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PREVALENCE OF *COXIELLAE BURNETII* ANTIBODIES IN SHEEP IN THE TERRITORY OF MONTENEGRO

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In this study, the results of serological research for the presence of specific antibodies against C.burnetii in sheep are presented. A total of 954 random blood serum samples from sheep from different areas of Montenegro was examined using the following immunological methods: microagglutination (MA) and micro-immunofluorescence (m-IFA). Serum samples in which the presence of specific antibodies against C.burnetii was confirmed by both serological methods were taken as surely positive samples. A total of 48 positive serum samples was found, representing 5.03% of all the sera examined. Since this kind of research has never been done before in the territory of Montenegro, either in humans, or animals, the first cases of Q-fever in sheep in this territory were diagnosed by this study. In order to obtain a complete epidemiological picture of Q-fever on the territory of Montenegro, wider research on other animal species liable to this disease, as well as humans, needs to be done.

Key words:Coxiella, Q-fever, sheep.

INTRODUCTION

Q-fever is an anthropozoonosis that, besides humans, most frequently affects sheep, goats, cattle, but the infection has also been found in cats, dogs, horses, camels, pigs and other domestic animals. The causative agent of this infection is *Coxiella burnetii*, an obligate intracellular parasite that performs its development cycle in the phagolysosomes of eukaryotic cells.

The first description of this agent was given in 1937, by Burnet, and therefore it was named after him (Burnett and Freeman, 1937). The previously unknown disease, was first described in 1935, in Australia (Derrick, 1937). Ever since, this disease has been known as Q-fever, Queensland fever and Balkan influenza, depending on the territory where it occurred, as well as the authors who described it. Q-fever is a widespread zoonosis that exists in each of the five populated continents, including this country. So far, the disease has not been found only in New Zealand (Hilbink *et al.*, 1993).

In the Mediterranean region, the disease occurs frequently, particularly in sheep and especially in the countries where these animals are intensively bred.

Q-fever is also frequently present in humans, with characteristic clinical signs. The disease frequently occurs in the states of Northern Africa (Botros *et al.*, 1995), Turkey (Cetinkaya *at al.*, 2000), Greece and Cyprus (Polydorou, 1981; Crowther, 1976), Spain (Pascual-Velasco *et al.*, 1998) etc.

On the territory of the Balkan peninsula, the first description of the disease was given as the Balkan flu among German soldiers in Bulgaria and Greece in World War II, and afterwards it spread throughout the local population. After successful isolation, the causative agent was identified as *C.burnetii* (Imhauser, 1943; Caminopetros, 1948 - quotation by Gerbec, 1957). Ever since, the disease -has been present in this territory, and occasional outbreaks occur in the form of minor epidemics, but wider surveys of its prevalence have not been conducted in any of the Balkan countries.

Literature data on the incidence of Q-fever in the territory of the former SFRY show that the disease was discovered quite early. In Zagreb, Mihaljević *et al.* serologically confirmed the first cases of Q-fever during 1948-49. The disease was described as a minor epidemics in Banat, in the municipal areas of Kovin, Pančevo, Alibunar and Ečka (Jovanović *et al.*, 1950), in Bosnia, in the district of Gračanica (Šimović *et al.*, 1951) and in Travnik, Zenica, Bugojno, Prozor (Vukšić *et al.*, 1953). In Sandžak (Novi Pazar, Sjenica and Tutin), the disease was discovered in sheep (39%) and humans (more than 50%) (Vukšić *et al.*, 1953). On the territory of Serbia the disease was detected as a minor epidemic in soldiers (Radoičić *et al.*, 1953) and 3 years later, Morelj and Gerbec described the specificity of the *Pirot* strain, isolated from stable dust, during one of the epidemics. In 1957 the disease occurred in the form of a major epidemic on the island of Uljan, Dalmatia (Vesenjak *et al.*, 1957). During the same year it was registered in dairy animals in Vojvodina (Šipka at al., 1957). Further evidence of Q-fever incidence can be found in the survey in sheep (Cvjetanović, 1959), in the town of Gacko, in humans in Croatia (Maretić *et al.*, 1962), in sheep in the territory of Bosnia and Herzegovina (Udovičić, 1964), in humans, in Pula (Maretić *et al.*, 1972). Further research on this disease was performed by Rašeta and Mihajlović (1983) in animals in Vojvodina, by Boričić *et al.*(1984) in humans in Croatia, by Vuković *et al.* (1984) in humans in Vojvodina and by Nevjestić *et al.* (1985) in sheep in Bosnia.

Wider research on the prevalence of Q-fever and its precise epidemiological outline has never been performed either on the territory of the former SFRY, or in the present FRY. No research has been done after 1985, therefore we have no reliable epidemiological data for the last 15 years.

Although it may seem that in animals the disease may not be caused by *C.burnetii*, it can cause abortion in sheep and goats, as well as reproduction complications in cattle, followed by shedding large numbers of the causative agent into the environment through placenta and birth fluids during abortion and parturition (Romvary *et al.* 1979., Bajalo *et al.* 1990). Considerably higher rates of seropositivity have been found, in several studies, in sheep and goats that miscarried than in those that did not (Crowther 1976). Therefore, there is a reasonable supposition that *C.burnetii* infection can cause abortion, which is, in the majority of cases, the only visible symptom of the disease in infected sheep and goats.

MATERIALS AND METHODS

This study of the presence of Q-fever in sheep in the epidemiological territory of Montenegro included sheep flocks from various parts of the Republic the majority of which were from municipal areas with well developed conditions for the sheep breeding. On the basis of free choice, 11 municipal areas were included, the major ones being in the northern part of Montenegro, in 6 municipal areas Pluzine, Zabljak, Pljevlja, Berane, Andrijevica and Mojkovac, where sheep breeding is well developed and where the majority of sheep flocks are situated. In the central part of Montenegro the research was conducted in 4 municipal areas- Nikšić, Danilovgrad, Podgorica and Cetinje, while in the southern (coastal) part, the southernmost municipal area of Montenegro - Ulcinj was chosen.

The method of random sampling was used for the selection of sheep flocks to be tested, but, at the same time preference was given to flocks that had recently registered cases of miscarriage. In the majority of cases, those were flocks from the sector of individual animal husbandry, with 40-60 sheep. Samples were taken from 20-25% of the total sheep number in each flock.

Blood samples were taken by punction of *v.jugularis* and stored for 20-24 hours at room temperature until spontaneous coagulation was completed. Then the separated serum was poured off and centrifuged for 10 minutes at 3000 r/min. The sera were stored at - 20°C until tested using the following immunological methods: microagglutination (MA) and micro-immunofluorescence (m-IFA) tests.

The microagglutination test was performed in microtitre plates using the *C.burnetii* phase II antigen (Institute of Virology Bratislava) in the working dilution 1:3 in PBS (pH 7.2). Before testing, sera had been inactivated in a water bath at 60° for 30 minutes, and then two-fold serial dilutions of sera in 1% negative calf fetal serum were examined in the microtiter plates. Working dilutions of sheep sera were made from 1:2 to 1:4096. After adding the working dilution of antigen the plates were placed in a humid chamber at room temperature for 24 hours. We then read the reaction. The formation of sediment (agglutinate of antibodies and coloured rickettia) with whittled edges, covering the whole bottom of the well was considered to be the positive antibody titer. The serum dilution giving 50% agglutination was taken as a negative reaction. The formation of an evenly shaped sediment, consisting of coloured rickettsia, with smooth edges, similar to a button at the bottom of the well. The serum dilution of 1:16 was taken as the positive (cut off) titer for Q fever, according to the antigen manufacturer's recommendation.

The m-IFA test was performed using the *C.burnetii* phase II antigen (Institute of Virology - Bratislava) in the working dilution of 1:5 in PBS (pH 7.2). The antigen working dilution was placed as a droplet on the microscope plate and left to dry overnight in the refrigerator at +4°C. Afterwards, microscope plates were fixed in cold acetone for 10 minutes and dried in air for 10 minutes. The sera examined were diluted in two-fold serial dilutions in PBS (pH 7.2) in microtiter plates. Diluted sera were placed on to the previously fixed and dried antigen in the microscope plate and incubated for 20 minutes in a humid chamber at room temperature. Afterwards, preparations were rinsed 3 times in PBS (pH 7.2) during a total of 20 minutes, the plates were irrigated with conjugate (anti-ovine IgG FITC antibodies INEP Zemun) and then incubated at room temperature, in the humid chamber during the next 30 minutes. The preparations were again rinsed 3 times in PBS (pH 7.2) during a total of 20 minutes, then buffered glycerin was poured over and

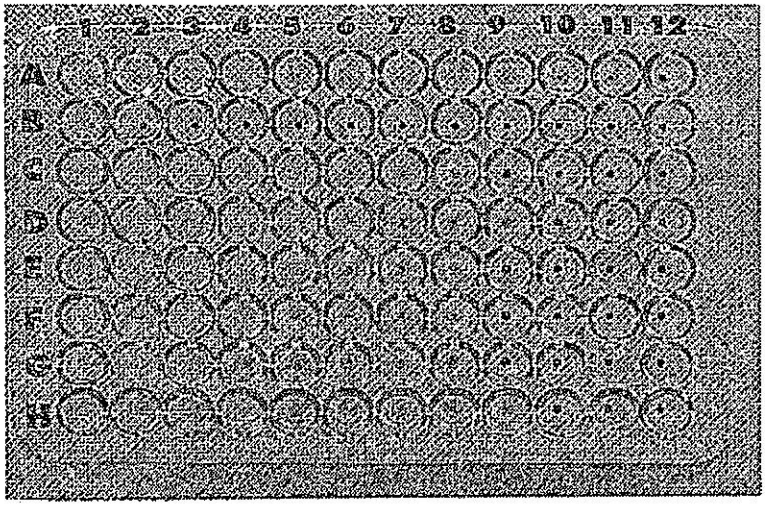

Figure 1. Microagglutination method in microtiter plates
A - positive contr
B - negative contro
C - H - tested sera

the plates observed under the fluorescence microscope at a magnification of 20x10 and 40x10. The serum dilution of 1:32 was considered as the positive (cut off) titer.

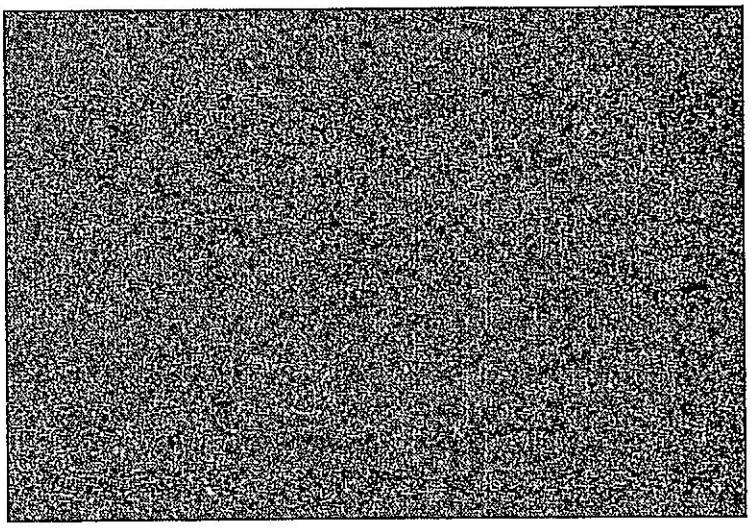

Figure 2. Microimmunofluorescence method - positive reaction

The tested blood serum were considered positive only when the presence of specific antibodies against *Coxiella burnetii* was detected by both serological methods.

RESULTS AND DISCUSSION

During this research, 954 blood sera samples taken from sheep from 11 municipal areas were tested. The total of positive samples was 48 (5.03%). Seropositive animals were found in all of the 11 municipal areas included in this study (11 out of 21 municipal areas in Montenegro). The highest incidence of seropositive sheep was registered in Pljevlja (21.85%) and Ulcinj (13.33%), and the lowest in Nikšić (1.17%). (Table1, Figure 3).

Table 1. The prevalence of seropositive sheep with antibodies against C. burnetii in different areas of Montegro

Montenegro	954	48	906	5.03
Plužine	255	12	243	4.70
Žabljak	219	10	209	4.56
Pljevlja	32	7	25	21.85
Berane	64	1	63	1.56
Andrijevica	64	1	63	1.56
Mojkovac	20	1	19	5.00
Nikšić	85	1	84	1.17
Danilovgrad	46	2	44	4.34
Podgorica	95	5	90	5.26
Cetinje	29	2	27	6.89
Ulcinj	45	6	39	13.33

The epidemiological map of FRY shows no literature data on the presence and prevalence of Q-fever in humans or animals for the area of Montenegro. The reason is that no research concerning this subject is being performed, rather than the good health of humans and animals. Taking into account data from the international and local literature on the prevalence of this disease in neighbouring countries, as well as free traffic of animals and humans in the former SFRY, the presumption that Montenegro too is infected with *C.burnetii* is quite acceptable. On the basis of the results obtained, it was shown that epidemiological presumptions for the existence of this disease in sheep on the epizootiological territory of Montenegro were correct. Thus, Q-fever was diagnosed for the first time on the territory of Montenegro, and in sheep.

The teritory included in the serological tests represents 66.78% of the total area of Montenegro. The total number of sheep on the examined territory is 211 710, which is 65.24% of the total number of sheep in Montenegro.

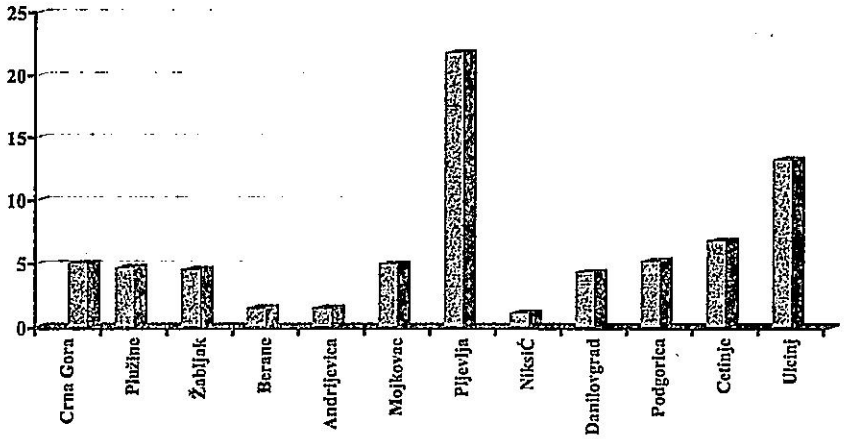

Figure 3. Percentage of seropositive sheep in the municipal areas included in this study

From the epidemiological viewpoint, the existence of *C.burnetii* infection in Montenegro was registered in 5.03% of the sheep. Similar surveys in the world showed the presence of specific antibodies against *C.burnetii* in blood sera of sheep in 3.9% of the cases examined in Switzerland (Metzler *et al.*, 1983), in 20.44% of the sheep examined in Bulgaria (Martinov *et al.*, 1989) and 10.5% of the sheep examined in Turkey (Cetinskaya *et al.*, 2000), while surveys conducted in Trinidad showed that Q-fever in sheep did not exist (Adesiyun and Cazabon, 1996). In Vojvodina (Yugoslavia), seroprevalence was found in 12.2% of the sheep (Vidić *et al.*, 1996).

Since the study included 20-25% of sheep from the selected flocks, it can be assumed that the real number of seropositive sheep in the flock is 4-5 times higher. Taking into account that the animals tested represent 0.45% of the total number of sheep in the territory of 11 of the total of 21 municipal area, it can be justifiably assumed that the real situation concerning the Q-fever infection, is unfavourable. This assumption is also justified by the fact that this infection exists in other species liable to it (cattle, humans, ticks, etc.) that were not included in this study.

Therefore, on the basis of the results obtained in this study, only a general idea of the prevalence of Q-fever in sheep in Montenegro can be seen, but further studies would have to include the rest of the sheep from the territory screened, as well as those from the territory that was not included in this study. Also, further studies should include other species liable to this infection. Moreover, in the diagnosis of this disease economic parameters and equipment in diagnostic laboratories should be considered, too, and an effort should be made to introduce other specific methods (ELISA, PCR, etc.) .

Although some authors deny the significance of Q-fever from the viewpoint of veterinary medicine, because of its most frequent latent form in domestic animals, the incidence of miscarriages, usually in sheep and goats, as the result of *C.burnetii* infection, and the subsequent great economic losses, support the

importance of Q-fever as a veterinary problem. Furthermore, the fact that veterinarians, together with cattle-breeders and abattoir workers, are most frequently exposed to the infection, shows that there are enough reasons not to leave Q-fever on the margins of veterinary profession interests.

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PRISUSTVO ANTITELA PROTIV *COXIELLAE BURNETII* KOD OVACA NA TERITORIJI CRNE GORE

D. LAUŠEVIĆ

SADRŽAJ

U radu su predstavljeni rezultati seroloških ispitivanja ovaca na prisustvo specifičnih antitela protiv *C. burnetii.* Na osnovu slobodnog izbora, ispitano je ukupno 954 uzorka krvnih seruma ovaca iz različitih krajeva Crne Gore. U laboratorijskoj obradi uzoraka korišćene su imunološke metode: mikro-aglutinacija (MA) i mikro-imunofluorescencija (m-IFA). Kao sigurno pozitivni uzorci seruma smatrani su oni u kojima je sa obe serološke metode utvrđeno prisustvo specifičnih antitela protiv *C. burnetii.* Ukupno je otkriveno 48 pozitivnih uzoraka što predstavlja 5.03% od ukupnog broja ispitanih krvnih seruma. Ovim ispitivanjem su kod ovaca dijagnostikovani prvi slučajevi Q-groznice na ovom prostoru.