

PRELIMINARY DATA ON SEROLOGICAL INVESTIGATION TO Q FEVER, LYME DISEASE AND ECHINOCOCCUS IN ALBANIA

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The aim of this study is to present the results on serological surveys of Lyme borreliosis, Q fever and echinococcus infection in northern Albania. In total we analyzed 200 sera blood samples collected from cattle, sheep and goats, and X fecal samples from red foxes. We used appropriate ELISA tests, commercially available to evaluate the presence of anti-Borrelia IgG, specific antibody against *Coxiella burnetii*, and coproantigen for *echinococcus*. In total 11.66% of sheep, 14.5% of goats and 6% of cattle had antibody against *Borrelia* sp; 13.3% of sheep, 17.7% of goats and 4% of cattle had specific IgG against *Coxiella burnetii*, and 27% of fecal red fox samples were positive to echinococcus. As a conclusion, both two above bacterial zoonotic diseases, and echinococcosis as an important parasitic zoonosis are present in Albania. They are a serious threat to public health, and may be largely underestimated in Albania. This paper includes the full results relating to Q fever and some recent, unpublished data from our group concerning with Borreliosis and echinococcus infections.

Key words: zoonosis; Q fever; Lyme borreliosis; ELISA tests; Echinococcus

ПРВИЧНИ ПОДАТОЦИ ОД СЕРОЛОШКИТЕ ИСПИТУВАЊА ЗА Q-ТРЕСКА, БОЛЕСТА LYME И ЕХИНОКОКУС (КУЧЕШКА ТЕНИЈА) ВО АЛБАНИЈА

Целта на ова истражување е да се прикажат резултатите од серолошките испитувања на Lyme borreliosis, Q-треска и ехинококни инфекции во северна Албанија. Вкупно се анализирани 200 примероци крвен срум од говеда, овци и кози и X фекални примероци од црвени лисици. Користени се соодветни ELISA-тестови, комерцијално достапни за проценување на присуството на anti-Borrelia IgG, специфично антитело против *Coxiella burnetii* и копроантигенот за ехинококус. Вкупно 11,66% од овците, 14,5% од козите и 6% од говедата имаа антитела против *Borrelia* sp; 13,3% од овците, 17,7% од козите и 4% од говедата имаа специфичен IgG против *Coxiella burnetii* и 27% од фекалните примероци од црвените лисици беа позитивни на ехинококус. Заклучок: двете горенаведени бактериски зоонотични болести и ехинококусот како значајна паразитска зооноза се присутни во Албанија. Тие претставуваат сериозна закана за јавното здравје и во Албанија генерално се потценети. Овој извештај ги вклучува и целосните резултати кои се однесуваат на Q-треската и некои неодамнешни необјавени податоци од нашата група кои се однесуваат на борелиозата и ехинококните инфекции.

Клучни зборови: зооноза; Q-треска; Lyme borreliosis; ELISA-тестови, ехинококус

1. INTRODUCTION

Nowadays it is clear that wildlife acts as reservoirs of infectious agents that cause disease in domestic stock, pet and captive animals and humans (Simpson 2002; Ghirotti et al., 1991; Tylew-

ska-Wierzbanowska, 1991). Lyme borreliosis is one important zoonosis which is spread worldwide (Radostits et al., 2007; Greene 2006; P. J. Quinn et al., 2002). *Borrelia burgdorferi* is a tick born infection which affects both wild and domestic ani-

mals. Four North American and two European species of Ixodes ticks harbor *Borrelia*. *Ixodes ricinus* and *I. persulcatus* feed on a wide range of hosts, including humans (Anderson, 1989).

In Europe there are identified approximately 40 mammals and birds as animal reservoir hosts of Bb. Infectious pathogens that originate in wild animals have become increasingly in recent decades (Bengis et al., 2004). It is reported that ticks are responsible for more than 100,000 cases of illness in humans every year (De La Fuente et al., 2008).

Study of IgG in sera blood samples of domestic animal species gives an opportunity to create a real picture of the presence of relationship between the possibility of infestation by infected arthropods such as *I. ricinus* and the possible spread of disease in the region (Trávníček, 2003; Walker, 1991).

Q fever is an important zoonosis which is due by *Coxiella burnetii*, an obligate intracellular organism that lives in phagolysosome of the host cell (Zvizdic et al., 2002; D. L. Heimann, 2004). It has two antigenic phases: the antibodies to phase I are found at lower level than phase II in the acute period and the reverse is true in chronic disease (Dorko et al., 2008). Since its first description in 1937, the disease has been found to be present in most countries of the world (Hirai and To, 1998). *Coxiella burnetii* has unusual stability; it can survive for 2 months in environmental conditions, and is highly resistant to many disinfectants. Q fever is spread in all countries except New Zealand, (Cracea, 1987; Cutler et al., 2007; DeForge and Cone, 2006).

Natural reservoirs of infection are a range of animals such as sheep, cattle, goats, cats, dogs, wild animals, and bandicoots, many species of feral rodents, birds and ticks (To et al., 1998). In ticks transovarial and transstadial transmission exist, so they participate in wildlife cycles in rodents, farm animals, wildlife and birds (Zvizdic et al., 2002). Most of infected animals are asymptomatic, but shed a large number of organisms in placental tissues at parturition (Cutler et al., 2007).

The disease can be transmitted by either airborne or ingested. The sources of infection are 1) dust from premises contaminated by placental tissues, birth fluids and excreta of infected animals in establishments processing infected animals or their products and in necropsy rooms, 2) milk, and 3) blood or bone marrow transfusion (David L. Heymann, 2004).

The organism can be spread by air over 1 km and the infection can be transmitted through direct contact or other infected materials, such as wool, straw, fertilizer and laundry. Factors predisposing to infection or exposure to *C. burnetii* included professional orientation and regular contact with farm animals and pets.

Clinical history of some seropositive subjects revealed substantial problems, such as fever of unknown origin, rheumatic disease, disease of heart, liver, respiratory tract (particularly atypical pneumonia), chronic fatigue syndrome and spontaneous abortion in females. Q fever is a profession-related disease and prevention of its spreading within the risk population groups requires observation of basic safety rules (Dorko et al., 2008).

2. MATERIALS AND METHODS

Animals

A number of cows, sheep and goats for Q fever were selected from different regions as they are shown in Table 1. The numbers of samples we calculated in accordance with proper epidemiological formula, had evidence of abortion of the animal population. To find any association between prevalence of infection in ruminants and ticks we have collected both ticks and blood from the animals.

The ticks are collected from goats, sheep and cows and are preserved in alcohol 70%. For technical reasons we did not finish analyzing the ticks and all sera blood samples for *B. burdorferi*. The flocks that have not any history of abortion were not selected for sampling. In total we collected samples from 9 goat flock, 4 sheep flock and 3 small cow farms.

Collection samples for ELISA

In order to investigate presence of *Coxiella burnetii* infection in the ruminants, collection of blood by jugular venipuncture and serum samples were collected and preserved at -20°C temperature. The samples were collected from aborted animals, animals in full term of gestation and animals in the last months of gestation.

Table 1

The sera blood samples according to the selected districts of Northern Albania

Districts	N° of cows	N° of sheep	N° of goats
Peshkopi	22	16	35
Kukes	28	16	35
Librazhd	No	15	55
Elbasan	No	15	45
Total	50	62	170

Q fever serology

In order to identify the positive animal for IgG against *C. burnetii* we used Q-fever (*Coxiella burnetii*) Antibody Test Kit (Chekit* Q-fever) IDEEX Laboratory. The microtiter plates are supplied coated with antigen. Dilutions of the samples (1:400) are incubated in the wells of these plates. Any antibody specific for *Coxiella burnetii* binds to the antigen in the wells and forms an antigen-antibody complex on the plate well surface. The unbound material is removed by washing the plates. A peroxidase-labeled-antiruminant IgG conjugate is added which binds to the ruminant's

antibodies complex with *Coxiella burnetii* antigen. The unbounded conjugate is removed by washing, and TMB substrate is added to the wells. The degree of color that develops (optic density measured at 450 nm) is directly proportional to the amount of the antibody specific to *Coxiella burnetii* present into the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD) that develops in well contained the samples with the OD from the wells containing the positive control. The calculation of the results was carried out in accordance with the Kit instruction using the formula

$$\text{Value (\%)} = \frac{(\text{OD 450 samples} - \text{OD 450 negative control})}{(\text{OD 450 positive control} - \text{OD 450 negative control})}$$

3. RESULTS

The interpretation of the obtained results was based on the Kit instruction and they are grouped as negative, positive and suspect samples:

Value	<30%	≥ 30% to <40%	≥40%
Interpretation	negative	suspect	positive

Table 2

Results of ELISA tests

Animal	Samples for <i>B. burdorferi</i>	Positive for <i>B. burdorferi</i>	% positive samples	Samples for presence of IgG against <i>C. burnetii</i>	Positive for <i>C. burnetii</i>	% positive samples
Cow	50	3	6	50	2	4
Sheep	60	7	11.66	60	8	13.3
Goats	62	9	14.5	170	30	17.7
Total	172	19	11.04	280	40	14.29

In total there are analyzed 280 sera blood samples from ruminants for antibodies against *C. burnetii* and 172 sera blood samples for *B. burdorferi*.

4. DISCUSSION

The main aim of this study was to determine the presence and distribution of Q fever infection in ruminants in the Northern Albanian districts. Very few studies are carried out in Albania related to this important zoonotic infection and little is

known about the role of *Coxiella burnetii* in ovine and bovine abortion and the importance of this infection related to the human health impact. In total 14.29% of samples were positive for Q fever, whereas 11.04% were positive for Borrelia infection. As for the animals all species have seroconversion against *C. burnetii*. The humoral response against *C. burnetii* was estimate according to OD of the samples measured at 450 nm (wave length). The cut off for positive samples was $\text{OD}_{450} \geq 0.347$: for samples negative $\text{OD}_{450} < 0.302$ and suspicious samples OD_{450} range from $\geq 0,302$ to <0.347 . The rate of *Coxiella burnetii* infection was

4% (2 samples), 13.3% (8 samples) and 17.7% (30) respectively in cows, sheep and goats (Fig. 1).

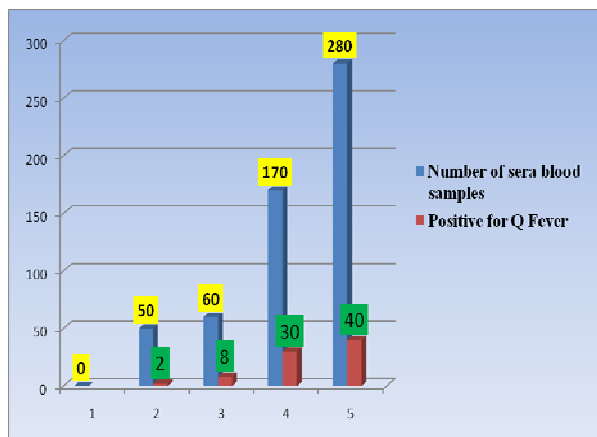


Fig. 1. Results of ELISA test performed in domestic ruminant's sera blood samples

The highest prevalence of Q fever was in goat's flock with the highest rate of abortion and was recorded in the Kukes District (Radostits et al.). The Q fever positive farms were respectively 1 cow farm, 2 sheep and 5 goat flocks (date not shown). We consider any farm with 2 samples *C. burnetii* positive.

Our data are closely related to the other reported study carried out in other countries such as Bulgaria, Sardinia (Italy), Greece, Slovakia, etc. We consider that the epidemiological pattern of distribution of Q fever infection is associated with large goat flocks managed in those areas, so the samples from goats are higher compare to cows and sheep. The goat samples are accurately representative for the flocks selected, so the data are supportive for estimating the prevalence of Q fever infection in the above districts.

The value of positive samples range from 54% to 112% (positive is considered any samples $\geq 40\%$), and 46.6% (14 of 30 samples) are with value over 80%. The results have shown that 2.1% were doubtful (OD_{450} range from ≥ 0.302 to <0.347). In order to discriminate the nature of infection (either acute or chronic) further study must be carried on by using the specific anti-*C. burnetii* phase I and phase II Immunofluorescence antibody IgG method (Ruiz-Fons et al., 2008, Whitney et al., 2009). The ELISA test is one of the most common tests used as screening for Q fever. It is reliable, fast, and relatively cheap and is safe to perform in ordinary laboratory conditions. The parameters related with specificity and sensitivity

are comparing other serological tests and the investigation has shown that the ELISA is superior and compatible with IFA (Immunofluorescence Antibody) and CFT (Compliment Fixation Test). Highly values of the positive samples ($>80\%$) indicate for implication of *C. burnetii* in abortion. A serological test cannot confirm *C. burnetii* as a cause of abortion (Rousset et al., 2007). The ELISA test as well as other serological tests cannot be used to determine shedder animals.

Possible control measures are difficult to apply and evaluate because of the lack of epidemiological information and simple tools to identify shedders (Rousset et al., 2009). Other studies must be undertaken in order to identify the *C. burnetii* shedder. Because of highly zoonotic isolation of such organism, it requires safe laboratory level 3 and well qualified personnel. We consider that molecular techniques must be applied in Albania, instead of *C. burnetii* isolation. The PCR method can be used to analyze an animal's feces, milk and vaginal mucus from both abortion and non abortion goat and sheep (Rousset et al., 2009).

Veterinarians have a high level of exposure to *C. burnetii*, the causative organism of Q fever, especially those veterinarians who treat livestock (Whitney et al., 2009). An updated veterinary curriculum is essential to empower future graduates to work in an international environment, applying international standards for disease surveillance, veterinary public health, food safety and animal welfare (Kahn et al., 2009).

In conclusion, Q fever disease is present in Northern Albania and cows, sheep and goats have a strong humoral immune response reflected on IgG against *C. burnetii*. The rate of *C. burnetii* infection varies from 4% in cows, 13.3% in sheep and 17.7% in goats. The importance of this disease is related much more with human health and must be considered by veterinary services as both economic and public health importance. A person with a high risk occupation must be knowledgeable of the sources of infection, the transmission method, appropriate disinfection of the environment, clothes and milk pasteurization.

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