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Clinical course of borreliosis in Bernese Mountain Dogs – a retrospective study

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Summary

The aim of this study was to perform a retrospective analysis of cases of Lyme borreliosis in Bernese Mountain Dogs (BMDs) in Poland. The observations were conducted on 111 Bernese Mountain Dogs with *Borrelia burgdorferi* antibodies detected in their serum. Clinical symptoms suggestive of Lyme disease were noted in 54 animals (group 1), while the remaining 57 dogs (group 2) were clinically healthy. PCR testing detected the presence of *Borrelia burgdorferi* DNA parallel in the blood and joint fluid of 4 dogs that showed symptoms of apathy, fever, urination disorders and lameness, in the blood and joint fluid of 25 animals that showed symptoms of apathy, fever and lameness, and in the blood of 2 dogs that showed symptoms of apathy, fever and enlarged lymph nodes. No *Borrelia* genetic material was found in the blood of any of the dogs showing no clinical symptoms, or in the blood of 23 dogs showing symptoms suggestive of Lyme disease. The presented observations indicate that BMDs can be infected with *Borrelia* spirochaetes and develop full-blown Lyme disease. Its diagnosis, however, should be supported not only by standard serological testing but also by PCR or Western blot testing. The presence of antibodies reacting with spirochaete antigens in a significant percentage of the BMD population indicates a high risk of overdiagnosing Lyme disease in this breed.

Keywords: *Borrelia burgdorferi*, Bernese Mountain Dog, PCR, ELISA

Lyme disease is a multi-organ disease caused by an excessive immune response to *Borrelia burgdorferi* sensu lato (19, 20), transmitted by ticks (4, 22, 23). Despite the development of numerous monitoring and prevention programs, the disease is still the most commonly diagnosed tick-borne infection in humans and animals in the Northern Hemisphere (18). The group of *B. burgdorferi* sensu lato currently includes 18 spirochaete subspecies (16, 17). The subspecies that are pathogenic for humans and animals mainly include *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto (s.s.), *B. bavariensis* (previously referred to as *B. garinii* OspA serotype 4) and *B. spielmanii*. The pathogenicity of other subspecies, such as *B. lusitaniae*, *B. valaisiana*, and *B. bissettii*, remains uncharacterized (13). Although the aforementioned pathogenic bacterial species may cause erythema migrans, they also often cause a range of other disorders. The vector transmitting disease-causing spirochaetes among animals and humans is *Ixodes ricinus*. The primary reservoirs of *Borrelia* spirochaetes in Europe are small rodents such as mice, rats, and squirrels, as well as rabbits and certain species of reptiles and birds, which serve as sources of these bacteria for ticks (18). Certain species of wild ruminants (deer) and sheep are considered by some researchers to be incompetent reservoirs of *Borrelia*, meaning that the arachnids feeding on them do not become infected (18). The course of Lyme disease varies considerably. Two forms of the disease are distinguished in dogs: articular and renal. The articular form is characterized by fever and shifting lameness. The pathological changes in the joints are generally progressive, and chronic polyarthritis may persist despite treatment. The renal form is a consequence of glomerulonephritis, leading to renal failure with proteinuria, uraemia, and peripheral oedema (12). Clinical

observations made by many authors indicate that the definition of Lyme disease as a multi-organ disease, or even a multi-system disease, is absolutely correct. Nevertheless, the most common disorders that occur during its course are related to the locomotor system; although symptoms from the circulatory system and the skin have also been reported (1, 3, 8, 9).

Numerous reports indicate that Bernese Mountain Dogs often test positive for antibodies against *Borrelia burgdorferi* in rapid diagnostic tests, even in the absence of clinical signs of disease (6, 7, 12, 16, 17). Additionally, quantitative analysis of immunoglobulins produced in response to spirochaete infections has indicated that BMDs may be associated with increased susceptibility to *Borrelia* infections of a hereditary nature (1).

The aim of this study was to perform a retrospective analysis of cases of Lyme borreliosis in Bernese Mountain Dogs in Poland.

Material and methods

Animals used in the study. The observations were conducted between 2020 and 2024 on 111 Bernese Mountain Dogs (68 males and 43 females) aged 0.5-9 years, in which rapid CaniV4 (VetExpert) serological tests detected antibodies to *Borrelia burgdorferi*. The medical history indicated that all dogs under observation had been in contact with ticks (the owners observed ticks on the dogs' bodies and removed them themselves). In the past the animals were not vaccinated against borreliosis Clinical symptoms were observed in 54 animals (group 1), and included: apathy in 54 dogs, fever (average body temperature of 39.5°C) in 54 animals, joint pain and lameness in 44 animals, urination disorders in 14 dogs, enlarged superficial lymph nodes (submandibular or popliteal) in 6 animals No other clinical symptoms were observed in patients of this group. Also no clinical symptoms were observed in the 57 dogs of group 2. In the group of diseased dogs, the simultaneous occurrence of symptoms including apathy, fever, lameness and urination disorders was observed in 12 animals, whereas apathy, fever and lameness were noted in 32 dogs; apathy, fever and lymph node enlargement in 6 dogs; apathy, fever and urination disorders in 2 dogs; and apathy and fever were observed in 2 dogs (Tab. 1).

Blood was collected from all dogs in the study for haematological, biochemical, serological, and molecular testing. After detailed orthopedical and neurological examination, in dogs with movement disorders (shifting legs lameness; n = 44), joint X-rays were performed, and synovial fluid was analyzed (cytology and PCR). An ultrasound examination of the abdomen was also performed in all dogs.

Tab. 1. Clinical findings from 54 dogs in group 1, with suspicion of borreliosis

Clinical symptoms	Number of dogs	%
Apathy, fever, lameness, urinary symptoms	12	22.23%
Apathy, fever, lameness	32	59.26%
Apathy, fever lymphadenopathy	6	11.11%
Apathy, fever, urinary symptoms	2	3.70%
Apathy, fever	2	3.70%

The Ethics Committee of the University of Life Sciences in Lublin (Poland) confirmed that no ethical approval was required for this study.

Haematological examination. Blood for haematological testing was collected in EDTA tubes and then analyzed on a Mythic 5Vet PRO CORMAY analyzer (Poland).

Blood chemistry testing. Blood for chemistry testing was collected in tubes with a coagulation activator. The concentrations of urea, creatinine and total bilirubin, and ALT and AST activity were determined in serum using an Accent-120 CORMAY analyzer (Poland).

Serological testing for selected tick-borne diseases. Serological testing for *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis* and *Dirofilaria immitis* was performed using CaniV4 rapid tests (VetExpert). The test material was whole blood collected in EDTA tubes. A volume of 20 µL of blood was applied to the assay and flooded with three drops of buffer. The results were read after 15 minutes.

PCR. Each blood sample (n = 111)/synovial fluid sample (n = 44) collected from dogs used in the study was labeled with a unique number, without identifying the dog's owner. All samples were analyzed in a BIONOTE Vcheck M10 analyzer (VetExpert, Poland), which isolated whole blood DNA and amplified the DNA of *Borrelia* spp., *Babesia* spp., *Mycoplasma haemocanis*, *Hepatozoon* spp., *Ehrlichia canis*, *Anaplasma* spp., and *Leptospira interrogans* in a real-time PCR (Canine Anemia 8 Panel).

All DNA samples positive in the BIONOTE Vcheck M10 analyzer were also amplified by standard real-time PCR using a Rotor-Gene thermocycler (Corbett Research, Mortlake, Australia). The list of primers used for all studied pathogens, along with the reaction conditions, is presented in Table 2. Real-time PCR with SYBR Green 1 dye was performed in thin-walled 100 µL test tubes. A DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Espoo, Finland) was used in the method, enabling a highly specific reaction. The reaction mixture with a volume of 20 µL consisted of the following components: 2 µL of the DNA matrix, 0.4 µL of each primer, 10 µL of Master Mix containing a hot start version of the modified polymerase Tbr (*Thermus brockianus*), buffer for the polymerase Tbr, dNTP, $MgCl_2$ and the intercalating SYBR Green 1 dye and water to 20 µL.

Cytology of the synovial fluid. Arthrocentesis was performed in 44 dogs with movement disorders under general anaesthesia and aseptic conditions. Synovia was obtained from the joints and analyzed using standard methods. Cytological examination of the synovial fluid was carried out using two staining methods. Staining with Mayer's haematoxylin and eosin was applied. At the same time, the specimens were stained with a Diff-Quick kit based on azure (basic stain) and haematoxylin (acidic stain). After collection, the synovial fluid aspirates were placed on a microscope slide. For the HE method, the specimens were additionally fixed with Cytofix. The specimens were viewed under a light microscope at 40 × magnification.

Tab. 2. Primers and PCR conditions for the detection and identification of *Anaplasma/Ehrlichia spp*, *Babesia canis*, *Borrelia* spp., *Hepatozoon canis*, *Mycoplasma haemocanis* and *Leptospira interrogans* in dogs used in the study

Pathogen	Primers	Target gene	Amplicon size (base pairs (bp)	PCR conditions	Reference
Borrelia burgdorferi s. l.	M1: (5'-ACG ATG CAC ACT TGG TGT TAA-3') M2: (5'-TCC GAC TTA TCA CCG GCA GTC A-3')	16S RNA	357 bp	30 cycles: denaturation at 85°C for 30 s, annealing at 50°C for 30 s, extension at 65°C for 60 s	Lee *et al.* (11)
Anaplasma/ Ehrlichia spp.	EHR 521: (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') EHR 747: (5'-GCA CTC ATC GTT TAC AGC GTG-3')	16S RNA	247 bp	35 cycles: denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 45 s	Pancholi *et al.* (15)
Babesia canis	BAB GF2: (5'-GTC TTG TAA TTG GAA TGA TGG-3') BAB GR2: (5'-CCA AAG ACT TTG ATT TCT CTC-3')	18S RNA	559 bp	50 cycles: denaturation at 92°C for 60 s, annealing at 52°C for 60 s, extension at 72°C for 90 s	Adaszek and Winiarczyk (2)
Leptospira interrogans	G1: 5'-CTG AAT CGC TGT ATA AAA GT-3' G2: 5'-GGA AAA CAA ATG GTC GGA AG-3'	16S RNA	298-320 bp	28 cycles: denaturation at 94°C for 30 s, annealing at 51°C for 180 s, extension at 72°C for 180 s	Ooteman *et al.* (14)
Hepatozoon canis	HepF: (5'-ATA-CAT-GAG-CAA-AAT-CTC-AAC-3') HepR (5'-CTT-ATT-ATT-CCA-TGC-TGC-AG-3')	18S RNA	666 bp	34 cycles: denaturation at 95°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 90 s	Inokuma *et al.* (10)
Mycoplasma haemocanis	SYBR_Forward (5'-AGC AAT RCC ATG TGA ACG ATG AA-3') SYBR_Reverse 1: (5'-TGG CAC ATA GTT TGC TGT CAC TT-3') SYBR_Reverse 2: (5'-GCT GGC ACA TAG TTA GCT GTC ACT-3')	16S RNA	103 bp	40 cycles: denaturation at 95°C for 30 s, annealing at 60°C for 60 s, extension at 72°C for 90 s	Willi *et al.* (21)

X-ray examination. A radiographic examination of the joints was performed in 44 dogs with lameness, using a Zoomax HF digital X-ray system in a direct digital radiography setup. The type of joint examined was determined by the observed walking disorder and the results of the clinical examination. The radiographs were performed in two views: medial-lateral and anterior-posterior. The images were evaluated using DICOM PACS DXR software.

Abdominal ultrasound examination. The examination was performed using an Esaote Mylab Twice ultrasound machine with a 3-11 MHz microconvex probe and a 4-15 MHz linear probe. Patients were examined in the supine and lateral positions. Patient preparation for the examination included shaving the abdominal area, disinfecting the skin with alcohol, and applying gel. The abdominal organs were imaged in longitudinal and transverse sections. In the kidneys, length and width were measured, and cortical-medullary differentiation, echogenicity, and parenchymal echotexture were assessed. Attention was paid to the contours of the renal capsule, the width of the renal pelvis, and the perirenal space.

Statistical analysis. Statistical analysis was performed using Statistica 13.3 (TIBCO Software Inc., USA). The association between the presence of clinical symptoms and PCR positivity for Borrelia burgdorferi was assessed using the chi-square (χ^2) test and Fisher's exact test where appropriate. The strength of association between categorical variables was evaluated using the Phi (φ) coefficient.

Additionally, univariate logistic regression analysis was performed to determine whether the presence of clinical symptoms was a predictor of PCR positivity. Due to quasi-complete separation of the data (absence of PCR-positive cases in asymptomatic dogs), Firth's penalized likelihood logistic regression was applied to estimate odds ratios with 95% confidence intervals.

Differences were considered statistically significant at $p \leq 0.05$.

Results and discussion

Results of rapid serological tests. Rapid serological tests (CaniV4 VetExpert) detected Lyme disease antibodies in all 111 animals under observation. No antibodies for *Anaplasma*, *Ehrlichia*, or *Dirofilaria immitis* antigens were found in the blood of any dog.

PCR test results. PCR testing detected the presence of *Borrelia burgdorferi* DNA in the blood and synovial fluid of 4 dogs that showed symptoms of apathy, fever, urination disorders and lameness; in the blood and synovial fluid of 25 animals that showed symptoms of apathy, fever and lameness; and in the blood of 2 dogs that showed symptoms of apathy, fever and enlarged lymph nodes. No *Borrelia* genetic material was detected in the blood of any of the dogs that showed no clinical symptoms, or in the blood of 23 dogs that showed symptoms suggestive of Lyme disease (Tab. 4).

Haematological and serum chemistry testing results. The results of haematological and serum chemistry tests are provided in Table 3. Leukocytosis was the most common abnormality found in haematological tests. In 60 dogs (54 dogs in group 1, and 6 dogs in group 2), the leukocyte count was elevated and ranged from 18-27 $\times$ 10^3 (reference range of 6-17 $\times$ 10^9). Leukopenia (WBC < 6 $\times$ 10^9) was noted in 3 dogs in group 2.

A decrease in haematocrit below 36% (lower limit of normal) was observed in 6 dogs in group 1 (4 dogs showed urinary tract disorders and signs of lameness, while 2 dogs showed only general and urinary tract symptoms), and 2 dogs in group 2. A decrease in erythrocyte count below 5.1 $\times$ 10^{12} (lower limit of normal) was found in 4 dogs in group 1 (all showed symptoms of lameness and urinary tract disorders), and in 5 dogs in group 2 (Tab. 4).

Serum chemistry tests showed an increase in aspartate aminotransferase (AST) activity above the upper limit of normal (37 > IU/l) in 12 dogs (8 dogs in group 1, 3 dogs showed urinary tract disorders and lameness symptoms, 5 dogs showed signs of lameness and general symptoms, and 4 dogs in group 2). An increase in alanine aminotransferase (ALT) activity above the upper limit of normal (> 50 IU/l) was found in 5 dogs (4 dogs in group 1 with general symptoms and signs of lameness, and 1 dog in group 2). Alkaline phosphatase (ALP) activity was elevated (> 155 IU/l) in 4 dogs in group 1 (all showed signs of lymph node enlargement). An increase in urea concentration above the upper limit of normal (> 45 mg/dl) was noted in 16 dogs in group 1 (in 12 dogs showing signs of lameness and urinary tract symptoms, 2 dogs showing general and urinary tract symptoms, and 2 dogs with enlarged lymph nodes), and in 6 dogs in group 2. Elevated creatinine concentration above the upper limit of normal (> 1.7 mg/dL) was observed in 16 dogs in group 1 (in 12 dogs showing signs of lameness and urinary tract symptoms, 3 dogs with lameness and general symptoms, and 1 dog with enlarged lymph nodes), and in 5 dogs in group 2.

Cytological examination results. In the cytological examination of synovial aspirates collected from 44 dogs with lameness, mucous secretion and morphotic elements, including neutrophils, synovial cells, macrophages, and monocytes, were observed in the material from 41 dogs. The nucleated cell count was elevated (> 1000 cells/μL), and neutrophils were predominant (80-90%).

Imaging test results. In 38 out of 44 dogs with lameness, X-ray examination of the joints affected by the disease showed an increased volume of the synovial cavity of the joint, a changed size of the joint gap and decreased bone saturation under the joint cartilage. In 29 of these dogs, PCR testing of both blood and synovial fluid confirmed the presence of *B. burgdorferi* DNA.

Abdominal ultrasound examination in 8 dogs in group 1 (6 dogs showing urinary tract and lameness symptoms, and 2 dogs showing general and urinary tract symptoms) revealed increased echogenicity of the renal parenchyma, and widening and thickening of the cortical layer. In 4 of the 8 dogs, PCR testing confirmed the presence of *B. burgdorferi* DNA in the blood. No changes were revealed in other abdominal organs on ultrasound.

Tab. 3. Haematological and biochemical test results

Examined parameter	Norm	Number of dogs (%) with decreased parameter	Number of dogs (%) with increased parameter	Number of dogs (%) with correct parameter values
WBC (× 10^9)	6.0-17.0	29.2% 33	67.3% 76	3.5%
RBC (× 10^6)	5.1-8.5	20.4% 23	2.6% 3	77%
Ht (%)	(36-56)	33.6% 38	–	66.4%
ALT (IU)	3.0-50.0	–	63.7% 72	36.3%
AST (IU)	1.0-37.0	–	53.1% 60	46.9%
BIL T (mg/dL)	< 0.6	–	31% 35	69%
ALP (IU)	20-155	–	41.6% 47	58.4%
UREA (mg/dL)	20.0-45.0	–	69% 78	31%
CREA (mg/dL)	1.0-1.7	–	36.3% 41	63.7%

Tab. 4. Clinical manifestation in relation to PCR, haematological and biochemical test results

Clinical symptoms (n = number of dogs)	Serological test results (n = number of dogs)	PCR test result (n = number of dogs)	WBC (n = number of dogs)	Ht (n = number of dogs)	RBC (n = number of dogs)	ALP (n = number of dogs)	ALT (n = number of dogs)	AST (n = number of dogs)	UREA (n = number of dogs)	CREA (n = number of dogs)
Apathy, fever, lameness, renal disorders n = 12	12	4	12	4	4	–	–	3	12	12
Apathy, fever, lameness n = 32	32	25	32	–	–	–	4	5	–	3
Apathy, fever, renal disorders n = 2	2	–	2	2	–	–	–	–	2	–
Apathy, fever, enlarged lymph nodes n = 6	6	2	6	–	–	6	–	–	–	1
Apathy, fever n = 2	2	–	2	–	–	–	–	–	–	–

Statistical analysis results. A highly significant association was found between the presence of clinical symptoms and PCR positivity for *Borrelia burgdorferi* DNA ($\chi^2 = 45.4$, df = 1, $p < 0.001$; Fisher's exact test, $p < 0.001$). PCR-positive results were observed exclusively in symptomatic dogs, whereas none of the clinically healthy animals were PCR-positive. The strength of this association, expressed as the Phi coefficient, was high ($\varphi = 0.64$), indicating a strong relationship between clinical status and PCR results. Logistic regression analysis confirmed that the presence of clinical symptoms was a strong predictor of PCR positivity. Symptomatic dogs were significantly more likely to be PCR-positive compared to asymptomatic animals (OR = 154.2; 95% CI: 9.1-2639; $p < 0.001$), although the wide confidence interval reflects the limited number of positive cases and the presence of separation in the dataset.

All dogs in group 1 were treated with doxycycline at 10 mg/kg p.o. for 4 weeks. As a result, clinical symptoms resolved in 9 of the 12 dogs that showed combined general, urinary tract and musculoskeletal symptoms (including 4 dogs with positive PCR results for *Borrelia*), in 28 of the 32 dogs that showed combined general and musculoskeletal symptoms (including 25 dogs with positive PCR results for *Borrelia*), in 6 dogs showing a combination of general symptoms and enlarged lymph nodes (including 2 dogs with positive PCR results for *Borrelia*), in 2 dogs showing only general symptoms, and in 1 of 2 dogs with general symptoms accompanied by urination disorders.

In the present study, among 111 dogs in which rapid serum serological tests revealed antibodies to *B. burgdorferi*, clinical symptoms were observed in 54. These were typical of Lyme disease and included apathy, weakness, enlarged lymph nodes, movement disorders and nephritis. PCR testing revealed spirochaete DNA in 25 dogs with general symptoms and lameness, in 4 dogs with general symptoms, lameness, and urination disorders, and in 2 dogs with general symptoms accompanied by enlarged lymph nodes. In each case, the clinical symptoms were accompanied by leucocytosis, which is a rather common haematological disorder in the course of canine Lyme disease (12). None of the dogs exhibited any cardiac problems that may accompany Lyme disease (5). The presence of spirochaete genetic material was demonstrated in the blood of 31 out of 54 BMDs with symptoms suggestive of Lyme disease, which provided the basis for the final diagnosis of Lyme disease in these animals. For the remaining 23 dogs, it cannot be ruled out that the observed disorders resulted from prior infection; however, relying solely on rapid ELISA-based serological tests should not be decisive for diagnosing Lyme disease in BMDs. These tests also yielded positive results in 57 clinically healthy representatives of this breed, which is a further confirmation that this breed is known to be predisposed to have antibodies to *B. burgdorferi*, and antibodies against spirochaetes may be present in the serum of the dogs that have not been exposed to the bacteria. This finding was provisionally confirmed by the results of Gerber et al. (7). Those authors tested 160 Bernese Mountain Dogs and 62 large-breed control dogs (all animals had similar fur and were kept under similar conditions) for the presence of antibodies against *B. burgdorferi*. Serological examination was performed using ELISA and Western blotting. Elevated antibody titres were found in 58% of Bernese Mountain Dogs and in only 15% of control dogs. The authors were unable to determine the cause of the large discrepancy between the groups and suspected that it might be a consequence of breed predisposition in Bernese Mountain Dogs. Similar conclusions were reached by German researchers who showed the presence of antibodies against *B. burgdorferi* in the serum of 43.3% of Bernese Mountain Dogs and in only 24.6% of control dogs (17). Another Bavarian study of dogs living in the same geographic area found spirochaete antibodies in 92% of Bernese Mountain Dogs (12 of 13 dogs were seropositive) and only 7% of other breeds (13 of 187 dogs were seropositive). Since it was excluded that these differences were due to increased exposure of Bernese Mountain Dogs to ticks (all animals used in the study came from the same geographical area), it was hypothesized that Bernese Mountain Dogs may by genetically predisposed to have in their serum the antibodies which react with *B. burgdorferi* antigen, and which did not develop as a response of the body to the contact with spirochaetes (6). Interestingly, Gerber et al. (6) found that *Borrelia burgdorferi* seropositive BMDs showed no increase in lameness or signs of renal disease over time. The results of the study indicate that antibodies against *B. burgdorferi* were neither associated with the development of lameness nor with signs of renal disease, such as azotaemia or proteinuria, characteristic of borreliosis.

The authors' earlier analysis enables comparison of the serum protein profiles of Bernese Mountain Dogs that tested positive for *Borrelia* in snap testing with those of dogs of other breeds (healthy and with clinical borreliosis) using MALDI time-of-flight (MALDI-TOF) mass spectrometry. The authors identified two protein fractions of approximately 7.630 and 15.260 kDa in all serum samples from BMD-positive individuals tested by a snap test, in both those exhibiting symptoms of borreliosis and seropositive individuals without symptoms. These two additional protein fractions may be used to distinguish seropositive from seronegative dogs infected with *B. burgdorferi* and may serve as a seropositivity marker (16).

The results of the statistical analysis demonstrate a strong and clinically relevant association between the presence of clinical symptoms and PCR-confirmed infection with *Borrelia burgdorferi*. Importantly, PCR

positivity was observed exclusively in symptomatic dogs, which supports the diagnostic value of molecular methods in identifying active infection.

In contrast, serological positivity was detected in both symptomatic and clinically healthy dogs, indicating limited specificity of antibody-based testing when used as a standalone diagnostic tool. This finding suggests that serological assays may reflect prior exposure or breed-associated seroreactivity rather than active disease in Bernese Mountain Dogs.

Therefore, the diagnosis of Lyme borreliosis in this breed should be based on a combination of clinical assessment and confirmatory molecular testing, particularly PCR, to improve diagnostic accuracy and reduce the risk of overdiagnosis associated with serology alone. The presented observations enable the conclusion that BMDs may become infected with *Borrelia* spirochaetes and develop a fully symptomatic disease with a course typical of Lyme disease. Its diagnosis, however, should be made with caution and supported not only by standard serological testing, but additionally by PCR or Western blot testing (12). In our study we didn't have access to Western blot technique, and we didn't perform this kind of analysis which is the weakness of the observations. It didn't change the fact, that a significant proportion of BMDs may have serum antibodies that react with spirochaetal antigens in rapid serological tests indicates that the risk of overdiagnosing Lyme disease in this breed is high.

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