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Studies on *Borrelia burgdorferi* in police dogs and their contacts

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List of Abbreviations

ACVIM	**American collage of veterinary internal medicine**
bp	**Base pair**
BSA	**Bovine Serum Albumen**
C	**Celsius(degree temperature)**
CDC	**Center of Disease Control**
CSF	**Cerbro spinal fluid**
CVBDs	**Canine vector born diseases**
DDW	**Deionized Distilled water**
DG	**Dark ground microscope**
dNTPs	**Deoxy nucleotide mix**
D.W	**Distilled water**
EDTA	**Ethylene Dinitrilo Tetraacetic Acid**
ELISA	**Enzyme Linked immune sorbent Assay**
IFA	**Immuno-fluorescent assay**
IIF	**Indirect Immune Fluorescent**
IgG	**Immune globulin G**
IgM	**Immune globulin M**
IRs	**Invariable regions**
LB	**Lyme Borellioses**
LD	**Lyme disease**
µ	**Micron**
µl	**Microliter**
MIA	**Ministry of interior affairs**
MLE	**Maximum likehood estimation**
MWD	**Military working dogs**
nm	**Nano meter**
NY	**New York state**
OD diff	**Optical density difference**
OIFs	**oil immersion fields**
OR	**Odd ratios**
***Osp*A**	**Outer surface protein A**
PBS	**Phosphate buffer saline**
PCR	**polymerase chain reaction**
PI	**Post infection**
POK	**Population in the republic of Korea**

Pro K	**Protienase K**
qPCR	**Quantitative polymerase chain reaction**
s.l	**Senso lato**
Sp	**Spices**
s.s	**Senso stricto**
TAE	**Trtate EDTAis Ace**
US	**United States**
UV	**Ultraviolet**
VBC	**Vector born diseases**
VIsE	**variable surface antigen**
WB	**Western Blot**

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INTRODUCTION

I. INTRODUCTION

Lyme disease is a multisystemic zoonotic disease caused by a tick borne spirochete *Borrelia burgdorferi* belonging to family *Spiorchetaceae* **(Font *et al.*, 1992).** Like other spirochetes, *Borrelia* spp are spiral shaped, Gram-negative, and have an outer sheath encasing endofibrils **(Wang *et al.*,1999)**. Unique to *Borrelia* spp are a singular linear chromosome (with additional linear and circular plasmids) and life cycles that require both arthropod vectors and mammalian hosts **(Roberts *et al.*,2002).** It occurs endemically and is borne by *Ixodes* type ticks, particularly by *Ixodes ricinus, I. pacificus, I. persulcatus* and *I. scapularis* **(Wodecka and Skotarczyk, 2000; Ambrasiene *et al.*, 2004; Cisak *et al.*, 2005; Foley *et al.*, 2007).** The genus *Borrelia* contains at least 37 species which are characterized into two groups; those causing relapsing fever, and those causing Lyme borreliosis. Dogs are only rarely affected by the relapsing fever *Borreliae* group, with the clinical significance of such infections not known **(Breitschwerdt, 1994).** The genospecies of *Borrelia burgdorferi* sensu lato is a bacterial group of at least 10 species that are the causative agents of borreliosis in Europe and the USA (Lyme disease). Organisms from this group are the causative agents of Lyme borreliosis **(Branton, 1998).** Within the *Borrelia burgdorferi* sensu lato group, most species are non-pathogenic for dogs. Three species, however, are clinically important as zoonoses in humans and dogs, *B. burgdorferi sensu stricto, B. garinii, and B. afzelii* **(Greene *et al.*, 2006).**

Diagnosis of canine Lyme disease can be challenging due to the variability in clinical signs, nonspecific clinicopathology, and serology using enzyme-linked immunosorbent assay, which may not distinguish between exposure and prior vaccination.

Borrelia burgdorferi infection in dogs may cause episodic fever, lethargy, anorexia, lymphadenopathy, and polyarthritis. Less commonly, effects on cardiac, renal, neurologic, and ocular systems may occur. The most common clinical features in canines are arthritis and arthralgia. Any combination and severity of these signs may be seen, making clinical diagnosis of Lyme disease difficult **(Appel *et al.*, 1993; Greene *et al.*, 1998).**

The diagnosis of Lyme borreliosis in both humans and dogs can be challenging. Clinical signs often are nonspecific and variable. Culture of the spirochete requires special media over a lengthy incubation period and often is unrewarding. Assays for circulating antibodies remain the most common means of laboratory confirmation despite recognized shortcomings. Enzyme immunoassays (EIA) and immunofluorescent assays (IFA) based on the whole cell or subunits of the spirochete generally lack specificity. The Centers for Disease Control and Prevention 1995 (CDC) advocates a "2-tier" testing system using an ELISA or immunofluorescence assay as a screening test, followed by a Western blot for confirmation if the result of the ELISA or immunofluorescence assay is positive **(Tugwell *et al.*,1997).**

The polymerase chain reaction (PCR) method of DNA detection is a sensitive and specific means of amplifying target DNA sequences.

(Desforges, 1990). It has been used to amplify segments of *B. burgdorferi* chromosomal and plasmid DNA from in vitro or clinical specimens **(Wise and Weaver, 1991).**

Avoidance of Lyme disease in dogs is based on vaccination or prevention of *I. scapularis* infestation, or at least limiting the duration of attachment to a period less than required for transmission of *B. burgdorfer*i. Although transmission of the spirochetes has been reported as early as 37 hours after tick attachment, these organisms were incapable of establishing an infection. However, infection was established when organisms were introduced 52 hours or more after tick attachment. Therefore, to reduce the potential of transmission of *B. burgdorferi*, ticks must be eliminated from the host within approximately 48 hours after attachment. Thus, the time interval within which ticks die on animals treated with acaricides is an important factor. **(Sood *et al.*,1997 and Straubinger *et al*.,1997).**

Because of the increasing risk of Lyme disease, the development of a safe and effective vaccine for this infection has been a high priority **(Edelman, 1991).** Vaccines are either based on a single antigen with or without adjuvants (*Osp*A subunit vaccine) or on a whole-cell bacterin, which contains all antigens of culture-grown and chemically inactivated *B. burgdorferi* organisms complemented with adjuvant **(Lovrich *et al.*, 1995).**

The aim of this work

This work was planned to study *Borrelia burgdorferi* in police dogs and their contacts. As *Borrelia burgdorferi* is a tick borne zoonotic

disease cause polyarthritis in dogs. Although it was proven that *Ripicephalus sanguinius* ticks incriminated in transmission of *Borrelia burgdorferi* among dogs in Egypt, there is no vaccination program against *Borrelia burgdorferi* in Egypt so we conduct this study to increase awareness about Lyme disease among physicians and veterinarians in Egypt and to strengthen laboratory capacity for its diagnosis. To achieve that, the following was done:

1. **Direct demonstration of *Borrelia burgdorferi* by Giemsa staining and dark field microscope.**
2. **Culture of *B. burgdorferi* from canine and human blood on BSK-H medium.**
3. **Detection of *Borrellia burgdorfei osp*A gene in human, canine sea and collected ticks by PCR.**
4. **Detection of IgM antibodies against *B. burgdorferi* in human and canine sera by ELISA.**

REVIEW OF LITERATURES

II.Review of Literatures

2.1. History

Lyme disease has been recognized in Europe for almost a century (**Afzelius, 1910 and Lipschütz, 1913)** but it was not described in humans in the United States until 1975 **(Steere *et al.*, 1977). Steere *et al.* (1983)** mentioned that Lyme first described in the late-1970s, and has become the most common vector-borne illness in the United States and parts of Europe. In 1983, *Borrelia burgdorferi* was identified as the causative organism**.** The disease occurs also in dogs, horses, cattle, and cats, while many wildlife mammals and birds become infected and serve as reservoirs for tick infection **(Donahue *et al.*, 1987; Parker and White 1992; Greene *et al.*, 1998 and Gylfe *et al.*, 2000).** During the 1980s, the reported disease incidence in both dogs and humans increased dramatically. Lyme borreliosis is now the most common arthropod-borne disease of humans in the United States (Center of Disease Control and Prevention; Division of Vector-Borne Infectious Diseases).

2.2. Taxonomy and classification

Breitschwerdt *et al.* (1994) stated that the genus *Borrelia* contains at least 37 species which are characterized into two groups; those causing relapsing fever, and those causing Lyme borreliosis. Dogs and cats are only rarely affected by the relapsing fever borreliae group, with the clinical significance of such infections not known.

Fraser *et al.* (1997) found that *B. burgdorferi* genome sequence

greater than 6% of the chromosomal genes are involved in motility and chemotaxi. The flagella of spirochetes traverse the length of the cell body and are “hidden” beneath the outer membrane, in contrast to other organisms that have external flagella radiating outward. A potential advantage to the flagellar arrangement of spirochetes is the shielding of the highly conserved and immunogenic flagella from the host immune system. Also, the morphology and motility of spirochetes allows these organisms to swim in highly viscous media that immobilize other bacterial species. The structural form of spirochetes may aid pathogenic species in penetrating host tissues and disseminating throughout the host. Authors also mentioned that the genome of *B. burgdorferi* is relatively small, probably reflecting its lifestyle as an obligate parasite. *B. burgdorferi* lacks the conventionally recognizable machinery for synthesizing nucleotides, amino acids, fatty acids, and enzyme cofactors, apparently scavenging these necessities from the host.

Branton (1998) mentioned that the genospecies of *Borrelia burgdorferi* sensu lato is a bacterial group of at least 10 species that are causative agents of borreliosis in Europe and the USA (Lyme disease). Organisms from this group are the causative agents of Lyme borreliosis.

Aguero-Rosenfeld *et al.* (2005) mentioned that *B. burgdorferi* is a helically shaped bacterium with multiple endoflagella. The cells, configured with 3 to 10 loose coils, are 10 to 30 µm in length and 0.2 to 0.5 µm in width. This spirochete possesses several morphological,

structural, ecologic, and genomic features that are distinctive among prokaryotes.

Tilly *et al.* (2008) mentioned that the causative agent of Lyme disease is a member of the eubacterial phylum*Spirochaetes*. Members of this group of organisms share a distinctive morphology that includes a spiral or wavelike body and flagella (organs of motility) enclosed between the outer and inner membranes.

Nolte (2012) mentioned that although the *B. burgdorferi senu lato* complex is large in terms of the number of described genospecies, only a few of them are indeed associated with LB/LD. These are the classical three genospecies *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, and *Borrelia afzelii*. In recent years it became evident, that a fourth species, *Borrelia spielmanii*, is causing disease, too. Three further species have been found in single LB cases, namely *Borrelia valaisiana*, *B. bissettii* and the recently described *B. bavariensis*. The taxonomic status of the latter, i.e. whether being a true species or only a subtype of *B. garinii* is still subject of debate.

2.3. Epidemiology and geographical distribution

Burgess (1986) suggested that contact transmission may have occurred between two dogs. However, the organism was not isolated from the in contact dog which would have provided the evidence that transmission had occurred.

Moody (1991) mentioned that there have not been any reports of sexual transmission of the disease and attempts to transmit it venereally in rats and hamsters failed.

Appel *et al.* (1993) investigated invetro transmission by testing the pups of infected dams failed to isolate *B. burgdorferi* and antibodies were not found in any puppy's heart blood. The same study failed to isolate the organism from infected dogs' urine or bladders concluding that urine is an unlikely source of infection. Keeping healthy dogs in direct contact with the infected dogs for up to a year did not lead to infection or seroconversion.

Kumi-Diaka (1995) stated that semen intended for artificial insemination might be considered a potential source of infection as the organism survives freezing and storage.

Piesman (1997) stated that borreliae cannot survive as free living organisms. Small mammals and birds are reservoir hosts and infection is transmitted by certain Ixodes species of tick. Haematophagous arthropods, including other tick species, fleas, flies and mosquitoes have been found to be infected in nature. These other arthropods are believed to have acquired infection from feeding on infected vertebrates but they have not been capable of transmitting infection to new hosts experimentally.

Hovius *et al.* (1999) monitored thirty-three family dogs for antibodies to *Borrelia burgdorferi* sensu lato over a 3-year period. Serum samples were collected before and during the season of high tick activity. Antibody levels were measured with an ELISA based on whole-cell antigens and an ELISA with a purified recombinant flagellin (r410). Antibody levels measured with the whole-cell ELISA increased after the first exposure to ticks. Following the first seasonal

period of tick quiescence, antibody levels decreased, and subsequently increased again in the second tick season. Thereafter whole-cell ELISA titres persisted at moderate levels and did not decrease between tick seasons. The recombinant flagellin ELISA did not show a strong response in the first tick season, but did in the second tick season and levels of antibodies continued to fluctuate thereafter. Authors concluded that most dogs in this study developed an antibody response against *Borrelia burgdorferi* sensu lato after their first tick infestation and were thereafter repeatedly immunologically stimulated, probably reinfected, during the consecutive tick seasons.

Woodrum (1999) mentioned that Natural transmission of the organism by any means other than by tick inoculation has not been reported. No references could be found indicating that relapsing fever borreliosis is of any clinical significance in dogs or that they are anything but dead-end hosts.

Steere (2001) suggested that the epidemiology of Lyme disease indicated transmission by an arthropod vector due to the geographic clustering of patients in rural areas and the seasonal occurrence of the symptoms.

Bratton and Corey (2005) mentioned that the bacterium has specific tropism for skin, musculoskeletal tissue, joints and the central nervous system depending on the species involved. Early symptoms of human borreliosis include a red, enlarging rash from the site of tick bite, and flu-like symptoms. Many complications may follow an untreated case, such as meningitis, Bell's palsy (paralysis of part of the

face), heart block and painful joints, muscles and bones.

Hovius (2005) mentioned that in an endemic area dogs may become infected but most remain asymptomatic with approximately 5% developing disease concurrent with a rising titre. Clinical signs include fever and polyarthritis. Cats appear to be asymptomatic and more resistant than dogs. Cats, dogs and humans are incidental hosts of Lyme borreliosis. Infection is associated with outdoor activities that result in exposure to tick vectors. Cats and dogs are not a direct source of infection to people. They may, however, bring infected ticks into the human household.

Wilske (2005) mentioned that Borreliosis is the most frequent tick-transmitted zoonotic disease in the northern hemisphere affecting humans (up to 155 cases per 100,000 individuals)**.**

Greene *et al.* (2006) stated that Within the *Borrelia burgdorferi* sensu lato group, most species are non-pathogenic for humans, dogs and cats. Three species, however, are clinically important as zoonoses in humans and dogs, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii.*

Ogden *et al.* (2006) mentioned that Seasonal tick activity under climate change scenarios was consistent with maintenance of endemic cycles of the Lyme disease agent in newly established tick populations. The geographic range of *I. scapularis*-borne zoonoses may, therefore, expand significantly northwards as a consequence of climate change in Canada.

Bowman *et al.* (2009) suggested that lyme disease agent

Borrelia burgdorferi may be present over a wider geographic area, and thus pose greater animal and public health risks, than is currently recognized. Dogs can serve as sentinels to identify the presence of *Borrelia burgdorferi* of both veterinary and public health significance.

Villeneuve *et al.* (2011) showed that the risk for vector-borne infectious agents in the Canadian canine population is low but widespread with foci of higher prevalence. *Borrelia burgdorferi* was the agent with the highest seroprevalence in Canada and expansion of *I. scapularis* into Canada may result in an increased risk of infection of the canine population to tick-borne infectious agents such as *B. burgdorferi.*

Elhelw *et al*. (2014) represented the first systematic study on animals associated with patients suffering from febrile illness to confirm the emerging of *Borrelia burgdorferi* in Egypt.

2.4. Method of transmission and zoonotic importance

Patrican (1997) mentioned that ticks most frequently acquire spirochetes from infected rodents during their larval feeding. After molting to the nymphal stage, infected ticks feed on a broad range of animals, including rodents, which become a new reservoir perpetuating the cycle. After the nymphs molt to the adult stage, they exclusively feed on larger mammals, which are often not competent hosts for *B. burgdorferi*. The spirochetes are rarely, if ever, transmitted trans-ovarially, so the larval and nymphal feedings are crucial to maintaining the spirochete. Both nymphs and adults occasionally feed on dogs, but the small size of the nymphs makes

them difficult to detect and, hence, more likely to feed long enough to transmit the spirochete and cause Lyme disease.

Straubinger (2000a) mentioned that hard shelled ticks of the genus Ixodes, transmitted *Borrelia burgdorferi* by attaching and feeding on various mammalian, avian and reptile hosts. Studies with dogs kept as pets in endemic areas have shown that approximately 5% of all infected dogs become ill. However, under experimental conditions, up to 75% of infected animals develop clinically apparent lyme arthritis.

Goossens *et al.* (2001) found that the seroprevalence of *B. burgdorferi* among hunting dogs (18%) was of the same order as the seroprevalence among pet dogs (17%) and hunters (15%). The seropositivity of a hunting dog was not a significant indicator of increased risk of Lyme borreliosis for its owner. No significant rise in seroprevalence was found in dogs older than 24 months. This indicated that seropositivity after an infection with *B. burgdorferi* in dogs is rather short, approximately 1 year. In humans this is considerably longer but is also not lifelong. Therefore, the incidence of *B. burgdorferi* infections among dogs was greater than that among hunters, despite a similar prevalence of seropositivity among hunters and their hunting dogs. Because no positive correlation was observed between the seropositivity of a hunter and the seropositivity of the hunter's dog, direct transfer of ticks between dog and hunter does not seem important and owning a dog should not be considered a risk factor for Lyme borreliosis.

Crippa *et al.* (2002) studied the efficiency of *Ixodes ricinus* ticks to transmit *B. afzelii* and *B. burgdorferi* sensu stricto (ss) and their infectivity for mice in relation to the duration of the blood meal. In addition, authors investigated whether these two *Borrelia* species can penetrate intact skin. Three modes of infection of mice were studied: tick-bite infection, inoculation of tick homogenates, and transcutaneous infection by topical application of tick homogenates on mouse skin. Transmission of *B. burgdorferi* sl from *I. ricinus* nymphs to mouse increased with duration of tick attachment. *B. afzelii*-infected ticks start to transmit infection earlier (< or = 48 h) than *B. burgdorferi* ss-infected ticks. As previously shown for *B. burgdorferi* ss in Ixodes scapularis, *B. burgdorferi* ss and *B. afzelii* in unfed I. ricinus were noninfectious for mice when tick homogenates were inoculated. However, the inoculation of homogenates of ticks fed for 24 h readily produced infection in mice. Therefore, *B. burgdorferi* ss and *B. afzelii* spirochetes are potentially infectious in the tick before natural transmission can occur. None of the mice (n = 33) became infected by transcutaneous transmission when tick homogenates were applied on mouse skin, showing that *B. burgdorferi* ss and *B. afzelii* are unable to penetrate intact skin, in contrast to relapsing fever spirochetes. This study also shows that *B. afzelii* is transmitted by *I. ricinus* to the host earlier than *B. burgdorferi* ss and that *I. ricinus* seems to be a more efficient vector of *B. afzelii* than *B. burgdorferi* ss.

Beklemishev *et al*. (2003) examined 151 adult female ticks Ixodes persulcatus Schulze, collected at three localities in eastern regions of West Siberia, where Lyme disease is endemic, for the

presence of the spirochete *B. burgdorferi* by polymerase chain reaction. Spirochetal DNA was detected in on average 15.2% of the ticks examined. The infection rate of adult ticks with *B. burgdorferi* at various localities ranged from 8.6% to 29.0%, being greatest in the northernmost site studied and decreasing southwards. Authors have not detected other genospecies, which were found in ticks in Europe, the Russian Far East and Japan.

Yang *et al.* (2004) mentioned that while infected nymphal ticks feed, spirochetes in the midgut respond in several ways to the incoming blood and increased temperature. The population of spirochetes expands and their protein synthesis alters. Then, spirochetes migrate from the midgut to the salivary glands, allowing transmission into a new host. The *B. burgdorferi* outer surface protein OspA was shown to be abundant on the surface of bacteria resident in ticks, but down-regulated during tick feeding and transmission to a mammal. Authors suggested that OspA is an adhesin, important for retaining spirochetes in the tick midgut until feeding.

Duncan *et al.* (2005) mentioned that Dogs have often served as effective sentinel animals to assess the risk of human *B. burgdorferi* infection.

Greene *et al.* (2006) mentioned that the role of ticks relative to the known tick vectors, and is considered insignificant. In North America, *B. burgdorferi* sensu stricto is the only pathogenic species found in dogs. *Ixodes scapularis*, *I. pacificus*, and *I. neotomae* are the tick vectors. In Japan, dogs may be infected with *B. japonica* and *B.*

garinii. In Europe, dogs are mainly infected with *B. burgdorferi* sensu stricto and *B. garinii*. *Ixodes ricinus* in Europe and *I. persulcatus* in Eurasia are the primary tick vectors. Distribution of infection corresponds to the habitat of the ticks. Author also mentioned that Blood transfusion could theoretically be a means of transmission, but this has not been reported in humans or animals.

Hovius *et al.* (2007) mentioned that *B. burgdorferi* have developed many strategies to adapt to the different environments that are present in the arthropod vector and the vertebrate host. Authors focused on *B. burgdorferi* genes that are preferentially expressed in the tick and the vertebrate host, as *B. burgdorferi* enhances expression of specific *Ixodes scapularis* genes, such as TROSPA and salp15. The importance of these genes and their products for *B. burgdorferi* survival within the tick, and during the transmission process.

Mead *et al.* (2011) suggested that canine seroprevalence >5% can be a sensitive but nonspecific marker of increased risk for human Lyme disease. Because dogs do not transmit infection directly to humans (or humans to dogs), this association reflects similar susceptibilities to tick-borne infection. In some circumstances, high canine seroprevalence appears to anticipate increasing rates of human infection at the county level. Conversely, canine seroprevalence <1% is associated with little to no local risk for human infection. Canine seroprevalence is a useful adjunct to human surveillance for Lyme disease.

Claerebout *et al.* (2013) collected 2373 ticks from 647

dogs. *Ixodes ricinus* (76.4%) and *I. hexagonus* (22.6%) were the predominant species. *Rhipicephalus sanguineus*(0.3%) and *D. reticulatus* (0.8%) were found in low numbers. All dogs infested with *R. sanguineus* had a recent travel history, but *D. reticulatus* were collected from a dog without a history of travelling abroad. Of the collected *Ixodes* ticks10.1% were positive for *Borrelia* spp. (*B. afzelii, B. garinii, B. burgdorferi s.s., B. lusitaniae, B. valaisiana* and *B. spielmanii)*.

Wang *et al.* (2014) demonstrated the presence of *B. burgdorferi* in tick populations at central Ohio location. Of 530 nymphal or adult *I. scapularis* analyzed by quantitative polymerase chain reaction (qPCR), 32 (6.1%) tested positive for the *B. burgdorferi flaB* gene, ranging from 36 to 390,000 copies per tick. Antibodies to *B. burgdorferi* antigens were detected in 2 of 10 (20%) field-captured *Peromyscus leucopus* from Tiverton Township, and in 41 of 355 (11.5%) dogs residing in Ohio. Collectively, these data suggested that the enzootic life cycle of *B. burgdorferi*has become established in Ohio, which poses risk of Lyme disease to people and animals in the area.

2.5. Diagnostic methods

The diagnosis of Lyme borreliosis in dogs is challenging. Clinical signs are often non-specific and variable. Culture of the spirochete requires special media over a lengthy incubation period and often is unrewarding. Assays for circulating antibodies remain the most common means of laboratory confirmation despite recognized

shortcomings. Enzyme immunoassays (EIA) and immunofluorescent assays (IFA) based on the whole cell or subunits of the spirochete generally lack specificity and may not distinguish between exposure and prior vaccination.

2.5.1. Clinical signs

Grauer *et al.* (1988) described the renal manifestations of Lyme borreliosis are histologically characterized by glomerulonephritis, tubular necrosis, and interstitial lymphoplasmacytic inflammation that are associated with a rapidly progressive and frequently fatal glomerular disease. Although B burgdorferi spirochetes have been identified in renal tissue, the pathogenesis of *B. burgdorferi*-associated renal disease is not well understood in some dogs.

Levy and Magnarelli (1992) examined the relationship between antibody production and the subsequent development of limb/joint disorders of borreliosis in dogs from south central Connecticut. Dogs without signs of illness, determined by physical examination, were selected from dogs being tested for *Dirofilaria immitis*. An ELISA was used to detect antibodies to *Borrelia burgdorferi* in 234 apparently healthy dogs during 1988. These dogs were monitored for 20 months after initial analyses to determine the prevalence of limb/joint disorder in seropositive and seronegative dogs. Of 234 dogs from which samples were initially obtained, 125 had antibodies to *B burgdorferi* and 109 were seronegative. The development of limb/joint disorder (eg, lameness, swelling, and signs

of pain) accompanied by lethargy, fever, and inappetence in each group was nearly equal. Rates of 4.8% (6/125) and 4.6% (5/109) were recorded for seropositive and serosurvey of dogs, respectively. Authors concluded that the serosurvey of apparently healthy dogs had no predictive value for the subsequent development of limb/joint disorder.

Azuma *et al*. (1993) mentioned that CNS dysfunction and heart block secondary to myocarditis have been attributed to B burgdorferi infection.

Dambach *et al.* (1997) demonstrated that a distinctive renal lesion consisting of glomerulonephritis, diffuse tubular necrosis with regeneration, and interstitial inflammation was found in 49 biopsy/necropsy cases obtained from 1987 to 1992. This lesion is manifested clinically as a rapidly progressive glomerular disease that was uniformly fatal. Immune-mediated membranoproliferative glomerulonephritis predominated (43/49, 88%). Membranous glomerulonephritis (5/49, 10%) and amyloidosis (1/49, 2%) were also noted. Subendothelial deposits, IgG, IgM, and C3 were present along glomerular basement membranes. IgA was absent. The exact cause of the tubular necrosis is unknown. Affected dogs were significantly younger (5.6 +/- 2.6 years) than dogs with other forms of glomerulonephritis (7.1 +/- 3.6 years) and amyloidosis (7.8 +/- 3.5 years) both in the studied population for the same period and in the reported canine population. Labrador and Golden retrievers were 6.4 and 4.9 times more likely, respectively, to develop this lesion. This is

the first report of a breed predilection for spontaneous canine glomerulonephritis. Previous reports have associated this lesion with *Borrelia burgdorferi* exposure. All dogs in this study were from Lyme disease-endemic areas. Of 18 dogs serologically tested, all were positive for exposure.

Callister *et al.* (2000) demonstrated that the presence of *B. burgdorferi* in skin- and joint biopsy samples of dogs was tightly correlated with the occurrence of lameness and fever.

Straubinger (2000a) mentioned that the clinical manifestations of Lyme borreliosis in dogs 2 to 5 months after being infected with *B burgdorferi,* dogs most commonly develop lameness, frequently with accompanying fever and anorexia. Arthritis is usually evident and confined to a single joint, most commonly the carpus or tarsus. In dogs experimentally infected by a single inoculation of *B. burgdorferi,* arthritis was self-limited, although recurrent episodes of 3 to 6 days'duration occurred for up to several weeks.

Chang *et al.* (2001) exposed 22 healthy Beagles verified to be free of borreliosis (Twenty 6-month-old dogs) to *Borrelia burgdorferi*-infected adult ticks and treated with dexamethasone for 5 consecutive days. Two dogs not exposed to ticks were treated with dexamethasone and served as negative-control dogs. Clinical signs, results of microbial culture and polymerase chain reaction (PCR) testing, immunologic responses, and gross and histologic lesions were evaluated 9 months after tick exposure. Predominant clinical signs were episodic pyrexia and lameness in 12 of 20 dogs. Infection with *B*

burgdorferi was detected in microbial cultures of skin biopsy specimens and various tissues obtained during necropsy in 19 of 20 dogs and in all 20 dogs by use of a PCR assay. All 20 exposed dogs seroconverted and developed chronic nonsuppurative arthritis. Three dogs also developed mild focal meningitis, 1 dog developed mild focal encephalitis, and 18 dogs developed perineuritis or rare neuritis. Control dogs were seronegative, had negative results for microbial culture and PCR testing, and did not develop lesions.

Fritiz and Kajemtrup (2003) stated that not all infected dogs will develop clinical signs of Lyme borreliosis, and younger dogs are more likely to do so than older dogs. Furthermore, dogs appear to lack the spectrum of clinical signs reported in humans with Lyme borreliosis, despite occasionally extensive systemic dissemination of spirochetes.

Korshus *et al.* (2003) mentioned that the most common clinical sign of early Lyme disease is sudden onset of lameness with swelling in the affected joints. Associated clinical signs of lethargy, fever, anorexia, arthralgia, lymphadenopathy, and generalized pain are also observed during this stage. During the early stage the spirochetes are susceptible to antibiotics. If left untreated, neurological, cardiac, renal disease, and arthritis, are potential manifestations of the late-stage disease as the organisms can take residence in areas not accessible to humoral and cellular immune responses or antibiotics. Remaining organisms may then multiply and be responsible for sites of inflammation resulting in recurrent episodes of arthritis and other

associated clinical signs.

Skarda (2005) mentioned that 28% of seropositive dogs arthritis was the most often and common form of borreliosis in dogs seen clinically with movement anomalies, reluctance to move, lethargy and so on. Clinical signs were recurrent and variable with a variety of complication in some of cases diagnosed months after the primary infection.

Skotarczak *et al.* (2005) examined 62 dogs delivered to the Veterinary Clinic in Szczecin and 30 from the Municipal Animal Shelter in Szczecin with varied clinical signs of borreliosis. In all cases the owners admitted frequent contacts of their dogs with ticks, both in the past, as well as shortly before the onset of sickness. Authors used two methods: PCR for detecting DNA of *B. burgdorferi* s.l. and ELISA test for detecting antibodies against the spirochete. Lameness, the principal symptom of canine borreliosis was the most frequent symptom of the group of 31 PCR-positive animals. The other most common symptoms in PCR positive dogs were fever, swelling of joints and loss of body weight.

Summers *et al*. (2005) induced experimental *borrelia* infection in 62 specific--pathogen-free beagle dogs by exposure to *Ixodes scapularis* ticks harboring the spirochaete *Borrelia burgdorferi.* Clinical signs of Lyme disease occurred in 39/62 dogs, the remaining 23 being subclinically infected. Clinical signs consisted of one to six episodes of transitory lameness with joint swelling and pain, most commonly affecting the elbow or shoulder joints. The polymerase

chain reaction and culture demonstrated that the dogs remained infected for up to 581 days. At necropsy, gross findings consisted of lymphadenopathy in the area of tick attachment. Microscopical changes consisted of effusive fibrinosuppurative inflammation or nonsuppurative inflammation, or both, affecting synovial membranes, joint capsules and associated tendon sheaths. Plasma cells dominated areas of chronic inflammation, with CD3(+) T cells being present in lesser numbers. Microscopical signs of arthritis were polyarticular and more widespread than indicated by clinical signs, and most of the subclinically affected animals also had synovitis. In areas of tick attachment to the skin, hyperkeratosis and a mixture of suppurative and nonsuppurative dermatitis were encountered. Lymphadenopathy in superficial lymph nodes resulted from follicular and parafollicular hyperplasia. In 14/62 dogs, lymphoplasmacytic periarteritis and perineuritis were noted.

Littman *et al*. (2006) mentioned that clinical syndromes known to be associated with canine Lyme disease include polyarthritis and glomerulopathy. Serological test results can be used to document exposure to *B. burgdorferi* but not prove illness. Although serum enzyme-linked immunosorbent assay/indirect fluorescent antibody assay titers can stay positive for months to years after treatment, quantitative C6 peptide antibody paired tests need more study. Serological screening of healthy dogs is controversial because it can lead to overdiagnosis or overtreatment of normal dogs, most of which never develop Lyme disease.

Jäderlund et al. (2007) evaluated whether seropositivity for the tick-transmitted bacterial species Borrelia *burgdorferi* sensu lato was associated with one or more specific categories of nervous system disorders in dogs. A total of 248 dogs with nervous system disorders were serotested for *Borrelia burgdorferi* and categorised into six main diagnostic categories: degenerative diseases of the spine, epilepsy, inflammatory diseases, neoplasia, peripheral neuropathies, and other diseases. Multivariable analysis using logistic regression was used to model whether a dog was diagnosed as being in any of these categories. The independent variables included were sex, age, year of serological testing, and whether the animal tested positive for *B burgdorferi* sensu lato. It was concluded that the association was not of clinical relevance.

Lovrich *et al*. (2007) mentioned that Canine Lyme disease manifests most often as subclinical polyarthritis and/or periarteritis but can progress to renal disease, cardiac disorders, or arthritis.

Speck *et al*. (2007) investigated samples from dogs suggestive of active canine borreliosis (group A) by culture and PCR and the detection of antibodies against *Borrelia burgdorferi* sensu lato in order to confirm a presumptive clinical diagnosis of canine borreliosis by laboratory results. Criteria for such a diagnosis were: history of tick exposure, lameness, neurological signs, nephropathy, lethargy, anorexia, and fever. A total of 302 samples comprising EDTA blood, urine, synovial fluid, cerebrospinal fluid, and tissue (skin, synovial membrane, kidney) from 98 dogs (26 with arthritis, 46 with neurological signs, 21 with nephropathy, 5 with non-specific symptoms) were collected and

examined. Moreover, 55 healthy dogs (group B) and 236 dogs with symptoms or injuries unlikely to be associated with borreliosis (group C) were included in this study. Blood serum samples collected from all individuals (n=389) were analysed by ELISA. Twenty-one (21%) out of 98 dogs from group A, 4 (7%) out of 55 from group B and 15 (6%) out of 236 dogs from group C were positive for antibodies against *B. burgdorferi* sensu lato. The seroprevalences between groups A, B and C differed significantly. None of the corresponding samples investigated by PCR and culture were positive for spirochetal DNA or viable spirochetes. *Borrelia afzelii* was grown from one EDTA-blood sample but the corresponding blood serum sample remained antibody-negative. Consequently, the etiologic role of *B. afzelii* in this case is unclear. In approximately 40% of the presumptive canine borreliosis cases, other lesions have been found to be responsible for clinical signs. This study affirmed that a definitive diagnosis of canine borreliosis cannot be made by clinical symptoms and serology based on a single consultation. Moreover, this study clearly revealed that the diagnostic sensitivity is enhanced by a thorough consideration and exclusion of other diseases.

Hutton *et al.* (2008) mentioned that Clinical signs of Lyme disease in dogs are fever, acute arthritis, arthralgia, lameness, and nephritis in some cases. Central nervous system involvement, heart block, and uveitis are less frequently reported in dogs.

Gerber *et al.* (2009) concluded that antibodies against *B. burgdorferi* determined by whole cell ELISA and confirmed by Western blot were neither associated with the development of lameness nor with signs of renal disease like azotemia or proteinuria

in dogs observed over a period of 2.5 to 3.0 years.

2.5.2. Culture and isolation of *Borrelia burgdorferi*.

Coulter *et al.* (2005) performed study to confirm the relative utility of culture and to identify laboratory testing algorithms that will supplement clinical diagnosis. Overall, 30 of 86 patients (35%) were culture positive, whereas an additional 15 of 84 (18%) were seropositive only (51% total sero- and culture positive), and PCR on skin biopsy identified 4 additional patients who were neither culture nor seropositive. Among 49 laboratory test-positive patients, the highest sensitivity (100%) for diagnosis was obtained when culture, skin PCR, and serologic tests were used, although serologic testing with skin PCR was almost as sensitive (92%). Plasma PCR was infrequently positive and provided no additional diagnostic value. Although culture was definitive and has a relatively high sensitivity, the results required a mean of 3.5 weeks to recovery.

Elhelw *et al*. (2014) failed to isolate *B. burgdorferi* from dogs in Egypt but only from cattle blood. Wet preparation from BSK-H purified culture showed characteristic spirochetal morphology and motility under the dark-field microscope.

2.5.3. Serodiagnosis

Magnarelli *et al.* (1987) obtained blood samples during 1984 and 1985 from 271 dogs that were suspected of having borreliosis. The dogs lived in areas known to be infested with ticks and had been examined because of limb/joint disorders or for unknown illnesses marked by fever, anorexia, or fatigue. Lameness had been the most

frequently reported clinical manifestation. Analyses of serum specimens, by an indirect fluorescent antibody (IFA) method or by an ELISA, detected antibodies to *Borrelia burgdorferi*, the etiologic agent of borreliosis in dogs and of Lyme disease in human beings. Antibody to *B. burgdorferi* was detected in 76.3% of 114 specimens from dogs living in the lower Hudson Valley region of New York State (predominantly Westchester County), in 66.5% of 155 specimens from dogs from southern Connecticut, and in single specimens from dogs from Rhode Island and California. Geometric mean antibody titers peaked during the winter. Results of IFA tests and ELISA were in agreement, but the latter method yielded less variable results, had greater sensitivity, and was more easily standardized. Five dogs from New York State and Connecticut seropositive to *B. burgdorferi* had developed kidney disorders during or after episodes of intermittent lameness. Application of murine monoclonal antibody in an IFA procedure verified the presence of *B. burgdorferi* in renal cortical tissues from one dog.

Cohen *et al.* (1990) examined 2,409 canine serum samples submitted to the Texas Veterinary Medical Diagnostic Laboratory between Jan 1, 1988 and Dec 31, 1988 and tested by immunofluorescent antibody technique for antibody to *Borrelia borgdorferi*, 132 (5.5%) had positive results. Clinical and epizootiologic characteristics of seropositive dogs from Texas (n = 110) were examined. Male dogs were more likely than female dogs to be seropositive for *B burgdorferi*. The most frequent clinical sign of disease described in seropositive dogs was lameness; neurologic,

ophthalmologic, dermatologic, renal, and hepatic signs also were reported by referring veterinarians.

Lindenmayer *et al.* (1990) used ELISA, indirect immunofluorescent-antibody assay (IFA), and Western immunoblot to test serum samples from 128 dogs for the presence of antibody to Borrelia *burgdorferi*. Sera included 72 samples from dogs suspected of having Lyme disease, 32 samples from dogs residing in areas in which Lyme disease was not considered endemic, and 24 samples from dogs with clinical and serologic evidence of immune-mediated disease (n = 10), Rocky Mountain spotted fever (n = 5), or leptospirosis (n = 9). Results of Western immunoblotting were used as the standard against which performances of ELISA and IFA were measured. ELISA was significantly more sensitive than IFA (84.8 versus 66.7%), although both tests were equally specific (93.5%). Eight samples that were positive by Western immunoblot were simultaneously negative by ELISA and IFA. Of these eight, four were from dogs suspected of having immune-mediated disease, two were from dogs suspected of having leptospirosis, and two were from dogs suspected of having Lyme disease. These results may indicate that sera from dogs with immune-mediated disease, and to a lesser extent sera from those with leptospirosis, cross-react with B. burgdorferi antigens. Alternatively, Western immunoblot results may not truly reflect Lyme disease status, particularly in the case of dogs with immune-mediated diseases.

Magnarelli *et al.* (1990) found that the prevalences of serum

antibody against *B burgdorferi* did not differ between healthy dogs (89.6% seropositive) and those with joint or limb disorders compatible with Lyme borreliosis (92.9% seropositive). Serodiagnostic testing should be reserved for dogs with a history and clinical presentation that are highly suggestive of active Lyme borreliosis.

Falco *et al.* (1993) collected a total of 1446 blood samples from resident dogs and tested by modified enzyme-linked immunosorbent assay. Equivocal samples were further tested by immunoblot. A mean number of 57.8 samples were collected from each of 25 towns and cities. Seroprevalence rates for municipalities ranged from 6.5% to 85.2%. County seroprevalence was 49.2%. There was a significant difference among the rates for the northern (67.3%), central (45.2%), and southern (17.3%) regions. Multiple range analysis indicated homogeneity between the southern and central regions and the central and northern regions in the USA.

Barthold *et al.* (1995) concluded that dogs with clinical borreliosis are seropositive and remain seropositive after antibiotic treatment, emphasizing that serologic testing is not a useful means of measuring clinical response. Serologic responses of infected dogs can be discriminated from those of vaccinated dogs by means of immunoblot analysis, and recombinant P39 is a potentially useful antigen for that purpose.

Jacobson *et al.* (1996) mentioned that serology can be a very useful aid in the diagnosis of Lyme disease, but it requires that the assays used have been subjected to rigorous validation criteria. When

that is not performed, an unacceptable level of false-positive and false-negative test results is virtually assured.

Wittenbrink *et al.* (1996) examined sera from 665 apparently healthy dogs for antibodies to the Lyme disease spirochete *Borrelia burgdorferi* by using an ELISA with a whole cell sonicate of *B. burgdorferi* sensu stricto reference strain B31 (ATCC 35210) as antigen. To discover false positive reactions due to the unsatisfactory specificity of conventional enzyme-linked immunosorbent assays for *B. burgdorferi*, sera were absorbed in parallel with both *B. burgdorferi* and a heterologous sorbent consisting of whole cells of *Escherichia coli, Salmonella typhimurium,* and eight serovars of *Leptospira interrogans.* The difference between optical densities obtained in the ELISA after serum absorption with the heterologous sorbent and the *B. burgdorferi* sorbent was defined as a new value, "ODdiff", for ELISA reactivity specific for *B. burgdorferi*. ELISA results were confirmed by immunoblot studies. By testing unabsorbed sera, 48 of 665 serum samples (7.2%) were considered ELISA positive. 37 of these 48 sera (77.1%) were apparently false positive: here a similar reduction of ELISA reactivity was obtained after absorption with *B. burgdorferi* antigen and with the heterologous sorbent (ODdiff approximately equal to 0). None of these 37 sera gave immunoblot patterns characteristic for canine *B. burgdorferi* infection.

Magnarelli *et al.* (1997) analyzed serum samples from dogs suspected of having canine borreliosis in polyvalent enzyme-linked immunosorbent assays (ELISAs) with whole-cell or recombinant

antigens of *Borrelia burgdorferi* sensu stricto. Purified preparations of recombinant antigens included outer surface protein A (OspA), OspB, OspC, OspE, OspF, and p41-G (a fragment of flagellin). Of the 36 dog sera that reacted positively to whole-cell antigen, 32 (88.9%) contained antibodies to one or more recombinant antigens. Reactivities to OspF (88.9% positive) and p41-G (75% positive) were most prevalent. In parallel tests of eight canine sera, there was good agreement in results of Western blot (immunoblot) analyses and ELISAs. Although dog sera with antibodies to whole-cell *B. burgdorferi* frequently reacted positively to one or more recombinant antigens, the inclusion of OspF and p41-G antigens in ELISAs was most useful in the serologic diagnosis of canine borreliosis.

Stefançíková *et al.* (1998) compared three antigens of *Borrelia burgdorferi* sensu lato (*B. burgdorferi* sensu stricto--Slovak strain Ir 105, *B. garini* - Slovak strain K 24 and *B. burgdorferi* sensu stricto--American strain B 31) by ELISA on a group of dogs from urban agglomeration of Kosice, eastern Slovakia. Of 256 serum samples from dogs examined for the presence of anti-*Borrelia* IgG antibodies, 128 (50%) were positive with Ir 105 antigen, 107 (41.7%) with K 24 and 74 (28.9%) with B 31. The seroprevalence between strains B 31 and K 24 and B 31 and Ir 105 differed statistically significantly (test chi2, $p < 0.05$), however, the difference between strains K 24 and Ir 105 was insignificant. A significantly higher seroprevalence of all the strains examined was detected in hunting dogs (test chi2, $p < 0.05$) when compared with service and pet dogs. The seroprevalence correlated with the frequency of outing the dogs in woody areas with

the occurrence of *borreliae* in ticks (R = 0.5 or 0.7) as well as with the frequency of finding engorged ticks (R = 0.5). An epizootiological anamnesis showed a fair specificity in all the strains examined. The Slovak strains showed the higher consistency of positive and negative findings (70%) but statistically lower specificity than the American strain.

Gauthier and Mansfield (1999) mentioned that Western immunoblotting may be used to distinguish between dogs naturally exposed and those vaccinated against *Borrelia burgdorferi*. Vaccinated and natural exposed dogs produce differing antibody responses, whereas dual-status dogs produced the full antibody response of both types of exposure.

Callister *et al.* (2000) Infected 13 pathogen-free beagles, 12 to 26 weeks old with *Borrelia burgdorferi* by tick challenge. Dogs were monitored for clinical signs and symptoms of Lyme disease along with borreliacidal antibody production against *B. burgdorferi* sensu stricto isolates 297 and 50772. Ten (77%) dogs developed lameness in one or more legs within 210 days after attachment of *Ixodes scapularis* ticks. Eight (80%) of the lame animals had concurrent fever of >38°C. Spirochetes were also recovered from the skin and joints of 12 (92%) dogs, but rarely from other organs. Borreliacidal antibodies against *B. burgdorferi* isolate 297 were detected in only four (31%) dogs, and the levels of killing antibodies remained low for the duration of the infection. In contrast, borreliacidal antibodies against *B. burgdorferi* isolate 50772 were detected in 13 (100%) dogs

within 21 days of infection. Furthermore, the borreliacidal antibody levels correlated with the severity of *B. burgdorferi* infection. Detection of borreliacidal antibodies, especially against *B. burgdorferi* isolate 50772, is also a reliable serodiagnostic test for detection of Lyme disease in dogs.

Guerra *et al*. (2000) assayed serum samples obtained from healthy, asymptomatic dogs in areas of Wisconsin and northern Illinois for antibodies to *Borrelia burgdorferi* by enzyme-linked immunosorbent assay (ELISA), and positive results were confirmed by immunoblot assay. Authors found that 56.9% (562 of 1,077) of the samples were positive by ELISA and 82.0% (461 of 562) were positive by immunoblotting.

Hovius *et al*. (2000) compared the antibody responses of symptomatic dogs and asymptomatic controls in an area where Lyme disease is endemic in The Netherlands all dogs had positive titers by whole-cell enzyme-linked immunosorbent assay and appeared to be naturally infected by *Borrelia burgdorferi* sensu lato. Authors performed Western blots and in vitro immobilization assays to study antibody-dependent bactericidal activity. Strains from three different genospecies were employed as the antigen source: *B. burgdorferi* strain B31, *Borrelia garinii* strain A87S, and *Borrelia afzelii* strain pKo. Antibodies against flagellin (p41) and p39 for three strains were found in sera from both symptomatic and asymptomatic dogs and were therefore considered to be markers of exposure. Antibodies against p56 and p30 of strain B31, against p75, p58, p50, OspC, and

p<19 of strain A87S, and against p56, p54, p45, OspB, p31, p26, and p<19 of strain pKo were found significantly more frequently in sera from symptomatic dogs younger than 8 years when the first symptoms were observed than in those from age-matched controls (P<0.01). These antibodies were not found in preclinical sera and appeared during development of disease. Antibodies against OspA of strains B31 and A87S were only seen in acute-phase and convalescent sera from three dogs that recovered from disease. Incubation with 25% normal canine serum did not result in the immobilization of strains B31 and pKo, but partial immobilization of strain A87S (61%+/-24% [standard deviation] at 5 h) occurred. Seven of 15 sera from symptomatic dogs but none of the sera from 11 asymptomatic dogs had antibody-dependent immobilizing activity against one of the strains. Consecutive sera from one of these dogs immobilized two different strains. Antibody-mediated bactericidal serum was not seen before onset of disease, was strongest in the acute phase of disease, and fluctuated during chronic disease. From seven out of eight symptomatic dogs *Borrelia* DNA was amplified by PCR; in three of them the bactericidal activity was directed against one of the genospecies amplified from that dog; however, four PCR-positive dogs lacked bactericidal activity. In conclusion, dogs with symptomatic canine borreliosis have more-extensive antibody reactivity against *Borrelia*, as shown by both Western blotting and immobilization assays.

Lianq *et al.* (2000) collected sera from dogs experimentally infected with *Borrelia burgdorferi* by tick inoculation were analyzed

for an antibody response to each of the six invariable regions (IRs; i.e., IR(1) to IR(6)) of VlsE, the variable surface antigen of *B. burgdorferi*. Six synthetic peptides (C(1) to C(6)), which reproduced the six IR sequences were used as peptide-based, enzyme-linked immunosorbent assay (ELISA) antigens. Two IRs, IR(2) and IR(6), were found to be immunodominant. Studies with serially collected serum samples from experimentally infected dogs revealed that the antibody response to IR(6) appears earlier and is stronger than that to IR(2). Thus, the IR(6) sequence alone appeared to be sufficient for serodiagnosis. When C(6) alone was used as antigen, the peptide-based ELISA was positive in 7 of 23 dogs (30%) as early as 3 weeks postinfection. All dogs (n = 33) became strongly positive 1 or 2 weeks later, and this response persisted for the entire study, which lasted for 69 weeks. Of 55 sera submitted by veterinarians from dogs suspected of having Lyme disease, 19 were also positive by the C(6) ELISA, compared to 20 positives detected by immunoblot analysis using cultured *B. burgdorferi* lysates as antigen. The sensitivity of using C(2) and C(6) together for detecting specific antibody in both experimentally infected and clinically diagnosed dogs was not better than sensitivity with C(6) alone, confirming that C(6) suffices as a diagnostic probe. Moreover, the C(6) ELISA yielded 100% specificity with serum samples collected from 70 healthy dogs, 14 dogs with infections other than *B. burgdorferi*, and 15 animals vaccinated with either outer surface protein A, whole-spirochete vaccines, or the common puppy-vaccines. Therefore, this C(6) ELISA was both sensitive and specific for the serodiagnosis of canine Lyme disease

and could be used with vaccinated dogs.

Turk *et al*. (2000) examined Sera from 120 apparently healthy dogs in the Zagreb area (Croatia) by enzyme-linked immunoadsorbent assay (ELISA) for antibodies to *Borrelia burgdorferi*. During triple i/v immunization of dog at 1, 7 and 14 day, on each occasion with 5 ml 1.4×107 cells/ml of B. burgdorferi sensu stricto, strain B31 ATCC 35210, a positive control serum was obtained at day 28 p.i., while negative control serum was obtained prior to immunization. The antigen for ELISA was prepared from the same bacterial strain sonicated on ice. The sonicate was quantified by sodium dodecyl sulphate polyacrilamid gel electrophoresis (SDS PAGE). IgG antibodies to *B. burgdorferi* were estimated in 6 (5%) samples of dog sera in titre ranging from 1:100 to 1:2000 (Optical density-OD=0.650 + 36.49, n=6). Antibodies to *B. burgdorferi* were found in two females and four males, two Labrador retrievers and in one German shepherd, a Hungarian viszla, one cocker spaniel, and a German wirehaired pointer at 2.5 to 8 years of age. Of 114 seronegative samples three samples were found to be very close to the margin of optical density that determined seropositive result (OD=0.562). Estimated seroprevalence to *B. burgdorferi* in dog sera suggested that dogs in the Zagreb area are infected with *B. burgdorferi* and that the Zagreb area is part of a wider Central European Lyme boreliosis endemic area.

Guerra *et al.* (2001) conducted a seroprevalence survey for *Borrelia burgdorferi* among the healthy canine pet population in selected counties of Wisconsin and northern Illinois to determine the

distribution of Lyme disease and associated risk factors. Information obtained for each dog included place of residence, Lyme disease vaccination status, history of travel and tick exposure, signalment, and medical history. Serum samples were screened by enzyme-linked immunosorbent assay and confirmed by an immunoblot procedure. Seroprevalence by county ranged 0-40%, with the highest estimates from west-central Wisconsin. The spatial pattern was significantly correlated with human incidence of Lyme disease and with abundance of the tick vector, Ixodes scapularis.

Joppert *et al.* (2001) tested dogs' sera samples collected from Cotia County, São Paulo using indirect immunoenzymatic test (ELISA) in order to study Lyme disease serology in dogs. Positive results were confirmed employing the Western blotting technique. Because of the possibility of cross reactions, sera were also tested for different serological strains of *Leptospira interrogans* and *L. biflexa* using microscopic sera agglutination test. Twenty-three of 237 (9.7%) serum samples were positive in the ELISA; 20 of them (86.9%) were confirmed by the Western blotting, what suggests that Cotia may be a risk area for Lyme disease. Although 4 samples (1.7%) were positive for Lyme disease and leptospirosis, no correlation was found between the results what suggests absence of serological cross reactivity.

Magnarelli *et al*. (2001) used ELISAs with separate preparations of 10 purified recombinant antigens of *Borrelia burgdorferi* sensu stricto to test sera from 36 dogs not vaccinated with whole cells of this agent and from five dogs vaccinated with whole-

cell *B. burgdorferi* bacteria. All dogs lived in tick-infested areas of Connecticut and south-eastern New York State, USA. The non-vaccinated dogs had limb or joint disorder, lameness and fever during the period 1984-1991 and had antibodies to *B. burgdorferi*, as determined by a polyvalent ELISA with whole-cell antigen. In re-analyses of sera for total immunoglobulins in ELISAs with recombinant antigens, reactions were most frequently recorded when outer-surface protein (Osp) F, protein (p)35, p37, p39 and p-41G (a flagellin component) were tested separately. Western immunoblots of a subset of 16 sera, positive by ELISA with whole-cell antigen and representing a range of antibody titres (640-40960), verified immune responses to these or other lysed whole-cell antigens. Sera from vaccinated dogs contained antibodies to OspA, OspB, p22, p37 and p41-G. Therefore, serological reactions to OspF, p35 and p39 were the most important indicators of natural exposure to *B. burgdorferi*. Authors concluded that serum reactivities to these recombinant antigens in ELISAs can be used to help identify possible natural infections of canine borreliosis in dogs not vaccinated with whole-cell *B. burgdorferi* and to provide information on the geographic distribution of this bacterium.

Skarda (2005) examined 650 dogs, 38.6% of dogs showed the presence of antiborelia IgG antibodies. Clinical signs did not correlate always with the presence of antiborrelial IgG antibodies.

Skotarczak *et al*. (2005) examined 62 dogs delivered to the Veterinary Clinic in Szczecin and 30 from the Municipal Animal

Shelter in Szczecin with varied clinical signs of borreliosis. ELISA tests specific for IgG antibodies were positive in 37 of 92 sera (40.2%) taken from examined dogs. Lameness was observed in 15 of 37 IgG seropositive dogs and in 25 of 55 seronegative animals. In 54% of dogs with the antibodies, swelling of instep- and wrist joints was observed compared to only 24.4% in seronegative dogs.

Pejchalová *et al.* (2006) examined serum samples obtained from healthy, asymptomatic military dogs from 12 different areas in the Czech Republic for IgG antibodies to *Borrelia burgdorferi* sensu lato (s.l.). The total of 399 serum samples were tested by a whole-cell ELISA. Specific antibodies to *Borrelia burgdorferi* s.l. were detected in 26 cases (6.5%). In different localities, the seroprevalence varied from 0.0% to 28.6%. Two local isolated strains Br-75 (*Borrelia afzelii*) and Br-97 (*Borrelia garinii*) were used as antigens. A total of 22 (5.5%) were positive for antibodies to *Borrelia afzelii* and 19 (4.8%) were positive for antibodies to *Borrelia garinii*. Fifteen cases were positive for both antibodies. A significantly higher seroprevalence was found in younger dogs (1-3 years) than in older ones ($p < 0.05$). An analysis of seroprevalence by months of sampling showed no significant difference ($p > 0.05$).

Gerber *et al.* (2007) examined 160 Bernese Mountain Dogs and 62 control dogs (large breed dogs with long hair). All dogs were considered healthy according to a questionnaire filled out by the owner, complete blood count, chemistry panel, urine analysis and urine culture. Bernese Mountain Dogs and control dogs were kept in

similar environments. Seroprevalence of *B. burgdorferi* was assessed by ELISA and Western blot and was 58% in Bernese Mountain Dogs compared to 15% in control dogs. This difference was significant. Neither antibodies against leptospires nor vaccination or hair coat color influenced the results. The authors concluded that a breed predisposition can be suspected in Bernese Mountain Dogs.

Beall *et al.* (2008) examined a population of 731 naturally exposed pet dogs at a private practice in Baxter, Minnesota, an area endemic for Lyme disease, was tested by serological and molecular methods for evidence of exposure to or infection with selected vector-borne pathogens. Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for *Borrelia burgdorferi* antibodies. Based on the owner history and the attending veterinarian's physical examination findings, dogs exhibiting illness compatible with borreliosis were considered clinical cases, and their results were compared to the healthy dog population. Antibodies to only *B. burgdorferi*, in 80 (11%) dogs; and seroreactivity to both *Anaplasma phagocytophilum* and *B. burgdorferi* , in 188 (25%) dogs. Of 89 suspected cases of canine borreliosis, *B. burgdorferi* antibodies were detected in 8 dogs (9%), whereas antibodies to both organisms were found in 38 dogs (43%).

Tinoco-Gracia *et al.* (2008) examined 39 (40%) Out of a total of 98 active private clinics in Mexicali for *Borrelia burgdorfei* in canine. Blood samples of 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analyzed with

96% sensitivity and 95% specificity. An adjusted prevalence of 6.8% was obtained. This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus*.

Hamer *et al,* (2009) evaluated 353 serum samples and 78 ticks obtained from dogs brought to 18 veterinary clinics located in the lower peninsula of Michigan from July 15, 2005, through August 15, 2005. Serum samples were evaluated for specific antibodies against *Borrelia burgdorferi* by use of 3 serologic assays. Ticks from dogs were subjected to PCR assays for detection of pathogens. Of 353 serum samples from dogs in 18 counties in 2005, only 2 (0.6%) contained western blot analysis-confirmed antibodies against *B. burgdorferi*. Ten of 13 dogs with *I. scapularis* were from clinics within or immediately adjacent to the known tick invasion zone. Six of 18 *I. scapularis* and 12 of 60 noncompetent vector ticks were infected with *B. burgdorferi*. Serosurvey in dogs was found to be ineffective in tracking early invasion dynamics of *I. scapularis* in this area. Tick chemoprophylaxis likely reduces serosurvey sensitivity in dogs. Ticks infected with *B. burgdorferi* were more common and widely dispersed than seropositive dogs. In areas of low tick density, use of dogs as a source of ticks is preferable to serosurvey for surveillance of emerging Lyme disease.

Adaszek *et al*. (2010) recognized the first cases of *Borrelia burgdorfei* in Poland among dogs.The etiological factor of diseases

with symptoms of lameness and subcutaneous tissues oedema, which occurred in 4 dogs after invasions of ticks. The results of serological examinations, and the reaction of sick animals on tetracycline therapy revealed, that in all four cases an etiological factor of the diseases was bacteria *Borrelia burgdorferi.*

Chandrashekar *et al.* (2010) evaluated the sensitivity and specificity of the in-clinic ELISA to detect antibodies against *Borrelia burgdorfer* in comparison with immunofluorescence assay. Sensitivity and specificity of the in-clinic ELISA for detection of antibodies against *B. burgdorferi* was (98.8% and 100%, respectively), compared with results for an immunofluorescence assay. The authors cocluded that the commercially available in-clinic ELISA could be used by veterinarians to screen dogs for exposure to tick-borne pathogens.

Leschnik *et al.* (2010) concluded that despite serological evidence of infection/immunization against *B. burgdorferi* , no clinical signs of disease were observed. The antibody patterns in a single Western blot did not permit differentiation between the different antigen sources (vaccine versus natural infection). However, repeated Western blot analyses may be useful for the confirmation of infection or vaccination status, since the time courses of the levels of specific antibodies seem to be different.

Menn *et al*. (2010) examined german dogs serological for *Borrelia burgdorferi*. In 64/212 *B. burgdorferi* was detected.

Icen *et al.* (2011) determined the prevalence of *Borrelia burgdorferi* antibodies using ELISA in 82 mixed breed dogs in turkey.

None of the tested dogs were positive for *Borrelia burgdorferi* antibodies.

Wagner *et al.* (2011) developed and validated a new bead-based multiplex assay for the detection of antibodies to *B. burgdorferi* in canine serum which combined the testing by ELISA and WB in a single quantitative test. *B. burgdorferi* outer surface protein A (OspA), OspC and OspF were expressed in *E. coli*. The recombinant proteins were coupled to fluorescent beads providing the matrix of the assay. Two sets of canine sera were used for validation of the multiplex assay. First, sera from 79 dogs with known ELISA and WB results were used to establish the conditions of the assay. These samples were selected to provide similar numbers of pre-tested sera ranging from negative to high positive results and included sera from vaccinated and/or naturally infected dogs. A high correlation was observed for detection of antibodies to *B. burgdorferi* in the single and multiplex assays (n=79). Spearman's rank correlations were 0.93, 0.88 and 0.96 for OspA, OspC and OspF, respectively. Second, a total of 188 canine serum samples that were not tested previously were used for further multiplex assay validation. All samples were also blindly analyzed for antibodies to *B. burgdorferi* antigens by WB. The WB results provided a 'relative gold standard' for each antigen and were used to perform a receiver operating curve analysis. The areas under the curves were 0.93 for OspA, 0.82 for OspC, and 0.89 for OspF. Multiplex assay interpretation ranges for antibodies to all three *B. burgdorferi* antigens in canine serum were established by likelihood analysis. The diagnostic sensitivities of the individual OspA, OspC

and OspF bead-based assays were 83%, 62% and 82%, respectively, and the diagnostic specificities were 90%, 89% and 86%, respectively. The new multiplex assay provides a sensitive and fully quantitative platform for the simultaneous evaluation of antibodies to *B. burgdorferi* OspA, OspC and OspF antigens and distinguishes between antibodies that originated from vaccination or natural exposure to *B. burgdorferi*.

Bell *et al.* (2012) determined the seroprevalence of tick-borne infections in the military working dog (MWD) population in the Republic of Korea (ROK). Our sample population consisted of 182 serum samples from MWDs for 3 different years (1996, 2002, and 2007). In addition, 63 whole blood samples from 2007 were available for polymerase chain reaction (PCR). Serum samples were evaluated by a commercially available enzyme-linked immunosorbent assay (ELISA) for *Borrelia burgdorferi*. PCR amplification of DNA was performed to screen *Borrelia burgdorferi* using previously published primers and probes. ELISA testing for *Borrelia* yielded 2 (1.1%) positive results. There was no significant differences in seroprevalence based on location, year, breed, or sex of the MWD. No MWD sample had a positive PCR result. MWDs stationed in Korea had serologic evidence of exposure to several tick-borne pathogens, but PCR testing did not identify any active infections.

Cardoso *et al*. (2012) sampled 120 veterinary medical centers from all the regions of mainland and insular Portugal, 557 apparently healthy and 628 Canine vector-borne diseases CVBD-suspect dogs.

Serum, plasma or whole blood was tested for qualitative detection of antibodies to *B. burgdorferi* s. l with commercial in-clinic enzyme-linked immunosorbent assay kit. Odds ratios (OR) were calculated by logistic regression analysis to identify independent risk factors of exposure to the vector-borne agent. Total positivity levels to *B. burgdorferi* 0.2% in the healthy group, and 0.5% in the clinically suspect group.

Wagner and Erb (2012) determined incidence risks of new companion animal infection in 2011 with *B.burgdorferi* in USA in 451 dog; the samples were non-randomly collected by referring veterinarians in NY State between June 15, 2011 and January 31, 2012 because of suspicion of infection with *B. burgdorferi* or during annual health checks. The samples were submitted from 50 out of 62 counties in the state. Incident infections were determined by measuring antibodies to outer surface protein C (OspC; a marker of early infection that is detectable in serum from 3 weeks to 5 months after infection). Incident infections with *B. burgdorferi* were detected in 23% (95% confidence interval (CI): 19, 27) of canine samples. Recognition of incidence infections in dogs might serve as a sentinel for infected ticks in different NY State counties; detection of the OspC antigen can provide a sensitive, new tool to allow recognition of risk for possible human and animal infection with *B. burgdorferi* by geographic region.

Wagner *et al.* (2012) evaluated antibody responses to Osp antigens to aid the diagnosis of early infection and the management of

Lyme disease. The authors analyzed antibody responses during the first 3 months after the experimental infection of dogs using a novel multiplex assay. Results were compared to those obtained with two commercial assays detecting C6 antigen. Multiplex analysis identified antibodies to OspC and C6 as early as 3 weeks postinfection (p.i.) and those to OspF by 5 weeks p.i. Antibodies to C6 and OspF increased throughout the study, while antibodies to OspC peaked between 7 and 11 weeks p.i. and declined thereafter. A short-term antibody response to OspA was observed in 3/8 experimentally infected dogs on day 21 p.i. Quant C6 enzyme-linked immunosorbent assay (ELISA) results matched multiplex results during the first 7 weeks p.i.; however, antibody levels subsequently declined by up to 29%. Immune responses then were analyzed in sera from 125 client-owned dogs and revealed high agreement between antibodies to OspF and C6 as robust markers for infection. Results from canine patient sera supported that OspC is an early infection marker and antibodies to OspC decline over time. The onset and decline of antibody responses to *B. burgdorferi* Osp antigens and C6 reflect their differential expression during infection. They provide valuable tools to determine the stage of infection, treatment outcomes, and vaccination status in dogs.

Volgina *et al.* (2013) examined specific population of dogs (Group 1) that had never been treated against ticks and mosquitoes. Moreover, the territory occupied by this population has also never been treated, because it is a protected area--Voronezh Natural Reserve. Canine patients from veterinary clinics (Group 2) that had been treated against VBD vectors were studied for

comparison. Eighty-two dogs (Group 1) were enrolled in June, 2008. Blood samples were tested using the IDEXX SNAP(®) 4Dx(®) test. The antibodies to *Borrelia* C6 peptide were detected in 2.4% of the samples. Almost all dogs with infections had no clinical signs. Only 3 mixed-infected dogs showed non-specific clinical signs. During the tick season, 358 *Ixodes ricinus* were collected; the prevalence of *Borrelia burgdorferi* s.l. was 21.9%. Four hundred and forty dogs (Group 2) were studied for comparison. Antibodies to *B. burgdorferi* s.l. were detected only in one dog.

Krämer *et al*. (2014) tested 3,094 serum samples taken from dogs throughout all 16 Polish provinces using a commercial kit for the detection of circulating antibodies against *Borrelia burgdorferi* sensu lato. A total of 3.75 % (116/3,094; 95 % CI: 3.11–4.48 %) of the dogs were positive for *B. burgdorferi* s.l. antibodies. The highest percentages of *B. burgdorferi* s.l. were recorded in Łódź Province. Co-infections with A. *phagocytophilum* and *B. burgdorferi* s.l. were recorded in 1.71 % of all examined dogs (53/3,094; 95 % CI: 1.29–2.23 %). One dog even had a triple infection, testing positive for *E. canis* too. *B. burgdorferi* s.l. has previously been reported in Poland and were confirmed in the present study by positive samples from all 16 provinces.

Pérez *et al*. (2014) determined anti *Borrelia burgdorferi* antibodies in 340 Finnish pet dogs and 50 healthy hunting dogs using the 4DX Snap®Test (IDEXX Laboratories). The overall seroprevalence was (2.9%). Seropositivities to and *Borrelia*

burgdorferi were significantly higher in the Åland Islands ($p < 0.001$), with prevalence of *Borrelia burgdorferi* antibodies of 20%. In healthy hunting dogs, seropositivity rates of 2% (1/50) were recorded for *Borrelia burgdorferi*.

2.5.4. Molecular diagnosis

Bauerfeind *et al.* (1998) worked on detection of DNA of *B. burgdorferi* in urine samples from dogs by a nested polymerase chain reaction and demonstrated that this method and the urine are useful in diagnosing *B. burgdorferi* infections in dogs.

Hovius *et al.* (1998) determined the prevalence of *Borrelia* species infection by polymerase chain reaction (PCR) in 138 ticks collected from dogs which were walked regularly in the wooded areas near the city of Eindhoven, the Netherlands. The PCR amplified the spacer region between the 5S and 23 S rRNA genes, and the *Borrelia* species was identified by hybridization with specific probes. *Borrelia burgdorferi* sensu lato was present in 20 of 138 (14.5%) ticks. Four species were identified: *B. burgdorferi* sensu stricto (n = 8), *B. afzelii* (n = 4), *B. garinii* (n = 2), and *B. valaisiana* (n = 2). One PCR product was non-typeable. Three ticks contained more than one species, all including *B. burgdoferi* sensu stricto, and one tick even contained four species. There was a significant difference ($P < 0.05$) in prevalence of *B. burgdorferi* sensu stricto between non-engorged ticks (either questing or attached) and semi-engorged ticks, 12% (10 of 85) and 2% (1 of 53), respectively.

Hovius *et al*. (1999b) tested tissues from Dutch family dogs

symptomatic for borreliosis according to established criteria and from infected but asymptomatic dogs for *Borrelia burgdorferi* sensu lato DNA using a polymerase chain reaction. Subsequently, *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana* were identified by hybridization. Symptomatic dogs showed a higher prevalence of *Borrelia* in liver samples (9 of 15) than asymptomatic dogs (9 of 43) p = 0.0049. Overall, *B. garinii* was the most prevalent species and occurred together with up to three other species in on liver sample. *B. burgdorferi* sensu stricto however, was predominantly detected in samples of synovial membranes, skin, cerebrospinal fluid, bladder, heart, and bone marrow. Nine out of 10 symptomatic dogs with a very high antibody titre were positive for *Borrelia* DNA by PCR in one or more of these tissues. Authors concluded that dissemination in naturally infected European dogs occurs and that the two most prevalent species, *B. burgdorferi* sensu stricto and *B. garinii*, differ in their tropism.

Schouls *et al.* (1999) developed a sensitive and specific PCR hybridization assay for the simultaneous detection and identification of *Borrelia burgdorferi* sensu lato. *B. burgdorferi* sensu lato were amplified and labeled by PCR. These PCR products were used in a reverse line blot hybridization assay in which oligonucleotide probes are covalently linked to a membrane in parallel lines. Hybridization of the samples with the oligonucleotide probes on this membrane enabled the simultaneous detection and identification of B. burgdorferi in 40 different samples. The application of the assay to DNA extracts from 121 Ixodes ricinus ticks demonstrated that 13% of

the ticks were infected with one or more *B. burgdorferi* genospecies. In five of the ticks both *Ehrlichia* and *B. burgdorferi* species were detected.

Wang *et al.* (1999) mentioned that whole DNA-DNA reassociation analysis is the most robust approach by which *B. burgdorferi* sensu lato can be classified based on phylogenetic relationships since the results are ultimately based on the entire genome sequence of the organism. Generally, this method is useful for the study of bacterial genetics, evolution, taxonomy, and epidemiology. However, DNA sequence analysis of some highly conserved gene loci can be used as a suitable alternative method. For example, *rrs*, *fla*, and *ospA* have been used for this purpose with *B. burgdorferi* sensu lato.

Banerjee *et al.* (2000) amplified *B. burgdorferi* DNA from 7/121 (5.8%) ticks, and 9/9 dogs tested were seroreactive to *Bo. burgdorferi* antigens by IFA and western blotting.

Skotarczak (2000) tested ticks collected in 1997 year in forest areas of Szczecin by PCR on the flagellin structural gene fla of *Borrelia burgdorferi* sensu stricto. The flagellin PCR primer set reaction was conservative for *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. The overall prevalence of *B. burgdorferi* sensu lato, in tick population studied was 8.8%. The female, nymphs and larves of *Ixodes ricinus* were infected almost just the same--about 10%, when the male 2.5% only.

Skotarczak and Wodecka (2003) demonstrated that the

northwestern part of Poland is an endemic area for *Borrelia burgdorferi* sensu lato and therefore sick dogs, at the time of the highest activity of ticks, should be suspected for having borreliosis. They carried out a preliminary PCR survey of the blood of 15 dogs naturally exposed on ticks for the presence of the DNA of *B. burgdorferi* using primers complementary to the fragment of the gene encoding 16S rRNA of the small ribosome subunit. 6 out of 15 dogs were infected, although 2 dogs had a lameness – the attribute of canine borreliosis were PCR-negative. Our findings suggest that the exposure to *B. burgdorferi* is common in dogs in the region declared an endemic area of borreliosis, and that this disease should be important to local veterinarians.

Courtney *et al.* (2004) developed a multiplex real-time PCR assay for the simultaneous detection of *Borrelia burgdorferi*. The assay was tested on various *Borrelia*, the assay was found to be highly specific for the *Borrelia* species tested (*B. burgdorferi*, *B. parkeri*, *B. andersonii, and B. bissettii*). The analytical sensitivity of the assay is comparable to that of previously described nested PCR assays (*B. burgdorferi*, fla gene), amplifying the equivalent 50 *borrelia* spirochetes. The dynamic range of the assay for *B. burgdorferi* was >/=4 logs of magnitude. Purified DNA from *B. burgdorferi* was spiked into DNA extracted from uninfected ticks and from negative control mouse and human bloods, and these background DNAs were shown to have no significant effect on sensitivity or specificity of the assay. The assay was tested on field-collected *Ixodes scapularis* ticks and shown to have 100% concordance compared to previously

described non-probe-based PCR assays. This is the first report of a real-time multiplex PCR assay that can be used for the simultaneous and rapid screening of samples for *Borrelia* species, two of the most common tick-borne infectious agents in the United States.

Shaw *et al.* (2005) used PCR analysis to determine the prevalence of tick-transmitted infections in 120 systemically ill dogs recruited over a period of three months from 52 veterinary practices in the UK. The animals had not travelled outside the UK and had one or more of the following clinical criteria: acute or recurrent pyrexia, anaemia and/or thrombocytopenia, polyarthritis/muscle pain, splenomegaly/lymphadenopathy, and intraocular inflammation with systemic signs. Blood samples from the animals were tested for the presence of DNA from *Borrelia burgdorferi* sensu lato by using simple PCR targeting. *B. burgdorferi* sensu lato was detected in five dogs. There were no statistically significant associations between the infections and the clinical signs shown by the dogs.

Skotarczak and Wodecka (2005) determined the prevalence of infection by *Borrelia* species by nested polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) with the enzyme Fsp4H I in the blood of dogs naturally infested by ticks in an endemic region of Poland. Blood samples were collected from 98 dogs of various breeds, delivered to the Veterinary Clinic in Szczecin (northwestern Poland) for various reasons. Nested PCR revealed the presence of DNA characteristic of only 1 genospecies, i.e. *B. burgdorferi* sensu stricto (s.s.), in all PCR-positive samples. Digestion

of PCR products from a fragment of the fla gene amplified with primers FLA1 and FLA2 gave only one band pattern consistent with the pattern obtained from sequence analysis of the fla gene from a reference isolate of *B. burgdorferi* s.s. GeHo (X15660) from GenBank.

Skotarczak *et al*. (2005) examined 62 dogs delivered to the Veterinary Clinic in Szczecin and 30 from the Municipal Animal Shelter in Szczecin with varied clinical signs of borreliosis. DNA of *B. burgdorferi* s.l. was most frequently detected in the blood of dogs of the group 2-5 years old (13/54.1%). An attempt to correlate the PCR results with the results of tests detecting antibodies against B. burgdorferi s.l. revealed that fewer than half (45.1%) of the dogs with presence of DNA of the spirochete, developed an immune response. Therefore the transfer of *B. burgdorferi* s.l. form, the primary lesion to the target tissues, is possible in dogs which did not develop immune response or develop an insufficient response. Among 92 borreliosis-suspected dogs 54 (over 58%) were diagnosed positively using laboratory methods. In most cases there was a correlation between clinical symptoms of borreliosis and presence of DNA of *B. burgdorferi,* thus PCR may contribute to improving to a large extent diagnostic of canine Lyme disease.

Chou *et al.* (2006) indicated that detection of *B. burgdorferi* DNA in formalin-fixed, paraffin-embedded tissues is feasible, but that intact *B. burgdorferi* DNA is rarely found in tissues from naturally infected dogs, even tissues from dogs with presumptive Lyme

borreliosis. Further, findings support the contention that Lyme nephritis may be a sterile, immune complex disease.

Gary *et al.* (2006) examined 108 canine samples from veterinarians in southern Ontario and Quebec in Canada. The polymerase chain reaction (PCR) prevalence was 1.85% for *Bo. Burgdorferi.*

Hiraoka *et al.* (2007) examined ticks removed from 1136 dogs all over Japan for *Borrelia* infection by PCR and sequencing. The 5S-23S rDNA intergenic spacer of *Borrelia* was detected from two *Ixodes persulcatus* ticks from two dogs and two unidentified *Ixodes* spp. from another two dogs in Hokkaido. Infected ticks carried by companion animals can be introduced into the human environment.

Zygner *et al.* (2008) collected 590 ticks from dogs, 209 were identified as *I. ricinus*, and 381 as *Dermacentor reticulatus*. they found that 6.2% of *I. ricinus* ticks harbored *B. burgdorferi* s.l. specific DNA. In these samples sequencing of the detected *Borrelia* amplicon confirmed infection with *Borrelia afzelii* genospecies. New sequences were submitted to the GenBank database (accession no. EU152128, EU152127, EU152126). This work is the first detection of B. *afzelii* in ticks from Warsaw in central Poland.

Kybicová *et al.* (2009) examined blood samples from 296 dogs and 118 engorged ticks. Samples were tested for *B. burgdorferi* s. l. using PCR amplification of the 16S rDNA and restriction fragment length polymorphism analysis of the 5S-23S rDNA intergenic spacer. In addition, blood samples were screened for antibodies to these bacteria. Infection with *Borrelia garinii* was detected by PCR in a dog with meningoencephalitis.

DNA of *B. burgdorferi* s. l. (*B. garinii* or *Borrelia afzelii*) was detected in 8.5% and 6.8% of ticks, respectively. IgM and IgG antibodies to *B. burgdorferi* s. l. were detected in 2.4% and 10.3% of dogs, respectively. Our findings suggest that the exposure to *B. burgdorferi* s. l. exists in dogs in the Czech Republic.

Wodecka, *et al.* (2009) monitored the presence of *B. burgdorferi* DNA before (study I), during (study II), and after (study III) completion of treatment of dogs with clinical borreliosis. The presence of DNA of *B. burgdorferi* were noted, in study I, in the blood of 7 dogs (63.6% dogs), in study II in 3 dogs, while in study III all blood samples were negative. In 6 out of 7 PCR+, the first study was carried out during week 1. Therefore, the PCR method is useful for monitoring early canine infections with spirochetes *B. burgdorferi*.

Leschnik *et al.* (2010) allocated 23 dogs raised under tick-free conditions to two groups. The 11 dogs in the first group were vaccinated with a commercial *borrelia* vaccine and subsequently developed detectable antibody titers. The 12 dogs in the second group were walked on two consecutive days in an area where ticks were endemic. On day 5 after exposure, engorged ticks were removed from the 12 dogs and were analyzed for *Borrelia* DNA by a real-time PCR assay. Blood samples were taken before exposure/vaccination and at defined time points thereafter. Antibody responses were evaluated using an immunofluorescence antibody test (IFAT) and Western blotting. Seven dogs from which *Borrelia*-positive ticks were removed seroconverted and developed individual immune responses. Blood and urine samples taken from the tick-

exposed group at weeks 1 and 3 for real-time PCR analysis and culture were always negative for bacterial DNA.

Krimer *et al.* (2011) evaluated canine brains for evidence of *Borrelia burgdorferi* infection. Twelve Beagles were experimentally challenged with *B. burgdorferi*-infected ticks at 18 weeks of age, and 2 unexposed dogs served as controls. One of the uninfected dogs and 6 infected dogs were each given 5 daily immunosuppressive doses of dexamethasone starting at 153 days post-infection. Eleven dogs were confirmed as infected by skin punch biopsy polymerase chain reaction (PCR) and serology. Neurological signs were not seen in any dogs through the end of the 190-day study. Whole blood, serum, cerebrospinal fluid (CSF), and brains from all dogs were collected. DNA was extracted from blood, CSF, and brain and evaluated by PCR for *B. burgdorferi*. Formalin-fixed brain tissue was examined by histopathology, immunohistochemistry, and PCR. Immunohistochemical staining for *B. burgdorferi* antigen was negative in all cases. The CSF analysis was normal, and PCR was uniformly negative for *B. burgdorferi* in alldogs. Six of the 11 (45%) infected dogs had mild to moderate lymphoplasmacytic choroid plexitis, which was more pronounced in the immunosuppressed dogs. The lack of *B. burgdorferi* DNA and immunohistochemical evidence of organisms, including within the choroid plexus lesions, suggests that *B. burgdorferi* does not have a direct role in the etiopathogenesis of canine central nervous system pathology.

Fryxell *et al.* (2012) collected ticks from canines. After which,

ticks and their hosts were screened for the presence of *Borrelia* using PCR to amplify the flaB gene. A subset of the positive samples was confirmed with bidirectional sequencing. In total ten (6%) canines, and 583 (27.5%) *Ixodid ticks* (252 *Ixodes scapularis*, 161 *A. americanum*, 88 *Rhipicephalus sanguineus*, 50 *Amblyomma maculatum*, 19 *Dermacentor variabilis*, and 13 unidentified *Amblyomma* species) produced a *Borrelia* flaB amplicon. Of the positive ticks, 324 (22.7%) were collected from canines (151 *A. americanum*, 78 *R. sanguineus*, 43 *I. scapularis*, 26 A. maculatum, 18 *D. variabilis*, and 8 *Amblyomma* species). None of the larvae were PCR positive. A majority of the flaB amplicons were homologous with *B. lonestari* sequences: 281 of the 296 sequenced ticks, 3 canines. Only 7 canines, and 15 tick flaB amplicons (12 *I. scapularis*, 2 *A. maculatum*, and *1 Amblyomma* species) were homologous with *B. burgdorferi* sequences. Data from this study identified multiple *Borreliae* genotypes in Arkansas ticks and canines including *B. burgdorferi* and *B. lonestari*; however, *B. lonestari* was significantly more prevalent in the tick population than *B. burgdorferi*. Results from this study suggest that the majority of tick-borne diseases in Arkansas are not *B. burgdorferi*.

Jenkins *et al.* (2012) constructed a consensus TaqMan real-time PCR test targeting the chromosomal flaB gene of *Borrelia burgdorferi* sensu lato. On material consisting of 242 *Ixodes ricinus* ticks collected from dogs and cats in Northern Norway (n = 139) and Telemark County in Southern Norway (n = 103). Ticks positive in either test were further tested by nested PCR amplification of the 5S-

23S rRNA intergenic-spacer region followed by sequencing for species identification. A tick was defined as *Borrelia* positive if two of three tests were positive. Thirty-four of the 242 (14 %) ticks satisfied this definition of positivity. Of these ticks 32 were positive both in the rRNA and flaB test, while two were positive only in the rRNA test. One tick was positive only in the rRNA test and was considered false positive since PCR for sequencing failed. The sensitivity of the flaB test was 94 % and the specificity 100 %. It was possible to determine the species present using Tm analysis. Among ticks from Northern Norway the prevalence of *Borrelia* was 13 %, whereas the prevalence in Telemark was 16 %. Among identified species (n = 33) *B. afzelii* was found in 16 (47 %), *B. garinii* in 15 (44 %) and *B. valaisiana* in 2 (6 %) ticks, respectively. The flaB test is a rapid, sensitive and specific test for detection and quantification of *Borrelia burgdorferi* s.l. in *I. ricinus* ticks. This is the first report on *Borrelia* prevalence in *I. ricinus* in Northern Norway.

Nolte (2012) mentioned that PCR for the detection of *Borrelia* genetic material in clinical specimens is a highly important diagnostic tool to aid the clinician or general practitioner/office physician in finding or ensuring a definite diagnosis of LB/LD in the suffering patient. Due to the lack of commercially available and sufficiently validated assays, many different PCR protocols are in use. While each published protocol may have its benefit for a specific patient population, a rigorously validated and standardized PCR assay is needed in order to face the actual challenges in diagnosing vector borne infectious diseases. Currently,

caution is required when choosing a PCR protocol from the published ones, since – as shown for the *ospA* targeting PCRs – some primers may only detect a subset of the known *B. burgdorferi* strains. An *ospA* PCR with primers designed on older *B. burgdorferi* s.s. *ospA* sequences may be sufficiently specific when used in the US but not in Europe.

Smith *et al*. (2012) detected the prevalence of *B. burgdorferi* in ticks attached to pet dogs using them as sentinels for human disease risk. Dogs give a good indication of the exposure of their human owners to infected ticks, since they largely share the same environment and visit the same outdoor areas. PCR was used to test 739 tick samples collected from 3534 dogs selected at random as they visited veterinary practices over a period of six months. Overall, the prevalence of infected ticks on all dogs was 0.5% giving an estimated 481 infected ticks per 100,000 dogs. The data suggest that the prevalence of *Borrelia* in the UK tick population is considerably higher than most recent estimates indicate.

Susta *et al.* (2012) mentioned that *Borrelia burgdorferi* is mainly characterized by lameness in dogs. More than 95% of naturally infected dogs are asymptomatic or subclinical; however, in experimental studies, histologic synovial lesions are consistently observed in asymptomatic dogs inoculated with *B. burdgorferi*. This study investigated the ability of a synovial histopathologic scoring system, clinicopathologic data, and polymerase chain reaction (PCR) testing to differentiate between *B. burgdorferi*-infected and

uninfected dogs. Eighteen 18-week-old beagles were subject to challenge with *B. burgdorferi*-infected wild-caught ticks (*Ixodes scapularis*), and 4 uninfected dogs served as controls. Infection was confirmed by serology (ELISA) and PCR amplification of *B. burgdorferi*-specific DNA of skin biopsies taken at the tick attachment site. A synovial scoring system from human medicine was adapted and implemented on postmortem synovial samples to discriminate infected and non-infected animals. Application of this system to elbows and stifles with a cumulative joint score cut off > 4 showed a sensitivity of 88.2% and a specificity of 100%, with a positive likelihood ratio of infinity and a negative likelihood ratio of 0.12. Complete blood count, serum biochemistry, urinalysis, urine protein: creatinine, urine PCR, synovial and lymph node cytology, and synovial PCR were evaluated but were not reliable indicators of clinical disease.

Galaviz-Silva *et al*. (2013) documented the geographic distribution of *Ixodes tick* species in dogs and the prevalence of *Borrelia burgdorferi* s.l. in adult ticks and blood samples by amplification of the ospA region of the *B. burgdorferi* genome. The study area included nine localities in Nuevo León state. DNA amplification was performed on pools of ticks to calculate the maximum likelihood estimation (MLE), and the community composition (prevalence, abundance, and intensity of infestation) was recorded. A total of 2,543 adult ticks, representing four species, *Rhipicephalus sanguineus, Dermacentor variabilis, Rhipicephalus (Boophilus) annulatus*, and *Amblyomma cajennense*, were recorded

from 338 infested dogs. Statistically significant correlations were observed between female dogs and infestation (P = 0.0003) and between *R. sanguineus* and locality (P = 0.0001). Dogs sampled in Guadalupe and Estanzuela were positive by PCR (0.9 %) for *B. burgdorferi. Rhipicephalus sanguineus* had the highest abundance, intensity, and prevalence (10.57, 7.12 and 94.6, respectively). PCR results from 256 pools showed that four pools were positive for *D. variabilis* (1.6 %), with an MLE of 9.2 %; nevertheless, it is important to consider that in the area under examination probably other reservoir hosts for *D. variabilis* and *B. burgdorferiare* present that, very likely, play a much more important role in the ecology of Lyme borreliosis than dogs, which could be considered in future studies.

Inokuma *et al.* (2013) diagnosed two dogs that exhibited sudden astasia, anorexia and fever higher than 40°C were suspected of having Lyme disease in July 2011. Clinical symptoms gradually improved with antibiotic treatment in both cases. Polymerase chain reaction and sequence analysis revealed *Borrelia garinii* DNA fragments in the peripheral blood in the acute disease phase. Serological tests, including enzyme linked immunosorbent assay and Western blot analysis, showed an increased IgG antibody titer against *Borrelia* pathogens in one of the dogs.

Rhodes *et al.* (2013) assessed *Ixodes scapularis* ticks collected from the field in Rhode Island for infection with *B. burgdorferi*. Ticks were fed on purpose bred beagles to repletion

and infection of the dogs which assessed through serology and PCR. Tissue biopsies (n=2) were collected from each dog 49 days post-tick infestation (dpi) and the ospC genotype of the infecting strains determined by direct PCR of DNA extracted from tissue or by PCR after cultivation of spirochetes from biopsy samples. The dominant ospC types associated with *B. burgdorferi* canine infections differed from those associated with human infection, indicating a relationship between ospC sequence and preferred host range. Knowledge of the most common ospC genotypes associated specifically with infection of dogs will facilitate the rational design of OspC-based canine Lyme disease vaccines and diagnostic assays.

Maggi *et al.* (2014) compared Canine vector-borne diseases (*Borrelia burgdorferi* in them) serological and molecular testing as the two most common methodologies used for screening healthy dogs or diagnosing sick dogs in which a vector-borne disease is suspected. Paired serum and EDTA blood samples from 30 clinically healthy dogs (Group I) and from 69 sick dogs suspected of having one or more canine vector-borne diseases (Groups II-IV), were tested in parallel to establish exposure to or infection with the specific CVBDs targeted in this study. Among all dogs tested (Groups I-IV), the molecular prevalences for individual CVBD pathogens ranged between 23.3 and 39.1%. Similarly, pathogen-specific seroprevalences ranged from 43.3% to 59.4% among healthy and sick dogs (Groups I-IV). Among these representative sample groupings, a panel combining serological and molecular assays run in parallel resulted in a 4-58% increase in the recognition of exposure to

or infection with CVBD. Authors concluded that serological and PCR assays should be used in parallel to maximize CVBD diagnosis.

Montandon *et al.* (2014) evaluated the presence of *Borrelia burgdorferi* infection in domestic and wild vertebrates and ectoparasites in endemic areas from the state of Minas Gerais, Brazil. A total of 445 serum samples were examined by ELISA, which used the *Borrelia burgdorferi* strain G39/40 U.S. source and 3,821 tick samples were tested by polymerase chain reaction (PCR). *B. burgdorferi* antibodies were found in 30 serum samples (6.74%); nine in dogs (6.25%). Nested-PCR performed in DNA samples obtained from collected ticks demonstrated negative results. Although attempts to amplify *B. burgdorferi* DNA from ticks had been not successful, the presence of seroreactive vertebrates suggests the possibility the *Borrelia* species circulating in these regions.

2.6. Prevention and control

Chang *et al.* (1995) showed that vaccination with recombinant OspA protected dogs against infection and disease after an experimental challenge with *B. burgdorferi* by exposure to ticks. Twenty-two specific-pathogen-free beagles were vaccinated with recombinant OspA (ospA gene derived from *Borrelia burgdorferi* B31) alone or with adjuvant (QuilA, Montanide ISA25, or aluminum hydroxide) at 6 weeks of age. Thirteen dogs were used as nonvaccinated controls with or without adjuvant. Three dogs were kept as contact controls and received neither vaccine nor challenge.

Six weeks or 6 months after the first vaccination, the vaccinated (20 of 22) and nonvaccinated dogs (13) were challenged by exposure to adult ticks (*Ixodes scapularis*) naturally that were infected with *B. burgdorferi* (tick infection rate, > or = 60%) and that were collected from Westchester County, N.Y. Protection from infection was evaluated by culture for *B. burgdorferi* from skin biopsies taken near the sites of tick bites. Skin biopsies were taken at monthly intervals for 3 months. *B. burgdorferi* was not isolated from any of the vaccinated dogs. In contrast, 12 of 13 control dogs challenged by exposure to the ticks were culture positive. The histopathology of the joint capsules 3 months after the challenge was used to evaluate protection from arthritis. Eight of 13 control dogs showed synovitis in single or multiple joints, while only 1 of the 22 vaccinated dogs had a single focus of mild inflammation in a single joint. At the time of the challenge, the vaccinated dogs had antibody to *B. burgdorferi*, which was demonstrable by kinetic enzyme-linked immunosorbent assay, Western blotting (immunoblotting), and a serum growth inhibition assay. The vaccinal antibody declined gradually after the challenge, especially in dogs vaccinated with OspA without adjuvants. Antibodies in the challenge control dogs were only detectable by 4 to 6 weeks after the challenge and remained at high levels until the termination of the study. Contact control dogs showed no antibody responses or histopathologic lesions and were culture negative. By Western blot analysis, antibodies to OspA first appeared in the sera of vaccinated dogs 3 weeks after the first vaccination. The absence of additional bands after the challenge suggests that infection in

vaccinated dogs was blocked.

Conlon *et al*. (2000) divided *Borrelia burgdorferi* negative, mixed-breed dogs 10 to 12 weeks of age into three groups of ten animals each for the purpose of evaluating a recombinant nonadjuvanted *B. burgdorferi* OspA vaccine for efficacy and safety. Two groups received two doses of two different lots of a non adjuvanted, OspA, recombinant vaccine; the third group served as non vaccinated controls. All dogs were challenged 3 weeks after the second vaccination with blacklegged deer ticks (*Ixodes scapularis*) harvested from a *B. burgdorferi* endemic area in Rhode Island. Clinical signs, antibody titers by ELISA, Western blot assays, and isolation and polymerase chain reaction analyses of spirochetes from biopsy specimens were used to evaluate vaccine efficacy. Direct fluorescent antibody assay was used to evaluate the infection rate in the challenge ticks and in naïve ticks allowed to feed on study dogs after the dogs were infected. Vaccinates responded with high levels of antibodies (determined by ELISA), which did not rise after challenge. Vaccinates demonstrated no clinical signs, negative isolation of spirochetes on biopsy, only an OspA antibody pattern on Western blot assay, and negative isolation of spirochetes on biopsy, confirming that the vaccine blocked infection with *B. burgdorferi* in all vaccinated dogs (20/20). Control dogs demonstrated clinical signs (2/10), antibodies characteristic of infection with *B. burgdoferi* (10/10), isolation of spirochetes (10/10), and polymerase chain reaction (PCR) detection of spirochetes (9/10). The recombinant, non adjuvanted *B. burgdoferi* vaccine protected 100% of vaccinates against infection

after a severe challenge that infected 100% of control dogs. The OspA vaccine proved to be highly safe and effective in this study.

Straubinger *et al.* (2000) mentioned that Antibiotic administration, orally or intravenous, fails to eliminate a persistent infection with *Borellia burgdorferi* in dogs but may prevent and cure joint disease. However, persistently infected dogs when subsequently treated therapeutically with an immunosuppressant (e.g., prednisone) may develop severe polyarthritis.

Littman (2003) did not recommend treating asymptomatic *Borrelia* carrier dogs, but did recommend screening them for proteinuria and for exposure to other agents. A positive Lyme titer is a marker of exposure to *Ixodes* ticks and the agents they carry. The risk/benefit of vaccination will be understood better as the symptomatology and immunopathogenesis of Lyme disease are defined. Meanwhile, tick control is highly recommended for all dogs in Lyme-endemic areas.

Blagburn *et al.* (2005) demonstrated that administration of K9 Advantix 25 days before infestation prevented attachment and subsequent feeding of infected ticks. Treatment with Frontline Plus prevented transmission of *B burgdorferi* to 6 of 7 treated dogs.

Littman *et al*. (2006) mentioned that tick control can include a product that repels or protects against tick attachment, thereby helping to prevent transmission of coinfections as well as *Borrelia* spp. Seropositive dogs with clinical abnormalities thought to arise from Lyme disease generally are treated with doxycycline (10 mg/kg q24h

for 1 month). Proteinuric dogs might need longer treatment as well as medications and diets for protein-losing nephropathy. The ACVIM diplomates believe the use of Lyme vaccines still is controversial and most do not administer them.

Wikle *et al*. (2006) found that the canine OspA vaccine of *Borrelia burgdorferi* is both safe and efficacious with long-lasting protection from clinical signs and spirochete proliferation for at least 1 year (366 days) after vaccination as the anti-OspA antibody produced by the vaccine enters the tick upon feeding and kills the spirochete in the tick's midgut preventing transmission to the host.

Levy *et al.* (2008) measured changes in antibody levels following antibiotic treatment of *B. burgdorferi* antibody-positive nonclinical dogs. One hundred thirty-two client-owned dogs were used in the study; 64 were negative, 53 of 68 positive animals received treatment, and 15 were untreated controls. Test sera were collected at 3, 6, and 12 months from seropositive dogs receiving treatment and untreated controls. Dogs in the treated group were assigned to moderate-to-high (> or =29 U/ml)- and low (<29 U/ml)-C6-level groups because the change in the C6 level after treatment was dependent on the level prior to treatment. There were significant declines in the 30 dogs with moderate-to-high initial C6 levels that exceeded the maximal declines of the untreated control dogs in all cases at 6 months (16 data points) and 12 months (29 data points) post treatment. There was little change in C6 level following antibiotic therapy in the 23 dogs with low initial C6 levels. The quantitative C6

antibody test can be used to measure changes in C6 antibody levels following treatment of antibody-positive nonclinical dogs.

Knauer *et al*. (2011) mentioned that Systemic antibiotic treatment of Lyme borreliosis is effective during the early stages of the infection, while chronic manifestations of the disease may remain refractory and difficult to treat. However their data indicate that topical treatment with a formulation containing azithromycin is a promising approach to prevent Lyme borreliosis shortly after a tick bite in order to eliminate the spirochaetal organisms in the skin of the freshly bitten host and thereby prevent Lyme borreliosis.

McCall *et al*. (2011) randomly assigned healthy, purpose-bred laboratory beagle dogs that had not been exposed to ticks and were seronegative for *Borrelia burgdorferi* in to four groups of eight dogs each. Control group 1 was not treated. Groups 2, 3 and 4 were treated with a single topical application of a new formulation of fipronil, amitraz and (S)-methoprene (CERTIFECT™, Merial Limited, GA, USA) at 28, 21 or 14 days prior to tick infestation, respectively. Each dog was infested with 25 female and 25 male field-collected adult *Ixodes scapularis* ticks that had infection rates of 66% for *B. burgdorferi* sensu stricto, as determined by polymerase chain reaction. Two and five days after tick infestation, control dogs had an average of 9.5 and 13.9 attached adult female ticks, respectively, whilst the 24 treated dogs remained tick-free aside from a single tick on the 2nd day after infestation. Serial serological tests demonstrated that the ticks successfully infected 8/8 control dogs with

B. burgdorferi . *B. burgdorferi* infection also was confirmed in most control dogs by culture (6/8) and PCR (7/8) of skin biopsies. In contrast, CERTIFECT protected all 24 treated dogs against infection by *B. burgdorferi* as demonstrated by its negative serological tests throughout the study and the absence of any positive skin biopsy culture or PCR in these dogs.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

3.1. Materials:

3.1.1. Samples:

A total of 100 samples of [70 dog blood samples, 15 human blood samples, 15 hard ticks (*Rhipicephalus sanguineus*)] collected from different sections in the general department of K9 during period from 2012 till 2013 as shown in **(Table 1)**. Selected dogs were suffering from signs of Lyme disease which were fever, acute arthritis, arthralgia, lameness, and nephritis in some cases.

Table (1): Types and Number of the examined samples.

Dog blood	Human blood	Hard ticks	Total of samples
70	15	15	100

3.1.2. Giemsa stain:

According to **Mylonakis *et al.*, (2003).**

Giemsa stain powder (GS500 SIGMA).

3.1.3. Medium used for cultivation and Maintenance of *Borrelia burgdorferi* Strain (B 31):

BSK-H medium (Sigma):

A purchased commercially BSK-H medium (Sigma) was used. The package was 100 ml and sterilized by filtration.

3.1.4. Filter and Disks:

Millipore filters and different Millipore disks were used for purification of contaminated cultures and sterilization of some ingredients of media used such as Tween 80 and oleic acid. Syringe filters were used for sterilization of approximate amounts of 10 ml each time. Millipore filters (presterilized 250 ml capacity, 0.45 µm porosity size).

3.1.5. Materials used in the extraction of DNA (Bloos *et al*., 2010).

- **TE buffer (Tris EDTA) pH 8:**

10mM Tris Hcl (pH 8.0).

1 mM EDTA (pH 8.0).

This buffer was prepared in a batch of 100ml, autoclaved and stored at 4oC.It was used for dissolving of DNA.

- **Lysozyme (10 mg/ml TE):**

Enzyme used for poring of cell membrane (lysing of the cell).

- **SDS 10%:**

It was prepared by dissolving 10g SDS (Invitrogen cat # 15525) in 100 ml distilled water. The solution was clear and stable at room temperature. Used as detergent for gentle lysis of cell.

- **Protinase K:**

Prepared as 20 mg/ml distilled water and used for denaturation of protein.

- **Equilibrated phenol:**

Used for removal of protein from cell lysate (purification of DNA) and pH was adjusted at 8.0.

- **Phenol: Chloroform: Isoamyle (25:24:1):**

Used for purification of DNA from any protein impurities.

- **Chloroform: Isoamyl alcohol (24:1):**

Used for purification and washing DNA from phenol residues.

- **Absolute ethanol (100%):**

Used for precipitation of DNA.

- **0.2M Sodium acetate pH 5.2:**

Used for precipitation and purification of DNA.

- **Ethanol 70%:**

Used for washing of DNA pellet from residues of absolute ethanol.

- **RNase:**

RNase (Stratagene) was used at a concentration of 10 mg/ml of deionized water for digestion or RNA which found after lysis of cells.

3.1.6. Materials used for PCR (Bloos *et al* 2010):

- **The DNA template:**

The DNA was isolated from each sample, purified and then used for different molecular tests.

- **10 x reaction buffer containing 15mM MgCl:**

Promega (Cat. No. M1861).

- **Deoxynucleotide mix (DNTPs) 25mM/each:**

Stratagene, Cat. No. 200415 was diluted before use from stock concentration (100mM "25mM each") to the working stock 4mM (1mM each) by mixing 20µl for dNTPs mix with 480 µl distilled water and stored at -20˚C till use.

- **MgCl (25mM):**

Used as cofactor.

- **Taq DNA Polymerase 5U/µl:**

Promega (Cat. No. M1861):

- **Biotechnology grade water (Roth):**

- **Primers :** See **Table (2):**

3.1.7. Oligonucleotide primers used for amplification of the DNA recovered from blood samples and hard ticks isolates (Postic *et al*., 1994):

The sequence, specificities, the primer combination and the size and length of the amplified products were summarized in **Table (2)**.

Table(2). Oligonucleotide primers sequences and size of the PCR-targeted products PCR for *Borrellia burgdorfei.*

Primer name	Primer sequence		Amplification product
Osp A	**F**	**5′-AATGTTAGCAGCCTTGACGAGAA-3′**	**309 bp**
	R	**5′-GATCGTACTTGCCGTCTTTGTTT-3′;**	

3.1.8. Buffers and reagents used for agarose gel electrophoresis (van Dam *et al*., 1993):

- **Agarose powder (Rocco, Spain):**

Free from DNAase and RNAase was prepared in 2% concentration.

- **Tris Acetate EDTA (TAE) electrophoresis buffer (50X) stock solution, pH 8.0:**

Tris-HCl (Sigma)		10.0 mM
EDTA (Sigma)		1.0 mM
Distilled water	to	1000ml

pH was adjusted to 8.0 and diluted to 1X in DDW to be used in

the preparation of the gel.

- **Ethidium bromide solution (Sigma):**

Ethidium bromide powder 10mg

Sterile DDW 1.0ml

Was mixed and stored covered at 4ºC.

- **Gel loading buffer (6X stock):**

Bromophenol blue 0.25%

Xylene cyanol 0.25%

Glycerol 30.0%

The components were dissolved in sterile DDW and stored covered with aluminum foil at room temperature.

- **DNA ladder (Molecular marker):**

M15 set of 100bp DNA ladder with stain (Sib Enzyme)

- Storage buffer: 10 mM Tris HCl (pH 8.0), 1.0mM EDTA, 50mM NaCl Stored at - 20ºC.
- Concentration: 0.2 mg DNA/ ml
- Size range: 100 – 1000 bp
- Number of fragments: 10 discrete fragments (in base pairs), 1000,900, 800, 700, 600, 500, 400, 300, 200, 100.

3.1.9. Materials for ELISA testing.

- ab108711—anti-B. *burgdorferi* IgM Human ELISA Kit, abcam, UK.

- DBGM96-Dog EIA *Borrelia* IgM, Test Line, Czech Republic.

3.1.10. Other Materials and Apparatuses used in the Present Investigation:

1. Dark-ground (DG) microscope, dry clean slides and cover slips were used for the examination of both leptospires and borrelias using high power lens (40 X) or low power (10 X).
2. Sterilized glass pipettes (1ml and 2 ml).
3. Pipetting aid.
4. Screw capped tubes with two different sizes, namely 16 X 125 mm (for leptospires) and 13 X100 mm (for borrelias).
5. Spectrophotometer, SPECOL 11, at WL = 600 nm.
6. Cooling centrifuge (15,000 rpm) (Model : 2-15, Sigma, West Germany).
7. Centrifuge (4,000 rpm) (Model : 202C, Sigma, West Germany).
8. Incubator (SHEL-Lab Sheldon Manufacturing Incubator , Model : 1545).
9. Hot air oven (Model : 40050 – IP20, Memmert – Germany).
10. PCR Tubes: PCR-05-C. 0.5ml Thin wall, Clear, flat cap. Manufactured by Axygen USA.

11. FPTC-100 Programmable Thermal Controller. Peltier – Effect Cycling, MJ Research Inc.
12. Homogenizer(Laboratory Aid, Poland).
13. Digital automatic micropipettes : 2-20 µl ; 10-100 µl and 20-20µl.(Eppendorf, Hamburg, Germany).
14. Power Supply.
15. Electrophoreses apparatus(Bio-Rad GS).
16. Eppendorf tube (AB gene) (1,1.5 and 2ml).

3.2. Methods:

3.2.1. Collection of samples:

The blood samples were collected in clean sterilized equipment and taken under aseptic condition, good handling of products to avoid contamination and transferred to lab in an ice box. The samples were refrigerated at 0-4 ˚C and DNA extracted as soon as possible, maximum within 36 h. Dogs examined for presence of ticks, which picked up using forceps and collected in specific containers, **Photo (1)**.

3.2.2. Direct demonstration:

3.2.2.1. Giemsa Staning Technique:

According to **(Mylonakis *et al.*, 2003)**. The Giemsa staining technique was performed by fixation of the blood film with methyl alcohol for 30 sec. The diluted stain was applied for 45 min, then rinsed with distilled water and air dried. The thin blood slides were examined for at least 100 oil immersion fields (OIFs), when spiral shape was detected *Borrelia burgdorferi* will be recorded.

3.2.2.2. Dark field Microsopy:

A drop of blood was placed on a microscope slide under a glass cover slip to keep it from drying out. The slide is then viewed at low power magnification with a dark-field microscope.

3.2.3. Trials to culture *B. burgdorferi* from blood:

The growth of *B. burgdorferi* was studied by inoculation into the patent BSK-H (Sigma) medium, incubation at 33ºC for 10-15 days.

To obtain the microaerophilic condition, the medium was dispensed in 13 X 100 mm screw capped tube (capacity 9 ml / tube), 8.5 ml of the medium were dispensed into each tube, and then 0.5 ml of medium were removed from the tube and 0.5 ml of the inoculum (well grown culture of *B. burgdorferi*) were added to the remaining 8 ml of the medium.

The strain which was obtained was then immediately subcultured into 3 tubes of patent BSK-H medium (Sigma). Cultures were incubated at 33ºC and all tubes were examined weekly under the DG microscope. A heavy growth was observed 10 days post-inoculation. From this well grown culture, only 1 ml was transferred into 2 tubes containing BSK-II supplemented with R.S (0.5 ml to each). Also, 1 ml of the same culture was transferred into two tubes containing BSK-II medium with swine serum (0.5 ml to each), and then incubated and examined as mentioned before.

3.2.4. Extraction of DNA from blood samples (Bloos *et al*., 2010):

1ml of blood was placed in a 1.5 ml tube and chop into small. Centrifuged for 5-10 minutes at maximum speed(3000 - 4000 rpm). twice the volume of PBS buffer were added, vortexed and centrifuged at max speed for 2-5 min. 330µl of STE buffer and 40ul of 20% SDS were added with 20ul of 20mg/ml Pro K . Mixed by vortex or by pipette and incubated at 60°C for 4hrs (mix occasionally to aid in

digesting). Another 5-10µl of ProK (20mg/ml) were added and incubated overnight at 37°C. Samples were centrifuged at max speed for 15-30min. to pellet cellular debris. Supernatant poured into a clean 1.5ml tube.400ul of phenol were added and mixed by hand. Samples left on ice for 10 min, then centrifuged at maximum speed for 2 min. the top layer (containing DNA) was removed by cutting a 200µl pipette tip. Layer was expelled into a clean 1.5ml tube. 400µl of chloroform/ isoamyl alcohol (24:1) were added and mixed by hand then centrifuged at maximum speed for 2 min. top layer was removed by cutting a 200µl pipette tip. Layer was expelled into a clean 1.5ml tube, 2 volumes of cold 95% ethanol were added and inverted. 4% 3M sodium acetate (NaAc) were added and inverted then placed in at -20°C freezer overnight to precipitate DNA. Tubes then Centrifuged for 15-30 min. at maximum speed to pellet DNA. Supernatant were discarded and 500µl of 70% EtOH added to wash the DNA pellet, inverted and centrifuged for 2min. at max speed. Supernatant discarded and dried DNA pellet by air, DNA resuspended in 50-200µl of sterile distilled water. Water added and mixed gently by pipette, then incubated at 37°C for 1-12hrs. Mixed gently and incubated at 4°C overnight. Mixed and stored at -20 to -80°C. **(Bloos *et al*., 2010).**

3.2.5. Estimation of purity and concentration of the DNA:

The concentration and purity of the DNA that had been extracted were determined by estimating the optical density at a wave length of 260 and 280nm using the spectrophotometer. The concentration was calculated as follows:

1OD. 260 nm = 50 µg/ml

The purity of DNA = OD. At 260/OD. At 280 nm.

The purity of DNA had a value of 1.8 where the optimum ranged between 1.8 – 2.

3.2.6. Extraction of DNA from hard ticks samples (Wallace, 1987).

The ticks were mechanically crushed using a Dounce mortar in 1 mL lysis buffer (NaCl 0.1M, Tris-HCl 0.21M, pH8 EDTA 0.05M, SDS 0.5%). Enzymatic digestion by proteinase K (100 µg/mL) was performed for 16 h (56 °C). DNA extraction was then carried out using phenol-chloroform extraction The DNA was then precipitated with absolute ethanol (two volumes) and resuspended in 200 µL of 1 × TE buffer (Tris 10 mM, EDTA 1 mM, pH8).

3.2.7. DNA amplification PCR for the detection of *Borrellia burgdorfei* by using *ospA* gene (Sparagano *et al*., 1999):

- The amplified reactions were performed in 50 µl volumes in micro-amplification tubes (PCR tubes). The reaction mixture consisted of 10 µl (200 ng) of extracted DNA template from blood and Hard ticks , 5 µl 10X PCR buffer, 0.375 µl MgCl2 (1.5 mM), 1.25 µl dNTPs (250 µM), 0.25 µl (1.25 Unit) Ampli Taq DNA polymerase, 0.25 µl (0.5 µM) from each primer pairs and the volume of the reaction mixture was completed to 50 µl using DDW.
- Each reaction mixture was overlaid with 30 µl mineral oil
- PCR amplifications were performed with a

thermal cycler.

- the thermal cycler was adjusted as follows:

Initial denaturation	94°C for 4 min
Amplification (30 cycles of)	
Denaturation	94°C for 1 min
Annealing	55°C for 1 min
Extension	72°C for 1 min
Final extension phase	72°C for 7 min

The PCR products were stored in the thermal cycler at 4°C until used.

3.2.8. Screening of PCR products by agarose gel electrophoresis (Schwartz *et al*., 1997):

Two grams agarose was added to 100 ml Tris acetate EDTA (TAE) buffer. The agarose was autoclaved for 10 minutes and 0.5 µg/ml ethidium bromide was added and then left to cool to room temperature. The gel tray was tapped and the warm agarose was poured in. The comb was inserted in the agarose which was left to polymerize. After hardening, the tray was untapped, the comb was removed and the gel was applied to the electrophoresis cell. The cell was filled with TAE buffer. 10 µl of each of the PCR product samples were applied to the gel along with 5 µl molecular weight marker after mixing each with 1µl loading buffer on a piece of parafilm. Each mixture was applied to a slot using 10µl micropipette. The electrophoresis cell was covered and the power supply was

adjusted at 10 Volt/cm. The gel was taken out from the cell and examined under short wave UV transilluminator. The gel was photographed in order to obtain a permanent record using digital camera (Acer CR-5130, China).

3.2.9. ELISA testing:

Serum samples of human were tested for the presence of IgM class antibodies against *B. burgdorferi* using (ab108711—anti-*B. burgdorferi* IgM Human ELISA Kit, abcam, UK). The sensitivity of the test is 93% and the specificity is 98.8%, while sera from dogs were tested for the presence of IgM class antibodies against *B. burgdorferi* using (DBGM96-Dog EIA Borrelia IgM, TestLine, Czech Republic).

RESULTS

IV. Results

4.1. Direct demonstration:

Fixed blood films were examined by oil immersion fields (OIFs) and wet preparations from the same samples were demonstrated by dark field microscope. Neither fixed films nor wet preparation showed any Spirochetes.

4.2. Culture results:

None of the dogs, ticks or human samples showed positive results for *B. burgdorferi*. After 8 weeks wet preparation from BSK-H purified culture did not show characteristic spirochetal morphology and motility under the dark-field microscope.

4.3. PCR results:

The results observed in **Figure (1)** revealed that *osp*A gene (309 bp) was only detected in four out of 100 extracted DNA from different samples (human blood ,dog blood and hard ticks) **Tables (3, 4 and 5)**.

4.4. ELISA testing:

Seventeen out of 70 canine sera were positive by ELISA, while 3 out of 15 human sera samples were positive **Tables (3, 4 and 5)**.

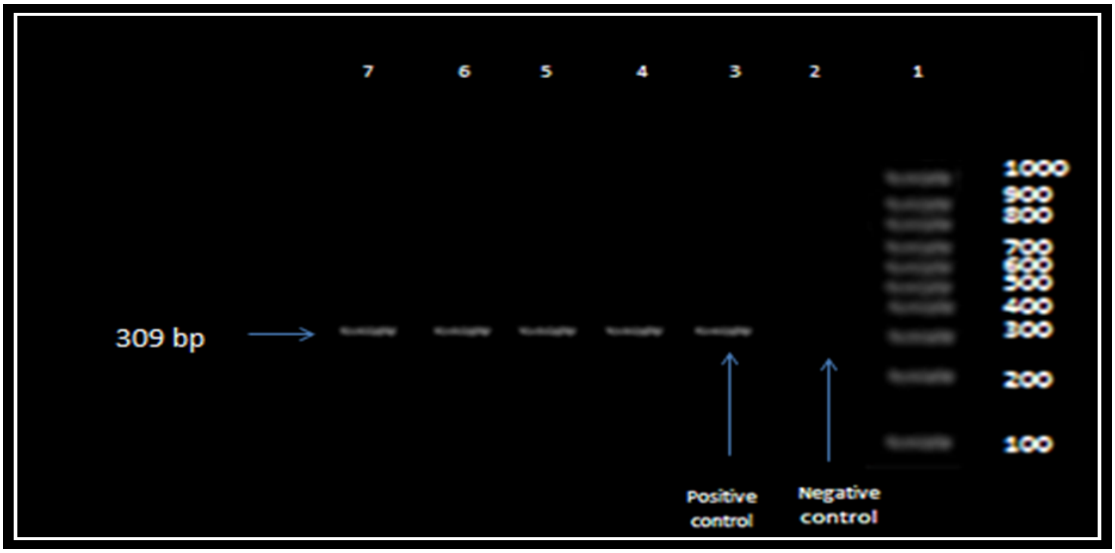

Figure (1):- Amplification of *B. burgdorferi osp*A gene showing PCR products (309 bp): Lane (1): represents the 100 bp DNA Marker, Lane (2): negative control, Lane (3): positive control, Lanes (4, 5, 6 and 7): positive samples.

Table (3): Positive cases of *Borrelia burgdorferi* and their percent among dogs, ticks and contact human.

sample	No	Direct demonstration		Culture (%)	ELISA (%)	PCR (%)
		Geimsa	DFM			
Dogs	70	0	0	0	17	3 (4.2)
Ticks	15	NA	NA	0	NA	1 (6.6)
human	15	0	0	0	3	0

NA: Not Applicable.

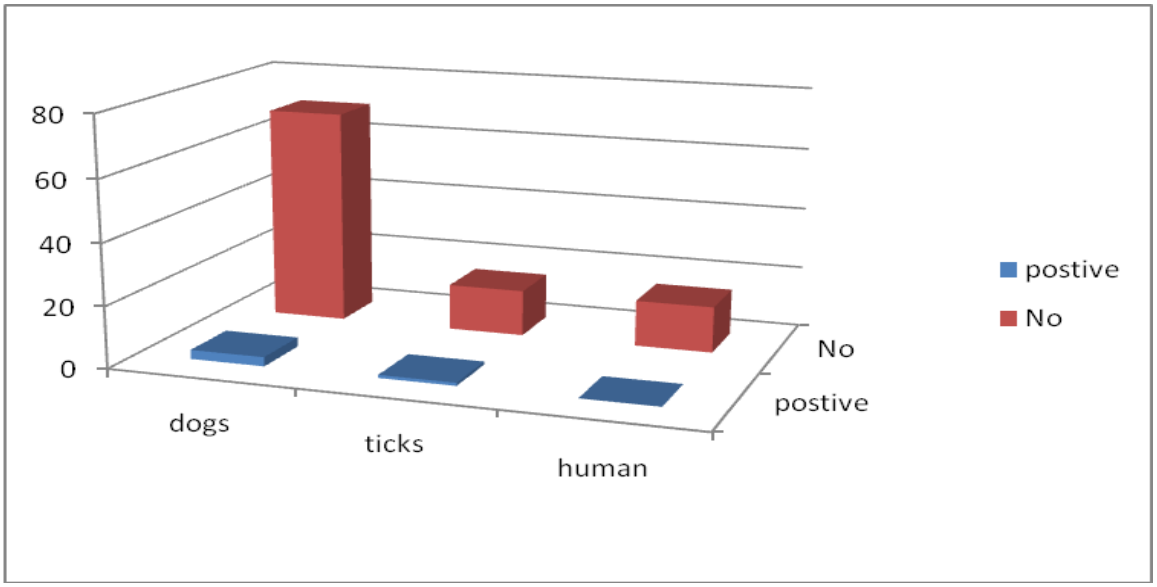

Figure (2): positive PCR cases of *Borrelia burgdorferi*

Table (4): *Borrelia burgdorferi* in human cases by culture, ELISA and PCR.

No	ID	Culture	ELISA	PCR
A	Human sample 1	Negative	Negative	Negative
B	Human sample 2	Negative	Negative	Negative
C	Human sample 3	Negative	Negative	Negative
D	Human sample 4	Negative	Negative	Negative
E	Human sample 5	Negative	Negative	Negative
F	Human sample 6	Negative	positive	Negative
G	Human sample 7	Negative	Negative	Negative
H	Human sample 8	Negative	Negative	Negative
I	Human sample 9	Negative	Negative	Negative
J	Human sample 10	Negative	positive	Negative
K	Human sample 11	Negative	Negative	Negative
L	Human sample 12	Negative	Negative	Negative
M	Human sample 13	Negative	Negative	Negative
N	Human sample 14	Negative	positive	Negative
P	Human sample 15	Negative	Negative	Negative

Table (5): *Borrelia burgdorferi* in dog cases by culture, ELISA and PCR.

No	Breed	culture	ELISA	PCR
1	Malinoa	Negative	Positive	Negative
2		Negative	Negative	Negative
3		Negative	Negative	Negative
4		Negative	Negative	Negative
5		Negative	Negative	Negative
6		Negative	Negative	Negative
7		Negative	Negative	Negative
8		Negative	Negative	Negative
9		Negative	Positive	Negative
10		Negative	Negative	Negative
11		Negative	Negative	Negative
12		Negative	Negative	Negative
13		Negative	Positive	Negative
14		Negative	Negative	Negative
15		Negative	Positive	Negative
16		Negative	Positive	Positive
17	Labrador	Negative	Positive	Negative
18	German Shepherd	Negative	Positive	positive
19		Negative	Negative	Negative
20		Negative	Negative	Negative
21		Negative	Positive	Negative
22		Negative	Positive	Negative
23		Negative	Negative	Negative
24		Negative	Negative	Negative
25		Negative	Negative	Negative
26		Negative	Negative	Negative
27		Negative	Negative	Negative
28		Negative	Negative	Negative
29		Negative	Positive	Negative
30		Negative	Negative	Negative
31		Negative	Negative	Negative
32		Negative	Positive	Negative

33		Negative	Negative	Negative
34		Negative	Negative	Negative
35		Negative	Negative	Negative
36		Negative	Negative	Negative
37	German Shepherd	Negative	Negative	Negative
38		Negative	Negative	Negative
39		Negative	Negative	Negative
40		Negative	Negative	Negative
41		Negative	Negative	Negative
42		Negative	Negative	Negative
43		Negative	Positive	Negative
44		Negative	Negative	Negative
45		Negative	Negative	Negative
46		Negative	Negative	Negative
47		Negative	Negative	Negative
48		Negative	Positive	Negative
49		Negative	Positive	Negative
50		Negative	Negative	Negative
51		Negative	Negative	Negative
52		Negative	Negative	Negative
53		Negative	Positive	Negative
54		Negative	Negative	Negative
55		Negative	Negative	Negative
56		Negative	Negative	Negative
57		Negative	Negative	Negative
58		Negative	Negative	Negative
59		Negative	Negative	Negative
60		Negative	Negative	Negative
61		Negative	Negative	Negative
62		Negative	Negative	Negative
63		Negative	Negative	Negative
64		Negative	Negative	Negative
65		Negative	Negative	Negative
66		Negative	Negative	Negative
67		Negative	Positive	positive
68		Negative	Negative	Negative
69		Negative	Negative	Negative
70		Negative	Positive	Negative

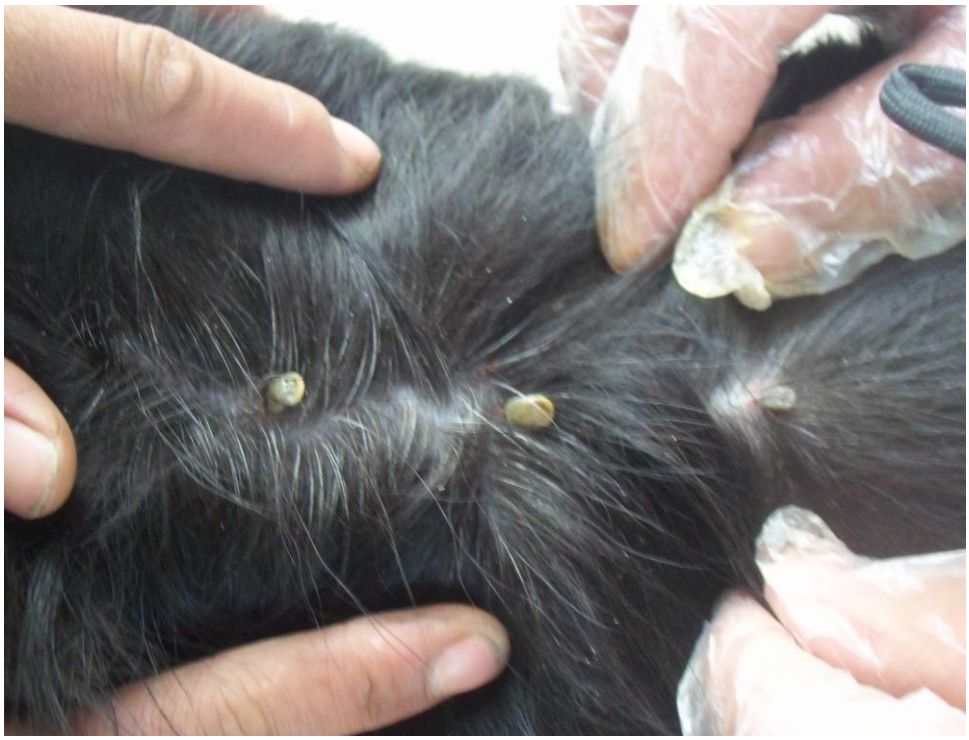

Photo (1): Dog infested with ticks.

Photo (2): *Rhipicephalus sanguineus* engorged female

DISCUSSION

V. Discussion

Lyme borreliosis in dogs is a systemic, multiorgan disease caused by spirochetes *Borrelia burgdorferi* belonging to family *Spiorchetaceae* **(Font *et al.*, 1992).** The aim of the current study was to determine the occurance of *Borrelia burgdorferi* among dogs, ticks and human contacts collected from different sections in the general department of K9.

No spiral shape bacterium was found in any blood smear of the examined dog. These results owed to that blood samples were taken randomly after the spirochetemia stage. Spirochetemia of the blood is a temporary phenomenon, and therefore a high level of detection is possible in a short period of primary infection only **(Coburn *et al.*, 1993; Goodman *et al.*, 1995).**

Borrelia burgdorferi could not be recovered from all the given samples by culturing on BSK-H medium **(Tables 3, 4 and 5)**, and this is could be due to the low sensitivity of culturing **(Schmidt, 1997; Adham *et al.*, 2010)**. Furthermore, by most conventional bacteriologic standards, borrelial cultures are more labor-intensive, more expensive, and much slower, requiring up to 12 weeks of incubation before being considered negative. The rapidity of identifying a positive culture, however, is directly dependent on the frequency with which the culture supernatant is examined microscopically, since macroscopic changes in the appearance of the culture medium tend to occur later, if at all.

Results in **Table (3)** showed that from the 70 examined dogs included in the trial, only 3 dogs showed positive results by PCR.

Clinical signs did not correlate always with the presence of positive results .The common findings included locomotory system disorders with clinical manifestations of chronic arthritis. Data on **(Table 5)** showed that 6.3% (one out of 16) of the Malinoa dogs are confirmed to be infected with *B. burgdorferi* by PCR, while 3.7% (2 out of 53) of the German Shepherd dogs were infected.

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 23% in canine cases at the veterinary clinics of police, the general department of K9 in Egypt, while seroprevelance in contact human was (20%). These higher results than that of PCR may be due to a possibility of cross reactions between *B. burgdorferi* and other bacteria, especially non-pathogenic spirochetes of *Borrelia* and *Leptospira* **(Dumler, 2001).** Moreover, dogs may remain immunologically active and this resulted in maintenance of positive antibody titers but not indicated presence of live *B. burgdorferi*. A correct timing of serologic examinations is important in order to determine whether active or past infection is responsible for the seropositivity. Early serodiagnostic results are usually negative because the immune response to borreliae develops gradually. Antiborrelial antibodies IgM are produced one to two weeks after the infection **(Hovius *et al.*, 1999a),** correlate with the onset of the clinical illness, and remain elevated for two months **(Greene *et al.*, 2006)**. In all three of our dog case studies, the immune response and PCR findings indicated an early disseminated stage of borrelial infection.

The occurance of *B. burgdorferi* infection in dogs is closely related to the tick density in a given area, the rate of tick infection and

outdoor or indoor habits of dogs. Also, to the sensitivity and specificity of the surveillance method employed **(Lindenmayer *et al.*, 1990)**.

The results of this study indicated an adjusted prevalence to *B burgdorferi* of 4.2% in canine cases at veterinary clinics of police, the general department of K9. Since there is no vaccination system against Lyme disease included in current immunization programs for dogs in Egypt, the results of this research will not be affected by the presence of false vaccine antigen.

Furthermore, the prevalence in this study is the first record of *B burgdorferi* in dogs in Egypt tested by PCR. Absence of previous records for *B burgdorferi* in Egypt may be due to the fact that the known vectors for this spirochete, *I scapularis, I pacificus, Dermacentor variabilis*, and *Amblyomma americanum* in North America, have not been found in Egypt. The only species of tick found in Egypt is *R sanguineus*, which has not been considered as a vector of borreliosis in other regions of the world but this was in agreement with **(Tinoco-Gracia *et al.*, 2008)** in Mexico as *R sanguineus* was the only ticks present on the area.

Lyme disease in dogs has been reported in several countries, with an occurance ranging from very low to 0.9 % in Mexico (**Galaviz-Silva *et al.*, 2013)**, 6.74% in Brazil **(Montandon *et al.*, 2014)**, 1.85% in Canada **(Gary *et al.*, 2006)**, 13/54.1% in Poland **(Skotarczak *et al.*, 2005)**, 2.9% in Finland **(Pérez *et al.*, 2014)**, 3.75 % in Poland **(Krämer *et al.*, 2014)**, 2.4% in Russia **(Volgina *et al.*, 2013)**, 23% in NY USA **(Wagner and Erb 2012)**, 0.2% -0.5% in Portugal **(Cardoso *et al.*, 2012)**, 30.1% in Germany **(Menn *et al.*, 2010)**, 6.8% in Mexico **(Tinoco-**

Gracia *et al.*, 2008), 9%-43% in Minnesota USA **(Beall *et al.*, 2008)**, 6.5% in the Czech Republic **(Pejchalová *et al.*, 2006)**, 40.2% in Poland **(Skotarczak *et al.*, 2005)**, 38.6% **(Skarda 2005)**, 9.7% **(Joppert *et al.*, 2001)** in São Paulo, Brazil, 0-40% in Wisconsin and northern Illinois USA **(Guerra *et al.*, 2001)**, 5% in Croatia **(Turk *et al.*, 2000)**, 50% in eastern Slovakia **(Stefançíková *et al.*, 1998)**, 7.2% **(Wittenbrink *et al.*, 1996)**, 6.5% to 85.2% in USA **(Falco *et al.*, 1993)**. While not detected in Turkey **(Icen *et al*., 2011).**

Table (6): Incidence of *Borrelia burgdorferi* in canine serum and ticks.

Country	Reference	Canine	Ticks
Mexico	(**Galaviz-Silva *et al.*, 2013**)	0.9 %	-
	(Tinoco-Gracia *et al.*, 2008)	6.8%	-
Brazil	(**Montandon *et al.*, 2014**)	6.74%	-
	(Joppert *et al.*, 2001)	9.7%	-
Canada	**(Gary *et al.*, 2006**)	1.85%	-

	(Banerjee *et al.*, 2000)	-	5.8%
Poland	**(Skotarczak *et al.*, 2005)**	13/54.1%	-
	(Krämer *et al.*, 2014)	3.75 %	-
	(Zygner *et al.*, 2008)	-	6.2%
	(Skotarczak 2000)	-	8.8%
	(Skotarczak *et al.*, 2005)	40.2%	-
Finland	**(Pérez *et al.*, 2014)**	2.9%	-
Russia	**(Volgina *et al.*, 2013)**	2.4%	-
USA NY	**(Wagner and Erb, 2012)**	23%	-

Minnesota **Wisconsin**	**(Beall *et al.*, 2008)**	9%-43%	-
	(Guerra *et al.*, 2001)	0-40%	-
	(Falco *et al.*, 1993)	6.5% to 85.2%	-
Portugal	**(Cardoso *et al.*, 2012)**	0.2% -0.5%	-
Germany	**(Menn *et al.*, 2010)**	30.1%	-
the Czech Republic	**(Pejchalová *et al.*, 2006)**	6.5%	-
Croatia	**(Turk *et al.*, 2000)**	5%	-
Slovakia	**(Stefançíková *et al.*, 1998)**	50%	-
Turkey	**(Icen *et al.*, 2011)**	0	-

UK	**(Smith *et al.*, 2012)**	-	0.5%
Japan	(**Hiraoka *et al.*, 2007)**	-	0.17%
Netherlands	**(Schouls *et al.*, 1999)**	-	13%
	(Hovius *et al.*, 1998)	-	14.5%

Incidence of Borrelia burgdorferi in ticks from dogs varied from 0.5% **Smith *et al.*, (2012)** in UK, 6.2% **Zygner *et al.*, (2008)** in central Poland, 0.17% **Hiraoka *et al.*, (2007)** in Japan, 8.8% **Skotarczak (2000)** in Poland, 5.8% **Banerjee *et al.*, (2000)** in southern Ontario, 13% **Schouls *et al.*, (1999)** in Netherlands, 14.5%**Hovius *et al.*, (1998)** in Netherlands.

Weather is a critical factor in the prevalence of the disease. Transmission depends on the intermediate host, which have certain climate requirement. Hot weather and suitable temperatures are necessary for development of ticks. In this study the lower prevalence of vector borne disease in dogs that live in such area can be attributed to less opportunity for exposure to the ticks due to tick control programs employed by the municipalities.

CONCLUSION AND RECOMMENDATIONS

VII. Conclusion and Recommendations

From the achieved results the following can be concluded:

- The tropical climate of our region favors the development of the brown dog- tick (*Rhipicephalus sanguineus*)
- The existence of dogs infected with *B. burgdorfei* in Egypt. The prevalence of canine Lyme disease was 4.2% by PCR.
- *Rhipicephalus sanguineus* are the only ticks present on the dogs with incidence of *B. burgdorfei* of 6.6%.
- This study confirms the existence of *B burgdorferi* infection in dogs in an area where the only identified tick is *R sanguineus*.
- *B. burgdorfei* could not detected in serum of contact humans by PCR.
- No spiral shape bacterium was found in any blood smear of the examined dog. That correlated to the spirochetemia of the blood is a temporary phenomenon.
- PCR has the advantage that it is extremely sensitive and specific. However, unless additional modifications are implemented into the detection protocol, the technique does not allow the differentiation between life and dead organisms. Furthermore Egypt does not apply a vaccination program against *B. burgdorfei* thus there is no result interference between life and dead bacteria.

- Clinical signs of Lyme disease in dogs are fever, acute arthritis, arthralgia, lameness, and nephritis in some cases. Central nervous system involvement, heart block, and uveitis are less frequently reported in dogs.

Based on the obtained results in the current study, the following are recommended:

- Avoiding tick infested locations in endemic areas, and preventing tick bites with insect repellents. Dogs should be checked frequently for ticks and remove them as soon as possible; gloves are recommended during tick removal.
- Incineration of ticks locations, hidden areas and cracks.
- Every effort should make to keep dogs free from tick infestation.

SUMMARY

VI. Summary

A total of 100 samples of (70 dog blood samples, 15 human blood samples, 15 hard ticks) collected from trained athletics dogs, belonging to dogs guarding section, transportation unit, K9, ministry of Interior Affair (MIA) were used to study the prevalence of *Borrelia burgdorferi*. All animals were imported and native purebred German shepherd dogs and of various age ranges (1-10 years old) of both sexes. Blood films were prepared, stained with Giemsa stain and examined for at least 100 oil immersion fields (OIFs), for detection of helically shaped *Borellia burgdorferi*. No spiral shape bacterium were found in any blood smear of the examined dog but morulae of *Ehrlichia canis, Babesia and trypanosome* were found in examined slides.

Borrelia burgdorferi could not be recovered from all the given samples by culturing on BSK-H medium, while seroprevalence to *B burgdorferi* of 23% in canine patients, while seroprevelance in contact human were (20%).

DNA was extracted from both blood samples of dogs and human and ticks for detection of *Borrelia burgdorferi* DNA by PCR. After amplification each PCR products was screened of by agarose gel electrophoresis and then examined under short wave UV transilluminator. The gel was photographed in order to obtain a permanent record using digital camera (Acer CR-5130, China).

The results observed revealed that the gene of *osp*A (309 bp)

was present only in four extracted DNA from different samples(human blood ,dog blood and hard ticks). Three dogs samples were positive with prevalence to *B burgdorferi* of 4.2% in canine patients, only one tick sample was positive by PCR with 6.6% incidence in ticks. However, *B burgdorferi* not detected in contact human blood.

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ARABIC SUMMARY

الملخص العربـــى

في هذه الدراسه تم بجميع مائه عينة دم (٧٠ عينه دم من الكلاب ، ١٥ عينة دم الإنسان و ١٥من القراد الصلب) عينات الكلاب تم جمعها من كلاب مدربة رياضيه ينتمون إلى كلاب حراسة وحدة النقل ، K9 ، وزارة الداخلية (MIA) لدراسة انتشار البورليا برجدورفيري . وقد تم استيراد جميع الحيوانات من الفصيله الأصيلة كلاب الراعي الألمانية و هي من الفئات العمرية المختلفة (من سنه الي عشره سنوات) من كلا الجنسين . تم إعداد أفلام الدم ، وصبغها بصبغه الغيمزا وفحصها تحت الميكروسكوب ، للكشف عن وجود الشكل الحلزوني للبورليابرجدورفيري. لم يتم العثور على الشكل الحلزوني للبكتيريا في أي لطخة دم للكلاب تحت الدراسه.

لم يتم الاستدلال على البورليا برجدورفيري في جميع العينات التي قد تم زراعتها على BSK -H ، في حين أن الانتشار المصلي للبورليا برجدورفيري في الكلاب المرضى٢٣ ٪ ، كانت نسبته في البشر المتصلين (٢٠ ٪) .

تم استخراج الحمض النووي من كل من عينات دم من الكلاب و الانسان و القراد للكشف عن وجود البورليا برجدورفيري بواسطة PCR . بعد التضخيم تم عرض كل منتجات PCR من قبل agarose gel electrophoresis ومن ثم فحصها تحت موجة قصيرة للأشعة فوق البنفسجية . تم تصوير الجل من أجل الحصول على السجل الدائم باستخدام كاميرا رقمية.

وكشفت النتائج أن هذا الجين من *ospA* كان موجودا فقط في أربع عينات من الحمض النووي المستخرج من عينات مختلفة (الدم البشري ، والكلب الدم و القراد الصلب) . وكانت ثلاثة عينات كلاب إيجابية بنسبه انتشار٤,٢٪ في الكلاب ، وكانت عينة واحدة فقط أيجابية باستخدام PCR بنسبه ٦,٦ ٪ في القراد. ومع ذلك ، الحمض النووي للبورليا برجدورفيري لم يتم الكشف عنه في الدم البشري .

الملخص العربي