Abstract
Lyme disease or Lyme borreliosis is an emerging infectious disease produced by Borrelia burgdorferi sensu lato, which is a bacteria transmitted to the host organism by the bite of Ixodes ticks. In this report we present the general knowledge about the etiological agent, clinical manifestations of the disease and diagnostic laboratory tests. We offer cumulative information about Lyme disease in Mexican children treated at two children’s hospitals in Mexico City during the last 10 years.

Key words: Borrelia burgdorferi, Erythema migrans, Lyme borreliosis, Neuroborreliosis

Introduction
Lyme disease or Lyme borreliosis is an emerging infectious disease transmitted by the bite of ticks with important consequences in North America and Europe because of the number of cases and its chronic weakening effect in affected patients. It is produced by Borrelia burgdorferi sensu lato, a bacteria transmitted by the bite of Ixodes ticks. Lyme borreliosis infection risk is directly related with the prevalence of ticks, infected reservoirs and contact with them in endemic areas.

Lyme borreliosis is found in most European countries, some Asian countries and in three enzootic areas in the U.S. (northeast coast, Minnesota and California). There are reports from Australia and South America (Chile and Brazil) where a disease similar to Lyme borreliosis has been detected without isolating B. burgdorferi from ticks, animals or patients.

The infection is endemic in Europe with a prevalence of 8-27% in forest workers in Germany and Sweden and 4% in the general population. In the U.S., the disease is confined to three enzootic areas (northeast coast, midwest and California) where infection prevalence ranges between 1 and 10%; however, there are reports from other areas with affected patients.

In 2002 the disease prevalence in the U.S. was 8.2 cases/100,000 inhabitants. In the states of Connecticut, Minnesota and New Jersey, prevalence is higher with 100 cases/100,000 inhabitants, whereas prevalence is lower in California.

Epidemiology in Mexico
The first suggestive cases of chronic migratory erythema were reported in 1991 in the Mexican states of Sinaloa and Nuevo Leon. Our group reported the results of the National Serum Epidemiological Survey in 1999 where the prevalence of B. burgdorferi in the general population reached 1.1%
and 3% in deer from Mexico’s northeast area.\textsuperscript{10,11} The first patients with neurological symptoms in Mexico City were also reported in that year.\textsuperscript{12,13} In 2003, we reported the serum prevalence of \textit{B. burgdorferi} infection in the general population in the northeastern area of Mexico (6.3%) and in Mexico City (3.4%).\textsuperscript{14} The first confirmed cases involving cutaneous and neurological disease were reported in 2007 from Mexico City and from the state of Quintana Roo.\textsuperscript{15,16}

**Clinical profile**

Lyme disease is a multisystemic disorder that involves skin, nervous system, heart and joints.\textsuperscript{1,17}

One third of the patients are children <18 years old and the age group with the highest risk is children between 5 and 14 years old.

Lyme borreliosis in humans has a wide spectrum of symptoms. It can be asymptomatic or have multisystemic clinical manifestations. This clinical diversity is associated with genetics and antigenic heterogeneity from species of \textit{B. burgdorferi} sensu lato. Arthritis and erythema chronicum migrans (ECM) occur in 80% of cases in the US, being associated with \textit{B. burgdorferi} sensu strict, which is the only species prevalent in that country. Neuroborreliosis is associated with \textit{B. garinii} (65%); acrodermatitis chronica atrophicans (ACA), lymphocytoma cutis and scleroderma are associated with \textit{B. afzelii}; facial paralysis and ECM are associated with \textit{B. valaisiana} and \textit{B. spielmanii}. All of the latter are prevalent species in Europe.

Lyme disease presents the following progressive stages:\textsuperscript{1,17} A) early localized stage, B) early disseminated stage, C) chronic stage.

**A) Early localized stage** – Manifests with erythema migrans (EM) and is present in 60-80% of cases as an initial sign. It is usually one homogenous or circular, popular and painful erythematous lesion with a centrifugal dissemination. It develops between 3 days to 16 weeks (1.5 weeks on average) after tick bite and clears spontaneously in 3-6 weeks.\textsuperscript{17} In children, the lesion is located in the head, arms, legs, and back. It is accompanied by fatigue and migrane in 50-60% of cases as well as fever and arthralgia in 30% of patients. Multiple EM lesions are smaller and develop if the patient has not received treatment.

**B) Early disseminated stage** – Several organs/systems are affected 3-12 weeks after EM if the patient does not receive treatment possibly because of hematogenous dissemination with neurological and/or cardiac manifestations.\textsuperscript{17,18}

**Lyme neuroborreliosis**

Lyme neuroborreliosis begins 3 weeks after tick bite and has a prevalence of 20% in the U.S. compared to 40% in Europe.\textsuperscript{18} In children, the most frequent neurological manifestations are facial nerve palsy, sixth cranial pair palsy and lymphocytic meningitis; peripheral neuropathies, radiculopathies and Bannwarth’s syndrome are infrequent.

**Cranial neuropathy**

Peripheral palsy of facial nerve (VII cranial pair) is the most common neurological manifestation in the US and is present in 25-50% of Lyme neuroborreliosis cases. It can be bilateral and occasionally is accompanied by III, IV, V and VI cranial palsy or as mononeuritis multiplex.\textsuperscript{18,19} Prevalence of idiopathic facial palsy from \textit{B. burgdorferi} infection is 1% in children.\textsuperscript{19,21}

**Meningoradiculoneuritis**

Meningitis manifests through mild meningism and intermittent migraine. It can be associated with cranial neuritis and radiculopathy (Garin-Boujadoux-Bannwarth Syndrome). Cerebrospinal fluid presents pleocytosis (10-316 cells/mm\textsuperscript{3}) with >70% lymphocytes, increased proteins and normal glucose.\textsuperscript{20} It is frequently accompanied by palsy of cranial nerves VI and VII, papilledema and increased intracranial pressure or EM. Prognosis is good and
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95% of children recover after management with antibiotics.

**Radiculoneuropathy**
Radiculoneuropathy is infrequent in children. It begins with an acute, localized and intense radicular pain and/or motor weakness with or without sensory loss. Sensory loss and neurological deficit can be multifocal and asymmetrical. Radiculoneuropathy may manifest as Garin-Boujadoux-Bannwarth Syndrome and has a higher prevalence in Europe. Other infrequent symptoms include hepatitis, testicular edema, encephalitis, myelitis and brain vasculitis.

**Peripheral neuropathy (Chronic axonal neuropathy)**
Symptoms include paresthesias, uncommon radicular pain and sensory deficit. Muscular strength and tendinous reflexes are preserved. Usually there is no meningitis or cranial neuritis. Antibodies in serum against *B. burgdorferi* are positive. Electromyography shows mild axonal neuropathy and normal cerebrospinal fluid without intrathecal antibodies. Sural nerve biopsy reveals distal axonal loss and perivascular infiltration without immune complex deposits. *B. burgdorferi* is not observed in nerve.

**Cardiac manifestations**
Cardiac manifestations are present in <1% infected adults and 0.5% pediatric patients. They start between the second and third week after infection and present with arrhythmia, dyspnea, thoracic pain or syncope secondary to second- and third-degree atrioventricular block; myopericarditis with effusion is rare and cardiac failure is usually transitory and self-limited in 10 days.

**Borrelial lymphocytoma**
Borrelial lymphocytoma begins between 6 and 180 days (30 days on average) after the tick bite. This is a rare presentation of Lyme borreliosis with a prevalence of 1.1-3% in Europe and only one case reported in the U.S. Average age of onset is 23 years (range: 2-72 years) and 44% of patients are children <15 years old. It is a reddish-purple nodular cutaneous lesion with a 0.5- to 2.5-cm diameter located with a higher frequency in the earlobe and areola mammæ and at times in the face and arms. Of the cases, 25% present this symptom at the same time as EM. If borrelial lymphocytoma is not treated, it may persist for months. It is histologically characterized by a dense polyclonal lymphocytic infiltration of the skin and/or subcutaneous tissue, sometimes with germination centers.

**Ocular alterations**
 Conjunctivitis has been observed in <5% of patients with EM. Direct eye affection (uveitis, keratitis, chorioiditis, panophthalmitis and optic neuritis) has been associated with identification of *B. burgdorferi* in culture.

**C) Chronic Stage** – Starts between 2 weeks and 2 years (6 months on average) after infection. Sixty percent of pediatric patients with EM or other disease manifestation who do not receive treatment present chronic arthritis; encephalomyelitis and ACA are rare.

**Arthritis**
Arthritis manifests in large joints. Of children, 90% present arthritis in knee and 10% in hip, ankle, elbow and/or wrist. Arthritis is accompanied by fever in 50% of pediatric cases compared with 25% of cases in adults. Episodes of recurrent arthritis occur in 50% of children and can affect the same joint or a different joint. Leukocytes in peripheral blood have normal levels but globular sedimentation velocity is >20 mm/h in 75% of cases. Synovial fluid shows 38,000 cells/mm³ on average with neutrophil predominance in 75% of cases. All children who present arthritis have positive serum for *Borrelia burgdorferi*. Prognosis at 4 years is good in 80% of cases; however, it may be associated with inflammatory lesions in eyes, including panophthalmitis, ischemic optic atrophy or inter-
stitial keratitis. Of children, 10% present chronic musculoskeletal pain and 3% of cases require synovectomy because of arthritis persistence.

Arthritis is migratory, monoarticular or oligoarticular, and asymmetrical and with an average duration of 3 months (range: 3 days-11.5 months). The knee is affected in the evolution of most patients and other large joints may also be affected. Temporomandibular joint is affected in 11% of cases. Synovial fluid presents a mild increase of proteins and leukocytes with an average of 24,250 cells/mm³ with predominance of polymorphonuclear cells and normal glucose. Myositis can also manifest with localized or generalized lymphadenopathy.17,22

**Encephalomyelitis**

Encephalomyelitis is a localized or multifocal disease with slow progression and affects the white matter more frequently than the gray matter. Serum and intrathecal antibodies against *B. burgdorferi* can be detected. Cerebrospinal fluid can present pleocytosis, hyperprotein at spinal level or positive PCR. Magnetic resonance is nonspecific and may suggest a white-matter disease, a rare manifestation.19,21

**Acrodermatitis chronica atrophicans (ACA)**

ACA is an atrophic skin lesion that occurs between 6 months and several years after the tick bite at lesion site. ACA and borrelial lymphocytoma are well documented in European Lyme borreliosis patients; however, patients in the U.S. are infrequently affected by this symptom, which is associated with *B. afzelii* infections.23

**Scleroderma**

*B. burgdorferi* has been implicated in the development of some scleroderma onsets as well as lichen sclerosus (LS). Scleroderma is classified according to the shape and affected site in linear scleroderma (*en coup de sabre* or on limbs), segmentary (hemifacial atrophy) and in plaques (morphea). Scleroderma etiology was regarded as unknown; however, histological studies show characteristics similar to those found in ACA. The bacterium has been found in histological tests and PCR trials as well as in serum response, although results have been contradictory in patients from the U.S. and Europe. This pathology has been recently associated with infection from *B. afzelii*.23

There is a larger clinical diversity in Europe because Lyme disease is associated with different species. Neuroborreliosis is more prevalent, especially in children, and is associated with *B. garinii* and *B. valaisiana*. Comparatively, arthritis in the U.S. is the most frequent clinical manifestation and is associated with *B. burgdorferi* sensu stricto.

**Borrelia burgdorferi sensu lato**

Phylogenetic studies indicate that the genus *Borrelia* belongs to the family *Spirochaetaceae*, order *Spirochaetales*.1,27

Genus *Borrelia* comprises >20 species. These *Borrelia* species have been classified into two higher categories based on their ecological and genetic characteristics.27 Relapsing *Borrelia* fever and the complex *Borrelia burgdorferi* sensu lato is formed by 13 genospecies; five are pathogenic for humans and produce Lyme borreliosis.

This is a mobile helical microorganism with a length of 5-25 μm and 0.2-0.5 μm width and is covered by an external cell membrane. It has between 7 and 11 flagella with a similar structure as found in other bacteria. It is microaerophilic and differs from other bacteria and spirochaeta because of its genomic structure with one linear chromosome and several linear and circular plasmids. It is sensitive to β-lactam antibiotics, tetracyclines, chloramphenicol and erythromycin. It is resistant to metronidazole, rifampicin, sulfonamides and 5-fluorouracil.1,27

*B. burgdorferi* has six large proteins on its surface: Osp A, B, C, D, E and F. The first protein is used to develop vaccines. OspC is a 20- to 25-kDa protein with variations among species and is useful
for serological diagnosis as a seroreactive antigen during early stages of the disease.26

**Diagnostic methods**

Diagnosis of Lyme disease is based on microbiological, serological and molecular biology tests.27,29,30 Bacteria culture is problematic because of the low isolation percentage; therefore, serological techniques have been included as diagnostic criteria and in some cases PCR has been useful.29,30

**Microbiology**

*B. burgdorferi* sensu lato culture from clinical specimens is useful in skin biopsies or cutaneous EM washings and blood of patients with early disseminated disease. Primary EM lesions present a culture sensitivity of 50%, which can reach 85% in secondary EM lesions. Moreover, the bacteria has been found in 48% of blood/plasma cultures in patients with early Lyme disease.27 *B. burgdorferi* identification from other sites such as cerebrospinal fluid and synovial fluid is rare; the low recovery rate is because of the small number of viable organisms presents in these anatomic sites.

**Histopathology**

Histopathology is used in biopsies from EM lesions and lymphocytoma cutis. We observe edema in this test from superficial and deep dermis, mucin deposits and perivascular infiltrate of macrophages, lymphocytes and in some cases plasma cells. These findings are not specific for Lyme borreliosis, and silver staining has been used to locate spiral organisms in skin and other tissues (Warthin-Starry, modified Dieterle, or modified Steiner stain), Fas or immunoperoxidase. The interpretation of this test is difficult; therefore, these techniques are limited to research and specific clinical cases.27

**Serology**

It is difficult to interpret ELISA and IFA (immunofluorescent assay) tests in skin biopsies with immunofluorescent antibodies against *B. burgdorferi*.29 Results depend on the clinical stage of the disease and its interpretation is complex because of cross-reactivity with other spirochaeta such as Treponemas and *Borrelia* spp., which are autoimmune diseases, infectious mononucleosis and Rocky Mountain spotted fever.28,29

This is because a) there is a large immune response variability among patients,29 b) early antibiotic treatment can abort an immune response and provide false negative results,29 and c) there are genospecies variability and different *B. burgdorferi* strains worldwide.28,29,31

In order to study questionable cases, VDRL or RPR (rapid plasma reagin card) tests are carried out; *B. burgdorferi* is not reactive to them. To increase serodiagnosis specificity, purified antigens or antigenic recombinant fractions of *B. burgdorferi* have been used for ELISA and Western-blot tests such as 39-kDa and 83-kDa proteins, flagellin, OspA, OspB, OspC, OspE, OspF, p22, BBK32 and V1sE.

Recently, an antigen for ELISA and Western blot has been used with recombinant protein from three common European *B. burgdorferi* sensu lato species: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. This antigen reports a sensitivity of 100% in patients with neuroborreliosis, 93% in patients with Lyme arthritis and 90% in patients with EM. Most serums reacted only to one species.31,32

To confirm a positive case after ELISA with complete antigen, we should carry out immune transfer test (Western blot), which increases specificity >95%.28,31,32 Currently, this is the most widely accepted diagnostic criterion.26

**Molecular biology**

The use of molecular biology techniques to identify Lyme borreliosis has an important role in cases with early neuroborreliosis symptoms and negative serology, in ocular borreliosis, or in newborns from mothers who presented active LD during pregnancy as
well as in difficult diagnostic cases such as those where infection from \textit{Babesia} and \textit{Ehrlichia} coexist with LD.\textsuperscript{30,33}

PCR can be three times more sensitive than early-stage Lyme borreliosis culture and may be a marker for disseminated disease. In skin biopsies from patients with EM, sensitivity is increased to 75\% with a 97\% specificity by amplifying the \textit{ospA} gene. In patients with late onset such as ACA, PCR has a 57\% sensitivity compared with the culture, where sensitivity is 19\% and specificity is 100\%. In patient with arthritis, bacteria have been cultured from synovial fluid only in one case; therefore, PCR is the gold standard to confirm diagnosis with 88\% sensitivity and 100\% specificity, amplifying flagellar and \textit{ospA} genes.\textsuperscript{32,34}

PCR has also been used to detect bacteria in vector because of its power to identify this hard-to-culture microorganism. It can also detect killed bacteria in paraffin-preserved samples and collected specimens.\textsuperscript{33}

\textbf{Phenotype typification methods}

The same phenotype typification methods used for other bacteria have been used to identify \textit{B. burgdorferi} such as biotyping, phagus typification and antibiotic susceptibility analysis; however, they are not feasible because of low bacterial growth. Therefore, electrophoretic analysis of SDS-PAGE proteins and fatty acid profile have been used, but conclusions based on these methods are not accurate.\textsuperscript{33}

1. Serum typification – Serum typification is the most commonly used phenotypic method to identify \textit{B. burgdorferi} sensu lato and is based on the reaction to specific monoclonal antibodies against OspA and OspC. Serum typification with OspA is able to identify eight different serotypes when studying 112 strains from humans and ticks from Europe, North America and Japan.\textsuperscript{34,35} Serotypes 1, 2 and J11 match with \textit{B. burgdorferi} sensu stricto, \textit{B. afzelii}, and \textit{B. japonica}, respectively, and serotypes 3-8 match with \textit{B. garinii}. Serotype 2 has been found in >50\% patients with Lyme borreliosis and serotype 4 has been detected in cerebrospinal fluid of patients with neuroborreliosis. On the other hand, >50\% of tick analyses identify serotype 6.\textsuperscript{34} Using OspC, 16 serotypes have been defined for European and North American cultures. Of these, six match with \textit{B. burgdorferi} sensu stricto, and four serotypes match with \textit{B. afzelii}. The remainder have not been identified. When comparing \textit{B. burgdorferi} sensu lato serotypes OspA and OspC, we observe a higher heterogeneity with OspC.\textsuperscript{35}

2. Multilocus enzyme electrophoresis (MLEE) – This method is based on protein typification with an electrophoretic pattern and differentiates electrophoretic motility of metabolic enzymes from each bacterial strain.\textsuperscript{35}

\textbf{Genotyping typification methods}

Molecular typification methods are based on the genetic characteristics of the microorganism and provide more precise information on bacteria pathogenic diversity. The study of genetic diversity in \textit{Borrelia} has epidemiological, clinical and diagnostic implications.\textsuperscript{35}

The following methods have been used in order to study genetic diversity of \textit{B. burgdorferi} sensu lato strains:

1. DNA-DNA hybridization analysis – This is one of best procedures to study the taxonomic relationship in \textit{Borrelia}. Using this method, \textit{Borrelia} spp. strains that cause relapsing fever have a homology level of 30-44\% with \textit{Borrelia burgdorferi}. Homology level among different \textit{B. burgdorferi} sensu lato subspecies is 48-70\%. Currently, DNA-DNA hybridization is acknowledged as the reference method to identify \textit{B. burgdorferi} sensu lato at subspecies level.\textsuperscript{35-37} Based on this method, \textit{B. burgdorferi} sensu lato has been subdivided into five subspecies: \textit{B. burgdorferi} sensu stricto, \textit{B. garinii}, \textit{B. afzelii}, \textit{B. japonica} and \textit{B. andersoni}.\textsuperscript{37}

Six species have recently been described: \textit{B. valaisiana}, \textit{B. spielmanii} and \textit{B. lusitaniae} in Europe as well as \textit{B. bisettii} in America\textsuperscript{38,39} and \textit{B. turdae} and \textit{B. tanu-
ki in Asia.\textsuperscript{40} Currently, there are 11 \textit{B. burgdorferi} sensu lato subspecies recognized, and only five are acknowledged as pathogen agents for humans.

2. Polymerase Chain Reaction (PCR) – This is a DNA-subtyping molecular method that is easy, quick and requires few cells from the microorganism. It is used to typify strains and identify species in clinical and in vector samples. The most common genes for amplification and gene sequence are gene \textit{fla} (flagellar) and \textit{ospA} (protein from external membrane). Initiators for gene \textit{fla} have been designed to amplify preserved regions as well as an internal gene fragment to amplify variable central regions and identify different genospecies.\textsuperscript{41,42} Gene \textit{ospA} is located in a 49-kb plasmid from \textit{B. burgdorferi} named Ip54; initiators have been designed for amplification in clinical and vector samples and are highly specific.\textsuperscript{33,41,42}

3. Real-time PCR – New PCR methods have recently been proposed. This technique not only detects bacteria but also quantifies it in clinical and vector samples. Additionally, it is able to typify \textit{B. burgdorferi} based on fusion temperature (Tm).

Studies carried out using DNA from human skin as well as vector samples (\textit{Ixodes ricinus}) have reported Tm results for \textit{B. garinii} of 2°C below \textit{B. burgdorferi} sensu stricto and \textit{B. afzelii}; therefore, this is considered as a quick identification and detection alternative for \textit{B. burgdorferi} sensu lato genospecies.\textsuperscript{42}

4. Sequencing – This is a nucleic acids molecular typification method that compares DNA sequences obtained from bacterial strains or from clinical and vector samples. This is the best quantitative method to determine the similarity or differences among strains. It allows analyzing short segments of DNA base sequences.

Sequenced genes from \textit{B. burgdorferi} include gene \textit{fla} (flagellar) and \textit{ospA} as well as intergenic space between ribosomal genes 5S and 23S. Its diversity has been used to typify strains, documenting different fragments originated by restriction enzymes (RFLP-PCR) based on the unique organization of \textit{B. burgdorferi}.\textsuperscript{33,42,43} Some authors suggest this method may be used for molecular epidemiology studies.\textsuperscript{43}

Other methods can be used to identify strains at species level and among strains of the same species; however, they have been little used to identify \textit{Borrelia burgdorferi} complex because of difficulties to isolate this bacteria. These include ribotyping, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) and plasmid fingerprinting.\textsuperscript{33,43-48} The latter compares strains from \textit{B. burgdorferi} with relapsing \textit{Borrelia} fever.

\textbf{Lyme Borreliosis in Mexican Children}

Lyme borreliosis represents >90\% of infections transmitted by ticks in the U.S. and Europe.\textsuperscript{9} There is evidence of genetic heterogeneity from \textit{B. burgdorferi} strains worldwide, which demonstrates the high migration level of Lyme borreliosis agents. Also, it presents a high clinical polymorphism related with genetic diversity of bacteria.

In Mexico, vectors are distributed in Baja California, Pacific Coast, northeast region, Gulf of Mexico and Yucatan Peninsula.\textsuperscript{6} In 1999 our group reported infection from \textit{Borrelia burgdorferi} in several Mexican states. We later detected the northeast region of Mexico and Mexico City as areas of high prevalence. We recently confirmed the presence of clinical cases of LD acquired in wooded areas near Mexico City.\textsuperscript{5}

Our research group has maintained a surveillance system to identify pediatric cases from March 1999 that were referred to the Pediatric Hospital, Centro Medico Nacional Siglo XXI (IMSS) and the Hospital Infantil of Mexico “Federico Gomez”.

Cases have been included using clinical, histological and immunodiagnostic criteria from the Centers for Disease Control and Prevention (CDC): erythema migrans or chronic manifestation and ELISA + Western blot positive tests. Data collection includes visit to wooded areas and/or exposure to ticks bite. Blood samples were taken from suspected patients to obtain serum. In patients with neuro-
logical manifestations we carried out lumbar puncture to obtain cerebrospinal fluid. IgM and IgG antibodies were measured using ELISA and confirmed using Western blot against three *B. burgdorferi* sensu lato species (*B. burgdorferi* s.s., *B. afzelii* and *B. garinii*).

Genotyping was carried out using the three following molecular methods applied to obtain DNA from clinical samples including skin biopsies, cerebrospinal fluid and synovial fluid from seropositive cases.

1) Southern blot was carried out using specific oligonucleotides from *flagellar* gene for the three most important pathogen species from *Borrelia burgdorferi* sensu lato: *B. burgdorferi* s.s., *B. garinii* and *B. afzelii*

2) Real-time PCR – We designed an initiator to amplify a 230-bp fragment from *flagellar* gene of *B. burgdorferi* and tubes with high specificity for each one of 11 genospecies from *Borrelia burgdorferi* sensu lato complex. We used Lightcycler 2000 (Roche Diagnostics, Indianapolis, IN) with TaqMan technology or double-hybridization tubes and locked nucleic acid (LNA) chemistry,

3) Sequencing¾We carried this out for *flagellar* and *ospA* genes from *B. burgdorferi* sensu lato, using capillary sequencing from Beckman Coulter (Fullerton, CA).

We studied 72 children with an average age of 8 ± 2 years. Of these patients, 55% (40) presented cutaneous manifestations, 27% (19) had neurological manifestations and 18% (13) presented arthritis. In 23 children, ELISA test was positive, confirming Lyme disease in 20 cases (28%) through Western blot test (Table 1).

In the remaining cases the following diagnoses were confirmed: polymorphous erythema associated with medication, granuloma annulare, systemic scleroderma, localized scleroderma with positive antinuclear antibodies, cranial neuropathy from herpes virus, Guillain-Barre syndrome, leukemia, juvenile rheumatoid arthritis and reactive arthritis.

Of 20 children with positive result, seven (34%) were bitten by a nonflying insect, finding a tick in three of these children. Exposure to domestic and wild animals (potential hosts for ticks) occurred in 12 patients who visited inhabited forest or rural areas in the Mexican states of Mexico, Morelos, Hidalgo and Tabasco. Of 20 patients, 75% presented early manifestations and 25% late manifestations (Table 2).

Early manifestations were present in 15 children with an average of 28 days evolution.

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>n</th>
<th>WB (+) (%)</th>
<th>PCR fla (+) (%)</th>
<th>SB fla (+)</th>
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<tbody>
<tr>
<td>Cutaneous</td>
<td>40</td>
<td>6</td>
<td>4/6 (67)</td>
<td>5/6 (83%)</td>
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<td>EM</td>
<td>3</td>
<td>1</td>
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<td>Lymphocytoma cutis</td>
<td>3</td>
<td>3</td>
<td>1/3</td>
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<tr>
<td>ACA</td>
<td>3</td>
<td>1</td>
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<td>Morphea</td>
<td>31</td>
<td>1</td>
<td>1/1</td>
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<tr>
<td>Neurological</td>
<td>19</td>
<td>11</td>
<td>3/7 (42)</td>
<td>3/7 (42%)</td>
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<td>7</td>
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<td>9</td>
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<td>Total</td>
<td>72</td>
<td>20 (28)</td>
<td>8/14 (57)</td>
<td>9/14 (64%)</td>
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</table>

WB, Western blot; SB, Southern blot; PCR, polymerase chain reaction; EM, erythema migrans; ACA, acrodermatitis chronica atrophicans.

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quent manifestation was lymphocytoma cutis in three cases and one case of EM. Of neurological manifestations, we found seven cases of meningopolyradiculoneuropathy and four cases of cranial neuropathy. Children with meningopolyradiculopathy presented cerebrospinal fluid with mild pleocytosis (10-20 cells), normal glucose and mildly increased proteins. Four children presented cranial pair affection (VI and VII). Mixed polyradiculoneuropathy with generalized weakness occurred in three cases. Of patients, 70% required respiratory assistance, with full recovery within 75 days.

Late manifestations were present in three children (9 years old) and there was one case with knee and ipsilateral hip affection. Cutaneous affectations comprised one case of ACA and one case of morphea.

The following complementary tests were carried out.

**Histopathology**

For EM cases, biopsy using hematoxylin-eosin stain revealed a perivascular lymphocyte infiltrate in superficial dermis and in lymphocytoma cutis we observed a mononuclear infiltrate with germinal centers (Fig. 1).

Warthin-Starry stain showed spiral bacteria for both pathologies (Fig. 2). Immunohistochemical test for monoclonal antibodies vs. anti-CD45 and CD20 specific for B-cell lymphocytes and T-cell lymphocytes was positive, showing a polyclonal infiltrate in lymphocytoma cutis.

**Serology**

Of patients with confirmed diagnosis using Western blot for *B. burgdorferi* sensu lato, 17 were confirmed using IgM WB and three were confirmed with IgG vs. *B. burgdorferi*. Positive patients with IgM vs. *B. burgdorferi* sensu lato were reactive to one of the following: nine (45%) to *B. garinii* and eight (40%) to *B. afzelii*. The other three patients (15%) positive to IgG reacted to *B. burgdorferi* s.s.

Of 20 seropositive children, we obtained DNA from 14 clinical samples (six skin biopsies, seven cerebrospinal fluid, one synovial fluid). PCR for *fla* gene was positive in eight cases (57%) and positive for gene *ospA* in three cases. Using Southern blot, nine DNA were positive for *fla* and four were positive for *ospA* (Fig. 3).

This yet to be published information shows general characteristics of children with Lyme disease.
acquired in Mexico. In this sample, *Borrelia burgdorferi* sensu stricto was the predominant species; this genospecies is the one most frequently reported in the U.S. However, Mexicans pediatric patients presented more diverse clinical manifestations and involved genospecies similar to European cases.

Evidence found in serological tests and reported cases in Mexico\(^9\text{-}^{16}\) should alert all pediatricians to include differential diagnosis for Lyme borreliosis in cutaneous and neurological disease cases as we have described in our study.

**Table 2. Demographic and epidemiological characteristics of Lyme disease in pediatric patients**

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Early stage n = 15 (75%)</th>
<th>Chronic stage n = 5 (25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutaneous</td>
<td>Neurological</td>
</tr>
<tr>
<td>Patients n (%)</td>
<td>20</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Age (years) mean ± SD</td>
<td>8 ± 2</td>
<td>NA</td>
</tr>
<tr>
<td>Gender M:F</td>
<td>1.7:1</td>
<td>NA</td>
</tr>
<tr>
<td>Insect bite</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Host exposure</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Evolution at admission (average)</td>
<td>NA</td>
<td>28 days</td>
</tr>
</tbody>
</table>

SD, standard deviation; NA, data not available

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**References**

Lyme disease: experience in Mexican children


