

Lyme and associated tick-borne diseases: global challenges in the context of a public health threat. Running title: Global challenges of Lyme disease

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Opinion

**Lyme and associated tick-borne diseases: global challenges in the
context of a public health threat.**

Running title :
Global challenges of Lyme disease

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1995 words

Key-words

Lyme disease, *Borrelia burgdorferi*, *Borrelia miyamotoi*, diagnosis, coinfections, tick borne disease, occult infection

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46 Lyme disease, caused by *Borrelia burgdorferi* and transmitted by ticks, was initially considered a
47 recent, rare and regional occurrence. We now have evidence that very similar bacteria infected
48 humans in Europe during the ice age (1). Evidence-based data are scarce therefore many aspects
49 of the disease remain controversial (2,3,4), but in 2013 the Centers for Disease Control and
50 Prevention (CDC) revised their annual estimates from 30,000 cases to 300,000 cases in the USA
51 alone. Having dramatically increased their numbers, the CDC are now calling Lyme disease “a
52 tremendous public health problem in the United States” (5).

53 The lack of a gold standard for diagnosis makes producing accurate statistics difficult. Some
54 pathogenic strains belonging to the *B. burgdorferi* sensu lato complex have a worldwide
55 distribution, yet they are rarely considered or tested for (6,7,8,9,10,11,12,13). *Borrelia*
56 *miyamotoi*, for instance, phylogenetically close to relapsing fever borreliae, is now recognized as
57 a cause of Lyme-like disease and relapsing fever in Asia, Europe and North America. It usually
58 does not cross react with *B. burgdorferi* tests (12,13). A novel isolate of *Borrelia* has been
59 isolated by PCR in a post-treatment serum from a patient with neurologic Lyme disease (13).

60
61 These recent historical, geographical and microbial data should prompt the medical community to
62 realize that cases of persisting post tick-bite syndromes are probably due to multiple pathogens
63 and that these occult infections will require a new approach if not an actual paradigm shift.

64 65 **Diagnostic pitfalls in routine practice**

66 Classical forms of Lyme disease are usually easy to manage, but these medical conditions with
67 pleomorphic non specific symptoms may prove confusing to physicians (14). Lyme disease may
68 mimic chronic inflammatory or degenerative diseases, including a wide range of auto-immune
69 diseases. Although practitioners from every medical specialty are likely to have encountered
70 cases of Lyme disease, they may have failed to recognize it, no matter how skilled they are. A
71 major obstacle is that only 30% of the patients report a history of tick bite and only 70 to 80%
72 present with a primary erythema migrans, the pathognomonic initial lesion. This lesion may go
73 unrecognized, or be mistaken for an “insect bite” or an “allergic rash”. Mini-erythema migrans
74 are less likely to be diagnosed. Secondary erythema migrans are observed in approximately 50%
75 of cases. Bacteriologic and pathologic analogies have been reported between tertiary
76 neuroborreliosis and tertiary neurosyphilis (15). Syphilis, once well-known as the great imitator,
77 gives us a good historical model for the concept of occult infection.

78 79 **Occult infections and their role in the pathophysiology of some diseases of unclear etiology**

80 Charles Nicolle, working at the Institut Pasteur in Tunis and Nobel prize winner in 1928, showed
81 great interest in the concept of occult infections (“les infections inapparentes”) like typhus,
82 syphilis and relapsing fever (*Borrelia recurrentis*) (16). Relapsing fever due to another species of
83 *Borrelia* (*B. crocidurae*) is still a public health concern in some parts of Africa, and the recently
84 discovered *B. miyamotoi* may also become a similar problem in Asia, Europe and America
85 (12,13,17). Peptic ulcer disease is another example of the hidden link between an occult infection
86 with another spiral-shaped bacterium, *Helicobacter pylori*, and a chronic disorder. *B. burgdorferi*
87 may persist in tissues even after antibiotic treatments, as animal models have shown
88 (18,19,20,21,22). In fact dormant persister cells of bacteria from different genera can escape the

89 bactericidal effect of antibiotics and be responsible for latent infections (13,23,24,25). Clinicians
90 have no diagnostic tests to check for the persistence of live borreliae. *B. burgdorferi*, having a
91 complex genetic structure, is a highly adaptable organism capable of evading immune response
92 through different processes. It can survive extracellularly and intracellularly (26,27). The
93 complexity of Lyme disease requires high quality diagnostic methods, yet serology is the only
94 diagnostic tool widely used.

95 96 **Serology, the current main diagnostic method**

97 Physicians should be made aware that, in the presence of primary erythema migrans, serology
98 will often be negative therefore diagnosis should be clinical (28). However, many practitioners
99 are still under the misconception that a positive serology is required for early stage diagnosis. For
100 later stages of the disease serology remains the main diagnostic tool. The Infectious Diseases
101 Society of America (IDSA) and the European Concerted Action on Lyme Borreliosis (EUCALB)
102 are recommending a two-tier testing approach, the first step being an ELISA using whole
103 sonicate of the in vitro cultured tick-derived strain B31 of *Borrelia burgdorferi* (29,30). If
104 positive, confirmation by immunoblot testing IgG and IgM is required. According to these
105 guidelines, immunoblot is not to be performed if the ELISA is negative. However, in 2011, the
106 CDC modified their case definition and included single-tier IgG immunoblot seropositivity as a
107 diagnostic criterion for Lyme disease (31). But most practitioners still use the two-tier system
108 despite the poor sensitivity of ELISA tests, ranging from 34% to 70.5% (32,33,34,35).
109 Calibration of the tests is a crucial issue.

110 111 **Calibration of serology**

112 When Lyme serology was developed, no reliable method was available to be used as a gold
113 standard for comparison. As most of the signs and symptoms are non-specific, no reliable clinical
114 diagnostic score could be established. The low yield of culture and the difficulty involved in
115 using the technique routinely were another major obstacle. A pragmatic cut-off level for the
116 serologic tests had to be determined arbitrarily on blood donors (30,36). In the late seventies,
117 when Lyme disease was first discovered, it was understandably thought to be a rare and regional
118 phenomenon. Therefore, a low prevalence was set as experts were afraid the serologies would
119 produce too many false positive diagnoses (30,36). Patients and control populations are ill-
120 defined with a high variability in predictive positive and negative values from one test to another.
121 Culture of *B. burgdorferi* or detection of its genome by polymerase chain reaction (PCR) may
122 occasionally confirm the clinical diagnosis in seronegative patients, however none of these
123 methods are sensitive enough to be considered reliable diagnostic methods, especially in routine
124 practice (32,36,37,38,39,40,41,42,43). As a result, many patients suffering signs and symptoms
125 compatible with Lyme disease, but whose test is negative, are falling by the wayside.

126 127 **Clinical and epidemiological consequences of negative serology**

128 Modern medical practice expects to rely on evidence. Most physicians would not consider
129 diagnosing Lyme disease without serological proof. Yet the failure to diagnose seronegative
130 neuroborreliosis, especially the acute or severe forms, can have dire consequences including
131 chronic neurologic sequelae or even death. A review of the literature shows that a diagnosis of
132 Lyme neuroborreliosis is often difficult to prove (44,45,46,47). The sensitivity of intrathecal
133 antibody index (measuring specific antibodies within the cerebro-spinal fluid) ranges from 55%
134 to 80%. In a Swedish study, antibodies were present in serum of only 23% of children with
135 neuroborreliosis (47). Cognitive tests or SPECT brain imaging may help to provide objective

136 evidence (48,49,50,51). Pragmatic diagnostic criteria including response to empiric antibiotic
137 treatment are used to diagnose neuroborreliosis (44). Should this strategy be recommended in
138 other clinical presentations as well? In fact some clinicians will not hesitate to classify as Lyme
139 disease cases, seronegative patients with a highly compatible clinical picture, provided other
140 diagnoses have been ruled out. In a major clinical trial on Lyme disease, 40% of the enrolled
141 patients were seronegative. These patients had a history of erythema migrans, neurologic or
142 cardiac symptoms, radiculoneuropathy or arthritis (52). Clinicians, often unaware of the
143 difficulties involved in diagnosing Lyme disease, will fall back on “weak” alternative diagnoses
144 (“viral”, “idiopathic”, “auto-immune”, “degenerative”, “inflammatory” or “psychosomatic”) (53).
145 New techniques are needed to accurately assess these patients. This current over-reliance on
146 inaccurate testing procedures not only flaws the diagnosis of individual patients but it also has
147 epidemiological consequences especially as new species and variants continue to be identified on
148 all continents (54,55).

149

150 **Possible causes of seronegativity**

151 Several factors leading to seronegativity have been identified in confirmed cases of Lyme
152 disease: (i) the arbitrary cut-off level of tests, (ii) the sequestration of antibodies in immune
153 complexes, (iii) the wide variety of species and subspecies of *Borrelia* that co-exist in different
154 parts of the world and (iv) coinfections with other pathogens which may be responsible for some
155 or all of the symptoms or which may alter the immune response (37,43). The complex
156 *B. burgdorferi* sensu lato includes (Table 1): *B. burgdorferi* sensu stricto (including genetic
157 diversity), *B. afzelii*, *B. garinii* (several serotypes) and additional species isolated in different
158 parts of the world (7,54,56). Some of these species have been isolated in symptomatic patients
159 (6,7,8,9,10,11,12,13). *B. spielmanii* may cause early skin disease (8). *B. bavariensis*, *B. bisettii*,
160 *B. valaisiana*, *B. americana*, *B. andersonii*, *B. lonestari* and more recently *B. kurtenbachii* have
161 been isolated from patients with Lyme-like diseases (7,8,9,10,57). The pathogenic role of *B.*
162 *lusitaniae*, isolated in a case of vasculitis, remains to be substantiated (7). Despite such diversity
163 in strains, most of the commercially available tests still rely on the original 1982 Massachusetts
164 B31 isolate of *B. burgdorferi*. No diagnostic tool is available for routine detection of *B.*
165 *miyamotoi* (12,13). Coinfections with other microbes add to the complexity of these illnesses
166 (Table 1). Among patients with early Lyme disease in the USA, 2% to 12% were found to also
167 have human granulocytic anaplasmosis, and 2% to 40% babesiosis (29). In Brazil, a Lyme-like
168 syndrome, due to the tick *Amblyomma*, has been described and mobile non cultivable spirochetes
169 could be visualized in patients’ blood using a dark field microscope (58). A new tick-borne
170 bacterial pathogen, *Candidatus* Neoehrlichia mikurensis, was reported in Switzerland (59). An
171 illustration of the limits of serology is the Scottish example: the sensitivity of the immunoblot
172 was improved by using local Scottish strains of *Borrelia* (60,61).

173

174 **Conclusion and perspectives**

175 The numerous complexities of Lyme disease make it an extremely difficult illness to fully
176 comprehend. It remains a diagnostic challenge even for the best informed of clinicians. The lack
177 of a gold standard for diagnosis renders the management of patients difficult and seriously
178 hinders our ability to produce accurate statistics, especially as very similar syndromes could be
179 due to other species of *Borrelia*. In some patients suffering from syndromes of unclear origin,
180 following tick bite, other microbial agents could also be playing a role. Lyme disease has now
181 entered the political debate as shown by the amendment (Section 54.1-2963.2) voted in 2013 by
182 the State of Virginia, USA, that compels physicians to inform their patients that the “current

183 laboratory testing for Lyme disease can be problematic”. The fact that politicians are being called
184 upon to rule on these matters should prompt scientists to regain control of the situation.
185 Politicians should instead become aware of the necessity to fund research and facilitate the
186 setting up of independent international working groups. Reliable testing is essential to investigate
187 the many syndromes of unclear origin that may mimic many other medical disorders. Proper
188 fundamental and clinical research is urgently needed as it would be the most cost effective way of
189 ensuring that patients are accurately diagnosed and that the best therapeutic strategies are decided
190 upon (62). Development of new diagnostic methods is badly needed. New PCR methods and new
191 genomic techniques, such as high throughput sequencing, could prove promising in identifying
192 the complex mix of microbial agents that are probably involved (13,63). Next generation
193 sequencing allowed the identification of various bacteria from *Ixodes ricinus* ticks in France:
194 *Anaplasma phagocytophilum*, *Bartonella henselae*, *B. grahamii*, *Borrelia afzelii*, *B. garinii*, *B.*
195 *burgdorferi*, *B. miyamotoi*, *Candidatus Neoerlichia mikurensis*, *Ehrlichia canis*, *Rickettsia*
196 *canadensis*, *R. felis* and *R. helvetica* (63). These new techniques should be applied to human
197 samples. Other variables, such as genetic, environmental or auto-immune factors should also be
198 studied. The name “Lyme disease” is too restrictive as it focuses and fuels the controversy. A
199 new term should be agreed upon for these syndromes with possible infectious involvement, often
200 following tick bites. Closer collaboration between epidemiologists, microbiologists,
201 immunologists, geneticians, environmental scientists, veterinarians, entomologists and clinicians
202 is needed to identify the main agents that could be causing these occult infections and to
203 determine strain pathogenicity. A new multidirectional approach is crucial in order to widen the
204 field of research and to move forward.

205

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