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PREVELANCE OF DNA AND ANTIBODIES TO *BORRELIA BURGDORFERI* SENSU LATO IN DOGS SUSPECTED OF BORRELIOSIS*

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Abstract: The aim of the paper was an attempt to correlate clinical signs with the presence of DNA of *Borrelia burgdorferi* (sensu lato) s.l. and the antibodies against *B. burgdorferi* s.l. in the blood of dogs. Among the animals studied there were 62 dogs delivered to the Veterinary Clinic in Szczecin and 30 from the Municipal Animal Shelter in Szczecin with varied clinical signs of borreliosis. In all cases the owners admitted frequent contacts of their dogs with ticks, both in the past, as well as shortly before the onset of sickness. We used two methods: PCR for detecting DNA of *B. burgdorferi* s.l. and ELISA test for detecting antibodies against the spirochete. Lameness, the principal symptom of canine borreliosis was the most frequent symptom of the group of 31 PCR-positive animals. The other most common symptoms in PCR-positive dogs were fever, swelling of joints and loss of body weight. DNA of *B. burgdorferi* s.l. was most frequently detected in the blood of dogs of the group 2-5 years old (13/54.1%). ELISA tests specific for IgG antibodies were positive in 37 of 92 sera (40.2%) taken from examined dogs. Lameness was observed in 15 of 37 IgG-seropositive dogs and in 25 of 55 seronegative animals. In 54% of dogs with the antibodies, swelling of instep- and wrist joints was observed compared to only 24.4% in seronegative dogs. An attempt to correlate the PCR results with the results of tests detecting antibodies against *B. burgdorferi* s.l. revealed that fewer than half (45.1%) of the dogs with presence of DNA of the spirochete, developed an immune response. Therefore the transfer of *B. burgdorferi* s.l. form, the primary lesion to the target tissues, is possible in dogs which did not develop immune response or develop an insufficient response. Among 92 borreliosis-suspected dogs 54 (over 58%) were diagnosed positively using laboratory methods. In most cases there was a correlation between clinical symptoms of borreliosis and presence of DNA *B. burgdorferi*, thus PCR may contribute to improving to a large extent diagnostic of canine Lyme disease.

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INTRODUCTION

Lyme borreliosis is a zoonotic disease, targeting mainly humans, but also household pets and domestic animals. From the epidemiological point of view, dogs have been very important since they had been declared an effective factor of spreading human borreliosis [12]. The clinical picture of human Lyme disease has been described frequently in a number of Polish and foreign papers, whereas canine borreliosis is insufficiently known, despite

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substantial similarity. In humans, Lyme borreliosis at its early phase is manifested by erythema migrans and lymphocytoma of the skin around the tick bite. If untreated it transforms into the second- and third phase, where neurological disorders and arthritis are dominant symptoms [14]. The principal attribute of human borreliosis - erythema migrans does not occur in canine borreliosis. In dogs, the principal symptom is the migratory arthritis (i.e. affected limb joints, mainly wrist or instep; oedema of one or two joints, enlargement of groin and prescapular lymph nodes). These symptoms are accompanied by "malaise" (expressed by elevated body temperature, loss of appetite and fatigue) and lameness after few days. Myocarditis develops rarely in canine borreliosis. In older dogs, the renal form may occur with membranous glomerulonephritis and tubular necrosis [5, 6, 15, 16, 17, 19, 20, 25].

Canine borreliosis was described for the first time in the 1980s in the USA [24, 29, 32] and in recent years in almost all countries of western Europe. Dog infections caused by *B. burgdorferi* were recorded in Germany [3, 51], the Netherlands [14, 15, 20, 21] Belgium [35], France [8, 10, 13], Great Britain [34], Spain [9], Slovakia [44], Sweden [11], and Switzerland [42]. We have previously presented preliminary studies on detection of DNA of *B. burgdorferi* s.l. in the blood of diseased dogs [39], and on the presence of antibodies against *B. burgdorferi* s.l. in clinically healthy dogs, naturally exposed to hard-shelled ticks, Ixodes ricinus in Poland [41].

In this paper we present an attempt to correlate clinical signs with the presence of DNA of *B. burgdorferi* s.l. and the antibodies against *B. burgdorferi* s.l. in the blood of dogs naturally exposed to ticks.

MATERIAL AND METHODS

Blood samples were taken from 92 borreliosis-suspected dogs, naturally exposed to ticks. The dogs showed no symptoms of other diseases. Among the animals studied there were 62 dogs of various breeds delivered to the Veterinary Clinic in Szczecin and 30 from the Municipal Animal Shelter in Szczecin. The examination of the dogs, carried out by veterinarians, started with overall physical health assessment, followed by blood sampling. The overall health assessment was aimed at detection of clinical signs of borreliosis (i.e. temperature, lameness, tenderness of instep- and wrist joints, swelling of those joints, enlargements of groin- and prescapular lymph nodes, loss of body weight, and appetite loss), according to a questionnaire provided by the present authors. Also the age, sex, and breed of dogs were recorded, as well as cases of tick exposure, both past- and recent, known to the owner.

The blood samples were taken in the period of the highest tick activity i.e. from June to mid-July (46 samples) and from September to mid-October (46 samples). 1.5 ml blood samples were taken from the cephalic vein into 2 test tubes: with EDTA (1 : 9 ratio) for PCR and without EDTA for serological study.

PCR. DNA of the bacteria was isolated from the blood samples using the QIAamp® DNA Mini Kit (Qiagen, Germany). The PCR primers used were: SC1 (5'-GCT GTC AGT GCG TCT TAA G-3') and SC2 (SC1: 5'- CTT AGC TGC TGC CTC CGT A-3'),complementary to rrs gene area encoding 16S rRNA of the small ribosome subunit of *B. burgdorferi* sensu lato [33]. The second pair were FLA1 and FLA2, complementary to fla gene, conservative for 5 european genospecies of *B. burgdorferi* s.l. [52].

The reaction mixture (20 μl) contained 0.5 U of Taq DNA polymerase (Qiagen), 1x concentrated reaction buffer, 50 μM of each trideoxynucleotide, 400 pM of both primers SC1 and SC2 or FLA1 and FLA2, and 2 μl of DNA isolated from the blood. DNA of the strain Bo-148c/2 *B. burgdorferi* sensu stricto was used as a positive control. The PCR reaction was carried out in a non-oil, T-gradient thermocycler (Biometra, Germany). The course of the PCR reaction we described previously [41].

ELISA. Serologic study was carried out using recombinant ELISA tests (Microgen, Germany) for detection of specific antibodies against *Borrelia burgdorferi* in dogs in the IgG and IgM class (against antigens: p18, p41, p100 and OspC; 22kDa). The source of recombinant antigens were strains of *B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto (s.s.). Positive canine serum was used as a positive control. The test sensitivity was estimated as 100% for IgG and 96% for IgM and the specificity - 84.6% for IgG and 93.8% for IgM. The results are expressed in relative quantification units, RU/ml.

RESULTS

Based on the questionnaire data collected by veterinarians, the dogs were divided into 4 age groups. The age ranged from 0.5–14.5 years. Eighteen dogs were in the group of 0.5–1.5 years. Twenty-four dogs (26%) were assigned to the group of 2–5 years (There were no dogs aged 18–24 months.), while 26 dogs (28.2%) - to the group of 5-8 years. Twenty-four dogs (26%) were older than 8 years (18 nine-year-olds and 6 aged 10.5–14 years).

Among 92 dogs examined, there were 44 (47.8%) females and 48 (52.2%) males. In all cases, the owners admitted frequent contacts of their dogs with ticks, both in the past as well as shortly before getting sick. The results of the physical study of 92 borreliosis-suspected dogs are shown in Table 1.

The blood samples of all dogs turned out to be negative in PCR with primers for the fla gene. The positive reaction of PCR - proving the presence of DNA of *B. burgdorferi* s.l. - with primers complementary to the

fragment of the gene encoding 16S rRNA of the small ribosome subunit was observed in 31 dogs (33.7%).

Lameness - the principal symptom of canine borreliosis was stated in 20 out of 31 PCR-positive dogs. This constituted 64.5% and it was the most frequent symptom of the disease in this group of animals. On the other hand, among 61 PCR-negative dogs, there were only 20 (32.8%) with lameness (Tab. 1). The second most common symptom in PCR-positive dogs was fever (19/ 61.3%), followed by swelling of instep- and wrist joints (58%), loss of body weight (48.4%), loss of appetite, and enlargement of lymph nodes (29%). In the PCR-negative group of dogs, the second most common symptom - after fever - was loss of appetite (47.5%), followed by swelling of instep- and wrist joints (34.4%). The enlargement of lymph nodes in this group was stated in 12 dogs (19.6%), whereas in the PCR-positive group there were only 9 (29%, Tab. 1). Two the most frequent symptoms (i.e. lameness and fever), occurring concurrently, were observed in 11 (35.5%) out of 31 PCR-positive dogs.

The number and percentage of dogs showing 1-, 2-, 3-, or more symptoms of borreliosis is shown in Table 2. Among 31 dogs with detected DNA of *B. burgdorferi* s.l. in their blood, 3 dogs (9.6%) exhibited all earlier-mentioned symptoms. In 2 dogs there were 5 symptoms, while in 5 dogs - 4 symptoms. In 8 dogs (25.8%) in this group, 3 symptoms were noted, while in 22.6% (7 dogs) - 2 symptoms (Tab. 3). Similarly, in the group of 61 borreliosis-suspected dogs, where borrelia DNA had not been detected in the blood, the majority demonstrated 2 (63.9%), and 3 (21.3%) disease symptoms. Two dogs in this group showed all borreliosis-related symptoms (Tab. 2).

DNA of *B. burgdorferi* s.l. was most frequently detected in the blood of dogs of the group 2-5 years old (13/54.1%, Tab. 3). The spirochete DNA in the youngest group (0.5-1.5 years) was detected in 22.2% dogs, while in the group of 5-8 years - in 30.7%. The oldest group had 25% of PCR-positive dogs (Tab. 3).

The results of the presence of antibodies were given in U/ml and all values exceeding 24 U/ml were assumed positive. Values of 20-24 U/ml were considered doubtful, while those below 20 U/ml - negative.

A single positive result was found in a 4-year-old lame bitch, PCR-positive, with a negative result of IgG (against *B. burgdorferi* s.l.) examination.

ELISA test of 92 sera taken from borreliosis-suspected dogs demonstrated that 37 of them (40.2%) contained IgG antibodies. In this number, 12 were doubtful, which was also considered an indicator of a past exposure - an important factor in the epidemiological description.

The lowest percentage of seropositive dogs was in the youngest group (27.8%), the highest - in the group 5-8 years old (46.1%), followed by the group 3-5 years old (45.8%). IgG antibodies were detected, in the sera of 9 dogs older than 8 years, which constituted 37.5% of the seropositive dogs (Tab. 3).

IgG antibodies were detected in 18 (39.1%) of 46 serum samples taken from dogs within the spring-summer season, while in the summer-autumn season 19 (41.3%) of dogs, out of 46, demonstrated the presence of this antibody.

The group of 37 seropositive animals consisted of 44% of females and 56% of males. The group of 55 seronegative dogs was represented by 49.1% of females and 32.7% of males. Lameness - the principal symptom of

Table 1. Prevalence of clinical symptoms (n/%) in dogs suspected of borreliosis (n=92), in dogs PCR positive and PCR negative for presence of *B. burgdorferi* s.l. DNA, and depending on the presence of IgG antibodies against *B. burgdorferi* s.l. (seropositive, n=37 and seronegative, n=55).

No.	Clinical symptoms	n/%	PCR positive n/%	PCR negative n/%	Sero-positive n/%	Sero-negative n/%
1	Fever	62/67.4	19/61.3	43/70.5	27/73	35/63.6
2	Lameness	40/43.4	20/64.5	20/32.8	15/40.5	25/45.4
4	Carpal and tarsal arthralgia	39/42.4	18/58	21/34.4	20/54	19/24.5
5	Enlargement of lymph nodes	21/22.8	9/29	12/19.6	8/22	13/23.6
6	Appetite loss (anorexia)	44/47.8	13/41.9	29/47.5	16/44.4	28/50.9
7	Body weight loss	32/34.8	15/48.4	19/31.1	16/44.4	16/29
8	Infestation by ticks	92/100	31/100	61/100	37/100	55/100

Table 2. Amount of clinical symptoms in PCR positive (n=31) and PCR negative (n=61) dogs and in dogs seropositive for IgG antibodies against *B. burgdorferi* s.l. (n=37) and seronegative (n=55), suspected of borreliosis.

Amount of clinical symptoms	PCR positive n/%	PCR negative n/%	Seropositive n/%	Seronegative n/%
1	6/19.3	4/6.5	6/16.2	5/9.1
2	7/22.6	39/63.9	12/32.4	30/54.4
3	8/25.8	13/21.3	12/32.4	13/23.6
4	5/16.1	2/3.2	3/8.1	3/5.4
5	2/6.4	1/1.6	0/0	2/3.6
6	3/9.7	2/3.2	4/10.8	2/3.6

Table 3. Prevalence of *B. burgdorferi* s.l. DNA (PCR positive) and antibodies against *B. burgdorferi* s.l. (seropositive) in dogs suspected of borreliosis in 4 age ranges.

Age (years)	Number examined	PCR positive n/%	Seropositive n/%
0.5-1.5	18	4/22.2	5/27.8
2-5	24	13/54.1	11/45.8
5-8	26	8/30.7	12/46.1
> 8	24	6/25	9/37.5
Total	92	31/33.6	37/40.2

canine borreliosis - was observed in 15 (40.5%) of 37 IgG-seropositive dogs and in 25 (45.4%) of 55 seronegative animals. The symptom affecting as many as 73% of seropositive dogs, however, was fever. On the other hand, this symptom was stated in 35 animals (63.6%) of the group without the antibodies. In 54% of dogs with the antibodies, swelling of instep- and wrist joints was observed compared to only 24.4% in seronegative dogs. Appetite loss was stated in 16 dogs of the seropositive group. Also 16 dogs showed loss of body weight (44.4%). Those symptoms occurred in the seronegative dogs in 50.9% and 29% of dogs, respectively. The enlargement of the lymph nodes was observed in as few as 8 dogs (22%) in the seropositive group, while in the seronegative - 23.6%. DNA of *B. burgdorferi* s.l. was detected in the blood of 14 dogs with IgG antibodies (25.4%) and in 17 of seronegative dogs (31%). Among seropositive dogs, only 4 (10.8%) demonstrated all earlier-mentioned symptoms (Tab. 2). In 3 dogs there were 4 symptoms observed, in 12 - 3 symptoms, and also in 12 - 2 (32.4%) symptoms. In 16.2% of seropositive dogs a single symptom was observed (Tab. 2). More than half of 55 seronegative dogs showed 2 symptoms (54.4%) and only 2 dogs - all of the earlier mentioned symptoms (Tab. 2).

In 31 PCR-positive dogs, only 1 (3.2%) was tested positively for the presence of IgM antibodies and 14 (45.1%) - for the presence of IgG antibodies. In total, 48.3% developed antibodies against *B. burgdorferi* s.l., which translates into 15 dogs out of 92 borreliosis-suspected, tested positively in both types of this laboratory diagnostic procedure (16.3%). Because the ELISA tests detected IgG in 37 dogs and IgM in 1 dog and the PCR method detected borrelia DNA in 31 dogs, we can conclude (after subtracting 15 overlapping cases) that *B. burgdorferi* s.l. was detected in 54 (58.6%) animals.

DISCUSSION

In North America, Jacobson *et al.* [23] the putative diagnosis borreliosis is obtained by the presence or absence of the following factors: 1) The presence of typical clinical symptoms; 2) exclusion of differential diagnosis; 3) a distinct reaction to antibiotic; 4) evident contact with a tick or living in an endemic area; 5) the presence of antibodies in the blood serum. The latter criterion has been a serious diagnostic indication, however, even the presence of specific antibodies does not give explicit evidence about an active disease nor a prime exposure to the pathogen. In the majority (86%) of seropositive dogs examined by Goossens *et al.* [15] for the presence of IgG antibodies, no symptoms occurred that could be attributed to borreliosis. Wieler *et al.* [51] and many other authors indicated the deceptiveness of serological tests in the diagnostics, because a high percentage of positive sera have been detected in dogs with no clinical symptoms.

In the present paper, as many as 73% of seropositive dogs suspected of borreliosis had fever, whereas in the group without the antibodies, fever was detected in 35 animals, which constituted 63.6%. In more than half of dogs with antibodies, swelling of instep and wrist joints occurred, but this symptom was also visible in 25% of seronegative dogs. As for the number of symptoms recorded, in both seropositive- and seronegative dogs the majority constituted animals with 2–3 symptoms.

Concluding from the above - the detection of antibodies in the sera and their correlation with the clinical symptoms of dogs infected naturally as well as experimentally, indicate similar limitations of serological tests that exist in the diagnostics of human borreliosis, including late seroconversion or its lack [3, 15, 20, 49]. Moreover, the differences in the different study results, sometimes originating from the same areas, might have been caused by serological test used.

The most commonly used serological test in the diagnostics of canine borreliosis is the test of enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody (IFA). Magnarelli *et al.* [31] carried out a comparative study of those tests in detecting antibodies against *B. burgdorferi* in the blood serum of dogs, and concluded that the former test was more sensitive and easier in standardisation. The test results, however, even those carried out by the same method, may differ substantially because of ununiformly prepared antigens representing different strains of borrelia, and this may constitute a serious diagnostic problem [31, 46, 47, 50].

Considering the high variability and extensive changeability of antigen proteins of *B. burgdorferi* s.l., a study was carried out aimed at determining reference antigens for serological tests for detecting antibodies against *B. burgdorferi* [30]. The above-mentioned authors demonstrated that the serological diagnostics does not require a local strain and can be based only on finding response to a mixture of highly specific and pure antigen subunits of flagellin (p41) and surface proteins A and C.

In naturally infected dogs, Barthold *et al.* [2], most frequently detected antibodies against antigens p41, p39, and p22 (OspC). Hovius *et al.* [20] demonstrated that antibodies against flagellin (p41) are present in the serum of dogs with- and without symptoms of borreliosis. Wieler *et al.* [51], however, proved that if the dog serum identified only the flagellin protein (p41), such a result was not dependable. On the other hand, the serum can be considered explicitly positive when it detects p41 protein and two out of five immunodominant antigens, i.e. >94 kDa (p100), 60 kDa, 34 kDa and 29-31 kDa (OspB and OspA) and 20-22 kDa (OspC).

The present study was based on immunoenzymatic, a commercially available ELISA test against IgG and IgM antibodies, targeting flagellin protein (p41), immunodominant protein OspC (22 kDa), p18 and p100 specific for *B. burgdorferi* s.s., *B. garini*, and *B. afzeli*. Therefore, all conditions of specificity and sensitivity of the test for

detecting acute- and chronic cases in Europe were fulfilled.

While human borreliosis received a lot of attention and numerous publications, the canine borreliosis is poorly known. In pet dogs, borreliosis assumes its articulate form and is limited mainly to the joints of wrist and instep. The symptoms are associated with the swelling and tenderness of joints, with enlargement of prescapular- or groin lymph nodes. While in human infections articular limb problems develop in the late phase of the disease - in dogs they appear shortly after exposure [31]. Straubinger *et al.* [45] observed joint swelling, followed by lameness from day 60 post-exposure to tick. Elevated body temperature, loss of appetite, and bad mood have been listed among associated symptoms. After recovering from the acute phase of the disease only lameness remains. Very rarely a myocarditis develops. More often in household pet dogs after a natural infection, nephritis or neurological disturbances develop, which has been described by a number of authors [7, 16, 25].

In the present paper, the lameness of borreliosis-suspected dogs was recorded in 40 cases (43.4%). In 27 of the latter, borreliosis was confirmed by laboratory methods. Magnarelli *et al.* [31] studied a group of borreliosis-suspected dogs where as many as 91% suffered from lameness, and only 62% developed immune response in the from of antibodies. Because in an earlier study, Magnarelli *et al.* [32] obtained 37% seropositive cases among clinically healthy dogs, those authors question the value of lameness as the principal symptom in diagnostics of canine borreliosis.

Each year in Europe, North America, and Asia a high number of people and animals become infected, but not all infected individuals develop clinical symptoms of the disease [27, 48]. Berglund *et al.* [4], Levy *et al.* [26], Steere *et al.* [43] estimated that 5–50% of the infected develop the clinical symptoms. It is not clear what course the infection will take, although it was demonstrated that many borrelia cells in the tissues of an experimentally infected mouse, could cause the tissue inflammation [37].

According to many authors, the sex does not have effect on the frequency of the antibodies occurring in dogs naturally exposed to ticks [9, 18, 36, 44]. No such relationship was observed in our studies neither earlier- nor present [41]

The dynamics of immune response in dogs exposed to *B. burgdorferi* is associated, among other factors, with age of the animals [15, 22, 36], which was also demonstrated by our earlier study [41] as well as the present one. Among 92 dogs examined, the antibodies occurred the most rarely in the youngest age group (27.8%), while they were most frequent in the group of 5–8 year-olds (46.1%), followed by the group 3-5 year-olds (45.8%), similar to the group of clinically healthy dogs [41]. It is interesting that the proportions in the distribution frequency of IgG antibodies against borrelia is consistent with the DNA detection pattern in the animals studied.

Stefancikova *et al.* [44], similar, as Merino *et al.* [36] demonstrated that the threshold for dogs developing a stable immune response is their age of over 1 year. The results of Goossens *et al.* [15] indicate that seropositive response in dogs stabilises itself from their second year of life, which is consistent with Schultze *et al.* [38], Lindenmayer *et al.* [28], and Hovius *et al.* [22]. The above-mentioned authors emphasise that the infection must be repeated each year in order to maintain seropositive readings.

Hovius [21] suggests that certain cases of canine borreliosis can be detected, based on the clinical criteria defined as " malaise-being preceding lameness", although the majority of cases of this disease are associated with changeable symptoms, which can be observed in infections caused by various geno-species of *B. burgdorferi* s.l. or their concurrent infections, which has been proved to exist in dogs. Therefore, in the diagnosis of borreliosis, not only human variety, but also the canine one, a promising tool seems to be the test detecting DNA of bacteria [3, 15, 20, 49, 50]. Chang *et al.* [5] used 2 different genes as genetic markers for the detection of DNA of *B. burgdorferi* s.l. in dogs, and obtained different results from the same biological material. Therefore they suggested the necessity of using not less than 2 pairs of primers for a correct assessment of the result in canine borreliosis. In the present paper, genes fla and rrs encoding 16S rRNA were selected for comparing their effectiveness. The nucleotide sequences in genes fla and rrs exhibit the closed homology among all hitherto studied genes of *B. burgdorferi* s.l.

The present conducted study on the presence of DNA of *B. burgdorferi* s.l. in the blood of dogs, demonstrated the uselessness of primers complementary to fla gene in PCR technique, because all blood samples were negative. Also, our earlier study revealed that fla gene was a very useful genetic marker for detection of DNA from isolates of ticks, *I. ricinus*, while it was not good for human blood [40]. The results of studies on the presence of DNA of *B. burgdorferi* s.l. using primers to the gene rrs encoding 16S rRNA in the blood of dogs without symptoms of borreliosis, were negative [41]. However, 33.6% out of 92 borreliosis-suspected animals exhibited a positive PCR result with primers complementary to gene rrs (Tab. 1), which was the evidence for the presence of DNA of *B. burgdorferi* s.l. in their blood.

Pathogens transmitted by ticks may cause acute- or chronic diseases in sensitive hosts. Other hosts recover spontaneously through elimination of organisms otherwise causing infection or making their hosts - the carriers [1]. Consequently, the exposure to pathogens transmitted by ticks, may lead to elimination of the infective factor, or to the infection in its clinical- or subclical form [1].

Lameness, the principal symptom of canine borreliosis occurred in 20 dogs (64.5%) out of 31 with DNA of *B. burgdorferi* s.l. detected in their blood, and was the main symptom stated in this group of animals. The second most

common symptom in PCR-positive dogs was fever, followed by swelling of instep and wrist joints, loss of appetite, loss of body weight, and enlargement of lymph nodes. The majority of PCR-positive and PCR-negative dogs demonstrated 2-3 disease symptoms. Summing up - among 40 lame, borreliosis suspected dogs, 20 were PCR-positive and lameness was the most frequent symptom among PCR-positive animals. PCR-positive dogs were older than 2 years, mainly from groups 2-5 years and 5-8 year-old.

In order to learn the pathogenesis of borreliosis, Straubinger [45] exposed dogs through tick inoculation. Arthritis occurred between the 50th and 123rd days post-exposure. Numerous spirochetes occurred in biopsy material of the skin on day 60 post-exposure, which coincided with clinical signs of arthritis i.e. lameness. The latter receded within 6 months. The numbers of the bacteria detected in the skin biopsy material was adversely correlated with the antibody level. This experiment has shown that the number of borrelia cells changed during the tissue sampling, and these data suggest that the clinical signs of arthritis developed when the large number of *B. burgdorferi* cells was present in the skin (antibiotic therapy reduced their numbers in the host, but failed to eliminate them altogether).

Based on the presently described clinical picture, it can be concluded that the majority of dogs - where spirochete DNA was detected in the blood - were at the borreliosis stage where symptoms typical for the early infection occurred (swelling of joints and lameness). At this stage, the spirochetes dynamically propagated in the skin and therefore their transfer to the blood was possible.

Within the group of PCR-positive dogs, only 1 (3.2%) demonstrated the presence of IgM antibodies and 14 - IgG antibodies (jointly 48.3% of seropositive dogs). This means that fewer than half of the animals developed antibodies against *B. burgdorferi* s.l. associated with clinical signs of the disease. It is possible that the transmission of *B. burgdorferi* s.l. from the inoculation site to the tissues takes place in dogs which did not develop immune response, or developed insufficient response.

CONCLUSION

In most cases there was a correlation between clinical symptoms of borreliosis and presence of DNA *B. burgdorferi*. PCR may contribute to improving to a large extent, the diagnostic of canine Lyme disease.

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