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Brazilian borreliosis with special emphasis on humans and horses

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ABSTRACT

Borreliosis caused by *Borrelia burgdorferi* sensu lato is a cosmopolitan zoonosis studied worldwide; it is called Lyme disease in many countries of the Northern Hemisphere and Lyme-like or Baggio-Yoshinari Syndrome in Brazil. However, despite the increasing number of suspect cases, this disease is still neglected in Brazil by the medical and veterinary communities. Brazilian Lyme-like borreliosis likely involves capybaras as reservoirs and *Amblyomma* and *Rhipicephalus* ticks as vectors. Thus, domestic animals can serve as key carriers in pathogen dissemination. This zoonosis has been little studied in horses in Brazil. The first survey was performed in the state of Rio de Janeiro, and this Brazilian Borreliosis exhibits many differences from the disease widely described in the Northern Hemisphere. The etiological agent shows different morphological and genetic characteristics, the disease has a higher recurrence rate after treatment with antibiotics, and the pathogen stimulates intense symptoms such as a broader immune response in humans. Additionally, the Brazilian zoonosis is not transmitted by the *Ixodes ricinus* complex. With respect to clinical manifestations, Baggio-Yoshinari Syndrome has been reported to cause neurological, cardiac, ophthalmic, muscle, and joint alterations in humans. These symptoms can possibly occur in horses. Here, we present a current panel of studies involving the disease in humans and equines, particularly in Brazil.

Lyme disease (LD) or Lyme borreliosis (LB) is the most common tick-borne disease in temperate regions of the Northern Hemisphere and is caused by the spirochete *Borrelia burgdorferi* sensu lato.[1] LD is a multistage disease that can affect multiple organs but in humans manifests predominantly in the skin, joints, and nervous system.[2]

History

In 1976, children in a geographical region of the United States, specifically near the town of Lyme, Connecticut, were affected by a mysterious syndrome[3] that was initially diagnosed as

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juvenile rheumatoid arthritis.[1] In 1981, the entomologist and physician Willy Burgdorfer, along with Alan Barbour and Jorge Benach, found a spirochete in the midgut of ticks of the genus *Ixodes* in an area of New York, a known endemic focus of LD. The researchers cultivated samples from ticks in a culture medium developed for growing the relapsing fever spirochete (*B. hermisii*) and found a new species of *Borrelia*, subsequently named *B. burgdorferi*.[4] Later, the same bacterium was isolated and cultivated from the blood of patients with LD.

The diseases termed Lyme borrelioses are known to be caused by a large number of species related to *B. burgdorferi*, which are called *B. burgdorferi* sensu lato.[5] Of the 34 existing *Borrelia* spp., 20 are referred to as *Borrelia burgdorferi* sensu lato and cause LD, which is transmitted by ticks of the genus *Ixodes*. Of these 20 species, only nine have been isolated from humans in the Northern Hemisphere (*B. afzelii*, *B. bavariensis*, *B. bissetti*, *B. burgdorferi* stricto sensu, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. spielmanii*, and *B. valaisiana*).[6]

The first isolation of *B. burgdorferi* sensu lato in the Southern Hemisphere was performed by Barbieri et al. in Uruguay from *I. pararicinus* ticks. Thereafter, the bacterium was also identified in Argentina[7] and Chile,[8,9] where it was named *B. chilensis*. All three isolations revealed the bacterium in ticks of the *I. ricinus* complex using amplification of the 16S ribosomal gene, 5S-23S intergenic spacer, and flagellin gene (*fla*) for species identification.

B. burgdorferi sensu lato is a highly invasive gram-negative spirochete. Its pathogenicity depends on its mobility, cytotoxicity, antigenic variability, lymphocyte stimulation, and resistance to complement activation in the absence of specific antibodies.[10]

Transmission

The pathogen is mainly transmitted by ticks of the *I. ricinus* complex.[11] However, there are reports of *B. burgdorferi* s.l. transmission by *Amblyomma americanum* in Florida and Georgia in the United States.[12] It has also been identified in *Dermacentor nitens* in the state of Paraná, Brazil.[13]

The bacteria can infect the tick when it feeds on an infected reservoir host.[11] After molting to the nymph stage, the ticks are able to transmit the pathogen to the animal from which it obtains its next blood meal. As transtadial transmission is not always successful, transmission of the bacteria is ensured by an enzootic cycle in which the tick feeds on various vertebrate hosts.[14]

The spirochetes are deposited into the bite wound along with the tick saliva. For infection to succeed, the tick must feed for at least 24 h adhered to the host, a period after which there is reduced expression of Outer Surface Proteins A and B (OspA and OspB) and increased expression of Outer Surface Protein C (OspC). OspAs and OspBs are lipoproteins essential for the survival of Borrelia spp. in the tick midgut. OspC is crucial for establishing infection in the invertebrate host because the protein allows the bacteria to migrate from the tick midgut to the salivary glands, where they will be carried with the saliva to the vertebrate host.[15] OspC also has an important role in the vertebrate host because it induces immunosuppression, thereby favoring infection.[14] Tilly and co-workers[16] found that bacteria lacking OspC do not establish infection in mice by either bacterial inoculation via injection or by tick bite.

Immune response

In the vertebrate host, *Borrelia* spp. are recognized by several mechanisms of the immune response, including the complement system and diverse innate immune cells.[17] Despite being classified as gram negative, *B. burgdorferi* does not produce lipopolysaccharide (LPS) but does express OspC in vertebrates. Recognition of *Borrelia* spp. by dendritic cells leads to maturation of these cells and triggers transcription of a large set of genes, such as those expressing chemokines, apoptosis inhibitors, matrix metalloproteases and a large subset of cytokines, including proinflammatory mediators, neutrophils attractants, immunomodulatory cytokines.[18]

Following antigen presentation by dendritic cells, T_H1 and T_H2 lymphocyte helper T cells initiate the adaptive response, promoting the release of interferon IFN-γ and interleukin IL-4, which are directly related to the severity of acute symptoms.[17] Subsequently, the cytokines released by T_H cells induce B-lymphocyte proliferation of and consequently, immunoglobulin production.[18]

Although the immune system attempts to prevent *Borrelia* infection, the spirochete has its own mechanisms to avoid host defenses. Components of the tick's saliva (such as *Salp*15) are known to be able to suppress the dendritic cell response, increasing the pathogenic virulence of *Borrelia*.[17] The spirochetes can also inactivate the host complement system by binding to host complement regulatory proteins, thereby inactivating the C3b mechanism. Another mechanism employed by *Borrelia* to escape the immune response is antigenic variation. The variable major protein-like sequence gene locus (*vls*E) on plasmid 28-1 undergoes extensive variation, which is stimulated by tick feeding.[18]

The disease in humans (LD of the Northern Hemisphere versus Brazilian Baggio-Yoshinari Syndrome)

Acute LD is typically manifested by an expanding erythematous skin lesion. Late manifestations may include arthritis, acrodermatitis chronica atrophicans, lymphocytoma, myocarditis, conjunctivitis, uveitis, and neurological signs.[19]

The existence of borreliosis in humans in Brazil was first suggested by Dr. Yoshinari and co-workers[20]; however, the first case in the country was not diagnosed until 1992. The increasing number of cases identified in Brazil show differences from the disease that occurs in the Northern Hemisphere.[21–24] In Brazil, the occurrence of *Ixodes* ticks (*I. auritulus* and *I. loricatus*) is associated with parasitism of some birds and *Didelphis albiventris*,[25,26] which are not considered the preferential vectors for horses and humans. Clinically, despite the occurrence of signs such as erythema migrans and the usual systemic complications, the Brazilian disease progresses with recurrences, especially if antibiotic treatment is initiated later than three months after infection. Brazilian patients

have been reported to have a high frequency of antibodies against different autologous cell components. Therefore, Brazilian Borreliosis (BB) was initially called Lyme-like disease, Lyme-like borreliosis or Baggio-Yoshinari syndrome (BYS) to distinguish it from the classical disease.[27]

In addition, studies conducted in the Laboratory of Rheumatology of the Clinical Hospital of the School of Medicine, University of São Paulo (LIM-17 Hospital das Clínicas – Faculdade de Medicina, Universidade de São Paulo – FMUSP) showed the occurrence of microorganisms with morphological structures similar to *Mycoplasma* spp., *Chlamydia* spp., and non-flagellated spirochetes in the peripheral blood of patients with BYS who were seropositive for *B. burgdorferi* sensu lato. However, those patients exhibited negative serology for *Mycoplasma* spp. and *Chlamydia* spp., suggesting a morphological difference between *B. burgdorferi* sensu lato and the Brazilian microorganism identified as the possible causative agent of BYS.[28] Because motile and spiral spirochetes were never isolated or cultured in Brazil, researchers from LIM-17 assumed that the etiological agent in Brazil was present in cystic form.

Due to these reasons, BB is defined as an emerging tick-borne disease, different from LD, caused by *B. burgdorferi* sensu lato with atypical morphologies and transmitted by ticks not belonging to *Ixodes*. It is possible that *Borrelia* bacterial passage through ticks from *Amblyomma*, *Rhipicephalus*, and *Dermacentor* genera can result in spirochete morphologic and genetic modifications in both vertebrate and invertebrate hosts, thus originating a new disease similar to LD.[28]

BYS differs from LD because the disease has higher morbidity due to the presence of symptom recurrence, severe reactive manifestations such as autoimmunity, and the need for prolonged treatment. Laboratory diagnosis of BYS is difficult because serological tests (ELISA, enzyme immunosorbent assay, or western blotting) for *B. burgdorferi* show low sensitivity and specificity[27–32]; this is because these tests utilize antigens from *B. burgdorferi* stricto sensu from the Northern Hemisphere to evaluate immunoglobulins of Brazilian *B. burgdorferi* sensu lato.

BYS can cause some symptoms similar to those observed in LD, including erythema migrans in approximately 50% of patients, arthritis in 35%, neurological symptoms in 35%, and cardiac disease in nearly 5%. The disease is often unrecognized, especially at secondary or tertiary stages when patients do not remember what occurred months or years before the current disease. Certainly, many cases of unrecognized chronic neurological or articular disease are in fact cases of BYS not identified and treated at early stages.[28]

The first studies in Brazil to report the occurrence of *B. burgdorferi* sensu lato were published in 2010, when dermatologists used immunohistochemistry to identify the spirochete in skin biopsies of the erythema migrans from four patients.[33] In a subsequent study employing immunohistochemistry and focus floating microscopy, Talhari and co-workers[34] also identified the bacterium in skin lesions from 22 patients. Despite conducting nested-PCR (polymerase chain reaction) using a set of four primers (external primers, 1 – AAG ACG AAG ATA CTC GAT CTG TAA TTG and 2 – TTG CAG AAT TTG ATA AAG TTG G, and internal primers, 3 – TCT GTA ATT GCA GAA ACA CCT and 4 – GAG TAT GCT ATT GAT GAA TTA TTG), none of the 22 samples was positive. Therefore, standardizing a PCR technique for identification of the *Borrelia* bacteria occurring in Brazil remains a challenge.

Mantovani and co-workers[35] demonstrated the presence of spirochetes of genus *Borrelia* in the blood of human patients with BB by performing *flgE* gene amplification (470 FW 5′-CGCCTATTCTAACTTGACCCGAAAT-3′ and 470 Rev 5′-TTAGTGTTCTTGAGCTTAGAGTTG-3′) as well as in genus *Rhipicephalus* ticks. Similarly, Gonçalves et al. identified *B. burgdorferi* s.l. strain B31 in *D. nitens* ticks collected on a horse in Paraná State (nested-PCR targeting the 5S (rrf) 23S (rrl) intergenic spacer region, 99.9% BLAST similarity with *B. burgdorferi* s.s.). Additionally, the same research group identified *B. burgdorferi* sensu lato in the blood of humans from a rural area in Paraná State (nested-PCR targeting the 5S (rrf) 23S (rrl) intergenic spacer region, 100% BLAST similarity with *B. burgdorferi* s.s.).[36] However, phylogenetic analysis was not performed in either study, leaving many uncertainties about the classification of the Brazilian pathogen(s).

Recent studies show the possibility of ticks of the genera *Amblyomma* and *Rhipicephalus* being directly related to dissemination of the disease. Rezende and co-workers[37] reported that embryonic cells of *R. microplus* and *A. sculptum* (previously *cajennense* according to the new taxonomic status[38]) could serve as substrate for the growth of *B. burgdorferi* sensu stricto strain G39/40. *B. burgdorferi* sensu lato was identified in *A. americanum* ticks collected from LD patients diagnosed by ELISA and PCR in Florida, USA.[12]

Borreliosis in horses

B. burgdorferi sensu lato is capable of infecting wild and domestic animals. Additionally, domestic animals, such as dogs, cattle, and horses, can be carriers of this pathogen. Unlike the unapparent disease observed in wild animals, this etiological agent is capable of causing clinical symptoms in domestic animals.[39–42]

Based on indirect ELISA and Western blotting, it was observed that horses exposed to ticks have a higher frequency of seropositivity for *B. burgdorferi* sensu lato than horses subjected to strict tick control.[43] In addition, viable *Borrelia* bacteria have been found in the urine of healthy horses in an endemic region of the United States,[44] warning of the possibility agent transmission by contact routes in addition to tick bites. Chang and co-workers validated an equine LD model by exposing ponies to ticks harboring *B. burgdorferi* for seven days. They then evaluated the antibody response and treatment efficacy at 120 days after infection and found that the antibody levels of the treated animals returned to negative levels in 10 months.[45,46]

In Brazil, it was detected an average ELISA seropositivity of 9.8% in horses in the state of Rio de Janeiro, and in the municipality of Seropédica, the frequency was 42.8%.[43] Madureira and co-workers[47] observed a frequency of 28.4% of anti-*Borrelia* homologous ELISA antibodies in horses in the municipalities of Três Rios and Vassouras, Rio de Janeiro State, whereas the frequency was 26.7% in the municipality of Belém, Pará State.[48] Guedes Junior and co-workers[49] identified 54.9% of cattle as being seropositive in the state of Paraná. In

dogs, the rate of seropositivity was 48.25% in Rio de Janeiro city.[50] The occurrence of LD in wild animal veterinarians and found 6.4% seropositivity for *B. burgdorferi* s.l. in São Paulo city.[51]

Borreliosis in horses remains underdiagnosed and poorly understood by veterinarians. According to a survey conducted in Germany of 118 veterinarians, only 56% believe that Lyme borreliosis can affect horses. When asked about the number of animals diagnosed in Germany, 45% answered that no animals are being diagnosed. Regarding control of the parasite, 46.5% stated that owners rarely perform ectoparasite control measures. A relatively large percentage of the veterinarians (30.5%) said that they would confirm the diagnosis only by serology and would not perform serological monitoring over time. They also indicated that they would treat positive animals with antibiotics and anti-inflammatory medication (54%) and stated that 71% of the horse owners are unaware of the disease.[52]

Studies indicate that LB in horses has clinical signs similar to the disease in humans,[53] including fever and lethargy,[54] arthritis,[55] polysynovitis,[56] lameness, muscle stiffness,[57] abortion,[54] meningitis, cranial neuritis, radiculoneuritis and encephalitis,[58,59] uveitis,[55,60,61] and premature death of foals.[54]

To study spirochete distribution in horses, Chang and co-workers experimentally infected eight ponies using *I. scapularis* ticks infected with *B. burgdorferi*. The ponies were euthanized approximately nine months after infection, and samples from several tissues, such as lymph nodes, skin, muscles, synovial capsule, and meninges, were subjected to molecular tests using primers targeting the *ospA* gene and the 23S ribosomal gene as well as cell culture. *B. burgdorferi* was mainly detected from the skin, fascia, and muscle.[45]

Diagnosis

The gold standard for laboratory diagnosis of LB is serological tests (ELISA confirmed by Western blotting). However, an increasing number of studies demonstrate the potential of molecular and culture tests as a tool for diagnosis. The final diagnosis in humans is often based on symptoms, exposure to ticks and serological tests.[62] For many other species of bacteria, the gold standard for identification is culture. However, *Borrelia* species can only grow under specialized culture conditions, such as Barbour-Stoenner-Kelly (BSK) medium with incubation at 30 °C to 35 °C for as many as 12 weeks under microaerophilic conditions. If a PCR method is chosen, results obtained from skin biopsies from patients with classic erythema migrans are more sensitive than are samples of blood collected from the same patients.[63]

The laboratory methods used for diagnosis of LB are the following: culture in Kelly medium of clinical samples from skin, cerebrospinal fluid, synovial fluid, and blood; molecular detection using tissue specimens (targeting p66, 16S rRNA, flagellar gene *fla*, 23S rRNA, and *ospA* genes and the 5S rRNA-23S rRNA intergenic spacer using nested or real-time quantitative PCR); and antibody detection such as enzyme immunoassays (ELISA), indirect immunofluorescent antibody assays (IFAs), western blotting, and peptide-based immunoassays.[64]

Lyme disease in horses must be diagnosed based on (1) the possibility of exposure to ticks infected with *B. burgdorferi*, (2) clinical signs compatible with LD, (3) absence of other causes for the clinical signs, and (4) a high titer of anti-*B. burgdorferi* antibodies.[65] The most common technique used for diagnosing *B. burgdorferi* in humans and horses is antibody detection, and the most frequently used techniques are IFA, Western blotting, and ELISA, with the latter having greater applicability for horses because it is faster and cheaper.[66] Specificity tests for IFA and ELISA show minimal cross-reactivity with anti-*Leptospira* antibodies.[67,68] In general, due to the slow multiplication of the spirochete in the host, three to six weeks may be required for detection of immunoglobulin G (IgG) titers, with eight to sixteen weeks needed to reach their maximum.[43,45,46] However, because the specificity of IFA and ELISA tests is still questionable, a positive result must be confirmed by a second diagnostic method. Western blotting is typically used to detect antibodies against *Borrelia*-specific antigens[69]; alternatively, some researchers have used PCR, which has the highest sensitivity and specificity.[46,66]

Chandrashekar and co-workers evaluated the feasibility of using a commercial enzyme immunoassay kit developed for dogs (SNAP® 4Dx) for the detection of anti-C6 peptide antibodies in 160 horses infected by *B. burgdorferi* and previously tested by western blotting (QualiCode™ IgG/IgM Western Blot Kits, Immunetics Inc., Boston, MA). The kit showed 100% specificity and 95% sensitivity compared to the gold standard serological test, and the authors indicated that the kit is a rapid and safe test for the diagnosis of borreliosis in horses in the field.[70]

A subsequent study found that serological tests using an anti-C6 commercial kit (SNAP® 4Dx) could identify most of the horses infected; however, it also produced false positive and false negative results. In addition, serological tests for detecting anti-C6 peptide antibodies and OspC and OspF, which are associated with clinical signs, were found to consistently support the diagnosis of borreliosis in horses.[71]

Equine therapeutics

Treatment with oxytetracycline (6.6 mg/kg, IV, every 12 h) for three weeks was more effective than the use of doxycycline (10 mg/kg, VO, every 12 h) or ceftiofur (2.2 mg/kg, IM, every 12 h). Oxytetracycline was the only antibiotic that led to negative results both according to culture and tissue PCR (lymph nodes, skin, muscle fascia, synovial membranes, pericardium, and meninges) at the end of treatment. Oxytetracycline can be administered (5.0 mg/kg, IV, every 24 h) for four weeks, with high efficacy against *B. burgdorferi* in experimentally infected ponies.[46] Divers and co-workers found that tetracycline is effective for treating LB in naturally infected horses (6.6 mg/kg, IV, every 24 h).[65]

Final remarks

LD is a condition of extreme importance because it is a zoonosis that causes physical and psychological sequelae in affected individuals. It remains poorly investigated in Brazil, especially in the field of veterinary medicine. Therefore, studies

describing the unique aspects of the disease in Brazil and the etiological agents found are needed.

Conflicts of interest

The authors declare no conflicts of interest.

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