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# MOLECULAR EVIDENCE OF THE PRESENCE OF *BORRELIA BURGDORFERI* SENSU LATO IN BLOOD SAMPLES TAKEN FROM DOGS IN POLAND

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**Abstract:** Our earlier studies on ticks, *Ixodes ricinus* have demonstrated that the north-western part of Poland is an endemic area for *Borrelia burgdorferi* sensu lato and therefore sick dogs, at the time of the highest activity of ticks, should be suspected for having borreliosis. We carried out a preliminary PCR survey of the blood of 15 dogs naturally exposed on ticks for the presence of the DNA of *B. burgdorferi* using primers complementary to the fragment of the gene encoding 16S rRNA of the small ribosome subunit. We found 6 out of 15 dogs were infected, although 2 dogs had a lameness - the attribute of canine borreliosis were PCR-negative. Our findings suggest that the exposure to *B. burgdorferi* is common in dogs in the region declared an endemic area of borreliosis, and that this disease should be important to local veterinarians.

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## INTRODUCTION

Lyme disease or borreliosis is a zoonotic, tick-borne, disease caused by spirochetes, *Borrelia burgdorferi* sensu lato. Ticks of the genus *Ixodes*, acting as vectors of *B. burgdorferi*, transmit them to many vertebrate species, although under natural conditions the clinical form of borreliosis occurs only in species not associated with the forest habitat - mainly the dog and the human [3, 12, 13]. The canine borreliosis occurs most often in the articular form, affecting joints of limbs, usually wrist or instep. One or two joints become swollen and the inguinal- and prescapular lymph nodes become enlarged. The associated symptoms are: "malaise", expressed in elevated body temperature, loss of appetite, and fatigue, and lameness following in days. Dogs suffering from borreliosis rarely develop myocarditis, although the older ones develop the renal form of this disease [3, 5, 6, 14].

Canine borreliosis was first described in the 1980s in the USA [7, 8] and recently it occurred in almost all countries of Western Europe [1, 14]. In Poland, no dogs naturally exposed to ticks have been hitherto studied for borreliosis. In European countries and in the USA the canine borreliosis has been studied quite often.

In this work we present preliminary results of detecting DNA of *B. burgdorferi* s.l. using the technique of polymerase chain reaction (PCR) in the blood of dogs naturally exposed on ticks, admitted to the Animal Clinic in Szczecin within the period of the highest activity of ticks, *Ixodes ricinus* in the region declared an endemic area of borreliosis [10, 11].

## MATERIAL AND METHODS

Blood samples were collected from a total of 15 dogs, representing different breeds, admitted to the Animal Clinic in Szczecin (north-western Poland) within the month of June - the period of the highest tick activity. The dogs had been taken to a veterinarian for various reasons. Each blood sample was accompanied by a report on the

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major clinical syndromes observed at the time of blood collection. The age of the dogs (12 males and 3 bitches) ranged from 5 months to 12 years. The 1.5 ml samples were taken from the cephalic vein.

Polymerase chain reaction (PCR) was used for identification of the DNA of *B. burgdorferi* s.l. The DNA was isolated from the blood with the aid of QIAamp® DNA Mini Kit (Qiagen). The extracted DNA was stored at 70ºC until analysed. The primers used were SC1 and SC2 complementary to the area of the 16S rDNA gene encoding rRNA for the small ribosome subunit of *B. burgdorferi* sensu lato and yielding the product of 325 bp [11].

In each PCR run, DNA of *Borrelia burgdorferi* sensu lato (strain Bo-148c/2) was used as a positive control and distilled water as the negative control. The samples were initially denatured for 3 min at 95ºC and 35 cycles were performed (94ºC for 30 sec, 64 C for 45 sec, and 72 C for 45 sec). The PCR products were examined on 2% agarose gels in 1 × TBE-buffer with ethidium bromide. To minimize contamination, the reagent setup, extraction, sample addition, and the PCR and sample analyses, were performed in 3 separate laboratories.

## RESULTS

The presence of DNA of *B. burgdorferi* s.l. was revealed in the blood of 6 out of 15 dogs examined (4 male dogs and 2 bitches aged 1.5-12 years). The principal disease symptoms in PCR-positive dogs are shown in Table 1. Three of the 6 dogs exhibited elevated body temperature (39.7–40.5ºC), 3 of the 6 - lameness. Two distinctly lame dogs were PCR-negative. Joint aches of wrist and instep were stated in 3 of the 6, while oedema of these joints - in 2 of the 6. One dog (of the 6) had enlarged groin- and prescapular lymph nodes. Two showed decreased body weight and anorexia. All dogs with positive PCR result had been attacked by ticks many times in the past, and also quite recently.

**Table 1.** Major clinical symptoms found in dogs tested positively in a PCR reaction for the presence of DNA of *B. burgdorferi* s.l. (n = 6).

| Clinical symptom | Number of dogs |
|---|---|
| Fever | 3 |
| Lameness | 3 |
| Carpal and tarsal arthralgia | 3 |
| Joint swelling | 2 |
| Enlargement of lymph nodes | 1 |
| Body weight loss | 2 |
| Appetite loss (anorexia) | 2 |
| Infestation by ticks | 6 |

## DISCUSSION

The most common canine borreliosis symptoms are: fever, loss of appetite and lameness due to arthritis [1, 7, 14]. While in humans, the infections with these bacteria, lead to clinical symptoms in the late phase of the disease, the dogs develop limb/joint disorders including arthritis quickly after exposure to *B. burgdorferi* [3, 5, 6]. Five of the presently examined 15 dogs exhibited lameness, fever, and anorexia, but only 3 of them showed the presence of DNA of *B. burgdorferi* in blood. The presence of the spirochetes in the blood, however, is only temporary, because after transmission of the bacterium from the tick into the mammalian host, infection is established in the skin, and from there the spirochete disseminates through the body [3, 12, 13]. During the infection a variety of organs can be affected, especially the skin, joints, the heart and the central and peripheral nervous system [12, 13]. The results of our findings suggest, that PCR gives possibility to detect DNA of spirochetes when in early borreliosis stage multiplying on skin and spreading to the blood.

The studies on the antibody levels in the blood sera of dogs infected both naturally and experimentally and their correlation with clinical symptoms suggest the existence of similar limitations, as in the diagnostics of human borreliosis [1, 6, 9, 14]. Even the presence of the specific antibodies does not prejudge about the ongoing disease or an earlier exposure to the pathogen. The majority (86%) of the seropositive dogs tested by Goossens *et al.* [4] for the presence of antibodies of the IgG class, no symptoms attributed to borreliosis were visible. Wieler *et al.* [15], and many other authors, emphasises the deceptiveness of serological tests in the diagnostics, because a high percentage of seropositive results is detected in dogs without clinical symptoms.

Because of difficulties related to the isolation and culture of *B. burgdorferi* on artificial media and ambiguity of serological tests, studies are in progress on the employment of PCR techniques for routine diagnostics of borreliosis. No PCR protocol, however, recommended for this kind of diagnostics, has hitherto been published. No reliable assessment is available in which a fragment of DNA of *B. burgdorferi* s.l. might have been a good genetic marker for PCR in canine borreliosis. In view of the above, the PCR techniques for detecting the spirochete DNA in dogs still remains in its experimental phase [3, 6].

Callister *et al.* [2] demonstrated that the presence of *B. burgdorferi* in skin- and joint biopsy samples of dogs was tightly correlated with the occurrence of lameness and fever; however, the above study was based on an experimentally induced borreliosis. On the other hand, Bauerfeind *et al.* [9] worked on detection of DNA of *B. burgdorferi* in urine samples from dogs by a nested polymerase chain reaction and demonstrated that this method and the urine are useful in diagnosing *B. burgdorferi* infections in dogs. The collection of urine from dogs, and especially tissue samples from live, sick, dogs in Poland is very difficult or even impossible.

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