Borreliosis

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Cláudio Mafra and Carlos Emmanuel Montandon

Abstract

Bacteria of the genus *Borrelia* are responsible for causing numerous diseases that can affect humans and also animals. Among these diseases we have Lyme disease and relapsing fever. Lyme disease, whose etiological agents are bacteria of the *Borrelia burgdorferi* s.l., is considered a major emerging zoonosis, especially in the northern hemisphere. Very important in the past, but now with a reduced number of accounts, due to a decrease in its vector, the louse, relapsing fever is caused by at least 15 different *Borrelia* species. Several other borreliosis affect animals and deserve significant attention. Avian and bovine borreliosis can be cited as examples of these. Currently, the main method of control and prophylaxis of these diseases is the control of their vectors, since there are no vaccines for them. Epidemiological studies are also of great value for the control of borreliosis, since they allow monitoring the endemic areas for these ills.

Keywords

Borreliosis • *Borrelia* • Lyme disease • *Ixodes*

13.1 Introduction

Borreliosis are diseases caused by the *Borrelia* genus of microorganisms, which are bacteria of the Borreliaceae family. Among the infections brought by these organisms, Lyme disease is prominent, characterized by being an infectious multisystemic disorder affecting humans and also wild and domestic animals (Fonseca et al. 2005), with skin, joint, neurological, and heart problems. It is the main disease caused by the *Borrelia burgdorferi* s.l., which comprises a

C. Mafra (✉) • C.E. Montandon
Laboratory of Parasitology and Molecular Epidemiology, Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, MG, Brazil
e-mail: mafra@ufv.br; em.carlos1984@gmail.com

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group of at least 20 species (Clark et al. 2014). Lyme disease is considered an emerging zoonosis of great importance in the USA and Europe, being transmitted to humans by the bite of *Ixodes* ticks (Yoshinari 2009). *Borrelia* organisms are also responsible for many other diseases, among which can be cited: Brazilian Lyme-*simile* disease (Yoshinari et al. 2003), relapsing fever (El-Bahnsawy et al. 2012), avian borreliosis (Saif 2013), bovine borreliosis (Smith et al. 1978), and bovine enzootic abortion (Teglas et al. 2011).

13.2 History

Analyzing the history of borreliosis, when it comes to Lyme disease, although emerging, can be affirmed that it is certainly very old. The first borreliosis symptom reports were described by Buchwald in 1883 in Germany and by Marcus in 1910, demonstrating the relationship between these symptoms and the bite of *Ixodes* genus ticks. In 1922, the neurologists Garin, Bujadoux, and Bannwarth described a meningopolyradiculoneuritis frame, later known as Bujadoux-Garin-Bannwarth syndrome, now considered a typical form of neurologic manifestation of Lyme disease. Finally, in 1976, the disease was identified in the region of Lyme, Connecticut, USA, where several cases of acute arthritis in adolescents led to evidence of pathology, thereby leading it to be called Lyme disease[1]. Later, in 1981, Burgdorfer and colleagues isolated an unknown spirochete in *Ixodes scapularis* tick specimens collected in this region (Burgdorfer et al. 1982). Reviewing smears of *Ixodes ricinus* gut stained with Giemsa, listed in Switzerland, Burgdorfer showed similar spirochetes previously identified in *I. scapularis* ticks (Burgdorfer et al. 1983). These structures were also observed in skin lesions and blood samples obtained from patients, providing strong evidence that such a body was the causative agent of Lyme disease (Steere et al. 1983).

The demonstration about the success in growing spirochetes from material collected from the gut of the tick, designated as strain B31 (Barbour 1984) in Barbour-Stoenner-Kelly (BSK) medium, initiated intensive research on Lyme disease. After studies, the B31 strain was characterized and named *B. burgdorferi* s.s.

In the following years, several cases were reported in mainland Europe and Asia where *B. garinii* and *B. afzelii* spirochetes were described as etiological agents in these regions. In South America, positive serology results against bacteria of the *Borrelia* genus have been reported in humans at Argentina (Stanchi and Balague 1993) and Bolivia (Ciceroni et al. 1994).

In Brazil, the first reports of Brazilian Lyme-*simile* disease were through skin manifestations related to tick bites that occurred in 1987 in the city of Manaus, Amazonas State, during the XLII Brazilian Congress of Dermatology. In 1992, Yoshinari et al. described cases of patients with extracutaneous manifestations and positive serology using enzyme-linked immunosorbent assay (ELISA) and Western blotting targeting *B. burgdorferi* s.s. New cases of patients showing cutaneous involvement and positive serology results were also reported in São Paulo. The isolation and characterization of the causative agent were not possible for these cases (Yoshinari et al. 1993). More recently, in 2014, the presence of antibodies against *B. burgdorferi* s.s. was evidenced by ELISA in wild and domestic animals in the state of Minas Gerais, Brazil (Montandon et al. 2014).

A borreliosis described since the time of Hippocrates, relapsing fever, named after an outbreak in Edinburgh between the years 1843 and 1848, had its etiology described by Otto Obermeier in 1873. Since then many studies have been done. However, after the outbreak occurred in 1977 in the city of Lyme, an increasing number of studies on Lyme disease have been initiated, while relapsing fever has been largely forgotten and is currently regarded as a neglected tropical disease infection (Cutler 2009).

Avian borreliosis, in turn, was first described in an extensive outbreak among geese in Russia. A few years later in Brazil, Marchoux and Salimbeni incriminated *Argas* ticks as natural vectors of the disease, which was later confirmed

[1] See Chap. 3 for more information on the history of Lyme disease (CBM).

by several authors around the world. *Borrelia theileri*, the etiologic agent of bovine borreliosis, was identified over 100 years ago in South Africa and named in honor of its discoverer Arnold Theiler. This species is considered the least characterized *Borrelia* organism transmitted by ticks. Historically, these bacteria have been described in Africa, Australia, South America, and North America (McCoy et al. 2014). In the case of bovine enzootic abortion, it is believed that it was first reported in 1923 and later described in 1956. Subsequent occurrences of this disease were reported in parts of California (Hall et al. 2002).

13.3 Characterization

Borrelia organisms are bacteria belonging to the order Spirochaetales, Borreliaceae family, accounting for many different diseases that affect both men and animals (Fonseca et al. 2005). Among these diseases can be cited:

- Lyme disease
- Brazilian Lyme-*simile* disease, a variant of Lyme disease found in Brazil, transmitted by *Amblyomma sculptum* (*A. cajennense* complex) ticks (Yoshinari et al. 2003)
- Relapsing fever caused by *Borrelia recurrentis* when transmitted by lice and more than 20 species of the *Borrelia* genus transmitted by ticks (El-Bahnsawy et al. 2012)
- Avian borreliosis, whose etiologic agent is *Borrelia anserina*, causing anemia, fever, apathy, and high morbidity rates in birds (Saif 2013)
- Bovine borreliosis, caused by *B. theileri*, which determines a discrete process that generates anemia in ruminants and horses, being of low pathogenic action (Smith et al. 1978)
- Bovine enzootic abortion, a disease that can affect cattle, deer, and, occasionally, humans, caused by *Borrelia coriaceae* (Teglas et al. 2011)

Different species of the *Borrelia* genus can cause Lyme disease, constituting the *B. burgdorferi* s.l. group, with at least 20 species, including three with the greatest epidemiological importance, namely, *B. burgdorferi* s.s., *B. garinii*, and *Borrelia afzelii*. In the USA, *B. burgdorferi* s.s. is the main cause of Lyme disease, with *B. garinii* and *B. afzelii* incriminated as causative agents of disease in both Europe and Asia (Clark et al. 2014) (Fig. 13.1).

The *Borrelia* genus has a spiral shape without a rigid cell wall, with dimensions varying from 10 to 30 μm in length and 0.2–0.3 μm in diameter, having from 7 to 11 flagella on each end that confer mobility on these microorganisms. *Borrelia* organisms have a small linear chromosome, with up to 21 linear and circular plasmids. Its flagella

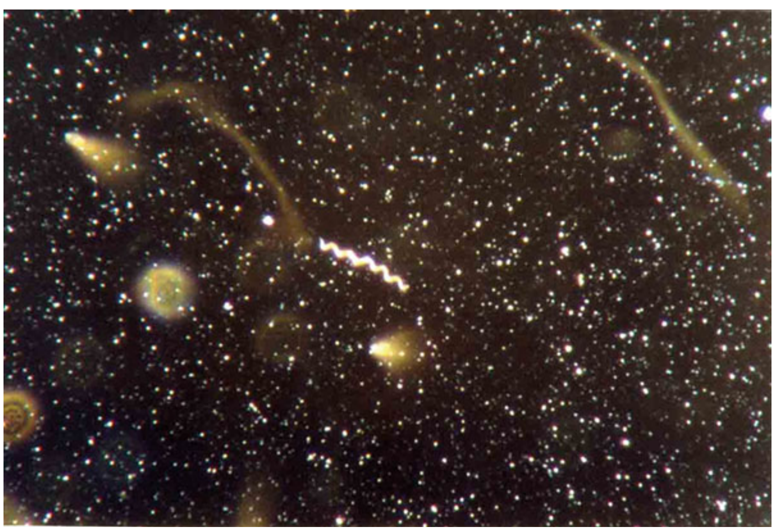

Fig. 13.1 A single *Borrelia* spirochete in vitro culture (Courtesy of Dr. Natalino Hajime Yoshinari)

have basically two types of flagellin (Fla), a smaller form, FlaA, 38 kDa, and another, larger form, FlaB, 41 kDa; both are conserved proteins in species of the *B. burgdorferi* s.l. complex. When cultured in vitro, borrelia organisms lose their flagella and spiral shape.

The composition of the *Borrelia* cell envelope is similar to that found in Gram-negative bacteria, highlighting as differences the absence of lipopolysaccharides and the abundance of lipoproteins in the outer membrane. Phosphatidylcholine, phosphatidylglycerol, and two atypical glycolipids: 1-*O*-palmitoyl-2-O-oleoyl-3-*O*-α-D-galactopiranosilglicerol and cholesteryl-6-O-palmitoyl-β-D-galactopyranoside, capable of stimulating the production of specific antibodies in rats, are included among the lipids found in *Borrelia* membranes. Cross-reactivity between these antibodies and the ganglioside component in neuron membranes is suggested as a possible cause for the neurological disorders observed in advanced cases of Lyme disease (Hossain et al. 2001).

Regarding genetic factors, take the example of the *B. burgdorferi* s.s. genome, a highly complex one with approximately 8 % of all open reading frames (ORFs) encoding lipoproteins and 6 % dedicated to motility and chemotaxis. Being fully sequenced, the *B. burgdorferi* B31 strain is composed of a small linear chromosome, with approximately 1 Mpb and 21 plasmids (12 circular and 9 linear) corresponding to 40 % of the total DNA bacteria. The main aspect of the *B. burgdorferi* s.s. genome is the large number of lipoprotein-coding sequences, including surface outer membrane proteins (outer membrane protein—Osp), which are subdivided into OspA to OSPF (Fraser et al. 1997). In contrast, this genome also encodes some enzymes required for few amino acid, fatty acids, nucleotide and biosynthetic pathways, and cofactors, explaining the dependency in relation to this spirochete on biomolecules being produced by the host cells. It was also found that the *B. burgdorferi* s.s. genome does not encode toxins to the host cells, with this extracellular pathogen causing infection due to its migration through the tissues, adhering to cells, and dodging the immune defenses (Steere et al. 2004).

With respect to molecular markers, the gene for the β-subunit of RNA polymerase (rpoB) is worth mentioning which is highly conserved in the *Borreliaceae* family, and the 16S-23S spacer of ribosomal DNA (rDNA) that, unlike rpoB, is quite divergent across *B. burgdorferi* s.l. complex species, allowing better differentiation between closely related species. Nevertheless, until now, using this gene for the detection of *Borrelia* genus bacteria in environmental samples has not generated satisfactory results, since this region has a size greater than 3 kb, which hampers its amplification by chain reaction polymerase (PCR), a limitation due to the low processivity of the Taq DNA polymerase enzyme (Lee et al. 2010).

13.4 Biology

Borrelia organisms are primarily transmitted by ticks although, experimentally and in rare cases, they can be transmitted by horse flies, mosquitoes, and fleas (Magnarelli et al. 1987). Within the *Spirochaetia* class, covering the *Borrelia*, *Leptospira*, and *Treponema* genera (Cavalier-Smith 2002), only borrelias are transmitted by blood-sucking ectoparasites.

In ticks, borrelia organisms usually develop as symbionts, acting as parasites when they infect humans and other animals (Hoogstraal 1985). Silva and Fikrig (1997) reported that in successive infections by *Borrelia* without involvement of the tick vector, there was a decrease in its pathogenicity, with the sample becoming apathogenic. A biochemical dependency was also found between *Borrelia* and their spirochete vectors, which occurs mainly in the intestinal tract of the tick during the development and multiplication of these organisms (Hoogstraal 1985). This phenomenon, as well as the chemotaxis of specific sites for spirochete in the digestive tract of the tick, is associated with the activation of genes responsible for certain phases of the *Borrelia* life cycle (Silva and Fikrig 1997).

The growth and multiplication of *Borrelia* in ticks are also influenced by the life cycle of this arthropod, with many spirochetes dying soon after the vector molts to the next stage and with the tick succumbing due to injuries to their internal organs caused by an excessive number of spirochetes (Smith et al. 1978).

In vector mode, *Borrelia* transmission by ticks may be transovarian or transstadial. In these *Borrelia* species, transmission occurs primarily by the transovarian route, being well characterized in *Argas* ticks with *B. anserine*. However, it is important to note that in these ticks, the transmission is intrinsically related to the strain, physiographic region, *Borrelia* species, and association with other pathogens (Hoogstraal 1985). Ixodid ticks can support both transmission modes (Smith et al. 1978), and this phenomenon is better understood in Lyme disease, with *I. scapularis* and other species of *Ixodes* vectors of *B. burgdorferi* s.l. (Burgdorfer et al. 1985) (Fig. 13.2).

For the host infection, the tick's fixing time is considered a highly relevant factor for *Borrelia* transmission efficiency, with some studies showing that a time of attachment longer than 48 h is necessary (Piesman et al. 1987). For ticks this is not a relevant parameter for transmission of the pathogen, since transmission to representatives of this family takes place both by saliva and coxal liquid, except at the larval stage that realizes a blood meal in a few minutes (Hoogstraal 1985).

Ticks have the potential to transmit almost all borrelias. In the five borrelia groups currently recognized, all are transmitted by ticks, with each one having a specific kind of spirochete and arthropod vector. In the Northern US and Europe, spirochete transmission of the *B. burgdorferi* s.l. group causing Lyme disease occurs mainly by the *Ixodes* tick genus. In warmer regions, such as in the Southern US, cases of Lyme disease are associated with infection by *B. lonestari*, which is transmitted by *Amblyomma americanum* ticks (Barbour et al. 1996). In South America, *Amblyomma* ticks are responsible for the transmission of *Borrelia* organisms (Yoshinari 2009).

As demonstrated in epidemiological studies, the principal natural reservoirs of *Borrelia* found in America, Europe, Asia, and South America are marsupials (opossum), rodents (bush rat, agouti, and capybara), insectivores (hedgehog), canids, equines, cattle, and deer (Costa et al. 2002).

Fig. 13.2 *Ixodes scapularis* (female) (Courtesy of Dr. Jose M. Venzal)

13.5 Clinical signs

Clinical signs in animals with borreliosis, including humans, may vary according to the species of bacteria causing the disease. Among the first clinical signs of Lyme disease, skin lesions called erythema migrans, which expand from the site of the tick bite, appear 8–9 days after the bite in the classic cases (Fonseca et al. 2005). In Brazil, this lesion appears, on average, 30 days after exposure to ticks (Yoshinari et al. 2000). Although erythema is a unique feature of the disease, it is not present in all cases, manifesting in 70 % of American patients (Fonseca et al. 2005) (Fig. 13.3).

The classical erythema migrans lesion is characterized in its early stage by presenting a ring-shaped macula that is reddish, in various shades, measuring between 0.5 and 2 cm, which may arise in the tick bite site with a papule that

Fig. 13.3 Erythema migrans lesion showing typical lesion characteristics in syndrome of Baggio-Yoshinari (Courtesy of Dr. Natalino Hajime Yoshinari)

corresponds to the center of the lesion (Berger et al. 1985). It slowly progresses as centrifuge dispersion, rapidly expanding and assuming, in a short period of time, a plate appearance. Typically, about 14 days after the tick bite, the erythema can reach a diameter greater than 15 cm, together with other morphological changes, such as an oval or elongated shape (Steere et al. 1983). Generally the lesion is seen in isolation; however, various injuries can occur due to the dispersion of bacteria through the vascular and lymphatic system, receiving the designation of secondary annular lesions (Berger 1989). *Borrelia burgdorferi* s.l. has been found in biopsies of lesions, suggesting that expansion of the injury is also determined by migration of the spirochetes through the subcutaneous tissue (Malane et al. 1991).

In studies of lesion biopsies, the main histopathological findings are proliferation and dilation of blood vessels with vasculitis, displaying primary infiltrates of lymphocytes associated with plasma cells. With the evolution of the erythema migrans, there is a reduction in the inflammatory process, with pronounced atrophy of the epidermis and dermis.

In addition to lymphocytes observed in the infiltrate in the site of the tick bite, the presence of macrophages, mast cells, and neutrophils, as well as a small number of eosinophils, also is observed (Koning and Hoogkamp-Korstanje 1986). According to Berger (1989), in cases where only a single lesion like erythema migrans occurs, it is considered as a localized form with milder symptoms. When multiple injuries are involved including symptoms of greater intensity, erythema is considered widespread. The distinction between these two presentations is important because in individuals with a localized lesion the response to antibiotics is positive, demanding long therapy, sometimes requiring repetitions, with a poor prognosis in relation to ultimate cure in patients with widespread erythema migrans.

The main extracutaneous symptoms observed in patients with Lyme disease are similar to those of a common cold, among which are fever, fatigue, musculoskeletal discomfort, and headache (Berger 1989).

Secondary manifestations may come to pass, with involvement of the nervous system manifesting as diffuse neuropathy or local radiculopathy or, less frequently, by meningitis or encephalitis. These neurological complications are often associated with infections caused by *B. garinii*, predominately in Europe. Cranial neuritis may also be observed, resulting in hearing loss, facial paralysis, and optic neuritis (Halperin 1997), as well as intraocular inflammation caused by infection with *Borrelia* (Flach and Lavoie 1990). Among the neurological manifestations associated with Lyme disease, several authors have suggested the infection's association with *Borrelia* neurodegenerative disorders such as Alzheimer's disease (MacDonald 2006). These secondary manifestations may recur, lasting months, or become chronic (Yoshinari et al. 2000).

Another recurring symptom of Lyme disease is European atrophying chronic acrodermatitis, which, as its name implies, occurs in the chronic phase of the disease. The characteristic of elderly people, the formation of a red and blue edema, can be observed, located in the feet or lower legs, progressing to an atrophic wrinkled lesion, allowing it show to through subcutaneous vessels (Berger 1989).

Another situation that intrigues researchers and doctors is whether *B. burgdorferi* s.s. persists after conventional antimicrobial treatments. In recent studies of patients with consecutive episodes of erythema migrans, some strains of *B. burgdorferi* s.s. demonstrated that they are distinct based on their genotypes, indicating the occurrence of reinfection (Nadelman et al. 2012; Shapiro 2015). Recently, success was obtained in cultivating strains of *B. burgdorferi* s.s. found in ticks fed on patients treated with antibiotics, showing the presence of bacteria even after conventional treatment, demonstrating both the possibility of reinfection and the persistence of *B. burgdorferi* in patients who have already had or maintained Lyme disease (Shapiro 2015).

Regarding the symptoms, the Brazilian Lyme-*simile* disease displays the same symptoms of Lyme disease observed in the northern hemisphere, such as erythema migrans, cardiac involvement, and cases of neurological complications, including associations with meningitis, facial palsy, and bilateral sudden deafness (Costa et al. 1996), differing mainly from Lyme disease recurrence in terms the symptoms and development of autoimmune disorders. It is noteworthy that, unlike Lyme disease, where ticks the *I. ricinus* complex are seen as the main vectors in the northern hemisphere, Brazilian Lyme-*simile* disease cases are associated with the bite of *A. cajennense* ticks (Fonseca et al. 2005).

Symptoms of relapsing fever differ on some points when transmitted by ticks or lice. When transmitted by ticks, it is characterized by presenting an initial episode of incessant fever, which lasts 3–6 days. When the transmission is due to lice, multiple febrile episodes may take 1–3 days each. However, in both situations an incubation interval occurs between 4 and 14 days, with fever lasting from several hours to 4 days, accompanied by chills, headache, nausea, vomiting, sweating, abdominal pain, arthralgia, and cough. Acute respiratory distress syndrome and specific neurological symptoms (hemiplegia, facial paralysis, myelitis, and radiculopathy) may occur in the disease transmitted by ticks, being a point of distinction between these two forms (El-Bahnsawy et al. 2012).

The onset of borrelias in other animal species such as birds shows that the youngest animals apparently are more susceptible to *B. anserina*, depression with cyanosis in the head being the main symptom in the acute form, leading to mortality in up to 30 % of the herd. Birds affected by this disease also present body temperature beginning to rise just after the infection, with rapid weight loss, water consumption increase, and production of green excrement due to bile excess. In subacute and chronic cases, birds demonstrate weakness that ends in paralysis and death[2]. Pathological features include grossly enlarged spleen and stains due to subcapsular hemorrhage occurring at the site, being this the predominant lesion. In some cases focal necrotic hepatitis also occurs.

In cattle and other animals, *B. theileri* infection causes smooth and variable clinical signs, including fever and anemia (McCoy et al. 2014).

Bovine enzootic abortion lesions develop gradually over a period of 3 months. At the end of this period, the fetus may be aborted or born alive with serious complications, such as bleeding in the mucosa and petechial lesions in the conjunctiva and oral cavity, among other symptoms (Hall et al. 2002). The pathogenesis of this disease consists of a generalized inflammation with vascular lesions in most organs. In the brain vasculitis and granulomatous meningitis occur. The lung is affected by alveolar septal infiltration with mononuclear cells and interlobular septum, with granulomatous focal infiltration. Hepatic occurrence, although less specific, consists of a granulomatous infiltration with centrilobular congestion variable and atrophy of hepatic cord.

13.6 Diagnosis

The diagnosis of borreliosis in domestic animals, such as avian borreliosis and bovine enzootic abortion, in addition to serological and molecular tests, also is based on the characteristics of the macroscopic and histological lesions, as well as additional information from the herd history, such as exposure to tick vector.

[2]See Chap. 34 for more information on paralysis caused by ticks (CBM).

From a clinical point of view, diagnosis in the early stage of borreliosis, regardless of the etiological agent involved, is inaccurate due to the lack of specific symptoms. When it comes to Lyme disease, in the first 3 weeks of illness, patients have similar signs of a cold frame, associated with reddish cutaneous complications spreading throughout the body from the tick bite site. Only after a few months, when the disease moves to a chronic condition, the symptoms do become more characteristic, coming to show joint, heart, and neurological complications. Although the diagnosis of Lyme disease may be difficult and even unfeasible, it needs to be done as early as possible, including based on clinical aspects (Steere et al. 2004).

The initial laboratory diagnosis for borreliosis in animals, including man, is done on peripheral blood smears stained by Giemsa or Fontana methods, though causative agents can be detected only in cases of high spirochetemia. Due to this, the gold standard procedure for diagnosis of borreliosis in general is bacterium isolation by culture, followed by PCR confirmation or another specific test. This method is very costly, since the culture requires special materials such as the complex BSK medium and highly trained staff to perform the procedure, so it is not a routinely performed technique. In addition, these procedures are very time-consuming, taking between 2 and 6 weeks before they are useful to the clinical decision-making.

Another factor to be taken into consideration during the diagnosis of Lyme disease is the sensitivity of the described procedures, which are successful with between 40 and 70 % of erythema migrans cases, in only 3–17 % when using cerebrospinal fluid samples, and still further reduced in efficacy when using synovial fluid and other tissues. This is due to the requirement for a relatively complex culture medium for the cultivation of *Borrelia* in the laboratory, since these spirochetes do not possess genes encoding enzymes necessary for amino acid, fatty acid, and nucleotide biosynthetic pathways or nucleotide cofactors. This difficulty has also been found in *Borrelia* isolation from vector samples.

Therefore, applications that rely on immunological techniques such as ELISA or those based on molecular biology—PCR, nested PCR, and real-time PCR—are increasingly sought for early reliable diagnosis of borreliosis (Dunaj et al. 2013).

It is known, however, that serological confirmation of infection can be impaired by several factors, particularly where the possibility of coinfection by other spirochetes is possible, e.g., of the genus *Leptospira*, with the occurrence of cross-reactions. This occurs mainly when using whole-cell extracts of *Borrelia* as antigen for the reaction, resulting in false positives (Rogers et al. 1999). Another problem is the production of anti-*Borrelia* antibodies. Typically detected only in the first or second week after infection, specific IgM and IgG antibodies against *Borrelia* can persist for years, making it impossible to distinguish between past and recent infections in some patients (Steere et al. 2008).

The improvement of techniques based on molecular biology afforded the advent of more sensitive methods for the detection of DNA pathogens in environmental or human samples. In this field, PCR has proven a very promising technique due to its high sensitivity and specificity in the early diagnosis of borreliosis and also to confirm diagnoses obtained by other techniques. Introducing advantages over immunological techniques, PCR allows amplified sequences of the target DNA pathogen to be obtained, post-sequencing of which enables the performance of in silico analyses against other sequences stored in biological databases. Then, they can be compared at the molecular level with sequences from different species/strains of *Borrelia*, and phylogenetic relationships between them can be established. For the molecular detection of these bacteria, the choice of DNA extraction processes, primers, and reaction conditions should not differ from the standard, which should be aware when selecting the sequence to be amplified and the PCR method (Dunaj et al. 2013).

Different patterns of molecular marker sequences are accepted for phylogenetic analysis of *Borrelia* DNA sequences, among them some for Lyme disease such as fla, recA, 16S rDNA, p66, hbb, rpoB, intergenic spacers in the 23S and 5S genes, and plasmid carriers (OspA, OspB, and ospCVlsE) (Dunaj et al. 2013). In choosing the sequence to be amplified, one must also be assured that it is not homologous to genetic material from another organism, e.g., *Treponema*, *Leptospira*, or *Escherichia coli*, or the human or animal DNA being analyzed. Nested PCR has been shown to be a very sensitive tool for diagnosis through the detection of DNA pathogens, amplifying an internal sequence of a previously amplified fragment, which increases the sensitivity over traditional PCR.

13.7 Treatment

Symptoms and signs of clinical borreliosis are commonly treated with antibiotics to prevent manifestations of other symptoms. The classes of antibiotics that demonstrate greater efficacy against *Borrelia* are β-lactam, in particular cephalosporins, and, to a lesser degree, macrolides. Definition of the best treatment still generates much debate, since not all patients and species or strains of this pathogen respond significantly to the same base.

For the treatment of Lyme disease, based on the evidence available from randomized clinical trials, either more lenient or harsh recommendations have been published by the Infectious Diseases Society of America (IDSA) and the American Academy of Pediatrics, among other national and supranational medical associations, including some from Europe.

According to the IDSA, the therapy recommended against the main signal of Lyme disease, erythema migrans, and to stop evolution of the disease to more complex clinical situations is the following:

- Daily, during 14 days, two doses of 100 mg of doxycycline, orally, or 2 mg/kg of body weight for children; three daily doses of 500 mg of amoxycillin, for 14 days orally, or 50 mg/kg for children; two daily doses of 500 mg of amoxycillin, for 14 days orally, or 30 mg/kg of body weight for children.
- For the treatment of relapsing fever, experts recommend 500 mg of tetracycline, orally, four times/day, during 10 days, or 12.5 mg/kg of body weight for children. In cases where tetracycline is not effectively recommended, erythromycin can be used under the same conditions.

Borreliosis treatment in domestic animals is effective, being the use of oxytetracycline or chlortetracycline administered 1–2 mg/kg body weight recommended for avian borreliosis (Hampson and Swayne 2013). The treatment of bovine enzootic abortion consists in the administration of antimicrobial therapy to pregnant animal in order to save the calf, since the treatment does not have effect death of the newborn occurs within 48 h of birth.

13.8 Epidemiology

Among the borreliosis, Lyme disease is the one with the largest number of epidemiological studies due to its importance to public health. It is considered a bimodal disease, with a higher incidence in children aged 5–9 years and in adults older than 50 years, having no preference for gender, as in other immune-mediated diseases.

This disease has occurred almost exclusively in the northern hemisphere, with most of the confirmed cases from the USA and Europe, and to a lesser extent in Asia and North Africa. In the USA the situation is so critical that notification of Lyme disease has been obligatory since 1991, a growing and steady increase in the number of reported cases having been observed, ranging from 9908 cases in 1992 to a peak of 30,000 cases in 2009, coming to stabilize at a level of 25,000 cases. In the majority of European countries, Lyme disease is not a reportable disease, without until recently standardized case definitions.

Perhaps the incidence observed in the USA and Europe, restricting the majority of reported cases to the northern hemisphere, highlight the efficiency and scope of surveillance in these regions. In South America, there are many reports of this disease, with cases reported in Argentina (Stanchi and Balague 1993), Bolivia

(Ciceroni et al. 1994), and Brazil (Yoshinari 2009; Montandon et al. 2014).

Relapsing fever has been a global disease, remaining endemic in many parts of the world. Humans sporadically contract relapsing fever transmitted by ticks when entering natural environments infested with these vectors. Transmission by lice may generate an epidemic situation. Usually relapsing fever epidemics transmitted by lice occur in areas of overcrowding and poverty, e.g., as in situations caused by wars. Although the number of cases of relapsing fever seems to be falling, this could be due to inattention to this disease; reports of lice infestation resurgence among certain groups such as the homeless have been done, and it is the cause of the infection in a focus that occurred in Ethiopia (Cutler 2009).

Epidemiological studies of borreliosis in animals are still very meager, requiring more attention, since many of these cases have been reported, e.g., in poultry in Europe, North America, and Australia (Saif 2013).

13.9 Control and Prevention

Currently the most effective method available to prevent infection by bacteria of the genus *Borrelia* and other pathogens transmitted by ticks is to avoid exposure to this vector. However, in cases where exposure to these vectors is inevitable, procedures to mitigate the risk of infection are recommended, among which are included a thorough check of one's own body and the bodies of pets, with direct removal of posted ticks, and the use of protective clothing and repellents against ticks.

Antimicrobial prophylaxis for Lyme disease after a tick bite is generally not recommended due to the low rate of infection, even if the tick proves positive for *B. burgdorferi* s.s. (Shapiro et al. 1992). However, in randomized controlled trials, as performed by Nadelman et al. (2001), there was some effectiveness in preventing the development of erythema migrans with the use of a single dose of 200 mg of doxycycline within 72 h after removing *I. scapularis* ticks. IDSA recommends the preventive use of a single dose of doxycycline in the following situations: (I) the tick was fixed for a time equal to or greater than 36 h, being identified as *I. scapularis*; (II) the treatment can be administered within 72 h even after removal of the tick; (III) sites in which the rate of *Borrelia* infection in ticks is equal to or greater than 20 %; and (IV) the use of doxycycline is not a contraindicated drug for the concerned patient.

It needs to be considered that this drug is contraindicated for use in pregnant women and children up to 8 years of age. People who have removed ticks from themselves, even those who received antibiotic prophylaxis, should be carefully monitored for clinical signs and symptoms of the disease for up to 30 days, with attention mainly to the development of skin lesions due to tick bites, which may correspond to erythema migrans. Those who have signs of the disease should be advised to be examined by a doctor immediately, to have appropriate action taken regarding the diagnosis and possible treatment.

As verified with other diseases transmitted by ticks that have wild and domestic vertebrates as links in their transmission cycle, knowledge of the frequency of seropositives in wild and domestic animals is of great value in epidemiological studies of this zoonotical disease, considering the region, as some species animals may act as sentinels (Lemos et al. 2001). Data on the frequency of positive DNA *Borrelia* samples in ectoparasites thus become of extreme relevance to support the development of epidemiological surveillance strategies and the actions of health professionals in assessing geographical risk areas for the emergence of potential infections arising from agents carried by ticks (Montandon et al. 2014).

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