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Diagnostic value of PCR analysis of synovial fluid for the diagnosis of Lyme borreliosis in dogs

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Lyme borreliosis, also known as Lyme disease, is a chronic multiorgan disease of humans and animals transmitted by ticks of the *Ixodidae* family and caused by a group of spirochetes *Borrelia burgdorferi sensu lato*. Dogs are one of the most susceptible animal species to Lyme disease. Symptoms of the disease in dogs can range from mild lameness and fever to more severe and potentially life-threatening conditions, such as kidney damage and neurological disorders. Due to the variety of clinical manifestations and the lack of a universal diagnostic approach, veterinarians often face difficulties in accurately diagnosing the disease. Therefore, improving the algorithms for diagnosing Lyme borreliosis in dogs remains an urgent issue for practical veterinary medicine. The article describes and analyzes data on two cases of dogs from Kyiv (Ukraine) that presented with musculoskeletal disorders, including lameness, swelling of the knee joints, and tenderness, which raised suspicion of Lyme borreliosis. The diagnostic tests used to confirm the diagnosis of Lyme borreliosis included clinical blood test, C-reactive protein test, cerebrospinal fluid cytology, enzyme-linked immunosorbent assay for IgM and IgG antigens to *Borrelia burgdorferi sensu lato*, computed tomography and PCR analysis of synovial fluid for the presence of *Borrelia burgdorferi sensu lato* DNA. As a result, the clinical blood test, C-reactive protein test, and cerebrospinal fluid cytology were not diagnostically important for the diagnosis of Lyme borreliosis. In both cases, serological tests for antibodies to *Borrelia burgdorferi sensu lato* were not positive. Computed tomography showed the development of an inflammatory process in the knee joints with the formation of osteophytes, enthesophytes, sclerosis of the articular surfaces, and an increase in the volume of synovial bursa. The final diagnosis of Lyme arthritis in both dogs was established by PCR analysis of synovial fluid from the affected joints for the presence of *Borrelia burgdorferi sensu lato* DNA.

This study emphasizes the importance of considering Lyme borreliosis as a potential cause of musculoskeletal disorders in dogs. We also propose an expanded algorithm for the diagnosis of Lyme borreliosis in dogs with a pronounced symptom complex of Lyme arthritis using the method of PCR analysis of synovial fluid to detect the DNA of *Borrelia burgdorferi sensu lato* spirochetes.

Key words: Lyme borreliosis in dogs, clinical cases, diagnosis, polymerase chain reaction, *Borrelia burgdorferi sensu lato*.

Problem statement and analysis of recent research. Lyme borreliosis (LB) is a widespread infectious, endemic disease caused by the group of spirochetes *Borrelia burgdorferi sensu lato* (*B. burgdorferi s.l.*) and is of both medical and veterinary importance. There are significant differences in the course of Lyme disease in humans and dogs. In humans, LB is considered the most common vector-borne disease characterized by an acute course and a primary local skin reaction in the form of «Erythema migrans» followed by chronic damage to the nervous system, musculoskeletal system, heart, and eyes. Laboratory confirmation of the diagnosis of Lyme disease in humans involves two steps, including enzyme-linked immunosorbent assay (ELISA) screening and immunoblotting for questionable samples. Only 10% of people infected with *B. burgdorferi s.l.* do not develop symptoms of LB [1]. Unlike humans, it is difficult to detect clinical signs characteristic of LB in dogs. They rarely have a characteristic erythematous rash associated with acute infection. Typically, the infectious process of LB in dogs is chronic with musculoskeletal damage in the form of polyarthropathy, and less commonly nephropathy, myocarditis, and nervous disorders [2–4]. These clinical signs are difficult to attribute to infection of dogs with *B. burgdorferi s.l.* In endemic areas, only up to 10% of seropositive dogs may show clinical signs of LB [3]. Therefore, the results of serologic tests for LB in dogs should be interpreted with caution, as they only confirm the presence of exposure to the pathogen and do not provide grounds for a clinical diagnosis of LB [4]. Conversely, exposure to borrelia does not always trigger an immune response, and the result of a serologic test may be negative [5, 6]. Previous studies indicate that up to 45.1% of dogs with clinical manifestations of LB, in which *B. burgdorferi s.l.* DNA was detected by PCR, do not have antibodies to borrelia antigens [5–7]. Currently, various commercial test kits are available to detect antibodies to *B. burgdorferi s.l.*, including: immunochromatographic assay (ICA), ELISA and immunoblotting kits, as well as molecular diagnostic tools for the identification of *B. burgdorferi s.l.* DNA, but the unwarranted use of these diagnostic tools and methods can lead to misdiagnosis, underdiagnosis and overdiagnosis of LB.

The development of an algorithm for the diagnosis of Lyme borreliosis in dogs remains relevant. Analysis of documented clinical cases is important for understanding the diagnosis of LB in animals and improving diagnostic algorithms.

The aim of the study. The purpose of our study was to analyze the clinical features of Lyme borreliosis in dogs on the example of two clinical cases and to improve the algorithm for the diagnosis of Lyme arthritis, justifying the use of PCR analysis in dogs with musculoskeletal disorders, taking into account the diagnostic tools available in Ukraine.

Material and methods. The object of the study was two clinical cases of Lyme borreliosis in dogs with manifestations of musculoskeletal disorders. The study collected and described data from medical records of dogs diagnosed with Lyme borreliosis. General and special research methods were used, including computed tomography (CT), detailed clinical blood tests, quantitative analysis of C-reactive protein, cerebrospinal fluid cytology (CSF), ELISA for IgM and IgG to *B. burgdorferi s.l.* and quantitative PCR analysis of synovial fluid samples to detect *B. burgdorferi s.l.* DNA. Examination, treatment, and sampling were carried out at the Zviropolis veterinary center, and laboratory tests were carried out at commercial veterinary laboratories Bald LLC and Biosoft LLC.

The collected data were analyzed to identify common clinical manifestations, abnormalities in laboratory parameters and CT scan results in dogs with Lyme borreliosis. Based on the analysis of two confirmed clinical cases of Lyme borreliosis in dogs and the analysis of previous studies from scientific sources, we improved the diagnostic algorithm.

Two clinical cases of Lyme borreliosis in dogs (case 1 – Dog 1; case 2 – Dog 2) are described in chronological order, including animal manipulations and research results.

Results. ***Case 1.*** The owner of the dog came to the veterinary center with complaints that the animal had been limping on the right hind limb for the last month. Initial data on Dog 1: female Rottweiler breed, 1.9 years old, 42 kg, Kyiv, Ukraine. The dog is kept in an enclosure, periodically walked in parks and forest parks of the city. The dog was vaccinated against infectious diseases, but was not vaccinated against Lyme borreliosis. According to the owner, he did not notice any ticks on the animal. The last preventive treatment against endo- and ectoparasites was carried out four months before the appeal. During the examination, it was found that the dog was in a depressed state, with a body temperature of 38.5 °C. The visible mucous membranes and superficial lymph nodes were within the physiological norm. Examination of the dog's limbs revealed tenderness and swelling of the right knee joint, a positive anterior drawer test of the knee, and crepitation when the limb was flexed. No other pathologies were found in Dog 1. According to the results of the examination, the primary diagnosis was a sprain or tear of the ligaments of the right knee joint. Additional diagnostic tests were prescribed, including a CT scan of the pelvic extremities, a complete blood count, and a C-reactive protein test. For treatment, the non-steroidal anti-inflammatory drugs (NSAID) Cimalgex (active ingredient cimicoxib) at a dose of 80 mg once daily for seven days and restriction of physical activity were prescribed. It was planned to check the effectiveness of the treatment in seven days.

Results of additional diagnostic tests.

Clinical blood test. The result of the analysis shows a slight tendency of increasing the average volume of red blood cells, hematocrit and lymphocytes. Detailed results of complete blood count are shown in Table 1.

Table 1– **Results of clinical blood test of a Dog 1**

Indicator name	Units of measurement	Study results for Dog 1	Physiological norms for dogs	
			min.	max.
Hemoglobin	g/L	187	110	190
Red blood cells	$x10^{12}/L$	7.8	5.5	8.5
Mean red blood cell volume	pg	73.1	62	72
Mean hemoglobin concentration in red blood cells	pg	23.9	20	25
Average haemoglobin concentration in red blood cells	g/L	328	300	380
Range for red blood cell	%	11.7	11	15.5
Hematocrit	%	57.0	39	56
White blood cells	$x10^9/L$	11.9	6	17
Neutrophils:				
young (myelocytes)	%	0	0	0
young (myelocytes)	$x10^9/L$	0	0	0
band neutrophils	%	1	0	3
band neutrophils	$x10^9/L$	0.11	0	0.3
segmented neutrophils	%	63	60	70
segmented neutrophils	$x10^9/L$	6.93	3	11.4
Lymphocytes	%	32	12	30
Lymphocytes	$x10^9/L$	3.52	1	4.8
Monocytes	%	3	1	7
Monocytes	$x10^9/L$	0.33	0.15	1.35
Eosinophils	%	1	0	5
Eosinophils	$x10^9/L$	0.11	0.1	0.75
Basophils	%	0	0	1
Basophils	$x10^9/L$	0	0	0.04
Erythrocyte sedimentation rate	mm/h.	1	1	22
Thrombocytes	$x10^9/L$	349	160	430

C-reactive protein test. The serum test for protein markers of the acute phase of inflammation showed that the quantitative content of C-reactive protein was 4.9 mg/L, which corresponds to the physiological norm and indicates the absence of active inflammation.

Results of computed tomography. CT examination of the pelvic extremities revealed signs of osteoarthritis and tendonitis in the right and left knee joints. The description of the CT results is given in Table 2.

Table 2 – **Results of computed tomography of the pelvic limbs in a Dog 1**

Indicator name	Description of CT scan results	
CT scan of the pelvic limbs:	**Right pelvic limb**	**Left pelvic limb**
Density of bone structures	not changed	not changed
Integrity of bone structures	not changed	not changed
Soft tissues of the joint	increased synovial volume; increased tissue density and displacement of the fat body in the knee joint area	increased synovial volume; increased tissue density and displacement of the fat body in the knee joint area
Knee joint	signs of formation of multiple osteophytes and enthesiophytes at the level of the knee joint with areas of sclerosis of the articular surface of the tibia	signs of formation of multiple osteophytes and enthesiophytes at the level of the knee joint

Seven days later, the effectiveness of the treatment was monitored and the results of laboratory tests and CT of the dog's pelvic limbs were analyzed. The results of additional diagnostic tests showed that the dog had osteoarthritis of the knee joints with signs of inflammation of the joint membrane. The initial diagnosis of sprain/tear of the right knee joint ligaments was erroneous, as the CT scan showed no signs of ligament damage. Clinical blood tests did not indicate the development of pathological abnormalities. The C-reactive protein test also showed no signs of active inflammation. During the examination, the dog's general condition improved, with a body temperature of 38.0°C. However, there were still symptoms of swelling, tenderness and crepitation in both knee joints. At this stage, chronic inflammation associated with an infectious process was suspected. To determine the cause of these persistent symptoms and for differential diagnosis, we proposed to perform an ELISA for antibodies to *Brucella canis* and *B. burgdorferi s.l.*, as well as a quantitative PCR analysis of synovial fluid from the affected joints to determine the presence of *B. burgdorferi s.l.* DNA.

Enzyme-linked immunosorbent assay and polymerase chain reaction. The results of the ELISA were negative for antibodies to *Brucella canis*, as well as IgM and IgG to *B. burgdorferi s.l.* However, a quantitative PCR test of synovial fluid from both joints revealed the presence of *B. burgdorferi s.l.* DNA.

Case 2. The second case occurred a week after the first. The owner of the dog came to the veterinary center and reported that over the past two months, the dog had been experiencing periodic short-term lameness of the pelvic limbs, accompanied by apathy and decreased appetite.

Initial data on Dog 2: male Labrador retriever, 2.5 years old, weighing 31 kg, kept indoors, actively walked with physical activity on specialized walking grounds, in parks, forest parks, forest plantations, etc. The dog was vaccinated against infectious diseases, but not against Lyme borreliosis. According to the owner, no tick infestation was observed on the animal. The last preventive treatment of the dog against endo- and ectoparasites was carried out four months before the animal was admitted to the clinic. Initial examination of Dog 2 revealed depression, body temperature of 38.8 °C, visible mucous membranes and superficial lymph nodes within the physiological range. Examination of the dog's pelvic limbs revealed hypermobility of the knee joints, tenderness and slight swelling in the area of these joints. The preliminary diagnosis was a sprain of the knee joint ligaments due to an impact. For treatment, the NSAID Cimalgex (active ingredient cimicoxib) at a dose of 80 mg once daily for four days and limiting physical activity.

Subsequent examination of Dog 2 revealed the following: the general condition of the animal was unchanged, visible mucous membranes and superficial lymph nodes were within the physiological norm, and the body temperature was 38.7 °C. An examination of the pelvic limbs revealed hypermobility of the kneecaps toward medial displacement, and the final diagnosis requires additional diagnostic tests. As in the first case, we recommended the same examinations, namely: clinical blood test, C-reactive protein test, CT scan of the knee joints, ELISA for antibodies to *Brucella canis*, ELISA for IgM and IgG to *B. burgdorferi s.l.*, PCR analysis of synovial fluid from the knee joints to detect *B. burgdorferi s.l.* DNA, and additional cytological examination of the cerebrospinal fluid (CSF). The course of treatment with the NSAID Cimagex at a dose of 80 mg once daily was extended for another seven days. It was also recommended to reduce physical activity and the duration of walks.

Results of additional diagnostic tests.

Clinical blood test. According to the results of the blood test, no clinical deviations from the physiological norm were detected in Dog 2. More detailed results of the general clinical blood test are shown in Table 3.

C-reactive protein test. A serum test for protein markers of the acute phase of inflammation showed that the quantitative content of C-reactive protein was 4.49 mg/L, which corresponds to the physiological norm and indicates the absence of active inflammation.

Computed tomography results. CT examination of the pelvic extremities revealed osteoarthritis of the knee joints with signs of synovitis (more pronounced on the left). A description of the CT results is given in Table 4. CT visualization of the knee joints of Dog 2 is shown in Figure 1.

Cytological analysis of the cerebrospinal fluid. According to the results of cytological analysis, no pathological changes were found in the sample of cerebrospinal fluid: a moderate number of leukocytes of varying degrees of maturity, a small number of erythrocytes, single mesothelial cells without features were found. No atypical cells were found.

Enzyme-linked immunosorbent assay and polymerase chain reaction. The results of ELISA in Dog 2 for antibodies to *B. burgdorferi s.l.* were negative for IgM and questionable for IgG. The ELISA result for antibodies to *Brucella canis* was also negative. As in the first case, the result of the quantitative PCR test of synovial fluid from both joints for the presence of *B. burgdorferi s.l.* DNA in Dog 2 was positive.

Table 3 – **Results of clinical blood test of a Dog 2**

Indicator name	Units of measurement	Study results for Dog 2	Physiological norms for dogs	
			min.	max.
Hemoglobin	g/L	173	110	190
Red blood cells	x10^{12}/L	7.26	5.5	8.5
Mean red blood cell volume	pg	72.5	62	72
Mean hemoglobin concentration in red blood cells	pg	23.8	20	25
Average haemoglobin concentration in red blood cells	g/L	328	300	380
Range for red blood cell	%	11.9	11	15.5
Hematocrit	%	52.6	39	56
White blood cells	x10^{9}/L	16.2	6	17
Neutrophils:				
young (myelocytes)	%	0	0	0
young (myelocytes)	x10^{9}/L	0	0	0
band neutrophils	%	1	0	3
band neutrophils	x10^{9}/L	0.16	0	0.3
segmented neutrophils	%	63	60	70
segmented neutrophils	x10^{9}/L	10.08	3	11.4
Lymphocytes	%	29	12	30
Lymphocytes	x10^{9}/L	4.64	1	4.8
Monocytes	%	3	1	7
Monocytes	x10^{9}/L	0.48	0.15	1.35
Eosinophils	%	4	0	5
Eosinophils	x10^{9}/L	0.64	0.1	0.75
Basophils	%	0	0	1
Basophils	x10^{9}/L	0	0	0.04
Erythrocyte sedimentation rate	mm/h.	2	1	22
Thrombocytes	x10^{9}/L	391	160	430

Table 4 – **Results of computed tomography of the pelvic limbs in a Dog 2**

Indicator name	Description of CT scan results	
CT scan of the pelvic limbs:	**Right pelvic limb**	**Left pelvic limb**
Density of bone structures	not changed	not changed
Integrity of bone structures	not changed	not changed
Soft tissues of the joint	increase in the volume of the synovial bag of the knee joint	increase in the volume of the synovial bag of the knee joint
Knee joint	a moderate amount of osteophytes is visualized, the articular surface is uneven with areas of sclerosis	a moderate amount of osteophytes is visualized, the articular surface is uneven with areas of sclerosis

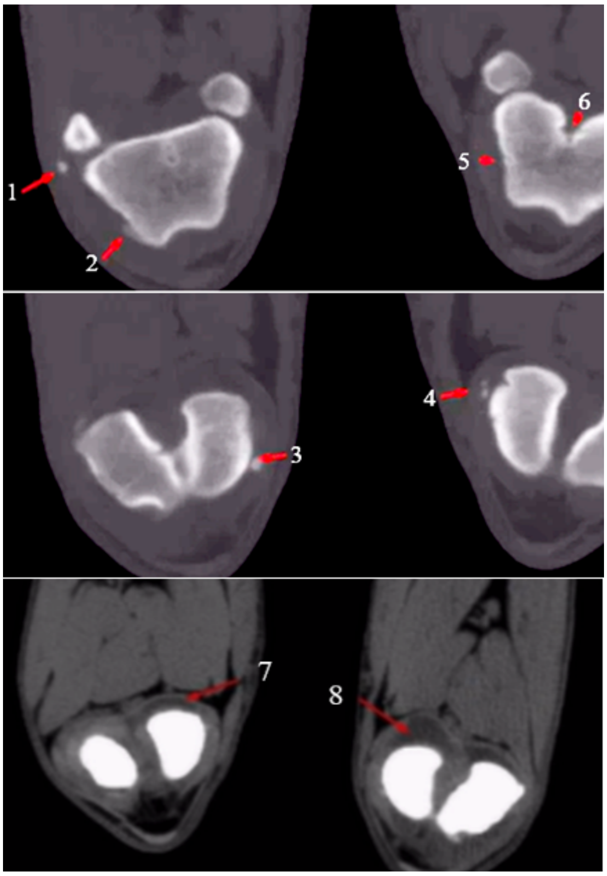

Fig. 1. **Osteoarthritis of the knee joint with signs of synovitis in Dog 2: 1-4 – osteophytes; 5-6 – unevenness of the articular surface with areas of sclerosis; 7-8 – increased volume of the synovial sac.**

Dog 1 and Dog 2 were finally diagnosed with Lyme borreliosis with a clear symptom complex of Lyme arthritis, taking into account the clinical manifestations of musculoskeletal lesions, lack of response to treatment with non-steroidal anti-inflammatory drugs, CT data and a positive PCR result of synovial fluid from the affected joints for the presence of *B. burgdorferi s.l.* DNA.

For the treatment of Lyme borreliosis in dogs, a course of the antimicrobial drug doxycycline at a dose of 100 mg once daily for 30 days, continued symptomatic treatment with the non-steroidal anti-inflammatory drug Cimalgex (active ingredient simicoxib) at a dose of 80 mg once daily and restriction of physical activity of dogs were prescribed. Two weeks later, the effectiveness of the treatment was monitored. Both dogs showed improvement in their general condition. In Dog 1, the volume of the right knee joint decreased, indicating a decrease in edema, a positive anterior knee drawer test and crepitation during flexion of the limb remained unchanged; the right knee joint was in the physiological range of normal, as at the initial examination. In Dog 2, the swelling of the knee joints also decreased, and the hypermobility of the knee joints remained the same as at the initial examination. As a result, both dogs showed positive dynamics of treatment of Lyme arthritis with the antimicrobial drug doxycycline.

Protocol for the diagnosis of Lyme arthritis in dogs and suggestions for its improvement. If a dog is diagnosed with mono- or polyarthropathy, accompanied by periodic or chronic lameness, or astasia, pain and swelling in the affected joints, such an animal may be suspected of Lyme borreliosis. In addition, in order to include Lyme disease in the list of differential diagnoses, when collecting anamnestic data, it is necessary to establish whether the dog was exposed to factors that contributed to the infection of the animal. In particular, the main risk factor for dogs being infected with *B. burgdorferi s.l.* is contact with Ixodes ticks. The risk is increased if dogs live in an endemic area, spend time in wooded or grassy areas, and are not treated with tick repellent. Young dogs under three years of age and certain breeds, such as Golden Retrievers and Labrador Retrievers, are also at increased risk of LB infection.

The next step is to exclude other diseases that are transmitted by blood-sucking insects or are related to the environment. For example, exposure to such pathogens as *Ehrlichia*, *Anaplasma spp*. and *Bartonella spp.* and the European tick-borne encephalitis virus. Symptoms of arthropathy can be caused by non-infectious etiologic factors. They are usually associated with animal trauma, excessive stress on joints and cartilage, as well as genetic factors (e.g., immune-mediated polyarthritis, systemic lupus erythematosus, joint dysplasia, etc.) and metabolic disorders, that contribute to the development of morphological and functional changes in bone and joint tissue (e.g., panosteitis, hypertrophic osteodystrophy, hypertrophic osteopathy, cartilage metabolic disorders, etc.).

The final confirmation of the diagnosis of Lyme arthritis in dogs requires the use of laboratory methods, in particular ELISA and PCR. Considering the data obtained during the study of clinical cases of Lyme borreliosis in dogs, as well as the fact that the diagnosis in both animals was confirmed by direct isolation of the pathogen DNA, we propose to expand the algorithm for the diagnosis of Lyme arthritis in dogs to include PCR analysis of synovial fluid samples from affected joints as the main method of confirming the diagnosis.

A detailed scheme for the diagnosis of Lyme borreliosis with severe symptoms of Lyme arthritis in dogs is described in Table 5.

Discussion. In February-March 2023, two cases of Lyme borreliosis were diagnosed in dogs from Kyiv (Ukraine). In both cases, knee joint lesions were initially thought to be the result of trauma, and the final diagnosis of Lyme borreliosis was made more than a month later. It is generally accepted that dogs are less susceptible to Lyme borreliosis than humans, and this belief may lead veterinarians to ignore Lyme borreliosis as a potential cause of the disease and not include it in the list of differential diagnoses. On the other hand, the diagnosis of Lyme borreliosis in dogs is complicated by the lack of highly specific laboratory tests and standardized diagnostic algorithms.

In general, both cases of LB in dogs were similar to each other. Both dogs had no vaccine immunity against LB. The dogs were last treated for endo- and ectoparasites approximately four months before the first complaint of lameness. The fact that the dog owners did not notice the tick infestation may be due to the small size of the larvae, nymphs or adults of the Ixodes ticks, which feed on animals and can infect dogs with *B. burgdorferi s.l.* It is difficult to assume when the animals were infected with borrelia, as the incubation period in dogs with LB can be from 2 to 6 months. The development of a borreliosis infection involves the attachment of an infected tick to the skin of a susceptible host and the beginning of its feeding on the animal's blood. After the contact of Borrelia surface proteins with mammalian blood, the expression of OspC metabolic proteins increases, which ensure the migration of *B. burgdorferi s.l.* from the tick gut into the blood or lymph of the mammalian host, followed by colonization of target tissues, where direct or indirect tissue damage occurs as a result of an inflammatory response or possibly due to the production of toxins by *B. burgdorferi s.l.* [8].

In both cases, the dogs developed inflammatory pathological processes in the knee joints of the pelvic limbs, which were clinically manifested by pain, swelling, lameness, crepitus during flexion and hypermobility of the knee joints. Computed tomography showed signs of osteoarthritis and synovitis, in particular the formation of osteophytes, enthesiophytes, sclerosis of the articular surface and an increase in the volume of the synovial bursa. Borrelia are considered highly invasive microorganisms with adhesive properties and tissue tropism that can cause inflammatory reactions in the connective tissues of the joints, causing pain, swelling, and lameness. Both in experimental conditions and in naturally infected dogs with *B. burgdorferi s.l.*, the clinical form of Lyme borreliosis often develops as a recurrent lameness associated with arthritis. In collagen-rich tissues, *B. burgdorferi s.l.* spirochetes can multiply and remain viable for years, which may explain the recurrent nature of lameness in dogs [9]. It has also been previously reported that *B. burgdorferi s.l.*, whose DNA was identified by PCR in synovial fluid samples from arthropathy in dogs, is a major pathogenic factor in inflammatory arthritis with knee joint instability and anterior cruciate ligament rupture [10].

Despite the development of inflammatory processes in the knee joints of Dog 1 and Dog 2, the clinical parameters of the blood tests of the animals were within normal limits, except for a tendency to a moderate increase in lymphocytes in Dog 2 – 32%, with an upper limit of 30%. The results of the acute phase protein analysis were also negative for both animals. Dog 2 underwent an additional CSF examination, the results of which were within the physiological normal range for dogs. Thus, in these cases of Lyme borreliosis in dogs, the data of the above analyzes were of no diagnostic value. In general, this coincides with the opinion of other authors who pointed out the absence of specific pathognomonic indicators of laboratory data of clinical blood test, cytological analysis of joint fluid and cerebrospinal fluid in the clinical form of LB in dogs [11,12].

Table 5 – **Algorithm for the diagnosis of Lyme borreliosis with severe symptoms of Lyme arthritis in dogs**

<table>
<tr><th colspan="7">DIAGNOSIS OF LYME ARTHRITIS IN DOGS</th></tr>
<tr><td colspan="7">PREREQUISITES FOR INCLUDING THE DIAGNOSIS OF «LYME BORRELIOSIS» IN THE LIST OF DIFFERENTIAL DIAGNOSES</td></tr>
<tr><td colspan="3">Mono- or polyarthropathy</td><td colspan="4">Risk factors for infection of dogs with B. burgdorferi s.l.:</td></tr>
<tr><td>Disease course: recurrent, sometimes chronic</td><td>Clinical signs: lameness, asthma, pain and swelling in the joints</td><td>Associated symptoms: fever, lymphadenopathy, lethargy, anorexia</td><td>The impact of ticks (infection of dogs with Ixodes ticks)</td><td>The presence of a dog in an area with an endemic status for LB</td><td>Age and breed (young dogs and some breeds: Golden Retrievers and Labrador Retrievers)</td><td>Lack of preventive treatment of dogs against ticks</td></tr>
<tr><td colspan="7">EXCLUSION OF DIFFERENTIAL DIAGNOSES</td></tr>
<tr><td colspan="3">Other infectious diseases transmitted by blood-sucking insects or related to the environment</td><td colspan="2">Genetic, metabolic and other systemic disorders of the body</td><td colspan="2">Traumatic factors</td></tr>
<tr><td colspan="3">Ehrlichiosis, anaplasmosis, bartonellosis, European tick-borne encephalitis, septic arthritis associated with other infectious agents</td><td colspan="2">Immune-mediated polyarthritis, systemic lupus erythematosus, joint dysplasia, panosteitis, hypertrophic osteodystrophy, hypertrophic osteopathy, cartilage metabolism disorders, etc.</td><td colspan="2">Limb injuries: bruises, dislocations, fractures, etc.</td></tr>
<tr><td colspan="7">DIAGNOSTIC TESTS TO ASSESS THE SEVERITY OF THE PATHOLOGICAL PROCESS</td></tr>
<tr><td colspan="2">Clinical blood test</td><td>C-reactive protein test</td><td>Rheumatoid factor test</td><td colspan="2">Cytology of CSF and synovial fluid</td><td>Computed tomography or magnetic resonance imaging of joints</td></tr>
<tr><td colspan="7">LABORATORY CONFIRMATION OF THE DIAGNOSIS OF «LYME BORRELIOSIS» WITH A SYMPTOM COMPLEX OF «LYME ARTHRITIS»</td></tr>
<tr><td colspan="4">Detection of antibodies to B. burgdorferi s.l.</td><td colspan="3">Direct isolation of the pathogen from synovial fluid</td></tr>
<tr><td colspan="4">Stage I – Immunochromatographic analysis or enzyme-linked immunosorbent assay</td><td colspan="3" rowspan="2">Polymerase chain reaction (conventional or quantitative)</td></tr>
<tr><td colspan="4">Stage II – Immunoblotting for samples with questionable ICA or ELISA results</td></tr>
</table>

Both clinical cases of Lyme arthritis in dogs were characterized by the absence of an appropriate immune response to the antigens of the LB pathogen. The ELISA for the presence of IgM and IgG to *B. burgdorferi s.l.* antigens in the sera was negative, except for a questionable IgG ELISA result in Dog 2. These results can be explained by the ability of *B. burgdorferi s.l.* spirochetes to avoid the host immune response by differential synthesis of lipoproteins on the outer surface. Spirochetes of *B. burgdorferi s.l.* can inhibit complementation with the help of the outer surface proteins OspS OspE, CspA and CspZ, which are complement factor regulators [6,14,15]. That is, borrelia evade the immune response due to high antigenic variability and resistance to complement. The clinical manifestations that develop in Lyme borreliosis can be caused partly by the pathogen *B. burgdorferi s.l.* and partly by the immune response of the animal [7,16,17]. Studies by other authors indicate that not all dogs with symptoms of Lyme arthritis develop an immune response to *B. burgdorferi s.l.* In addition, researchers point to a correlation between the age of dogs and the degree of immune response to *B. burgdorferi s.l.* antigens, where it is noted that dogs under three years of age have the lowest frequency of detection of antibodies to the causative agent of Lyme arthritis (Hovius *et al.*, 1999; Goossens, van den Bogaard and Nohlmans, 2001; Skotarczak *et al.*, 2005). It is possible that in the case of Dog 1 and Dog 2, the lack of immune response to *B. burgdorferi s.l.* antigens may also be related to their age.

ELISA and immunoblotting are the most commonly used tools for the two-step diagnosis of LB in humans. However, this standard of diagnosis of LB is not suitable for use in veterinary medicine [9]. A number of studies indicate that seropositive dogs to *B. burgdorferi s.l.* remain asymptomatic in most cases and only 5-10% of dogs may develop clinical LB [18-20]. Given that the immune system can often respond positively to the causative agent of Lyme borreliosis in asymptomatic dogs and that the spirochete *B. burgdorferi s.l.* can evade immune detection, reliance on serological tests alone for the diagnosis of Lyme borreliosis in dogs may not be conclusive. Therefore, positive, questionable, or negative results for antibodies to *B. burgdorferi s.l.* may not be sufficient to confirm or refute the diagnosis of Lyme borreliosis in dogs.

Therefore, any serologic diagnostic results should be accompanied by studies based on direct methods that allow the identification of the presence of *B. burgdorferi s.l.* The causative agents of LB can be identified using microbiological methods of cultivation and molecular methods for detecting borrelia DNA from blood or tissue samples. Microbiological cultivation can detect viable *B. burgdorferi s.l.* microorganisms in samples, but this method has low sensitivity, requires special equipment, 6-8 weeks of incubation of cultures under special conditions, and is therefore usually used for research purposes rather than in the laboratory diagnosis of Lyme borreliosis [21]. The best alternative to the microbiological identification of borrelia is another direct method – PCR analysis, which allows to establish the presence of bacterial DNA of *B. burgdorferi s.l.* spirochetes in the sample within a few hours, which directly indicates the presence of the pathogen [8]. In practice, veterinarians are much less likely to use PCR analysis for the diagnosis of LB in dogs compared to serological methods [22]. However, in our study, the diagnosis of Lyme borreliosis in Dog 1 and Dog 2 was confirmed by PCR analysis of synovial fluid from the affected knee joints.

Thus, the definitive diagnosis of Lyme borreliosis in dogs requires a comprehensive approach and none of the laboratory diagnostic methods can be used alone to confirm the clinical diagnosis of Lyme arthritis in dogs. Earlier, Littman et al. proposed five key criteria for the diagnosis of Lyme borreliosis in dogs: 1) clinical presentation consistent with LB; 2) history of tick exposure and/or increased risk of LB in endemic areas; 3) positive serologic test results; 4) exclusion of differential diagnoses; and 5) response to LB treatment [4]. But this protocol does not take into account the currently available PCR method, which, according to our data, has a high diagnostic value.

Based on the results of our studies and analysis of the results of studies by other authors, we propose to expand the algorithm for the diagnosis of Lyme borreliosis in dogs with Lyme arthritis symptom complex to include the method of PCR testing of synovial fluid for *B. burgdorferi s.l.* DNA.

Conclusions. 1. It is important to consider Lyme borreliosis as a potential cause of musculoskeletal diseases in dogs. Clinical cases highlight the problems of accurate diagnosis of Lyme borreliosis in dogs due to the variety of clinical manifestations and the lack of a universal diagnostic approach. PCR analysis of synovial fluid for the presence of *B. burgdorferi s.l.* DNA can be a valuable tool for the diagnosis of Lyme arthritis in dogs, and an expanded algorithm for the diagnosis of Lyme borreliosis can improve the accuracy of diagnosis and help in the timely treatment of animals.

2. The diagnostic value of clinical blood counts, cerebrospinal fluid cytology, and C-reactive protein tests have proven to be limited for the diagnosis of Lyme borreliosis. Methods for the detection of antibodies to *B. burgdorferi s.l.* in dogs cannot be effectively used as the only diagnostic test for

Lyme disease in dogs. A comprehensive diagnostic approach is required for an accurate diagnosis.

3. Computed tomography revealed inflammation in the knee joints of dogs with Lyme arthritis with the formation of osteophytes, enthesiophytes, sclerosis of the articular surfaces and an increase in the volume of synovial bursae, so it can be useful for assessing the severity of musculoskeletal symptoms in dogs with Lyme arthritis, but does not indicate the cause of the pathology.

4. PCR analysis of synovial fluid for the presence of *B. burgdorferi s.l.* DNA has proven to be a valuable diagnostic tool for the diagnosis of Lyme borreliosis in dogs with symptoms of arthropathy. This method can be particularly useful in determining the causes of musculoskeletal disorders in dogs.

5. An expanded algorithm for the diagnosis of Lyme borreliosis in dogs with severe musculoskeletal symptoms is proposed, which includes the use of PCR analysis of synovial fluid as the main method for the direct identification of the pathogen *B. burgdorferi s.l.*, the etiological factor in the development of Lyme arthritis. Further studies of clinical cases of Lyme disease in dogs are needed to evaluate the effectiveness of the PCR diagnostic method and to fully understand the degree of relationship between *B. burgdorferi s.l.* and arthropathy in dogs.

Information on bioethical compliance. All manipulations with animals were carried out as part of treatment at the veterinary hospital and did not involve additional impact on animals, all bioethical standards were observed in accordance with the bioethical rules of the veterinary hospital.

Conflict of interest disclosures. The authors declare no conflict of interest.

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Діагностична цінність ПЛР-аналізу синовіальної рідини для діагностики Лайм-бореліозу у собак

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Лайм-бореліоз, також відомий як хвороба Лайма – це хронічне, поліорганне захворювання людей та тварин, яке передається кліщами родини *Ixodidae* і зумовлюється групою спірохет *Borrelia burgdorferi sensu lato*. Собаки є одним з найбільш сприйнятливих видів тварин до хвороби Лайма. Ознаки захворювання у собак можуть варіювати від легкої кульгавості та лихоманки до більш важких і потенційно небезпечних для життя станів, таких як ураження нирок та неврологічних розладів. Через різноманітність клінічних проявів і відсутність універсального діагностичного підходу ветеринари часто стикаються з труднощами в точному діагностуванні захворювання. Тому покращення діагностичних алгоритмів Лайм-бореліозу у собак залишається актуальним питанням для практичної ветеринарії. У статті описано та проаналізовано дані про два випадки захворювання собак з м. Київ (Україна), у яких спостерігалися порушення опорно-рухового апарату, зокрема кульгавість, набряк колінних суглобів та болючість, що викликало підозру на Лайм-бореліоз. Діагностичні тести, використані для підтвердження діагнозу Лайм-бореліозу, передбачали клінічний аналіз крові, С-реактивний білок, цитологію спинномозкової рідини, імуноферментний аналіз сироватки крові на IgM та IgG до антигенів *Borrelia burgdorferi sensu lato*, комп'ютерну томографію та ПЛР-аналіз синовіальної рідини на ДНК *Borrelia burgdorferi sensu lato.* В результаті аналізи крові на клінічні показники, С-реактивний білок та цитологічне дослідження спинномозкової рідини не були діагностично важливими для встановлення діагнозу Лайм-бореліозу. В обох випадках серологічні дослідження на антитіла до *Borrelia burgdorferi sensu lato* не дали позитивного результату. Комп'ютерна томографія вказала на розвиток запального процесу в колінних суглобах з утворенням остеофітів, ентезіофітів, склерозом суглобових поверхонь, збільшення об'єму синовіальних сумок. Остаточний діагноз Лайм-артритів обох собак був встановлений за допомогою методу ПЛР-аналізу синовіальної рідини з уражених суглобів на наявність ДНК *Borrelia burgdorferi sensu lato.*

Це дослідження підкреслює важливість розгляду Лайм-бореліозу як потенційної причини розладів опорно-рухового апарату. Також пропонуємо розширений алгоритм діагностики Лайм-бореліозу у собак з вираженим симптомокомплексом Лайм-артриту з використанням методу ПЛР-аналізу синовіальної рідини для виявлення ДНК спірохет *Borrelia burgdorferi sensu lato.*

Ключові слова: Лайм-бореліоз у собак, клінічні випадки, діагностика, полімеразна ланцюгова реакція, *Borrelia burgdorferi sensu lato*.

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