of reproductive disorders and / or abortion cases in the last 12 months.

From September, 2015 to February, 2017 were collected 256 blood samples (136 sheep, 120 goats) in sterile vials. All blood samples were collected from the jugular vein, in accordance with the Directive 2010/63/EU of the European Parliament and of the Council. The blood samples were transported under chilled condition and processed in laboratory for sera separation. Until use serum samples were stored at -20°C.

Serological test

Serum samples from Braila (n=150), Constanta (n=10), Giurgiu (n=21), and Sibiu (n=75) were investigated by iELISA (Q-fever *Coxiella burnetii* Antibody test kit, IDEXX Laboratories, Liebefeld-Bern, Switzerland) as per the manufacturer's instructions. In brief, the pre-coated ELISA plates with inactivated *C. burnetii* antigens and all reagents supplied with the ELISA kit were brought to room temperature. The serum samples, positive control and negative control were diluted in accord with the manufacturer's instructions, and dispensed in volume of 100 µl in appropriate wells of the microtiter plate. Both controls were included in duplicate.

The microtiter plate was covered and incubated for 60 minutes (±5 min) at 37°C (±3°C) in humid chamber, and washed three times with 300 µl wash solution. Afterwards, the conjugate was dispensed into each well in volume of 100 µl, the microtiter plate was covered and incubated in the same conditions previously described, and washed three times with 300 µl wash solution. Then, 100 µl tetramethyl benzene (TMB) substrate N.12 was dispensed into each well, covered and incubated for 15 minutes (±1 min) at 18–26°C. The reaction was stopped by adding 100 µl Stop solution N.3 into each well. At the end, the absorbance values

(optical density-OD) were read at wavelength of 450 nm. Results were expressed in percentage by using the formula $(S/P)\% = 100 \times (S-N) / (P-N)$, where S, N and P are the optical densities of test sample, negative control, and positive control, respectively. The results were negative if S/P% is <30%, suspect if $S/P\% \geq 30\%$ and <40%, and positive if $S/P\% \geq 40\%$. The manufacturer provided 0.99 sensitivity and 0.98 specificity values of test accuracy.

Results and discussions

In this study, the indirect ELISA was used, a serological assay with high sensitivity and good specificity in Q fever diagnosis (Fournier *et al.*, 1998). In some studies indirect ELISA for *Coxiella burnetii* has been correlated very well with the complement fixation test (Schalch *et al.*, 1998) and in others proved to be more specific and/or sensitive than complement fixation (Field *et al.*, 1983) and immunofluorescence assay (Peter *et al.*, 1988; Cowley *et al.*, 1992). However, due to its simplicity and accuracy, immunofluorescence assay is still the reference method for Q fever diagnostic in human infections diagnosis, while ELISA was proposed as a good method for seroepidemiological surveys (Peter *et al.*, 1997; Fournier *et al.*, 1998).

In the presented study, the 256 serum samples belong of three of the four Romanian macroregions (Fig. 1). Macroregion 1 was represented by one flock from Sibiu County, Macroregion 2 by one flock from Braila County and one from Constanta County, and Macroregion 3 by one flock from Giurgiu County. The limited number of flocks included and their low distribution did not allow statistical assesment of the differences between the Macroregions or Counties studied, and just uncovered seropositive animals in three out of four flocks (Fig. 2).

The samples from Macroregion 1 (Sibiu County) revealed 6.66% (5/75) seropositive reactors. All samples were supplied by the same sheep farm. The samples from Macroregion 2 (Constanta County and Braila County) revealed 6.25% (10/160) seropositives. The samples from Macroregion 3 (Giurgiu County) revealed 47.62% (10/21) seropositives. Also, according to the geo-spatial distribution of seropositive small ruminants, the circulation of *Coxiella burnetii* is highly possible in Sibiu, Giurgiu and Braila Counties (Fig.

Figure 1. Macroregions of Romania. Macroregion 1 - Includes Northwest and Center; Macroregion 2 - includes North-East and South-East; Macroregion 3 - includes South-Muntenia and Bucharest-Ilfov; -Macroregion 4 - includes Southwest and West. Macroregions of Romania do not have a proper administrative status and a form of government or own administration, they only exist for the collection of regional statistics (https://en.wikipedia.org/wiki/NUTS_statistical_regions_of_Romania)

Figure 2. Geospatial distribution of serological positive and negative small ruminants tested with iELISA Q-fever *Coxiella burnetii* Antibody test kit (IDEXX Laboratories, Liebefeld-Bern, Switzerland). Samples collected during the period of September, 2015 to February, 2017.

Tabel 1. Results of serological investigation with iELISA Q-fever *Coxiella burnetii* Antibody test kit in Romanian sheep and goats herds

County	Sheep		Goats		Total	
	Positive	Negative	Positive	Negative	Positive	Negative
Sibiu	5	70	0	0	5	70
Constanta	0	0	0	10	0	10
Braila	10	45	0	95	10	140
Giurgiu	0	6	10	5	10	11
Total	15	121	10	110	25	231

2.). See the distribution of seropositive samples by species and by Counties in table 1.

Given the major zoonotic risk of *Coxiella burnetii* infections serological survey on representative and uniformly distributed flocks would be required, and an individual animal study should be performed in the seropositive ones.

Taking into account that the infected animal can excrete the organism through milk, urine, faeces, and mainly in genital fluids and placental membranes - up to a billion germs per gram (Porter *et al.*, 2011), the animal handlers as well as slaughter house workers should be aware of this risk too, and control measures to be set up.

CONCLUSION

Based on the results obtained using a diagnostic method in accordance with OIE and WHO standards, the presence of *Coxiella burnetii* infection in small ruminant flocks from Braila, Giurgiu and Sibiu Counties could be considered. In goat samples from Constanta County, *Coxiella burnetii* antibodies were not found. Prevalence of sero-positive results and their distribution do not provide enough data to design a surveillance program, but support the hypothesis of the presence of the pathogen. Further studies should be done by using statistically relevant sampling system.

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