frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

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Journal Name:	Frontiers in Cellular and Infection Microbiology
ISSN:	2235-2988
Article type:	Opinion Article
Received on:	25 Mar 2014
Accepted on:	19 May 2014
Provisional PDF published on:	19 May 2014
www.frontiersin.org:	www.frontiersin.org
Citation:	Perronne C(2014) Lyme and associated tick-borne diseases: global challenges in the context of a public health threat. Running title: Global challenges of Lyme disease. *Front. Cell. Infect. Microbiol.* 4:74. doi:10.3389/fcimb.2014.00074
/Journal/FullText.aspx?s=149&name=cellular%20and%20infection%20microbiology&ART_DOI=10.3389/fcimb.2014.00074:	/Journal/FullText.aspx?s=149&name=cellular%20and%20infection%20microbiology&ART_DOI=10.3389/fcimb.2014.00074 (If clicking on the link doesn't work, try copying and pasting it into your browser.)

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

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

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

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

Diagnostic pitfalls in routine practice

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

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

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

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

Serology, the current main diagnostic method

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

Calibration of serology

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

Clinical and epidemiological consequences of negative serology

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

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

Possible causes of seronegativity

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

Conclusion and perspectives

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

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

Acknowledgment

The author thanks Nelly Pointis for her help with editing.

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