

FIRST CASES OF DOG BORRELIOSIS IN EASTERN POLAND. SHORT COMMUNICATION

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Summary. The aim of this study was to recognize an etiological factor of diseases with symptoms of lameness and subcutaneous tissues oedema, which occurred in 4 dogs after invasions of ticks. The results of serological examinations, and the reaction of sick animals on tetracycline therapy revealed, that in all four cases an etiological factor of the diseases was bacteria *Borrelia burgdorferi*.

Keywords: *Borrelia burgdorferi*, ELISA, Western blott, tetracycline, dog.

PIRMASIS RYTŲ LENKIJOS ŠUNŲ SUSIRGIMAS BORELIOZE. TRUMPAS PRANEŠIMAS

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Santrauka. Šio tyrimo tikslas buvo nustatyti šlubavimą ir poodinę edemą sukeltantį etiologinį faktorių, atsiradusį keturiems šunims įkandus kraujasiurbėms erkėms *Ixodes ricinus*. Atlikus serologinius tyrimus ELISA nustatyta, kad susirgimą sukėlė mikroorganizmas *Borrelia burgdorferi*. Sergantiems šunims sėkmingai pritaikyta terapija antibiotiku tetraciklinu.

Raktažodžiai: *Borrelia burgdorferi*, ELISA, Western blotingas, tetraciklinas, šuo.

Lyme disease in dogs (borreliosis) is a systemic, multiorgan disease caused by spirochetes *Borrelia burgdorferi* belonging to *Spirochetaceae* (Font et al., 1992). It occurs endemically and is borne by *Ixodes* type ticks, particularly by *Ixodes ricinus* (Ambrasiene et al., 2004, Cisak et al., 2005, Foley et al., 2007, Wodecka & Skotarczyk 2000). In the course of the disease, the infected animals can show fever, apathy, arthritis (Appel et al., 1993), kidney damage (Grauer et al., 1988, Reusch et al., 1994), meningitis, encephalitis, neuritis (Chang et al., 2001) and myocarditis (Breitschwerdt et al., 1996). Clinical symptoms do not occur in all cases of infected dogs; it is estimated that in endemic areas merely 5-10% out of 75% sero-positive animals have shown clinical symptoms. The situation might be explained by the fact that some of the positive serological findings in animals result from non-pathogenic strain infection or a scant dose of *B. burgdorferi* cells infecting host with a well-functioning immune system.

Establishment of Lyme disease diagnosis is difficult and requires the presence at the same time at least four elements, that is clinical symptoms have to be accompanied by positive antibodies titer, together with

tick exposure and a positive reaction to antibiotic therapy. Demonstration of the bacteria by culture techniques or detecting its presence in tissues by microscopic methods is very difficult due to a small number of bacterial cells in an infected organism. Nevertheless, the highest probability of isolation borreliae in skin biopsy appears to be at the onset of the disease, then after having administered antibiotic therapy, the PCR is the method of choice.

The aim of the research was to determine the cause of the disease in four dogs, which had contact with ticks (September-November 2006). All the animals came from households located near forests. In the cases of two German shepherd dogs, there were symptoms of fever, strong apathy and lack of appetite. In the case of one pointer, high fever was observed and scrotum oedema, which expanded to subcutaneous tissue on the inner surface of thighs. In the case of the fourth dog – crossbreed, clinical examination revealed pyrexia – (body temperature 39.1°C was slightly raised above physiological standards), apathy, lack of appetite, left ankle joint oedema with minor lameness and scrotum skin oedema.

The observed clinical symptoms in dogs from eastern Poland, where the tick-born diseases occur endemically (Cisak et al., 2005, Winiarczyk et al., 2007) indicated borreliosis. To reveal the potential presence of *Babesia* and *Ehrlichia* organisms in erythrocytes and leucocytes respectively, microscopic examination of blood smear stained by Giemza method was conducted and a PCR

amplification was done in order to detect their genetic material. Neither method produced evidence of protozoan or *Rickettsia* in the examined material. No leucopenia or thrombocytopenia was discovered, which often accompany babesiosis and ehrlichiosis however in all animals a hematological examination revealed leucocytosis (Table 1).

Table 1. Results of hematological examination in dogs

Parameters	German shepherd (male)	German shepherd (female)	Pointer	Crossbreed dog
RBC ($10^6/\text{mm}^3$)	8.12	7.45	5.88	7.82
Ht%	53.1	46.5	39.4	48.4
WBC ($10^3/\text{mm}^3$)	16.8	15.5	14.4	14.1
Hb g/dl	18.7	16.4	15.6	16.0
PLT ($10^3/\text{mm}^3$)	434	340	295	303

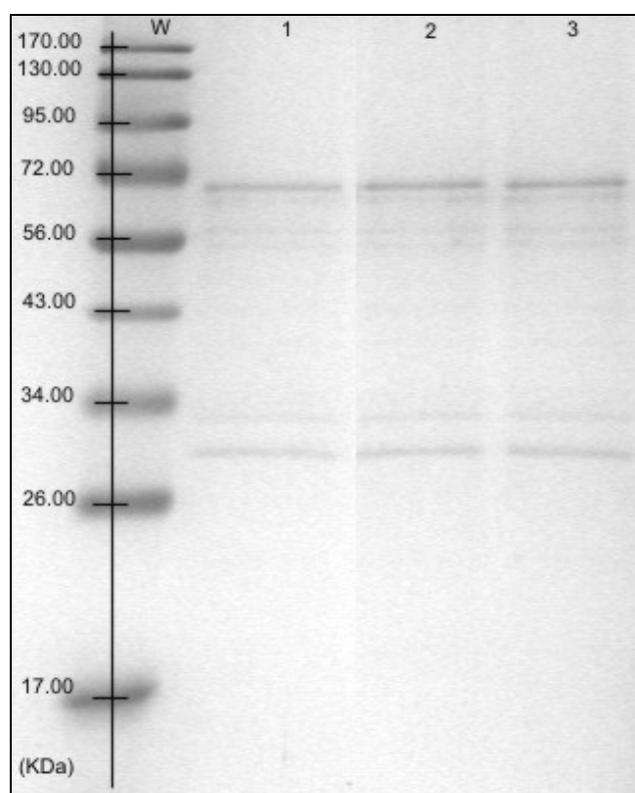


Fig.1. Immunoblot of dogs sera with local strain *Borrelia afzelii*

By means of ELISA test in which *Borrelia afzelii* cells were used as an antigen, an increased level of antibodies for the bacteria was revealed in all the dogs' sera. Absorbance value measured by wave 492 nm during the first examination ranged from 2.124 to 3.024. Due to a possibility of cross reactions between *B. burgdorferi* and other bacteria, especially non-pathogenic spirochetes of *Borrelia* and *Leptospira*, the findings had to be confirmed by Western blott method. Antibodies contained in the examined sera reacted with protein fractions of 30, 33, 44, 56, 64, 68 kDa mass (Figure 1). The presence of electrophoretic bands of 56, 44, 33 kDa mass *B. afzeli*,

which most probably represent protein p58, flagellin periplasmatic protein *B. burgdorferi* clearly indicate a natural infection. Molecular mass of protein fractions reacting in the Western blott test in our study differ slightly from those found in most papers concerning the issue. It mainly depends on the antigen which is used in individual research. In the present study it was *B. afzelii* strain isolated from ticks caught in area of eastern Poland, which could have differed from standard strains of *B. burgdorferi* routinely used in most of the investigations. For the same reason, a fraction of approximate mass of 22 kD corresponding to surface protein OspC, did not appear on the electrophoregram. It is worth noting here, that this specific protein undergoes expression only in higher temperatures in host organisms during the course of infection and does not occur in spirochetes cells multiplied on artificial media or isolated from ticks.

The antibiotic (doxycycline *per os* in 10 mg/kg dose within the period of three weeks) and corticosteroid (dexamethasone *s.c.* in dose 0.15 mg/kg) therapy resulted in major improvement. led to a marked regression of clinical symptoms, inner body temperature came back to normal, apathy regressed, appetite returned. A follow-up serological examination 4 weeks later done by ELISA test revealed a significant drop in antibodies level in blood serum compared with the first examination. Absorbation of sera samples taken from the sick dogs significantly dropped and ranged from 1.884 to 2.244 (Table 2) at the time. The disappearance of clinical signs followed by treatment with tetracycline combined with the drop of specific antibodies titer between acute and convalescent serum samples is indicative of recent infection with *Borrelia* organisms. Suspicion of that infection is strongly supported by the fact of exposition of the dogs to ticks in the endemic area for tick-born diseases.

In the light of the research, veterinary practitioners should keep in mind the presence of Lyme disease in Poland and include it in differential diagnosis for lameness in dogs especially when there are associated systemic signs and a history of exposure to ticks.

Table 2. Absorptions values of dogs sera in ELISA test

Dogs	Specimens taken at the admission of the dog to the clinic (day 0)	Specimens taken after three weeks therapy
German shepherd (male)	2.847	2.050
German shepherd (female)	3.024	2.344
Pointer	2.124	1.884
Crossbreed dog	2.766	2.010

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