sciendo

DOI: 10.2478/fv-2023-0009

FOLIA VETERINARIA, 67, 1: 75—90, 2023

LYME BORRELIOSIS IN DOGS: BACKGROUND, EPIDEMIOLOGY, DIAGNOSTICS, TREATMENT AND PREVENTION

Milkovičová, M.[1], Šimková, J.[1], Valko-Rokytovská, M.[1], Očenáš, P.[1], Salayová, A.[1], Bhide, M. R.[2]

[1]Department of Chemistry, Biochemistry and Biophysics; [2]Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

maria.milkovicova@uvlf.sk

ABSTRACT

Lyme borreliosis (LB) is a multisystemic tick-borne disease that can affect many organs and have various clinical manifestations in dogs. We attempted to summarise various aspects of Lyme disease: i. e., pathogenesis, epidemiology, benefits and risks of diagnostic approaches, treatment options, and prevention in dogs. Several diagnostic bottlenecks for LB in dogs and humans are compared. Because the occurrence of LB in both humans and dogs is closely related, monitoring its prevalence in dogs as sentinel animals is an excellent aid in assessing the risk of Lyme disease in a given geographic area. Although clinical symptoms in humans help clinicians diagnose LB, they are ineffective in dogs because canines rarely exhibit LB symptoms. Despite significant differences in sensitivity and specificity, serological two-step detection of antibodies against *Borrelia* spp. (ELISA and Western blot) is the most commonly used method in humans and dogs. The limitations of the assay highlight the need for further research to develop new clinical markers and more accurate diagnostic tests. Due to the lack of a specific all-encompassing LB test, a definitive diagnosis of LB remains a difficult and time-consuming process in human and veterinary medicine. Understanding the disease prevalence and diagnostics, as well as preventing its spread with effective and timely treatment, are fundamental principles of good disease management.

Key words: *Borrelia burgdorferi*; borreliosis; diagnostics; dogs; Lyme disease; treatment

INTRODUCTION

Lyme borreliosis (LB) or Lyme disease is a tick-borne zoonotic infection that primarily occurs in the temperate regions of Europe, North America, and Asia. Its geographic distribution is rapidly expanding due to climate change, which affects tick survival, host populations, and human behaviours [42]. Lyme disease is caused by *Borrelia burgdorferi* sensu lato (s.l.) complex genospecies and is generally transmitted by ticks of the *Ixodes* complex, with *I. ricinus* being the major vector in Europe and *I. scapularis* in the United States [84]. The frequency of *B. burgdorferi* infection in humans and dogs is related to the tick population density and biotope location [34]. Dogs are frequently used as seroindicators for risk assessment of Lyme disease in geographic areas [8, 28]. Sensitive de-

tection of tick-borne disease-causing organisms in dogs is diagnostically important for veterinarians, pets, and their owners, and epidemiologically important for public health surveillance [53].

The purpose of this review is to provide a more comprehensive understanding of: the pathogenesis, epidemiology, benefits and risks of diagnostic approaches, treatment options, and prevention of Lyme borreliosis in dogs. We attempted to highlight fundamental issues and differences in diagnostics in dogs versus commonly used human tests.

Etiologic agent and its characteristics

Lyme disease is caused by spirochetes *B. burgdorferi* sensu lato (s.l.) complex which was considered a single species after its discovery in the 1980s [42]. Currently, the complex consists of at least 22 recognized or proposed genospecies that can be naturally transmitted among different vertebrate species and ticks of the genus *Ixodes* [58]. The predominant tick vectors in Europe and Asia are *I. ricinus* and *I. persulcatus*, while in North America it is *I. scapularis* or *I. pacificus* [86],

Borrelia burgdorferi s.l. is a group of gram-negative, spiral-shaped, microaerophilic bacteria with irregular coils, 0.2–0.3 µm in diameter and 10–40 µm in length, included in the family *Borreliaceae*, within the order *Spirochaetales*, the class *Spirochaetia* and phylum *Spirochaetes*. In the recent past, there have been a controversial proposal to modify the taxonomy of the family *Borreliaceae* into two genera, *Borrelia* and *Borreliella*, in order to reflect their genetic and phenotypic divergence [5]. Some authors recommend that the spirochetes responsible for relapsing fever retain the generic name *Borrelia* and those causing Lyme borreliosis, including species of the *Borrelia* burgdorferi s.l. complex, should be given the new name *Borreliella* [6, 35]. However, proposed changes have been contested and remain under debate [60].

At least five genospecies (*B. garinii, B. afzelii, B. burgdorferi* sensu stricto (s.s.), *B. bavariensis* and *B. spielmanii*) can cause Lyme borreliosis in humans in Europe, of which only *B. burgdorferi* s.s. has been known to cause borreliosis in North America [84, 86]. Occasionally, *B. bissettii* and *B. mayonii* are reported in patients in the USA [32, 70]. *B. garinii* is most commonly associated with neuroborreliosis, which affects a higher proportion of human patients in Europe than in the United States. *B. afzelii* infections are mostly manifested with a rare chronic skin condition such as *acrodermatitis chronica atrophicans* while *B. burgdorferi* s.s. seems to be the most arthritogenic with Lyme arthritis as the most frequent form of borreliosis in the USA [83, 84]. Pathogenic significance of other species such as *B. lusitaniae, B. bissettii* and *B. valaisiana* in humans and animals are still questionable [18, 57, 59].

Three human genospecies have been identified as pathogenic to dogs [29]: *B. burgdorferi* s.s., *B. afzelii*, and *B. garinii* (Table 1). So far, no other species has been reported that infect dogs.

Table 1. Genospecies in the *B. burgdorferi* s.l. complex of relevant pathogenicity for humans and dogs

Genospecies	Typical vectors	Main reservoir	Pathogenicity	Epidemiological distribution
***B. burgdorferi* sensu stricto**	*I. scapularis, I. pacificus, I. ricinus, I. persulcatus (?)*	mammals, birds	humans, dogs	North America, Europe
B. garinii	*I. ricinus, I. persulcatus*	birds	humans, dogs	Europe, Asia
B. bavariensis	*I. ricinus, I. persulcatus*	small mammals, birds	humans	Europe, Asia
B. afzelii	*I. ricinus, I. persulcatus*	small mammals	humans, dogs	Europe, Asia
B. spielmanii	*I. ricinus, I. persulcatus*	garden dormouse	humans	Europe

Data reviewed from [55]

Spirochetes have several periplasmic flagella that are anchored to the ends of the bacterium, allowing them to move at a typical undulating and rotational speed of up to 2 mm per minute. *Borrelia* spp. can freely pass through the endothelium of blood vessels and overcome the blood-brain barrier. They are able to enter fibroblasts, dendritic cells and macrophages, in which they survive for a relatively long time [46]..

Various surface lipoproteins (OspA – OspF, DbpA, DbpB, CspA, VlsE, BptA, p13, p66, BesC, BamA, Lmp1, BB0405 and others) are found on the outer membrane of *B. burgdorferi*, which play important roles in virulence, host-pathogen interaction and in maintaining the enzootic cycle of *B. burgdorferi*. Several immunodominant surface lipoproteins have been identified that can recognize and bind host proteins, accelerate bacterial adherence to host cells, and evade the host immune response through antigenic variations as well as activation of the complement system [41]. *B. burgdorferi* s.l. is able to adapt under different environmental conditions and temperatures during the transition from the vector to the host (homothermic, poikilothermic, heterothermic vertebrates) which is reflected in different gene expression of some lipoproteins: OspA expressed mainly during colonization of the tick is replaced in the host by OspC, crucial in an early mammalian infection [72]. OspA and OspC are considered excellent candidates for the development of new vaccines, and understanding of their antigenic structure with its natural diversity essential for the correct interpretation of the immune response induced by vaccination or infection [19, 39]. Another outer surface lipoprotein VlsE (variable major protein-like sequence, expressed) is an antigenically variable protein that evades the host immune response by constantly changing its surface epitopes [66] and replaces the OspC protein on the outer surface of *B. burgdorferi* during persistent infection [92].

Under unfavourable conditions, *Borrelia* spp. are able to form extracellular membrane cystic forms (blebs) with reduced metabolic activity that probably have a defensive function against the penetration of antibodies and antibiotics. Cystic forms can reversibly change into metabolically active spirochetal forms and are responsible for "recurrent" or "dormant" LB. *Borrelia* can thus persist unrecognized in the host's body for several years [78].

Clinical manifestations

Lyme borreliosis is a multisystemic disorder with distinct spectra of clinical manifestations in humans and dogs. In humans, the infection can begin asymptomatically or with influenza-like symptoms (fever, headache, mild stiff neck, arthralgia, and myalgia), but typical LB symptoms can be divided into three stages: early localised (*erythema migrans*), early disseminated (neuroborreliosis, carditis, ocular manifestations), and late disseminated neuroborreliosis and carditis (*acrodermatitis chronica atrophicans*, arthritis) [55, 83]. *Erythema migrans*, a common skin rash of early localised infection that affects approximately 70–80 % of human patients [77], is rarely been detected under the dog fur, so the disease often goes unnoticed until later symptoms appear [4, 52]. Clinical symptoms of LB in humans can be very similar in Europe and in the USA, however, there are some differences due to the greater variety of *B. burgdorferi* s.l. genospecies in Europe. Neuroborreliosis is the most common disseminated form of human LB in Europe, followed by Lyme arthritis, and, on rare occasions, borrelial lymphocytoma, *acrodermatitis chronica atrophicans*, and Lyme carditis [83].

Because the majority of the infected dogs (95 %) show no clinical signs, bacteria can often spread to other tissues and cause more severe manifestations such as: polyarthritis, transient fever, anorexia, lethargy, neurological dysfunctions, and/or lymphadenopathy, particularly in the prescapular or popliteal nodes [4, 50, 52]. Arthritis is the most common syndrome, affecting one to a few joints, particularly the carpal joints, with or without swelling, causing lameness or shifting-leg. In dogs, signs of neurologic (facial paralysis, seizures, aggression) or cardiac manifestations (myocarditis or conduction abnormalities with bradycardia) are uncommon and poorly documented [82]. Furthermore, chronically infected dogs may develop serious Lyme nephritis, associated with rapidly progressive glomerulonephritis, that is less common than Lyme arthritis [51]. Because the pathogenesis of Lyme nephritis has not been experimentally replicated, it is thought to be caused by the deposition of antigen-associated immune complexes in the kidneys [38].

Incidence and prevalence

Canine vector-borne diseases, including LB, have been a matter of concern in Europe for several decades, with changes in prevalence and distribution observed. Further-

more, tourism, traveling with dogs, and importing dogs from endemic areas all contribute significantly to the spread of canine vector-borne diseases to new areas [74, 100].

Monitoring the prevalence of *B. burgdorferi* s.l. in ticks has been considered pivotal in the public health risk assessment of LB. *Ixodes* spp. which is an important vector of LB because its uninfected stages (larvae, nymphs, adults) can feed on infected wildlife reservoirs, become infected, and then transmit the infection to other mammals when taking their next blood meal. As ticks are indiscriminate in their choice of host, *B. burgdorferi* s.l. can be transmitted from wild animals to companion animals and humans [81]. Changes in climate and land use can have a major impact on the population size of many insect vectors, ticks and wildlife reservoirs, such as rodents or migratory birds [74], even though S t r n a d et al. [91] have found that the prevalence of *B. burgdorferi* s.l. in *I. ricinus* ticks remains reasonably constant over recent decades despite tick spreading into higher latitudes and altitudes. After summarising published data since 2010 to 2016 [91], the meta-analysis showed that the overall mean prevalence of *B. burgdorferi* s.l. in ticks in Europe is 12.3 % with the highest incidence in Central Europe (Austria, Czech Republic, Germany, Hungary, Poland, Slovakia, and Switzerland) and Balkan Peninsula (Romania, Serbia, and Bosnia and Herzegovina), with 19.3 and 18.5 %, respectively. Slovakia has a long-standing tradition in researching the ecology of ticks and the epidemiology of tick-borne diseases. *Borrelia* prevalence in questing ticks in Slovakia belongs to the highest in Europe, however, it varies significantly in suburban forests from 4.4 % in northern Slovakia [67] up to 53.2 % in eastern Slovakia [97]. In general, the prevalence of *B. burgdorferi* s.l. in ticks in urban habitats is lower, but they still pose a risk for disease transmission to humans and dogs [85].

Prevalence estimates of LB in dogs are often inaccurate due to a lack of visible clinical signs and no national surveillance system for companion animal diseases. However, screening tests for canine antibodies to *B. burgdorferi* are widely used in diagnostic laboratories and by veterinarians and the results are presented by the Companion Animal Parasite Council (CAPC) for estimating *B. burgdorferi* seroprevalence in the US and Canada [51]. Based on the data collected by the CAPC [13], 3.82 % of canine serum samples were positive for *B. burgdorferi* from over 11 million samples submitted in 2022 with the highest seroprevalence in the New England, mid-Atlantic and upper Midwest regions, ranging from 5.34 % up to 15.66 %. Moreover, CAPC estimates that the number of detected positive canine samples probably represents less than 30 % of total canine seropositivity to *B. burgdorferi* in the US. In general, overall *B. burgdorferi* seroprevalence among dogs in the US has been declining since 2016 (6.43 %) when compared to 2022 (3.82 %). Humans and dogs share many of the same risk factors for encountering *B. burgdorferi*-infected ticks due to their close association; thus, dogs serve as excellent sentinels for human Lyme disease risk. This link is highlighted by comparing maps of human Lyme disease prevalence with maps of *B. burgdorferi* seroprevalence in dogs [73].

Individual countries frequently monitor canine Lyme disease in Europe, but aggregated data may be useful in understanding its distribution on a larger scale. M i r o et al. [63] used point-of-care ELISA testing data to map the distribution and seropositivity of dogs for selected canine vector-borne diseases (*Anaplasma* spp., *Ehrlichia* spp., *Borrelia burgdorferi, Leishmania* spp., and *Dirofilaria immitis*) in Europe since 2016 through 2020. *Borrelia burgdorferi* antibody positivity was concentrated in Northern and Eastern Europe with higher positivity rates (> 5 %) in Austria, the Czech Republic, Estonia, Finland, Germany, Lithuania, the Netherlands, Norway, Poland, Slovenia, Sweden and Switzerland and lowest rates (< 1 %) in Andorra, Croatia, Greece, Hungary, Italy, Malta, Portugal, Romania and Spain. The highest positivity of the test was recorded in Sweden (13.3 %) and the lowest in Greece (< 0.1 %). Annual European test positivity rates decreased from 3.3 % in 2016 to 2.4 % in 2020 for *B. burgdorferi* [63]. G o o s s e n s et al. [33] tested 448 hunting dogs and 75 healthy dogs living in the countryside of the Netherlands for antibodies against *B. burgdorferi* by a whole-cell ELISA. The dogs were of different breeds and age. Antibodies against *B. burgdorferi* were detected in 18 % of hunting dogs and 17 % of pet dogs. In the group of hunting dogs, individuals older than 24 months appeared to have a greater risk of being exposed (22 %) than younger dogs (9 to 11 %), and in addition, the seroprevalence among hunting dogs over 24 months of age remained stable at approximately 22 %. No significant rise in seroprevalence in dogs older than 24 months may indicate that seropositivity after *B. burgdorferi* infection in dogs is rather short, approximately 1 year compared to humans whose seroprevalence

can last considerably longer [33]. Another study examined 846 dog sera for the presence of anti-*Borrelia* antibodies by using ELISA with a mixture of *B. garinii, B. afzelii, B. burgdorferi* s. s. antigens revealed 283 positive samples with mean seroprevalence 33.5 % in dogs [87].

Interestingly, there may have been a breed predisposition of Bernese Mountain Dogs for Lyme borreliosis due to their higher seropositivity against *Borrelia burgdorferi* s. l. in some regions in Europe [30, 69], although it was not sufficiently verified [30]. On the other hand, not having discovered any ticks on a small or medium dog breed can be used as a significant indicator for *Borrelia* seronegative status [9]. However, no correlation between the number of seropositive dogs and their size and gender was reported in some studies [23, 11].

In the United States, ~30,000 to 40,000 cases of human Lyme disease are reported to the Centers for Disease Control and Prevention (CDC) each year [15]. Similarly, the number of cases in Europe has increased steadily since 1990 and more than 360,000 cases having been reported over two decades. Between 1990 and 2010, the highest average incidence rates of human Lyme disease in Europe were found in Belarus, Belgium, Croatia, Norway and Serbia (< 5/100 000), Bulgaria, Finland, Hungary, Poland and Slovakia (< 16/100 000), the Czech Republic, Estonia, and Lithuania (< 36/100 000) and Slovenia (< 130/100 000) [102]. The incidence of human Lyme borreliosis has been slightly increasing in Slovakia since 2010 [85].

Methods of detection of Lyme disease

The diagnosis of canine borreliosis is based on: the epidemiological case history, duration of tick exposure, compatible clinical symptoms, exclusion of other diseases, response to antibiotics and laboratory evidence of infection. In dogs, it is difficult to definitively attribute a particular set of clinical signs to underlying *B. burgdorferi* infection because most dogs infected with *B. burgdorferi* will never develop signs. Since the *erythema migrans* is rarely found in dogs, the diagnosis is based on various laboratory techniques including culturing, histological examination of thin sections, serological tests and polymerase chain reaction (PCR) [56, 62, 89]. We are reviewing some of the routinely used diagnostic techniques for LB.

The direct detection methods

For many years, direct detection methods such as cultivation or microscopy have been the "gold standard" for detecting and classifying bacterial infections. As *Borrelia* spp. cannot be identified by standard optical microscopy or by Gram staining, a dark-field microscopy or phase-contrast microscopy have become a more accurate method in routine diagnostics [20].

In vitro culture is a relatively reliable method for the demonstration of *B. burgdorferi* in clinical samples even though it requires special growth media and long incubation periods. It is rarely sensitive enough because bacterial load in tissue samples or body fluids is generally low with the exception of skin samples from human patients. Accordingly, *B. burgdorferi* is detected most frequently in collagen-rich tissue including skin in experimentally infected dogs [47]. Cultivation is still widely used in the field of LB research during preparation of cultures for experimental work and for the preparation and control of materials in antigen production, and occasionally in clinical investigations [71].

The indirect detection methods

The indirect detection of *B. burgdorferi* by identifying specific antibodies in serum has become an important tool in diagnosing LB. However, the presence of antibodies can only indicate previous exposure to pathogen, but does not prove clinical disease in a given patient [45, 84, 93].

The indirect immunofluorescence assay (IFA) is one of the first established serological methods for the LB testing for Borrelia-specific immunoglobulins M (IgM) detectable within 7 days of infection and immunoglobulins G (IgG) in a few days later. Despite its limitations such as low specificity and cross-reactivity of the antibodies with other antigens, such as heat-shock proteins and flagellar antigens, which often lead to false-positive results, IFA is still used to detect antibodies against *Borrelia* spp. in veterinary practices in Germany and other European countries [2, 11]. In the study by B a r t h et al. [7] IFA-IgG antibodies directed against *B. burgdorferi* s. l. were detected in 51 of 200 serum samples, resulting in a prevalence of 25.5 %. The sensitivity and specificity of IFA-IgG were 76.6 % (95 % confidence interval [CI] 46.87–86.72) and 87.1 % (95 % CI 80.06–91.90), and 26.3 % (95 % CI 11.81–48.79) and 81.0 % (95 % CI 73.64–86.71) for IFA-IgM, respectively. Based on their data, both IFAs had very low sensitivity and specificity and should not be recommended for screening purposes.

In recent years, two-tiered test methods, including infection-specific tests using recombinant antigens, have been developed. The test has two components: a highly sensitive screening enzyme-linked immunosorbent assay ELISA to filter out negative samples with high fidelity and a confirmation assay Western blot (WB) used in a second step to further characterise positive samples or distinguish infected from vaccinated animals [10, 11, 45]. Serological tests are based on the detection of anti-*Borrelia* antibodies produced by patients against antigens mostly on the surface of *B. burgdorferi*. A flagellin B (FlaB or 41 kD flagellin), a major component of the periplasmic flagellar filament crucial for bacterial mobility and OspC, a lipoprotein needed for the establishment of early localised infection, were described as early immunodominant antigens after infection. The C6 antigen, a peptide based on the 6th invariable region (IR6) of a surface lipoprotein VlsE that provides an antigenic disguise of *B. burgdorferi*, is the foundation of the C6 peptide assay [48]. All of the antigens mentioned above are expressed during the early stages of infection and are currently used as target antigens in many serological tests to detect anti-*Borrelia* antibodies produced by patients. Some of their drawbacks are their cross-reactivity and high variability. FlaB immunologically cross-reacts with many other bacterial flagellins, and a high percentage of healthy non-*B. burgdorferi* infected individuals can have antibody reactivity with it. OspC, although less cross-reactive, is a highly variable protein with 24 serotypes. The least cross-reactive antigen expressed after infection is VlsE (IR6). However, IR6 does not bind IgM well, and has more variability than originally thought. The reality is that sensitivity of serological tests is lower in the early stage of the disease and they are incapable to prove an active infection due to a larger number of *B. burgdorferi* antigens recognized. There is a need to develop simpler, more sensitive, and more specific assays [22, 24]. C6 ELISA is a relatively reliable diagnostic tool using a specific synthetic peptide, the 25-mer C6 peptide of VlsE. The region is highly conserved among different *B. burgdorferi* s.l. genospecies and highly immunogenic in the canine host. C6 ELISA has clearly demonstrated that a peptide containing a specific epitope can improve both sensitivity and specificity when compared to whole protein-based assays. However, the C6 assay has limitations that have precluded its adoption as a stand-alone assay. Hence, improved serological assays are needed, and it is likely that a multipeptide assay based on peptides containing specific epitopes from multiple key *B. burgdorferi* antigens could solve many of the issues of current LB serodiagnosis [10, 22, 49].

The kinetic enzyme-linked immunosorbent assay (KELA) is a diagnostic method in ELISA format using whole-cell sonicates for the detection of *Borrelia* antibodies. Limitations of KELA include the possibility of cross-reactions with non-Borrelia-specific antibodies, and the inability to distinguish vaccination from natural infection. However, due to its convenient use and the possibility for automation, KELA has become more popular than other immunoassays despite some of its shortcomings [40, 54].

Many different assays are available as rapid tests in veterinary practice or as more sophisticated laboratory tests. Recently, VetScan FLEX4 Rapid Test (Abaxis, Inc., Union City, CA) and the VetScan canine rapid Lyme test (Abaxis, Union City, CA, USA) have been launched as new assays to detect tick-borne pathogen antibodies and heartworm antigen simultaneously. Both tests are able to detect antibodies reactive to C6 of the VlsE on the surface of *B. burgdorferi* [31, 48, 53]. The SNAP 4Dx Plus Test (IDEXX Laboratories, Inc., Westbrook, ME) can similarly identify antibodies or infection with multiple tick-borne pathogens and canine heartworm antigen in a single assay. Each test kit consists of a coated matrix with 5 blue spots in the result window. These spots contain specific peptides as antigens for the detection of antibodies against *B. burgdorferi* s.l., *Anaplasma phagocytophilum*, and *Ehrlichia canis*, as well as specific capture antibodies for the detection of *Dirofilaria immitis* antigen. Lyme disease detection is based on identification of anti-*Borrelia* antibodies with C6 ELISA technology in clinically and subclinically infected dogs. Out of 200 canine serum samples tested by SNAP 4Dx Plus Test, 10.5 % were determined as positive with a relatively high sensitivity and specificity (84.2 %, 98.5 %, respectively) [7]. G e t t i n g s et al. [31] have evaluated the cross-reactivity of five commercially available and reference laboratory *B. burgdorferi*-based tests (SNAP 4Dx Plus, VetScan canine Lyme rapid test, Lyme Quant C6, the *B. burgdorferi* titre indirect fluorescent antibody test (IFA) and Accuplex4) on six laboratory-raised dogs infected with *B. turicatae* a causative agent of tick-borne relapsing fever. Three of these tests reacted to anti-*B. turicatae* antibodies. Five of six seroconverted dogs to *B. tu-*

ricatae were tested positive on at least one of the tests. The highest magnitude of cross-reactivity was detected for the whole-cell IFA. The three most reactive dogs in the study had measurable antibody levels above 10 U.ml^{-1} with the quantitative C6 ELISA. However, these results are below the positive threshold for the test (30 U.ml^{-1}) and would have been reported as negative. Those three dogs also had colour development on the test line of the VetScan test which can be considered positive according to manufacturer´s instructions. The study has highlighted concerns in evaluation of the results obtained by *B. burgdorferi* diagnostic tests due to significant cross-reactivity to other *Borrelia* spp. that can complicate diagnosis determination and surveillance of Lyme disease in dog.

Polymerase chain reaction (PCR)

PCR has a higher diagnostic sensitivity comparable to that of culture in tissues [47]. Moreover, given the fact that the number of organisms in clinical samples is low and unequally disseminated, the chance of detection is reduced. Clinical manifestation, type of samples investigated and target genes used for PCR also influence sensitivity of PCR [2].

H o v i u s et al. [36] determined simultaneous infection of *B. burgdorferi* s.l. in organ tissues and skin from naturally infected dogs using PCR-coupled DNA-DNA hybridization. Chou et al. [17] used a quantitative PCR assay for the detection of *Borrelia burgdorferi* DNA in formalin fixed, paraffin-embedded tissues of 58 dogs (38 were classified as positive or equivocal for LB on the basis of clinical signs, serologic findings, and pathologic abnormalities) and compared the results with immunohistochemical staining of tissues from seropositive dogs. *Borrelia burgdorferi* DNA was amplified from tissue samples from only 4 dogs (7 %), all of which had been classified as having positive or equivocal results for Lyme borreliosis. They concluded that while it is possible to detect *B. burgdorferi* DNA in formalin-fixed, paraffin-embedded tissues, intact *B. burgdorferi* DNA is rarely found in tissues from naturally infected dogs, including those with presumptive Lyme borreliosis.

The potential of metabolomics in Lyme disease diagnostics

In the field of multi-omics with a focus on genomics, transcriptomics, proteomics and metabolomics, a significant development has been observed in research of various diseases, including infectious diseases, to comprehend relationships between molecular signatures and phenotypic manifestations of a particular disease [21]. Metabolomics involves the quantitative detection of multiple small molecule metabolites in biological fluids, cells and tissues which provides an efficient method for monitoring altered biochemistry that is closely related to the current disease or therapeutic status. Urine and blood serum or plasma are the most commonly used biofluids for metabolomics because both contain hundreds to thousands of detectable metabolites and can be obtained non- or minimally invasively [65, 95]. Despite the many benefits of metabolomics application in nutrition science, toxicology, environmental studies, and biomedicine, particularly for the identification of new disease biomarkers and novel insights into disease pathogenesis, metabolomics has not been fully utilized in veterinary medicine when compared to human medicine. Nonetheless, metabolite profiling in veterinary research can complement our understanding of pathogenesis, diagnostics and treatment of human diseases [94]. There are a few studies demonstrating metabolomics application for disease research in dogs such as: obesity, heart disease, intestinal dysbiosis, bladder cancer, lymphoma, diabetes mellitus, anxiety-related disorders [14], however, none of them investigates Lyme disease or other zoonotic infections. In the study by H o x m e i e r et al. [37] metabolomics approach was used to examine the dynamics of survival and multiplication of spirochetes in tick vectors prior to transmission to the vertebrate host by tick saliva. Using gas chromatography coupled to mass spectrometry (GC-MS), they identified statistically significant differences in metabolic profile between uninfected *Ixodes scapularis* nymphal ticks, *Borrelia burgdorferi*-infected nymphal ticks, and *Borrelia mayonii*-infected nymphal ticks by measuring metabolism every 24 hours over the course of their blood meals up to 96 hours. A study focused on the metabolites such as purines, amino acids, carbohydrates and fatty acids during a blood meal and statistically confirmed differences in their amounts.

Currently, there is a rapid development in the detection and monitoring of diseases using urinary metabolomics which can represent a great potential for the identification of specific biomarkers, reflects the current state of the organism and provides comprehensive information on non-invasive monitoring of disease [96, 104]. Recent-

ly, there have been some studies investigating metabolomics approach in diagnostics of Lyme disease in its early stage searching for potential biomarkers in humans. P e g a l a j a r-J u r a d o et al. [68] used a metabolomics approach to detect urinary metabolites in patients with early stage of Lyme disease, infectious mononucleosis, and healthy controls. Analysis and identification of metabolites revealed dysregulation of several metabolic processes in early stage of Lyme disease compared to healthy controls or mononucleosis, including tryptophan metabolism. Due to the increased catabolism of tryptophan by indoleamine 2,3-dioxygenase (IDO) in infectious diseases, including Lyme disease, tryptophan metabolites in the kynurenine metabolic pathway have been identified and quantified. Their study confirmed significantly elevated kynurenine levels in patients with early stage of Lyme disease compared to healthy controls and significantly reduced tryptophan levels in the patients with disseminated infection compared to patients with localised infection. The results of their study suggest that the metabolic pathway leading to quinoline acid production differs in patients with early-stage Lyme disease and infectious mononucleosis. The study provided further evidence for the use of urinary metabolic profiling to differentiate early stage borreliosis from related diseases. M o l i n s et al. [64] detected metabolites in serum samples from patients with early stage Lyme disease, other diseases and healthy individuals using liquid chromatography-mass spectrometry (LC-MS) method. The result of the study was a metabolic biosignature of 95 molecular features that distinguished patients with early Lyme disease from healthy controls. By statistical adjustment, the biosignature was reduced to 44 molecular features and patients with early Lyme disease and healthy controls were correctly classified with a sensitivity of 88 % (84–95 %) and a specificity of 95 % (90–100 %). In addition, metabolomic biosignature correctly diagnosed 77–95 % of patients with early-stage Lyme disease with a negative serological result.

Treatment

Treatment of LB is based on treating spirochetal infection and managing pain of Lyme arthritis. Since the discovery of Lyme disease causative agent, the antimicrobial therapy is recommended. Recently, many different classes of antibiotics have been described for eradication of the causative agent of LB (Table 2). *Borrelia* organisms are sensitive to tetracyclines, penicillins, macrolides and cephalosporins. They are used during the early and late stages of the disease, and may be given orally or intravenously. Beta-lactams and tetracyclines have also shown to be effective and are widely used in human and veterinary medicine to treat patients with Lyme disease. As the first choice for most sick dogs with suspected LB, doxycycline is recommended due to its easier administration and efficacy against coinfections. Doxycycline can be prescribed for puppies and kittens since the age of 4 weeks in some countries. However, some veterinarians in the field prefer amoxicillin for doxycycline-sensitive or growing dogs.

Table 2. Antibiotics used in the treatment of LB (data reviewed from [51])

Antibiotic		Route; dosage; frequency; duration of use
Cephalosporins	Cefovecin	SC; 8 mg.kg^{-1}; 2 times, 14 days apart; 28 days
	Cefotaxime	IV; 20 mg.kg^{-1}; 3 times daily; 14–30 days
	Ceftriaxone	IV or SC; 25 mg.kg^{-1}; once daily; 14–30 days
Tetracyclines	Doxycycline or minocycline	PO or IV; 10 mg.kg^{-1}; 1–2 times daily; 30 days
Penicillins	Amoxicillin	PO; 20 mg.kg^{-1}; 3 times daily; 30 days
Macrolides	Erythromycin	PO; 25 mg.kg^{-1}; 2–3 times daily; 30 days
	Azithromycin	PO; 22 mg.kg^{-1}, once daily; 10–20 days
	Clarithromycin	PO; 7.5–12.5 mg.kg^{-1}; 2 times daily; 30 days

po – peroral; sc – subcutaneous; iv – intravenous

Other classes of antispirochetal antibiotics are applied in the case of tetracycline intolerance [51].

Veterinarian has a more difficult task in the treatment of LB compared to human disease, since it is often difficult to determine the onset of infection due to the lack of clinical signs reported in humans. LB symptoms in dogs are mostly manifested as an acute monoarticular or polyarticular lameness with joint swelling, fever, lethargy, and mild local lymphadenopathy, usually in young, often large breed dogs with an active/outdoor lifestyle. A critical factor of successful treatment is its initiation, therefore it should start as soon as possible with a focus on suppressing infection and pain. However, in most cases veterinarians begin treatment at the stage where spirochetes have already spread to various tissues [45, 51, 90].

Parenterally and orally administered antibiotics show high efficacy in treating LB that can be seen as a rapid response of the patient to antibiotics typically occurring within 1–2 days. Many experimental studies have shown that antibiotics greatly reduce the number of spirochetes demonstrated by the low incidence of culture-positive tissues and temporary disappearance of *B. burgdorferi*-specific DNA. The most effective antibiotic classes for the supportive therapy of dogs are beta-lactams and tetracyclines with long treatment course (4 weeks) due to the protracted biological behaviour of *Borrelia*. The best drug, dosage and duration of treatment for affected dogs are unknown [45, 51, 90].

In the study by Straubinger [90], antibiotic-treated dogs with ceftriaxone, azithromycin, doxycycline revealed a decrease in antibody titers established by KELA and Western blot. On the contrary, titres in 4 untreated control dogs have shown rapid growth within the first 90 days after tick exposure and continued increasing slightly throughout the experiment.

Recently, Wagner et al. [101] reported a comparative study of cefovecin (2 injections, 14 days apart) efficacy along with 4 weeks treatment of doxycycline or amoxicillin. They compared the outcome of cefovecin (long-acting cephalosporin) treatment of beagles experimentally infected with *B. burgdorferi* to doxycycline and amoxicillin as recommended standard antibiotics. Clinical outcome associated with LB symptoms was low because transient lameness was developed only in 2 out of 32 infected dogs confirmed either serologically (SNAP, Quant C6 or Multiplex) or using PCR amplification of *Borrelia* DNA in skin biopsy. After infection, 12 out of 32 dogs had a detectable *Borrelia* DNA in their skin biopsies. In conclusion, all tested antibiotics were effective against *B. burgdorferi* as the rapid elimination of spirochetes was measured in the skin as well as levels of circulating antibodies to *B. burgdorferi* were reduced. A significant difference was detected in a decrease of joint lesions of cefovecin-treated dogs compared to untreated dogs.

Despite effective treatment in the majority of early cured Lyme disease cases, relapse may occur after antibiotic administration is discontinued. The causative agent *B. burgdorferi* s.l. is capable of establishing a persistent infection in the host. Some studies have shown that PCR positivity in absence of culture positivity may also occur in dogs after antibiotic treatment [4, 89]. It is not necessarily caused by insufficient treatment or improper dosing of the antimicrobial, but it can indicate that the spirochetes may have become non-pathogenic. However, it is not known whether the lack of pathogenicity is irreversible. It remains questionable whether there is enough evidence to predict disease relapse or reinfection or it is induced by more fundamental genotypic or phenotypic alteration of the pathogen [51, 103]. Patients with early infection who recover after antibiotic therapy are susceptible to reinfection [44].

Moreover, problems with treatment may arise due to several immune evasion tactics employed by *B. burgdorferi* [3, 79] and due to the mechanism of persister formation [75]. As a response to the altered conditions, the atypical pleomorphic forms of *Borrelia* can occur. Hostile environment signals activate conversion of spirochetes to their persistent forms. Multiple morphologies of *Borrelia* such as looped or ring shaped, blebs, round bodies (RB) or cysts and colonies aggregates have been described [75]. Persister formation is a reversible process that leads to the rise of a *Borrelia* spp. cell population, particularly biofilms, with different susceptibility to conventional antibiotics. However, successful antimicrobial treatment should eliminate all morphological forms of the microorganism [76]. It was found that most frequently used doxycycline and amoxicillin reduced the spirochetal structures comparably by ~85–90 %. On the other hand, doxycycline increased the number of round body forms about twofold. Metronidazole, tinidazole, and tigecycline significantly decreased both the spirochete and the round body forms of *B. burgdorferi*. Quantitative analysis of biofilm-like colonies showed only 30–40 % reduction of doxycycline or

amoxicillin compared to tinidazole (~50–55 %) [76].

F e n g et al. [27] studied the use of drug combination against *B. burgdorferi* persisters *in vitro* using a SYBR Green/PI viability assay. Currently recommended Lyme antibiotics such as doxycycline or amoxicillin in combination with other antibiotics proved to be more efficient, while the combination of daptomycin, cefoperazone (or cefuroxime) and doxycycline eradicated the most resistant microcolony forms of *B. burgdorferi* persisters. Another study indicated two other triple drug combinations against amoxicillin-induced round body model, artemisinin/cefoperazone/doxycycline and sulfachlorpyridazine/daptomycin/doxycycline [26].

The efficacy of antibiotic treatment used in dogs showing signs of acute arthritis should be rapid (1–3 days) if the clinical signs are a consequence of LB. If it is necessary, then analgesic treatment (e. g., gabapentin for neuropathic pain) is started. Treatment with nonsteroidal anti-inflammatory drugs is less preferred to avoid the necessary "wash-out" period and to reduce the risk of gastrointestinal ulceration if subsequent glucocorticosteroid therapy is indicated for suspected immune-mediated polyarthropathy in unresponsive dogs. If relapse occurs before or after finish of antibiotic treatment, secondary diagnoses should include other infectious diseases, immune-mediated diseases, soft tissue trauma (e. g., ligament or meniscal tears), septic arthritis, or degenerative joint disease [51].

Prevention

As Lyme borreliosis is transmitted via the tick bite, prevention of tick attachment and feeding must be seen as the first obligation of any tick-control agent. The final agent potency should be 100 % at killing the tick before it is able to transmit the pathogen [80]. Many formulations (e. g. collars, spot-ons and orals) and modes of action (contact vs. systemic efficacy) have been used for the transmission blocking of tick-borne pathogens and tick vectors within different transmission times. The capability of Seresto® collars (imidacloprid 10 % + flumethrin 4.5 %) to prevent transmission of *Borrelia burgdorferi* s. l. and *Anaplasma phagocytophilum* by naturally infected ticks was evaluated in two studies with 44 dogs. This collar is highly effective in preventing tick and flea infestations on cats and dogs and has also shown to successfully prevent transmission of a range of pathogens including *Ehrlichia canis* and *Babesia vogeli*. The Seresto® collar was tested for its ability to prevent transmission of *Borrelia burgdorferi* s. l. or *Anaplasma phagocytophilum* from *I. ricinus* at 2 months and from *I. scapularis* ticks at 1 and 7 months after application. Acaricidal efficacy as well as pathogen transmission blocking of *Borrelia burgdorferi* s. l. and *Anaplasma phagocytophilum* was shown to be 100 % for all time points evaluated [43].

The development of an effective vaccine would help preventing the spread of the disease in humans and dogs. However, it is necessary to take into account that immunity to the infection is strain-specific and decreases after one year of infection [25]. The main goal of current research is to detect and characterise a specific antigen that induces persistent protection of the immune system [1].

Borrelia vaccines for dogs are worldwide administered to dogs in endemic areas. Commercially produced vaccines induce strong antibody response to one or more outer surface proteins (Osp) and other antigenic proteins of *B. burgdorferi*, which can be detected by IFA, WB, and whole cell antigen-based assays. Nevertheless, it is not possible to distinguish a vaccinated dog from a naturally exposed one using serological methods.

In 2003 and 2011, vaccination with four vaccines (RECOMBITEK® Lyme, Boehringer-Ingelheim Animal Health; LymeVax®, Zoetis; Galaxy® Lyme, Merck Animal Health; Nobivac® Lyme vaccine, Merck Animal Health) were monitored. Despite characteristic immune response to vaccination in all monitored groups, all samples at all sampling times were negative for *B. burgdorferi* antibodies in the SNAP 4Dx Plus test and the Lyme Quant C6 assay which demonstrates the absence of test reactivity with serum antibodies of vaccinated dogs [88].

Several different types of *B. burgdorferi* s. s. vaccines are currently commercially available in the US, including several bacterins (e.g., LymeVax®, Zoetis; Nobivac® Lyme, Merck Animal Health), recombinant OspA subunit vaccines (e.g., RECOMBITEK® Lyme, Boehringer Ingelheim), and a chimeric recombinant OspA and OspC vaccine (VANGUARD® crLyme, Zoetis) [99]. So far, there are no available experimental field trials examining the efficacy of canine *B. burgdorferi* vaccines [98]. In Europe, lysate vaccines produced with *B. burgdorferi* s. s., *B. garinii* and *B. afzelii* are on the market, however, more pathogenic species may be present in ticks and complete cross-reactive protection of the vaccine-induced antibodies is not documented [16].

CONCLUSIONS

This study was written to summarise knowledge of the epidemiology, clinical manifestations, diagnostic approaches, treatment, and prevention of Lyme borreliosis in dogs, with a focus on the strengths and limitations of various assays used to diagnose borrelial infection. Subclinical infections are common in dogs and on top of that spirochetes are difficult to detect in canines. As a result, a definite diagnosis of LB remains a complicated and time-consuming process in human and veterinary medicine, resulting in many differential diagnoses due to the lack of a specific all-encompassing test for LB. Moreover, the late immune response with delayed antibodies production and late clinical manifestations often complicate diagnosis and efficient treatment. Commonly used serological tests (mostly ELISA and Western blot) show varying sensitivity and specificity due to cross-reactions with other pathogens and a lack of their standardisation. Another developing approach based on biomarker discovery specific for Lyme disease represents a potential for the identification of early disease stage and its differentiation from other diseases. In conclusion, the high prevalence of *Borrelia* spp. in our latitudes, as well as the need for early and targeted veterinarian intervention in the treatment of Lyme disease in dogs, necessitate further research into the remaining challenge of developing precise and rapid diagnostic tests.

ACKNOWLEDGEMENT

This publication is the result of the project implementation: "Open scientific community for modern interdisciplinary research in medicine (OPENMED", ITMS2014+: 313011V455 supported by the Operational Programme Integrated Infrastructure, funded by the ERDF.

REFERENCES

1. **Abbott, A., 2006:** Lyme disease: Uphill struggle. *Nature*, 439, 524–525. DOI: 10.1016/c2016-0-01430-4.
2. **Aguero-Rosenfeld, M., Wang, G., Schwartz, I., Wormser, G., 2005:** Diagnosis of Lyme Borreliosis. *Clin. Microbiol. Rev.*, 18, 484–509. DOI: 10.1128/CMR.18.3.484-509.2005.
3. **Anderson, C., Brissette, C. A., 2021:** The brilliance of *Borrelia*: Mechanisms of host immune evasion by Lyme disease-causing spirochetes. *Pathogens*, 10, 1–17. DOI: 10.3390/pathogens10030281.
4. **Appel, M. J. G., Allan, S., Jacobson, R. H., Lauderdale, T. L., Chang, Y. F., Shin, S. J., et al., 1993:** Experimental lyme disease in dogs produces arthritis and persistent infection. *J. Infect. Dis.*, 167, 651–654. DOI: 10.1093/infdis/167.3.651.
5. **Barbour, A. G., 2018:** Borreliaceae. In **Whitman, W. B., et al.:** *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley and Sons, Inc., Hoboken, NJ, 1–9.
6. **Barbour, A. G., Gupta, R. S., 2021:** The family *Borreliaceae* (*Spirochaetales*), a diverse group in two genera of tick-borne spirochetes of mammals, birds, and reptiles. *J. Med. Entomol.*, 58, 1513–1524. DOI: 10.1093/jme/tjab055.
7. **Barth, C., Straubinger, R. K., Krupka, I., Müller, E., Sauter-Louis, C., Hartmann, K., 2014:** Comparison of different diagnostic assays for the detection of *Borrelia burgdorferi*-specific antibodies in dogs. *Vet. Clin. Pathol.*, 43, 496–504. DOI: 10.1111/vcp.12213.
8. **Bhide, M., Trávniček, M., Čurlík, J., Štefančíková, A., 2004:** The importance of dogs in eco-epidemiology of Lyme borreliosis: A review. *Vet. Med.*, 49, 135–142. DOI: 10.17221/5687-VETMED.
9. **Bjurman, N. K., Bradet, G., Lloyd, V. K., 2016:** Lyme disease risk in dogs in New Brunswick. *Can. Vet. J.*, 57, 981–984.
10. **Branda, J. A., Body, B. A., Boyle, J., Branson, B. M., Dattwyler, R. J., Fikrig, E., et al., 2018:** Advances in serodiagnostic testing for Lyme disease are at hand. *Clin. Infect. Dis.*, 66, 1133–1139. DOI: 10.1093/cid/cix943.
11. **Bruckbauer, H. R., Preac-Mursic, V., Fuchs, R., Wilske, B., 1992:** Cross-reactive proteins of *Borrelia burgdorferi*. *Eur. J. Clin. Microbiol. Infect. Dis*., 11, 224–232. DOI: 10.1007/BF02098084.
12. **Burgdorfer, W., Barbour, A., Hayes, S., Benach, J., Grunwaldt, E., Davis, J., 1982:** Lyme disease – A tick-borne spirochetosis ? *Science*, 216, 1317–1319. DOI: 10.1126/science.7043737.
13. **Companion Animal Parasite Council, 2022:** *Lyme disease – prevalence map*. Available at https://capcvet.org/maps/#/2022/all-year/lyme-disease/dog/united-states. Accessed January 31, 2023.
14. **Carlos, G., dos Santos, F. P., Fröehlich, P. E., 2020:** Canine metabolomics advances. *Metabolomics*, 16, 1–19. DOI: 10.1007/s11306-020-1638-7.

15. **Centers for Disease Control and Prevention, 2022:** *Lyme disease: Data and surveillance*. Available at https://capcvet.org/maps#/2022/all-year/lyme-disease/dog/united-states. Accessed January 31, 2023.
16. **Chomel, B., 2015:** Lyme disease. *Rev. Sci. Tech.*, 34, 569–576. DOI: 10.20506/rst.34.2.2385.
17. **Chou, J., Wünschmann, A., Hodzic, E., Borjesson, D. L., 2006:** Detection of *Borrelia burgdorferi* DNA in tissues from dogs with presumptive Lyme borreliosis. *J. Am. Vet. Med. Assoc.*, 229, 1260–1265. DOI: 10.2460/javma.229.8.1260.
18. **Collares-Pereira, M., Couceiro, S., Franca, I., Kurtenbach, K., Schäfer, S. M., Vitorino, L., et al., 2004:** First isolation of *Borrelia lusitaniae* from a human patient. *J. Clin. Microbiol.*, 42, 1316–1318. DOI: 10.1128/JCM.42.3.1316-1318.2004.
19. **Comstedt, P., Schüler, W., Meinke, A., Lundberg, U., 2017:** The novel Lyme borreliosis vaccine VLA15 shows broad protection against *Borrelia* species expressing six different OspA serotypes. *PLOS ONE*, 12, 1–13. DOI: 10.1371/journal.pone.0184357.
20. **Cutler, S. J., Rudenko, N., Golovchenko, M., Cramaro, W. J., Kirpach, J., Savic, S., et al., 2017:** Diagnosing borreliosis. *Vector-Borne Zoonotic Dis.*, 17, 2–11. DOI: 10.1089/vbz.2016.1962.
21. **Dai, X., Shen, L., 2022:** Advances and trends in omics technology development. *Front. Med.*, 9, 1–25. DOI: 10.3389/fmed.2022.911861.
22. **Dattwyler, R. J., Arnaboldi, P. M., 2014:** Editorial commentary: Comparison of Lyme disease serologic assays and Lyme specialty laboratories. *Clin. Infect. Dis.*, 59, 1711–1713. DOI: 10.1093/cid/ciu705.
23. **Delgado, S., Cármenes, P., 1995:** Seroepidemiological survey for *Borrelia burgdorferi* (Lyme disease) in dogs from northwestern of Spain. *Eur. J. Epidemiol.*, 11, 321–324. DOI: 10.1007/BF01719437.
24. **Dolange, V., Simon, S., Morel, N., 2021:** Detection of *Borrelia burgdorferi* antigens in tissues and plasma during early infection in a mouse model. *Sci. Rep.*, 11, 1–13. DOI: 10.1038/s41598-021-96861-z.
25. **Embers, M. E., Narasimhan, S., 2013:** Vaccination against Lyme disease: Past, present, and future. *Front. Cell. Infect. Microbiol.*, 3, 1–15. DOI: 10.3389/fcimb.2013.00006.
26. **Feng, J., Shi, W., Zhang, S., Sullivan, D., Auwaerter, P. G., Zhang, Y., 2016:** A drug combination screen identifies drugs active against amoxicillin-induced round bodies of *in vitro Borrelia burgdorferi* persisters from an FDA drug library. *Front. Microbiol.*, 7, 1–12. DOI: 10.3389/fmicb.2016.00743.
27. **Feng, J., Auwaerter, P. G., Zhang, Y., 2015:** Drug combinations against *Borrelia burgdorferi* persisters *in vitro*: Eradication achieved by using daptomycin, cefoperazone and doxycycline. *PLOS ONE*, 10, 1–15. DOI: 10.1371/journal.pone.0117207.
28. **Galluzzo, P., Grippi, F., Di Bella, S., Santangelo, F., Sciortino, S., Castiglia, A., et al., 2020:** Seroprevalence of *Borrelia burgdorferi* in stray dogs from southern Italy. *Microorganisms*, 8, 1–8. DOI: 10.3390/microorganisms8111688.
29. **Gatellet, M., Vanderheyden, S., Abee, M., Adaszek, Ł., Varloud, M., 2019:** A Suspected case of Lyme borreliosis in a dog from Belgium. *Case Reports Vet. Med.*, 2019, 1–3. DOI: 10.1155/2019/3973901.
30. **Gerber, B., Haug, K., Eichenberger, S., Reusch, C. E., Wittenbrink, M. M., 2009:** Follow-up of Bernese Mountain dogs and other dogs with serologically diagnosed *Borrelia burgdorferi* infection: What happens to seropositive animals? *BMC Vet. Res.*, 5, 1–8. DOI: 10.1186/1746-6148-5-18.
31. **Gettings, J. R., Lopez, J. E., Krishnavahjala, A., Armstrong, B. A., Thompson, A. T., Yabsley, M. J., 2019:** Antibodies to *Borrelia turicatae* in experimentally infected dogs cross-react with *Borrelia burgdorferi* serologic assays. *J. Clin. Microbiol.*, 57. DOI: 10.1128/JCM.00628-19.
32. **Golovchenko, M., Vancová, M., Clark, K., Oliver, J. H., Grubhoffer, L., Rudenko, N., 2016:** A divergent spirochete strain isolated from a resident of the southeastern United States was identified by multilocus sequence typing as *Borrelia bissettii*. *Parasites and Vectors*, 9, 1–5. DOI: 10.1186/s13071-016-1353-4.
33. **Goossens, H. A. T., Van den Bogaard, A. E., Nohlmans, M. K. E., 2001:** Dogs as sentinels for human Lyme borreliosis in the Netherlands. *J. Clin. Microbiol.*, 39, 844–848. DOI: 10.1128/JCM.39.3.844-848.2001.
34. **Guerra, M. A., Walker, E. D., Kitron, U., 2001:** Canine surveillance system for Lyme borreliosis in Wisconsin and Northern Illinois: Geographic distribution and risk factor analysis. *Am. J. Trop. Med. Hyg.*, 65, 546–552. DOI: 10.4269/ajtmh.2001.65.546.
35. **Gupta, R. S., 2019:** Distinction between *Borrelia* and *Borreliella* is more robustly supported by molecular and phenotypic characteristics than all other neighbouring prokaryotic genera: Response to Margos' et al. "The genus *Borrelia* reloaded" (PLOS ONE, 13,12, E0208432). *PLOS ONE*, 14, 1–22. DOI: 10.1371/journal.pone.0221397.

36. **Hovius, K. E., Stark, L. A. M., Bleumink-Pluym, N. M. C., van de Pol, I., Verbeek-de Kruif, N., Rijpkema, S. G. T., et al., 1999:** Presence and distribution of *Borrelia burgdorferi* sensu lato species in internal organs and skin of naturally infected symptomatic and asymptomatic dogs, as detected by polymerase chain reaction. *Vet. Q.*, 21, 54–58. DOI: 10.1080/01652176.1999.9694992.

37. **Hoxmeier, J. C., Fleshman, A. C., Broeckling, C. D., Prenni, J. E., Dolan, M. C., Gage, K. L., et al., 2017:** Metabolomics of the tick-*Borrelia* interaction during the nymphal tick blood meal. *Sci. Rep.*, 7, 1–11. DOI: 10.1038/srep44394.

38. **Hutton, T., Goldstein, R. E., Njaa, B. L., Atwater, D. Z., Chang, Y. F., Simpson, K. W., 2008:** Search for *Borrelia burgdorferi* in kidneys of dogs with suspected "Lyme nephritis". *J. Vet. Intern. Med.*, 22, 860–865. DOI: 10.1111/j.1939-1676.2008.0131.x.

39. **Izac, J. R., Camire, A. C., Earnhart, C. G., Embers, M. E., Funk, R. A., Breitschwerdt, E. B., et al., 2019:** Analysis of the antigenic determinants of the OspC protein of the Lyme disease spirochetes: Evidence that the C10 motif is not immunodominant or required to elicit bactericidal antibody responses. *Vaccine*, 37, 2401–2407. DOI: 10.1016/j.vaccine.2019.02.007.

40. **Kalish, R. A., McHugh, G., Granquist, J., Shea, B., Ruthazer, R., Steere, A. C., 2001:** Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10–20 years after active Lyme disease. *Clin. Infect. Dis.*, 33, 780–785. DOI: 10.1086/322669.

41. **Kenedy, M. R., Lenhart, T. R., Akins, D. R., 2012:** The role of *Borrelia burgdorferi* outer surface proteins. *FEMS Immunol. Med. Microbiol.*, 66, 1–19. DOI: 10.1111/j.1574-695X.2012.00980.x.

42. **Kilpatrick, A. M., Dobson, A. D. M., Levi, T., Salkeld, D. J., Swei, A., Ginsberg, H. S., et al., 2017:** Lyme disease ecology in a changing world: Consensus, uncertainty and critical gaps for improving control. *Philos. Trans. R. Soc. B Biol. Sci.*, 372, 1–15. DOI: 10.1098/rstb.2016.0117.

43. **Krämer, F., Hüsken, R., Krüdewagen, E. M., Deuster, K., Blagburn, B., Straubinger, R. K., et al., 2020:** Prevention of transmission of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* by *Ixodes* spp. ticks to dogs treated with the Seresto® collar (imidacloprid 10 % + flumethrin 4.5 %). *Parasitol. Res.*, 119, 299–315. DOI: 10.1007/s00436-019-06394-8.

44. **Krause, P. J., Foley, D. T., Burke, G. S., Christianson, D., Closter, L., Spielman, A., et al., 2006:** Reinfection and relapse in early Lyme disease. *Am. J. Trop. Med. Hyg.*, 75, 1090–1094. DOI: 10.4269/ajtmh.2006.75.1090.

45. **Krupka, I., Straubinger, R. K., 2010:** Lyme borreliosis in dogs and cats: Background, diagnosis, treatment and prevention of infections with *Borrelia burgdorferi* sensu stricto. *Vet. Clin. North Am. Small Anim. Pract.*, 40, 1103–1119. DOI: 10.1016/j.cvsm.2010.07.011.

46. **Kybicová, K., Lukavská, A., Balátová, P., 2018:** Lyme borreliosis – cultivation of *Borrelia burgdorferi* sensu lato (In Czech). *Zprávy Cent. Epidemiol. Mikrobiol.* (SZÚ Prague), 27, 113–115.

47. **Lebech, A. M., Clemmensen, O., Hansen, K., 1995:** Comparison of *in vitro* culture, immunohistochemical staining, and PCR for detection of *Borrelia burgdorferi* in tissue from experimentally infected animals. *J. Clin. Microbiol.*, 33, 2328–2333. DOI: 10.1128/jcm.33.9.2328-2333.1995.

48. **Liang, F. T., Jacobson, R. H., Straubinger, R. K., Grooters, A., Philipp, M. T., 2000:** Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.*, 38, 4160–4166. DOI: 10.1128/jcm.38.11.4160-4166.2000.

49. **Liang, F. T., Philipp, M. T., 1999:** Analysis of antibody response to invariable regions of VlsE, the variable surface antigen of *Borrelia burgdorferi*. *Infect. Immun.*, 67, 6702–6706. DOI: 10.1128/iai.67.12.6702-6706.1999.

50. **Little, S. E., Heise, S. R., Blagburn, B. L., Callister, S. M., Mead, P. S., 2010:** Lyme borreliosis in dogs and humans in the USA. *Trends Parasitol.*, 26, 213–218. DOI: 10.1016/j.pt.2010.01.006.

51. **Littman, M. P., Gerber, B., Goldstein, R. E., Labato, M. A., Lappin, M. R., Moore, G. E., 2018:** ACVIM consensus update on Lyme borreliosis in dogs and cats. *J. Vet. Intern. Med.*, 32, 887–903. DOI: 10.1111/jvim.15085.

52. **Littman, M. P., Goldstein, R. E., Labato, M., Lappin, M. R., Moore, G. E., 2006:** ACVIM small animal consensus statement on Lyme disease in dogs: Diagnosis, treatment, and prevention. *J. Vet. Intern. Med.*, 20, 422–434. DOI: 10.1111/j.1939-1676.2006.tb02880.x.

53. **Liu, J., Drexel, J., Andrews, B., Eberts, M., Breitschwerdt, E., Chandrashekar, R., 2018:** Comparative evaluation of 2 in-clinic assays for vector-borne disease testing in dogs. *Top. Companion Anim. Med.*, 33, 114–118. DOI: 10.1053/j.tcam.2018.09.003.

54. **Liveris, D., Schwartz, I., McKenna, D., Nowakowski, J., Nadelman, R., DeMarco, J., et al., 2012:** Comparison of

five diagnostic modalities for direct detection of *Borrelia burgdorferi* in patients with early Lyme disease. *Diagn. Microbiol. Infect. Dis.*, 73, 243–245. DOI: 10.1016/j.diagmicrobio.2012.03.026.

55. **Lohr, B., Fingerle, V., Norris, D. E., Hunfeld, K. P., 2018:** Laboratory diagnosis of Lyme borreliosis: Current state of the art and future perspectives. *Crit. Rev. Clin. Lab. Sci.*, 55, 219–245. DOI: 10.1080/10408363.2018.1450353.
56. **Malloy, D. C., Nauman, R. K., Paxton, H., 1990:** Detection of *Borrelia burgdorferi* using the polymerase chain reaction. *J. Clin. Microbiol.*, 28, 1089–1093. DOI: 10.1128/jcm.28.6.1089-1093.1990.
57. **Margos, G., Lane, R. S., Fedorova, N., Koloczek, J., Piesman, J., Hojgaard, A., et al., 2016:** *Borrelia bissettiae* sp. nov. and *Borrelia californiensis* sp. nov. prevail in diverse enzootic transmission cycles. *Int. J. Syst. Evol. Microbiol.*, 66, 1447–1452. DOI: 10.1099/ijsem.0.000897.
58. **Margos, G., Fedorova, N., Becker, N. S., Kleinjan, J. E., Marosevic, D., Krebs, S., et al., 2020:** *Borrelia maritima* sp. nov., a novel species of the *Borrelia burgdorferi* sensu lato complex, occupying a basal position to North American species. *Int. J. Syst. Evol. Microbiol.*, 70, 849–856. DOI: 10.1099/ijsem.0.003833.
59. **Margos, G., Sing, A., Fingerle, V., 2017:** Published data do not support the notion that *Borrelia valaisiana* is human pathogenic. *Infection*, 45, 567–569. DOI: 10.1007/s15010-017-1032-1.
60. **Margos, G., Castillo-Ramirez, S., Cutler, S., Dessau, R. B., Eikeland, R., Estrada-Peña, A., et al., 2020:** Rejection of the name *Borreliella* and all proposed species comb. nov. placed therein. *Int. J. Syst. Evol. Microbiol.*, 70, 3577–3581. DOI: 10.1099/ijsem.0.004149.
61. **Merino, F. J., Serrano, J. L., Saz, J. V., Nebreda, T., Gegundez, M., Beltran, M., 2000:** Epidemiological characteristics of dogs with Lyme borreliosis in the province of Soria (Spain). *Eur. J. Epidemiol.*, 16, 97–100. DOI: 10.1023/A:1007690807637.
62. **Minkus, G., Breuer, W., Wanke, R., Hermanns, W., Reusch, C., Leuterer, G., et al., 1994:** Familial nephropathy in Bernese Mountain dogs. *Vet. Pathol.*, 31, 421–428. DOI: 10.1177/030098589403100403.
63. **Miró, G., Wright, I., Michael, H., Burton, W., Hegarty, E., Rodón, J., et al., 2022:** Seropositivity of main vector-borne pathogens in dogs across Europe. *Parasites and Vectors*, 15, 1–13. DOI: 10.1186/s13071-022-05316-5.
64. **Molins, C. R., Ashton, L. V., Wormser, G. P., Hess, A. M., Delorey, M. J., Mahapatra, S., et al., 2015:** Development of a metabolic biosignature for detection of early Lyme disease. *Clin. Infect. Dis.*, 60, 1767–1775. DOI: 10.1093/cid/civ185.
65. **Nagana Gowda, G. A., Zhang, S., Gu, H., Asiago, V., Shanaiah, N., Raftery, D., 2008:** Metabolomics-based methods for early disease diagnostics: A review. *Expert Rev. Mol. Diagn.*, 8, 617–633. DOI: 10.1586/147371 59.8.5.617.
66. **Norris, S. J., 2006:** Antigenic variation with a twist – The *Borrelia* story. *Mol. Microbiol.*, 60, 1319–1322. DOI: 10.1111/j.1365-2958.2006.05204.x.
67. **Pangrácová, L., Derdáková, M., Pekárik, L., Hviščová, I., Víchová, B., Stanko, M., et al., 2013:** *Ixodes ricinus* abundance and its infection with the tick-borne pathogens in urban and suburban areas of Eastern Slovakia. *Parasites and Vectors*, 6, 1–8. DOI: 10.1186/1756-3305-6-238.
68. **Pegalajar-Jurado, A., Fitzgerald, B. L., Islam, M. N., Belisle, J. T., Wormser, G. P., Waller, K. S., et al., 2018:** Identification of urine metabolites as biomarkers of early Lyme disease. *Sci. Rep.*, 8, 1–12. DOI: 10.1038/s41598-018-29713-y.
69. **Preyß-Jägeler, C., Müller, E., Straubinger, R. K., Hartmann, K., 2016:** Prävalenz von Antikörpern gegen *Borrelia burgdorferi, Anaplasma phagocytophilum* und bestimmte *Leptospira*-interrogans-Serovare bei Berner Sennenhunden. *Tierarztl. Prax. Ausgabe K: Kleintiere – Heimtiere*, 44, 77–85. DOI: 10.15654/TPK-140962.
70. **Pritt, B. S., Respicio-Kingry, L. B., Sloan, L. M., Schriefer, M. E., Replogle, A. J., Bjork, J., et al., 2016:** *Borrelia mayonii* sp. nov., a member of the *Borrelia burgdorferi* sensu lato complex, detected in patients and ticks in the upper midwestern United States. *Int. J. Syst. Evol. Microbiol.*, 66, 4878–4880. DOI: 10.1099/ijsem.0.001445.
71. **Pritt, B. S., Mead, P. S., Johnson, D. K. H., Neitzel, D. F., Respicio-Kingry, L. B., Davis, J. P., et al., 2016:** Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: A descriptive study. *Lancet Infect. Dis.*, 16, 556–564. DOI: 10.1016/S1473-3099(15)00464-8.
72. **Radolf, J. D., Caimano, M. J., Stevenson, B., Hu, L. T., 2012:** Of ticks, mice and men: Understanding the dual-host lifestyle of Lyme disease *spirochaetes*. *Nat. Rev. Microbiol.*, 10, 87–99. DOI: 10.1038/nrmicro2714.
73. **Reif, K. E., 2020:** Lyme disease in dogs: Signs and prevention. *Today's Vet. Pract.*, 10, 24–28. Available at https://todaysveterinarypractice.com/. Accessed January 31, 2023.

74. **Rosà, R., Andreo, V., Tagliapietra, V., Baráková, I., Arnoldi, D., Hauffe, H. C., et al., 2018:** Effect of climate and land use on the spatio-temporal variability of tick-borne bacteria in Europe. *Int. J. Environ. Res. Public Health*, 15, 1–15. DOI: 10.3390/ijerph15040732.

75. **Rudenko, N., Golovchenko, M., Kybicova, K., Vancova, M., 2019:** Metamorphoses of Lyme disease spirochetes: Phenomenon of *Borrelia persisters*. *Parasites and Vectors*, 12, 1–10. DOI: 10.1186/s13071-019-3495-7.

76. **Sapi, E., Kaur, N., Anyanwu, S., Luecke, D. F., Datar, A., Patel, S., et al., 2011:** Evaluation of *in vitro* antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*. *Infect. Drug Resist.*, 4, 97–113. DOI: 10.2147/IDR.S19201.

77. **Schwartz, A. M., Hinckley, A. F., Mead, P. S., Hook, S. A., Kugeler, K. J., 2017:** Surveillance for Lyme disease – United States, 2008–2015. *MMWR Surveill. Summ.*, 66, 1–12. DOI: 10.15585/mmwr.ss6622a1.

78. **Schwarzová, K., Ferko, M., Farkaš, P. (Eds.), 2019:** *Arbobactera, Leptospira, Kapnocytophages, Streptobacili – Bacteria with Pathogenic Potential Transmissible to Man* (In Slovak). OZ Preveda, Banská Bystrica, Slovakia, 54 pp.

79. **Smith, A. J., Oertle, J., Prato, D., 2014:** Chronic Lyme disease: Persistent clinical symptoms related to immune evasion, antibiotic resistance and various defense mechanisms of *Borrelia burgdorferi*. *Open J. Med. Microbiol.*, 4, 252–260. DOI: 10.4236/ojmm.2014.44029.

80. **Spencer, J. A., Butler, J. M., Stafford, K. C., Pough, M. B., Levy, S. A., Bledsoe, D. L., et al., 2003:** Evaluation of permethrin and imidacloprid for prevention of *Borrelia burgdorferi* transmission from blacklegged ticks (*Ixodes scapularis*) to *Borrelia burgdorferi*-free dogs. *Parasitol. Res.*, 90, 106–107. DOI: 10.1007/s00436-003-0904-8.

81. **Sperling, J. L. H., Sperling, F. A. H., 2009:** Lyme borreliosis in Canada: Biological diversity and diagnostic complexity from an entomological perspective. *Can. Entomol.*, 141, 521–549. DOI: 10.4039/n08-CPA04.

82. **Spickler, A. R., 2020:** *Lyme Disease*. Available at https://www.cfsph.iastate.edu/Factsheets/pdfs/lyme_disease.pdf. Updated January 2020. Accessed January 31, 2023.

83. **Stanek, G., Fingerle, V., Hunfeld, K. P., Jaulhac, B., Kaiser, R., Krause, A., et al., 2011:** Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clin. Microbiol. Infect.*, 17, 69–79. DOI: 10.1111/j.1469-0691.2010.03175.x.

84. **Stanek, G., Wormser, G. P., Gray, J., Strle, F., 2012:** Lyme borreliosis. *Lancet*, 379, 461–473. DOI: 10.1016/S0140-6736(11)60103-7.

85. **Stanko, M., Derdáková, M., Špitalská, E., Kazimírová, M., 2022:** Ticks and their epidemiological role in Slovakia: From the past till present. *Biologia*, 77, 1575–1610. DOI: 10.1007/s11756-021-00845-3.

86. **Steere, A. C., Strle, F., Wormser, G. P., Hu, L. T., Branda, J. A., Li, X., et al., 2016:** Lyme borreliosis. *Nat. Rev. Dis. Prim.*, 2, 1–18. DOI: 10.1038/nrdp.2016.90.

87. **Štefančíková, A., Derdáková, M., Škardová, I., Szestáková, E., Čisláková, L., Kováčová, D., et al., 2008:** Some epidemiological and epizootiological aspects of Lyme borreliosis in Slovakia with the emphasis on the problems of serological diagnostics. *Biologia*, 63, 1135–1142. DOI: 10.2478/s11756-008-0177-x.

88. **Stillman, B. A., Thatcher, B., Beall, M. J., Lappin, M., O'Connor, T. P., Chandrashekar, R., 2019:** *Borrelia burgdorferi* antibody test results in dogs administered 4 different vaccines. *Top. Companion Anim. Med.*, 37, 1–4. DOI: 10.1016/j.tcam.2019.100358.

89. **Straubinger, R. K., Summers, B. A., Chang, Y. F., Appel, M. J. G., 1997:** Persistence of *Borrelia burgdorferi* in experimentally infected dogs after antibiotic treatment. *J. Clin. Microbiol.*, 35, 111–116. DOI: 10.1128/jcm.35.1.111-116.1997.

90. **Straubinger, R. K., Straubinger, A. F., Summers, B. A., Jacobson, R. H., 2000:** Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: An experimental study. *J. Infect. Dis*., 181, 1069–1081. DOI: 10.1086/315340.

91. **Strnad, M., Hönig, V., Růžek, D., Grubhoffer, L., Rego, R. O. M., 2017:** Europe-wide meta-analysis of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks. *Appl. Environ. Microbiol.*, 83, 1–16. DOI: 10.1128/AEM.00609-17.

92. **Tilly, K., Bestor, A., Rosa, P. A., 2013:** Lipoprotein succession in *Borrelia burgdorferi*: Similar but distinct roles for OspC and VlsE at different stages of mammalian infection. *Mol. Microbiol.*, 89, 216–227. DOI: 10.1111/mmi.12271.

93. **Töpfer, K. H., Straubinger, R. K., 2007:** Characterization of the humoral immune response in dogs after vaccination against the Lyme borreliosis agent. A study with five commercial vaccines using two different vaccination schedules. *Vaccine*, 25, 314–326. DOI: 10.1016/j.vaccine.2006.07.031.

94. **Tran, H., Mcconville, M., Loukopoulos, P., 2020:** Metabolomics in the study of spontaneous animal diseases. *J. Vet. Di-*

agnostic Investig., 32, 635–647. DOI: 10.1177/1040638720948505.

95. Valko-Rokytovská, M., Očenáš, P., Salayová, A., Kostecká, Z., 2018: New developed UHPLC method for selected urine metabolites. *J. Chromatogr. Sep. Tech.*, 9, 1–8. DOI: 10.4172/2157-7064.1000404.

96. Valko-Rokytovská, M., Očenáš, P., Salayová, A., Titková, R., Kostecká, Z., 2020: Specific urinary metabolites in canine mammary gland tumours. *J. Vet. Sci.*, 21, 1–10. DOI: 10.4142/jvs.2020.21.e23.

97. Venczel, R., Knoke, L., Pavlovic, M., Dzaferovic, E., Vaculova, T., Silaghi, C., et al., 2016: A novel duplex real-time PCR permits simultaneous detection and differentiation of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato. *Infection*, 44, 47–55. DOI: 10.1007/s15010-015-0820-8.

98. Vogt, N. A., Sargeant, J. M., MacKinnon, M. C., Versluis, A. M., 2019: Efficacy of *Borrelia burgdorferi* vaccine in dogs in North America: A systematic review and meta-analysis. *J. Vet. Intern. Med.*, 33, 23–36. DOI: 10.1111/jvim.15344.

99. Vogt, N. A., Stevens, C. P. G., 2021: Why the rationale for canine *Borrelia burgdorferi* vaccination is unpersuasive. *Front. Vet. Sci.*, 8, 1–3. DOI: 10.3389/fvets.2021.719060.

100. Vrhovec, M. G., Pantchev, N., Failing, K., Bauer, C., Travers-Martin, N., Zahner, H., 2017: Retrospective analysis of canine vector-borne diseases (CVBD) in Germany with emphasis on the endemicity and risk factors of leishmaniosis. *Parasitol. Res.*, 116, 131–144. DOI: 10.1007/s00436-017-5499-6.

101. Wagner, B., Johnson, J., Garcia-Tapia, D., Honsberger, N., King, V., Strietzel, C., et al., 2015: Comparison of effectiveness of cefovecin, doxycycline, and amoxicillin for the treatment of experimentally induced early Lyme borreliosis in dogs. *BMC Vet. Res.*, 11, 1–8. DOI: 10.1186/s12917-015-0475-9.

102. WHO, Regional Office for Europe, 2006: *Lyme borreliosis in Europe*. Available at https://www.euro.who.int/__data/assets/pdf_file/0008/246167/Fact-sheet-Lyme-borreliosis-Eng.pdf. Accessed January 31, 2023.

103. Wormser, G. P., Schwartz, I., 2009: Antibiotic treatment of animals infected with *Borrelia burgdorferi*. *Clin. Microbiol. Rev.*, 22, 387–395. DOI: 10.1128/CMR.00004-09.

104. Zhang, J., Wei, S., Liu, L., Nagana Gowda, G. A., Bonney, P., Stewart, J., et al., 2012: NMR-based metabolomics study of canine bladder cancer. *Biochim. Biophys. Acta Mol. Basis Dis.*, 1822, 1807–1814. DOI: 10.1016/j.bbadis.2012.08.001.

Received January 15, 2023
Accepted February 16, 2023