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Modern diagnostic methods for Lyme disease in dogs

Oksana Kravchuk*

Graduate Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

https://orcid.org/0000-0002-5151-2239

Abstract. The article addressed the issue of diagnosing Lyme disease in dogs. The study aimed to analyse the effectiveness of contemporary diagnostic methods for Lyme disease in dog populations. A review of the literature was conducted, examining studies that explored various diagnostic approaches. Based on the literature analysis, a classification of diagnostic methods was developed. This classification encompassed general diagnostic methods – including molecular, serological, and bacterioscopic techniques – as well as the application of nanotechnologies for diagnosing the causative agent of this disease (*Borrelia burgdorferi*) in *Ixodes* ticks (*Ixodes ricinus*, *I. hexagonus,* and *I. persulcatus*). The general diagnostic methods for Lyme disease in dogs also include several advanced high-precision techniques. These molecular methods comprise polymerase chain reaction, quantitative real-time polymerase chain reaction, and polymerase chain reaction dissociation. Serological methods include enzyme-linked immunosorbent assay, immunofluorescent assay, and immunoblotting. Bacterioscopic methods involve cultural techniques, immunohistochemical analysis, and microfluidics. Among the nanotechnologies, complete analysis microsystems and electrochemical methods were identified. Tick diagnosis for Lyme disease includes techniques aimed at detecting the presence of pathogens, specifically *Borrelia* species, within the tick itself. The analysis of diagnostic methods provided their characteristics and highlighted promising approaches for identifying Lyme disease in dogs. Among these, enzyme-linked immunosorbent assay and immunofluorescent assay yielded the best results due to their cost-effectiveness and rapid output, requiring minimal pathological material obtained at various stages of the disease. It was established that one of the most promising diagnostic tools for Lyme disease in dogs is the use of biomarkers. Notable examples include protein markers of inflammation, cytokines, chemokines, and genetic biomarkers. The

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*Corresponding author

findings from the literature analysis of diagnostic methods for Lyme disease in dogs will be valuable for veterinary practitioners involved in the treatment of vector borne diseases

Keywords: vector-borne diseases; *Ixodes* ticks; *Borrelia*; spirochaetes; molecular, serological, and bacterioscopic studies; biomarkers

Introduction

Lyme borreliosis (LB) is a serious disease that can significantly impact the health of animals, particularly dogs, leading to severe complications. Thanks to ongoing technological advancements, new diagnostic methods are allowing for more accurate and rapid detection of this dangerous infection. Traditional methods of diagnosing LB in dogs involve identifying clinical symptoms such as lameness, joint inflammation, and general lethargy. Additionally, blood tests can detect the presence of antibodies to *Borrelia burgdorferi*, the bacterium that causes LB. The introduction of cutting-edge technologies in veterinary medicine offers vast opportunities for the effective diagnosis of LB in dogs. Detecting and treating LB in dogs requires a combination of several approaches: from the application of advanced diagnostic technologies to the development of effective treatment and prevention methods. Research in this field is crucial for improving the quality of life for animals and preventing the spread of disease.

L. Huggins *et al.* (2023) and Y. Paladsing *et al.* (2024) demonstrated that the intensity of animal and human infection with LB is determined by the tick population density and the location of the biotope. Important factors contributing to the emergence and spread of blood parasites among dogs include arthropod vectors and other vertebrates, dogs living in close proximity to humans, and environmental factors such as climate, landscape types, and habitats. Blood parasites in dogs can cause both mild and severe diseases. L. Huggins *et al.* (2023) conducted several studies on the presence of blood parasites in dogs using microscopy, molecular, and serological methods.

V. Nguyen *et al.* (2021) noted that in tropical regions, the significant distribution and abundance of ectoparasite vectors contribute to the year-round transmission of vector-borne diseases. For instance, stray and domestic dogs in Southeast Asia can serve as hosts and reservoirs for the transmission of vector-borne diseases. T. Rawangchue & S. Sungpradit (2020) also conducted research to determine the vector-borne transmission of infectious diseases by ectoparasites and the possibility of co-infection in dogs. N. Sontigun *et al.* (2022) noted that in Thailand, among blood parasitic diseases recorded in dogs, pathogens such as *B. canis vogeli*, *H. canis, E. canis*, and *Anaplasma* were isolated, which were also detected in dogs from other Southeast Asian countries. The authors concluded that collecting information from pet owners about their care practices is crucial for understanding the transmission routes of parasitic infections and developing and implementing strategies for their prevention and control.

P. Galluzzo *et al.* (2020) determined that dogs are often the first to be exposed to microorganisms that also cause diseases in humans. Dogs can act as carriers or reservoirs for vector-borne diseases, such as Lyme disease, due to their frequent contact with the environment, particularly shrubs and fields that provide natural habitats for ticks. The quantitative measurement of antibodies against *Borrelia* in

dogs can reflect the prevalence of the pathogen in the environment and the frequency of tick bites. Consequently, dogs are often studied as serological markers for assessing the risk of Lyme disease in various regions worldwide.

Given the above, scientific research into the diagnosis of this vector-borne disease in dogs is essential for the early detection and prevention of its spread among both humans and animals. This study aimed to analyse and justify approaches to the laboratory diagnosis of LB in dogs, determine their effectiveness, and provide recommendations for future applications.

Literature Review

V. Klius (2018) dedicated their study to the challenges of diagnosing LB in both animals and humans, focusing on the clinical and instrumental features of neurological involvement in chronic cases of the disease to improve diagnostic procedures and therapeutic strategies. The study also aimed to identify the disease in patients aged 20 to 77. I. Ben (2019) directed their research towards studying the epidemiological and clinical characteristics, including the diagnostic algorithm, of human granulocytic anaplasmosis. V. Levytska (2021) investigated the geographical distribution of *Dermacentor reticulatus* and *Ixodes ricinus* ticks and developed an improved system for protecting animals from vector-borne diseases. O. Panteleinko *et al.* (2021) described the key causes of LB, as well as the causative agent and genotypic composition of *Borrelia* species that cause the disease. The study outlined the link between the prevalence of LB and ecological factors, climate change, and anthropogenic impacts on biocenoses and biotopes, as well as the role of vectors and reservoir hosts in the spread of this disease. G. Margos *et al.* (2019) conducted diagnostics for LB in dogs after tick bites and recommended treatment measures for dogs with various forms of LB. M. Nepveu-Traversy *et al.* (2024) dedicated their research to describing the development of anti-tick vaccines and their effectiveness in combating tick-borne diseases that pose a threat to human health. M. Milkovičová *et al.* (2023) analysed the differences in diagnosing this disease in dogs compared to tests commonly used for human diagnosis. The researchers noted that this disease can be caused by *Borrelia burgdorferi* sensu lato, spirochetes that affect many animals. These bacteria are spread by ticks (*Ixodes ricinus*, *I. hexagonus*, and *I. persulcatus*) and affect both humans and animals, especially dogs. In 2022, scientists identified 11 species/genotypes within the *Borrelia* complex.

The *Borrelia burgdorferi* complex belongs to the bacterial genus *Borrelia*. G. Chiappa *et al.* (2022) demonstrated that this type of spirochete is known for its ability to cause Lyme disease – an infectious illness primarily transmitted through the bite of an infected tick. Within this complex, researchers have identified several species or genotypes of *Borrelia burgdorferi*. It is important to note that the phylogeny and nomenclature of these species may change over time as new research emerges. W. Burgdorfer *et al.* (1982) provided information about spirochetes within the *B. burgdorferi* s.l. complex and were the first to identify them in *Ixodes* ticks. Although it had been suspected since the early 20th century that tick-borne pathogens could cause symptoms now considered characteristic of LB.

R. Johnson *et al.* (1984) and G. Stanek *et al.* (2002) identified the causative agent as *B. burgdorferi*, considering it a single bacterial species. However, as the genetic and ecological heterogeneity of *Borrelia* in Europe, Asia, and North America became apparent, the diversity of this species complex became evident. Several new genotypes were discovered in these regions. Since then, the term *B. burgdorferi* s.l.

has been used to refer to this entire species complex, while *B. burgdorferi* s.s. specifically refers to the species first identified in the USA by W. Burgdorfer *et al.* (1982). Consequently, scientists have identified 11 species/genotypes within the *Borrelia burgdorferi* complex:

♦ *Borrelia burgdorferi* sensu stricto (*B. burgdorferi* s.s.): the primary species responsible for Lyme disease in North America;

♦ *Borrelia afzelii*: commonly associated with Lyme disease in Europe, particularly in central and northern regions;

♦ *Borrelia garinii*: predominantly found in Europe and Asia, and is often linked to neurological manifestations of Lyme disease;

♦ *Borrelia bavariensis*: closely related to *B. garinii* and identified in Europe, especially in Bavaria and Germany;

♦ *Borrelia spielmanii*: discovered in Europe and associated with cases of Lyme disease in this region;

♦ *Borrelia valaisiana*: found in Europe and Asia, and is a genospecies within the complex;

♦ *Borrelia lusitaniae*: discovered in Portugal and linked to Lyme disease in southern Europe;

♦ *Borrelia japonica*: primarily found in Japan and is a species within the complex;

♦ *Borrelia tanukii*: initially discovered in Japan and linked to Lyme disease;

♦ *Borrelia mayonii*: discovered in the United States of America and associated with cases of Lyme disease in western states;

♦ *Borrelia yangtzensis*: discovered in China and is a species within the *Borrelia burgdorferi* complex.

J. Gray *et al.* (2016) established that the transmission cycle of *B. burgdorferi* s.l. is closely linked to the life cycle of its primary vector, the *Ixodes* tick. This life cycle consists of four stages: egg, larva, nymph, and adult (female/male). Each of the three active stages (larva, nymph, and imago) must take a blood meal to progress to the next stage or, in the case of adult females, to lay eggs. Typically, the life cycle of *I. ricinus* is completed within three years. However, under certain environmental conditions, such as weather, the duration of cold periods, and temperature, the life cycle can be extended to up to six years, as these factors can cause pauses in tick development and behaviour. Depending on regional and microclimatic conditions, larvae are typically active from late April to late October. In Northern and Central Europe, nymphs and imagos are active from March to November. Their peak activity usually occurs in April or May, as well as during the summer months in cool and dry weather. Nymphs and imagos can be active at temperatures as low as +4°C. Cases of tick attacks are often reported during cool periods. Their activity tends to decrease temporarily at the end of winter and the beginning of spring. C. Bregnard *et al*. (2021) suggested that considering biotic and abiotic variables that significantly influence tick density allows for predictions of tick populations in the following year.

T. Hart *et al.* (2021) discovered that *Borrelia* species produce a diverse array of immune molecules that interfere with the complement system at various stages of activation during tickborne infection of mammals. These genetically unrelated and allelically distinct immune molecules exhibit a unique inhibitory effect on the activity of complement components. Using inactivation methods developed by Y. Lin *et al.* (2020) and J. Skare & B. Garcia (2020), these molecules were classified as proteins that indirectly suppress the complement system by capturing host regulatory proteins (C4BP, factor H (FH), factor H-like protein-1 (FHL-1), and factor H-related proteins (FHR)) from the fluid phase, or directly interacting with specific complement components (C1r, C4b, C7, C8, and C9) or the assembled membrane attack complex (MAC). This mechanism results in the specific inhibition of complement activation.

Materials and Methods

An analysis of scientific publications concerning the current state of LB diagnostics in dogs was conducted between 2023 and 2024 by the Department of Veterinary Epidemiology and Animal Health at the National University of Life and Environmental Sciences of Ukraine (Kyiv). The study encompasses the following: literature review – a comprehensive review of scientific literature indexed in Scopus and Web of Science databases; theoretical synthesis – a synthesis of global research on LB diagnostic methods in mammals; identification of the most effective diagnostic methods for LB currently available in the field of laboratory diagnostics; a justification for the relevance of diagnostic methods using blood and synovial fluid samples, including their specific characteristics; an analysis of the advantages and disadvantages of various diagnostic tests for detecting *Borrelia burgdorferi* in dogs.

During the study and analysis of literature data, the advantages and limitations of current diagnostic tests for detecting *Borrelia burgdorferi* in dogs were identified. This was achieved using the following scientific methods: review and systematisation of information from scientific articles and clinical guidelines related to the diagnosis of *Borrelia burgdorferi* in animals; comparative analysis of diagnostic methods, highlighting their advantages (e.g., specificity or sensitivity) and drawbacks (e.g., duration, cost, or risk of false-positive results); compilation of a list of primary diagnostic tests encompassing key approaches for diagnosing *Borrelia burgdorferi*, including molecular, serological, and bacterioscopic methods, the application of nanotechnology, and testing ticks for the presence of the LB pathogen.

The research was conducted in four stages. The initial stage involved a comprehensive analysis of clinical cases reported by researchers from various geographic locations. The second stage focused on identifying the primary diagnostic methods for LB based on the existing scientific literature. These methods included molecular, serological, bacterioscopic approaches, nanotechnology applications, and the testing of ticks for the presence of the causative agent. The third stage involved a critical evaluation of the effectiveness of laboratory diagnostic methods that utilise blood and synovial fluid samples, and the identification of their unique characteristics. The final stage involved a thorough analysis and synthesis of the research findings. This systematic approach to the analysis of literature on the subject enabled the scientific identification of the most effective laboratory diagnostic methods for LB in dogs and the formulation of recommendations for their practical application in veterinary medicine.

Results and Discussion

Based on an analysis of available literature, five diagnostic methods for LB in dogs have been identified: molecular, serological, bacterioscopic methods, the application of nanotechnology, and tick diagnostics for the presence of the pathogen (Fig. 1). Molecular diagnostic methods play a crucial role in the early and accurate detection of LB in dogs. These methods enable the identification of bacterial pathogens or their genetic material in tissues or body fluids, providing rapid and reliable results. Among these, the Polymerase Chain Reaction (PCR) stands out as a key method for detecting *Borrelia burgdorferi* DNA in biological samples from dogs. Samples suitable for PCR include blood, urine, synovial fluid, or tissue. PCR diagnostics for LB can detect bacterial contamination at early stages of infection, even before clinical symptoms appear (Fig. 2).

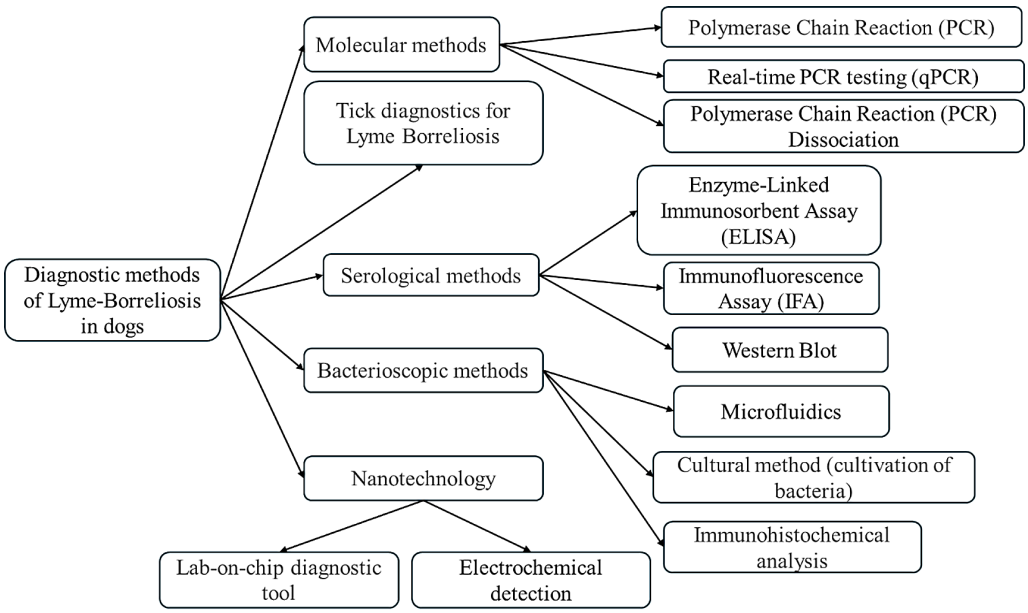

Figure 1. The most common diagnostic methods for Lyme borreliosis in dogs
Note: *classification of diagnostic methods for LB in dogs*
Source: *developed by the author based on the literature review*

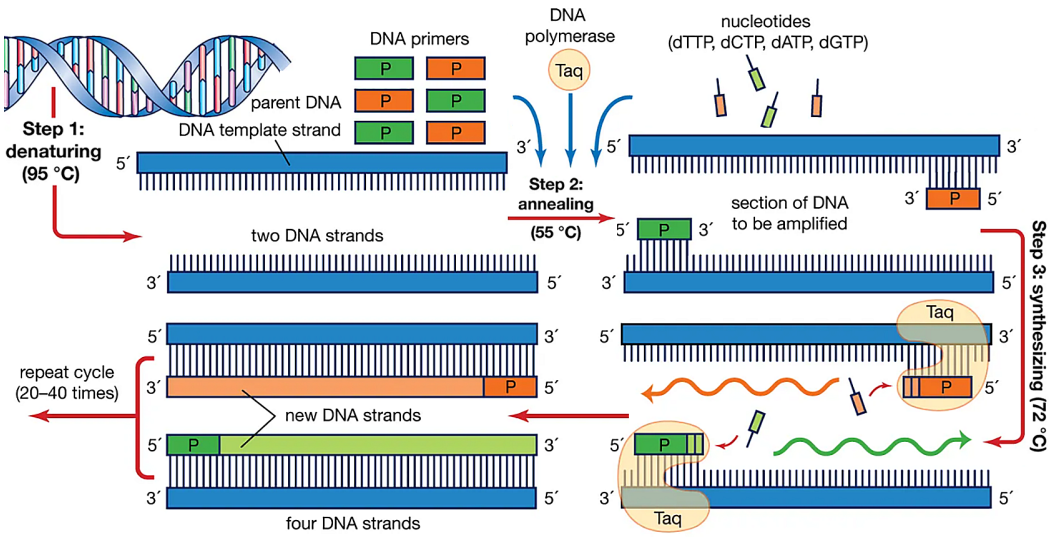

Figure 2. Polymerase Chain Reaction
Note: *the figure illustrates various stages of the chain reaction*
Source: *Encyclopædia Britannica (n.d.)*

Polymerase Chain Reaction Dissociation (PCRD) is a technique employed to detect and quantify nucleic acids. S. Cardenas-Cadena *et al.* (2023) have conducted studies using this method. PCRD can be valuable in identifying infections at various stages of the disease. Real-time PCR testing (qPCR) is another method used to detect and quantify the DNA of *Borrelia burgdorferi* in the tissues or fluids of a dog. qPCR offers a more precise method for detecting the presence of bacteria and determining their concentration in whole blood or serum. qPCR is a powerful tool for diagnosing LB in dogs, providing a highly sensitive and specific method for detecting infection. Unlike traditional diagnostic methods, which can take a considerable amount of time to produce results, qPCR offers faster and more accurate results, crucial for timely treatment. This method can also determine the level of bacterial contamination, making it useful for monitoring disease progression and evaluating treatment efficacy. Furthermore, qPCR can detect the presence of *Borrelia burgdorferi* at the preclinical stage of infection, increasing the chances of successful treatment. This technology is also used to distinguish between active infections and residual bacterial traces after treatment, helping to avoid the use of unnecessary antibiotics.

The following advantages of molecular diagnostic methods should be highlighted: accuracy – molecular methods are highly precise and specific, enabling the reliable identification of pathogens; early detection – these methods facilitate the diagnosis of infections even before the onset of clinical symptoms, at the early stages of disease development; quantification – certain molecular methods can quantify the amount of pathogen present, which is valuable for assessing the severity of infection and monitoring disease progression. Polymerase Chain Reaction Dissociation (PCRD) is a molecular diagnostic technique employed to detect and quantify nucleic acids like DNA or RNA. PCRD plays a crucial role in determining the presence of infectious agents at various stages of disease progression, including the early stages when symptoms may be subtle.

G. Miró *et al.* (2022) investigated serological methods for diagnosing LB in dogs, focusing on detecting antibodies produced in response to infection with the pathogen *Borrelia burgdorferi*. Among these methods, the most effective for identifying the pathogen include the Enzyme-Linked Immunosorbent Assay (ELISA), Immunofluorescence Assay (IFA), and Western blot. Specifically, the ELISA method detects antibodies to *Borrelia burgdorferi*. It is based on the interaction between antibodies and specific antigens presented on test plates. A positive reaction in the blood serum sample indicates the presence of antibodies against *Borrelia*.

J. Branda *et al.* (2021) demonstrated that the Immunofluorescence Assay (IFA) is also used to detect antibodies but differs by employing fluorescent probes to highlight the antigen-antibody reaction. This method can be beneficial in cases where ELISA does not yield a definitive result. Western blot is an additional confirmatory method employed to validate positive results obtained from ELISA or IFA tests. Furthermore, R. Schettig *et al.* (2021) highlighted that IFA is a valuable tool for recognising antibodies formed in response to the introduction of LB pathogen into the host organism.

Serological methods have been widely used for screening for antibodies against the bacteria and can be performed in many veterinary laboratories. However, detecting positive results for bacterial contamination through serological methods is possible only after a certain period following infection. This limitation increases the risk of false-positive results, as other infections may also lead to antibody production. Bacterioscopic diagnostic methods for LB in dogs have primarily been used to examine

samples containing *Borrelia burgdorferi* bacteria. Nevertheless, their application in veterinary medicine is limited because the bacteria are rarely found in the blood or other biological fluids of dogs.

M. Guérin *et al.* (2023) demonstrated that in specific cases of diagnosing LB, the following methods might be useful: cultural methods, immunohistochemical analysis, and microfluidics. Microfluidics is an advanced technology that involves manipulating fluids on a microscopic scale to conduct various biological tests and analyses. This approach enables the use of extremely small fluid volumes (typically in nanolitres or microlitres), making it an ideal tool for rapid and accurate diagnosis of infections such as Lyme disease in animals, particularly in dogs. Due to its high precision and sensitivity, microfluidic technology allows for swift results with minimal use of biological material, which is crucial for timely treatment.

The cultural method involves cultivating *Borrelia burgdorferi* bacteria from biological samples collected from dogs, such as blood, urine, synovial fluid, and tissues. While this method is considered the "gold standard" for many bacterial infections, its application for LB has limitations. *Borrelia* are slow-growing bacteria, and their isolation from a dog's body is often challenging due to the low number of pathogens in blood and tissues. Additionally, this process is time-consuming and requires specialised equipment.

Bacterioscopic examinations primarily involve analysing body fluids such as blood and synovial fluid. In some cases, *Borrelia burgdorferi* bacteria can be detected in a dog's blood using special techniques during bacterioscopy. However, this method is not very sensitive and is therefore not used as a first-line diagnostic tool. Nevertheless, for dogs with clinical symptoms of LB, veterinarians may perform joint punctures and examine the synovial fluid for the presence of borreliae. However, this method is invasive and has limited applications.

Immunohistochemistry is a technique used to detect bacteria within a dog's tissues using specific antibodies that bind to *Borrelia burgdorferi* antigens. After a tissue sample (e.g., biopsy) is collected, it is treated with antibodies capable of identifying the bacteria. Immunohistochemistry can reveal infection in specific tissues such as the skin or synovial membrane. However, this method has several drawbacks: it is time-consuming and often inaccessible due to the need for specialised laboratory conditions and reagents. Additionally, a low number of bacteria in the samples can lead to negative results. Bacterioscopic methods for detecting LB in dogs are less common and less sensitive compared to other methods, as borreliae do not circulate in the blood or biological fluids during the early stages of infection. Therefore, veterinary practitioners typically employ enzyme-linked immunosorbent assays (ELISAs) and molecular tests for diagnosing LB in dogs due to their greater reliability and sensitivity.

Given that ticks are the primary vectors for spirochetes, testing ticks can help prevent LB and avoid the complications of subsequent pathological processes in animals. J. Lewis *et al.* (2024) conducted a noteworthy study developing a quantitative PCR test that demonstrated high sensitivity and specificity for detecting pathogens in both wild and human ticks. This test enhances the monitoring and control of LB and contributes to effective epidemiological surveillance and prevention.

Veterinary medicine is continually evolving to find more effective and accurate methods for diagnosing LB in dogs. While bacterioscopic methods are not widely used, recent research and technological advancements offer the potential to improve these techniques. For example, in some cases, the development

of new microscopy technologies and improved methods for determining borreliae concentrations in blood may enable the detection of bacteria in biological samples from infected dogs. Additionally, combining bacterioscopy with other methods, such as immunodiagnostics or molecular diagnostics, could provide a more accurate and comprehensive picture of an animal's health.

J. Branda *et al.* (2021) demonstrated that one of the innovative technologies for detecting LB in dogs could be nanotechnology, specifically lab-on-chip diagnostic tools and electrochemical detection methods. C. Flynn *et al.* (2020) described a lab-on-chip study that allows for comprehensive biochemical analyses using minimal sample volumes and reagents. In the case of LB, such a chip can be used for the rapid identification of *Borrelia burgdorferi*. This makes the diagnostic process much faster and more convenient, as only a few drops of blood or another biological sample are required for testing. C. Flynn *et al.* (2023) found that the electrochemical method is used for the quick and accurate detection of pathogens in biological samples. The principle of this method involves detecting changes in electrical signals when certain molecules or biomarkers interact with electrodes coated with specific sensors. The electrochemical method can be applied to identify *Borrelia burgdorferi* DNA or other specific biomarkers in dogs, indicating the presence of infection. The method is characterised by its exceptional sensitivity, capable of detecting even the smallest concentrations of pathogens. Furthermore, the diagnosis of LB in ticks involves methods that focus on identifying the presence of pathogens, particularly *Borrelia*, within the tick itself. This is crucial as ticks are vectors for this disease and can transmit *Borrelia* to humans and animals during a bite. The authors noted that one of the most promising diagnostic approaches is the use of biomarkers to detect LB in dogs. Biomarkers are specific substances or indicators in an animal's body that can be used to diagnose the onset of a disease. The research of T. Casselli *et al.* (2021) focused on finding specific biomarkers in the blood or other tissues of dogs that could indicate the presence of LB. According to the researchers, such biomarkers can indicate the state of the immune system in response to an infectious disease, helping to quickly establish an accurate diagnosis.

T. Casselli *et al.* (2021) and C. Flynn *et al.* (2023) have identified specific biomarkers, such as cytokines, antibodies, and other molecules, that can be detected in the blood of infected animals. This discovery opens up the possibility of developing new diagnostic tests that could be faster and more accurate in detecting the disease. However, this area of research still requires further investigation to determine the most effective biomarkers and their application in veterinary medicine. The study of biomarkers could be a significant step towards accurate, early, and effective diagnosis of LB in dogs. The authors highlighted the identification of the following markers: inflammatory protein markers, cytokines, chemokines, and *Borrelia burgdorferi* DNA. In particular, protein markers are important because, during *Borrelia burgdorferi* infection in dogs, there are elevated levels of certain proteins, such as C-reactive protein (CRP), which is an indicator of systemic inflammation. An increase in CRP can serve as an indicator of the active phase of the infectious process and is used to assess the animal's condition. Additionally, during infection, the body produces specific cytokines (proteins that regulate the immune response). Measuring the levels of these cytokines, such as interleukin-6 (IL-6) or tumour necrosis factor-alpha (TNF-α), can help determine the stage of infection. Furthermore, genetic biomarkers, such as fragments of

Borrelia burgdorferi DNA, can be detected using PCR. This is a highly accurate method that can detect the presence of bacteria even when antibody levels or clinical symptoms have not yet appeared. PCR analyses are extremely sensitive, enabling the detection of pathogens at pre-clinical stages of infection.

O. Panteleinko & T. Tsarenko (2023) have developed a comprehensive diagnostic algorithm for LB, involving PCR analysis of synovial fluid to detect the DNA of spirochetes *Borrelia burgdorferi* sensu lato in dogs with pronounced symptoms of Lyme arthritis. The authors outlined the following diagnostic steps: 1) determining the preconditions for classifying Lyme arthritis among the differential diagnoses, which includes a preliminary diagnosis of mono- or polyarticular disease in dogs (the course of the disease is relapsing, sometimes chronic; clinical symptoms include lameness, shortness of breath, pain, and joint swelling; accompanying symptoms include fever, lymphadenopathy, lethargy, and anorexia), including the preliminary diagnosis; 2) identifying the risk factors for canine infection with *B. burgdorferi* s.l. (infection of dogs by *Ixodes* ticks), living in endemic Lyme disease areas, age and breed (young dogs and certain breeds, such as Golden Retrievers and Labradors), and the absence of preventive treatments against *Ixodes* ticks; 3) excluding differential diagnoses (blood-sucking insects), including the diagnosis of other infections transmitted or related to the environment (anaplasmosis, ehrlichiosis, European tick-borne encephalitis, bartonellosis, septic arthritis associated with other infectious agents), as well as metabolic, genetic, and other systemic diseases (immune-mediated polyarthritis, hypertrophic osteodystrophy, systemic lupus erythematosus, cartilage metabolism disorders, panosteitis, arthroplasia, hypertrophic osteopathy, etc.), and considering traumatic injuries (limb injuries: dislocations, bruises, fractures, etc.); 4) diagnostic tests to assess the severity of the clinical condition, including complete blood count, determination of C-reactive protein, tests for rheumatoid factor, computerised or magnetic resonance imaging of joints, and cytology of cerebrospinal and synovial fluids; 5) laboratory confirmation of the diagnosis of LB with symptoms of Lyme arthritis, including detection of antibodies to *B. burgdorferi* s.l. (Stage I – immunochromatography or enzyme-linked immunosorbent assay; Stage II – Western blotting for samples with doubtful ELISA results), and direct isolation of the pathogen from synovial fluid (PCR: quantitative or conventional).

A. Bajer *et al.* (2022) and S. Porcelli *et al.* (2024) established that, in addition to arthritis, dogs with LB may exhibit both general clinical symptoms, such as lethargy (appearing sluggish, inactive, and tiring easily), reduced appetite (refusing food or eating less than usual), fever (elevated body temperature ranging from 39.5-40.5°C), and neurological symptoms, including disorientation (difficulties with spatial orientation), impaired coordination (tremors, unsteadiness, difficulty walking), and seizures (rarely occurring due to nervous system involvement). Additionally, cardiovascular issues may arise, including bradycardia or arrhythmia (abnormal heart rhythms) and myocarditis (inflammation of the heart muscle, potentially leading to weakness and breathing problems); kidney involvement may occur, such as glomerulonephritis (inflammation of the renal glomeruli, which can lead to proteinuria, fluid retention (oedema), increased urination or thirst, and, in severe cases, kidney failure). Furthermore, musculoskeletal disturbances may arise, including myositis (muscle inflammation causing pain and limited mobility), intermittent lameness (shifting leg involvement, with lameness that may come and go); rare skin manifestations, such as

erythema migrans (redness or rash at the tick bite site, though this is more commonly observed in humans than in dogs); other symptoms include enlarged lymph nodes (regional lymph nodes may become swollen and painful), dyspnoea (in cases of heart involvement or significant weakness), abdominal pain, or diarrhoea (digestive complications related to kidney dysfunction).

Thus, the diagnostic algorithm for LB in dogs can be represented in three stages. The first stage involves the anamnesis and clinical examination: gathering information about possible tick exposure, particularly during walks in forested or grassy areas, determining the preventive measures in place, including the use of tick repellents, and identifying any clinical symptoms that have been diagnosed. The second stage is laboratory diagnostics: a complete blood and urine test (the complete blood count can identify leukocytosis or normal physiological leukocyte levels, while the urine test helps detect signs of kidney damage, such as proteinuria); serological tests: the ELISA test is used to detect antibodies to *Borrelia burgdorferi*. A positive result indicates contact with the pathogen but does not always confirm active infection; immunoblotting (Western Blot) is used to confirm a positive ELISA result, specifying the presence of antibodies to specific pathogen proteins; molecular methods: PCR can detect *Borrelia burgdorferi* DNA in blood samples, synovial fluid, or tissues, providing a highly specific test used to confirm the active phase of infection. The final, third stage is differential diagnosis (confirming the results of laboratory tests).

Therefore, exploring new technologies and methodological approaches in veterinary practice for the diagnosis of LB will help ensure timely treatment measures, leading to more effective management of the disease in pets. This, in turn, will contribute to the improvement of dogs' health and their overall well-being.

Conclusions

An analysis of the literature regarding methods for detecting LB in dogs reveals five main diagnostic approaches: molecular, serological, bacterioscopic, the use of nanotechnologies, and tick diagnostics for LB. Molecular methods are considered the most effective for the early and accurate detection of LB in dogs. These include PCR and PCR dissociation. Serological diagnostic methods for LB in dogs are based on detecting antibodies to *Borrelia burgdorferi*. Among these methods, enzyme-linked immunosorbent assay, which relies on the interaction of antibodies with specific antigens, and immunofluorescent assays, which can be useful when previous tests yield inaccurate results, are considered the most effective. The latter is particularly helpful when false-positive results complicate diagnosis. A drawback of these methods is that they do not provide rapid confirmation of LB in dogs. In contrast, the bacterioscopic method allows for confirmation of the presence of live *Borrelia* in pathological samples (blood, synovial fluid, urine), but it is costly and time-consuming, leading to delays in diagnosis. Based on the reviewed publications, the following recommendations can be made: the diagnosis of this disease should always be confirmed through laboratory testing; early diagnosis of LB is crucial for timely treatment, prevention of complications, and maintaining the animal's health. The approach to diagnosing LB should be comprehensive, taking into account all types of investigations – from medical history collection to laboratory diagnostics, including all its variations, as clinical symptoms and history are key components in making a diagnosis. If a tick bite is confirmed, the tick should be sent to the laboratory for further investigation of transmissible diseases, and the presence of LB in the dog can be immediately suspected. It is important to analyse symptoms such as sudden joint pain, periodic lameness, heart and kidney

dysfunction, neurological symptoms, and others. Among all the methods discussed, some stand out as the most successful, namely enzyme-linked immunosorbent assay and immunofluorescent diagnostics, as they are cost-effective, provide rapid results from a small amount of pathological material, and can be used at various stages of the infectious process. Future research will aim to experimentally evaluate the effectiveness of the methods described in this article to optimise the identification of the disease-causing agent and monitor the effectiveness of treatment.

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Conflict of Interest

None.

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Сучасні методи діагностики Лайм-бореліозу в собак

Оксана Кравчук

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-5151-2239

Анотація. Стаття присвячена проблемі діагностування Лайм-бореліозу в собак. Мета дослідження полягала в аналізуванні ефективності сучасних методів діагностики Лайм-бореліозу в собак. Проведено аналіз джерел літератури, в яких представлено результати дослідження вчених щодо методів діагностики Лайм-бореліозу в собак. За проведеним аналізом джерел літератури розроблена класифікація методів, до якої увійшли загальні методи діагностики – молекулярні, серологічні та бактеріоскопічні, а також нанотехнології для діагностики в іксодових кліщів (*Ixodes ricinus*, *I. hexagonus* і *I. persulcatus*) збудника цієї хвороби (бактерії *Borrelia burgdorferi*). Загальні методи діагностики Лайм-бореліозу в собак включають ще низку сучасних високоточних методів. До таких молекулярних методів відносяться: полімеразна ланцюгова реакція, кількісна полімеразна ланцюгова реакція в реальному часі, дисоціація полімеразної ланцюгової реакції; серелогічні: імуноферментний аналіз, імунофлуоресцентний аналіз, імуноблотинг; бактеріоскопічні: культуральний метод, імуногістохімічного аналізу, мікрофлюїдика. Серед нанотехнологій виокремлені мікросистеми повного аналізу та електрохімічний метод, а діагностика кліща на Лайм-бореліоз включає методи, які стосуються виявлення наявності патогенів, зокрема борелій у самому кліщі. У результаті проведеного аналізу методів надана їх характеристика та виокремлені перспективні методи визначення Лайм-бореліозу в собак, серед яких найкращі результати отримані за використання імуноферментного аналізу та імунофлуоресцентного аналізу, оскільки вони незатратні та сприяють швидкому отриманню результату з використанням невеликої кількості патологічного матеріалу, одержаного на різних стадіях захворювання. Визначено, що одним із найперспективніших діагностикумів Лайм-бореліозу в собак є використання біомаркерів, серед яких виділені протеїнові маркери запалення, цитокіни, хемокіни та генетичні біомаркери. Отримані результати аналізу джерел літератури щодо способів діагностики Лайм-бореліозу в собак будуть корисними для практикуючих лікарів ветеринарної медицини, які займаються лікуванням трансмісивних хвороб

Ключові слова: трансмісивні хвороби; іксодові кліщі; борелії; спірохета; молекулярні, серологічні та бактеріоскопічні дослідження; біомаркери