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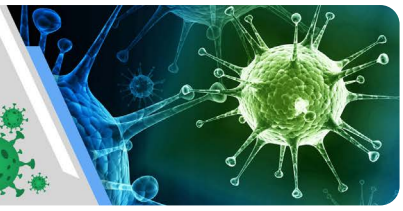

Lyme Disease, Tests and Treatment: A Review of the Controversy on the Ineffectiveness of Biological Tests and Proof of the Existence of a Chronic Form

Alexis Lacout*,[1] and Christian Perronne[2]

Abstract

Lyme disease is caused by an infection with the bacterium *Borrelia burgdorferi*. Other *Borrelia* species have been discovered and cause similar diseases. The first species described, *Borrelia burgdorferi* sensu stricto, was isolated in the United States. Lyme disease is a great imitator, resembling many conditions, including autoimmune diseases.

The diagnostic tests ELISA and Western blot, which some experts claim to have an almost 100% sensitivity, are often negative in many patients with genuine Lyme disease. These tests are poorly calibrated, of mediocre quality, with an arbitrarily defined antibody positivity threshold designed to ensure that no more than 5% of patients in any given region test positive.

There is controversy regarding the existence of the chronic form. However, chronicity is observed in many patients, and the mechanisms of *Borrelia* persistence are well documented in scientific publications. Recently, in 2018, the French National Authority for Health (Haute Autorité de Santé) defined SPPT (Syndrome persistant polymorphe après possible piqûre de tique), which allows for an empirical antibiotic trial in cases of persistent illness not explained by standard investigations, even when Lyme serology is negative.

Lyme disease is also frequently associated with numerous other asymptomatic infections, known as crypto-infections, whether parasitic, bacterial, viral, or even fungal. Treatment must be effective against *Borrelia* and other co-infections. Long-term antibiotic treatment lasting several weeks or months, often combined with antiparasitic drugs, may be necessary. Relapses are frequent upon stopping treatment, explained by *Borrelia*'s persistence mechanisms, and often require the rapid reintroduction of previously effective treatments.

Denying the published scientific realities described in this article results in hundreds of thousands of patients suffering from debilitating symptoms, left untreated, while an appropriate and low-cost anti-infectious treatment can bring remission in many cases. This review also highlights the political and military factors behind the "official" denial of the chronic form of this potentially highly debilitating disease.

Keywords: Post-treatment Lyme Disease Syndrome (PTLDS), Centers for Disease Control, Lyme disease, DNA, *Borrelia burgdorferi*, syndrome post-borréliose de Lyme (PTLDS), Centers for Disease Control, polymorphic persistent syndrome after a possible tick bite (SPPT), Lyme disease,

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Mycoplasma spp, *Rickettsia* spp, *Bartonella* spp. *Candida* spp, *Babesia* spp, *Theileria* spp, ELISA, Western Blot

Background

Lyme disease is caused by infection with the bacterium *Borrelia burgdorferi*. Other *Borrelia* species have been discovered that cause similar diseases. The first described species, *Borrelia burgdorferi sensu stricto* was isolated in the USA. The complex *Borrelia burgdorferi sensu lato*, includes *Borrelia burgdorferi sensu stricto* and other species. Ten species of this complex were identified in Eurasia: *Borrelia garinii*, *Borrelia afzelii*, *Borrelia bavariensis, Borrelia spielmanii, Borrelia lusitaniae, Borrelia japonica, Borrelia sinica, Borrelia valaisiana, Borrelia tanukii, Borrelia turdi* and *Borrelia yangtze*. Four species of this complex were identified in North America: *Borrelia americana, Borrelia andersonii, Borrelia californiensis*, *Borrelia kurtenbachii* and *Borrelia mayonii*. Three species of this complex, *Borrelia burgdorferi sensu stricto, Borrelia bissettii* and *Borrelia carolinensis* are present in Eurasia and the Americas (1). In addition to this complex of *Borrelia* species responsible for Lyme or Lyme-like diseases, many species of *Borrelia* are responsible for relapsing fever, some of them described decades before the discovery of *Borrelia burgdorferi*. The relapsing fever *Borrelia* group includes: *Borrelia crocidurae, Borrelia duttonii, Borrelia recurrentis, Borrelia hispanica, Borrelia coriacae, Borrelia lonestari, Borrelia miyamotoi, Borrelia parkeri, Borrelia turicatae, Borrelia hermsii, Borrelia anserina* and *Borrelia persica* (2). In 2020, a new species, Candidatus *Borrelia mahuryensis* has been isolated in French Guiana. *Borrelia miyamotoi* is known to be responsible for either Lyme-like syndrome or relapsing fever. It has been shown that a *Borrelia* species, genetically related to *Borrelia burgdorferi sensu stricto*, was isolated by PCR in the ice man «Ötzi», a more than 5,000-year-old mummy discovered in the Alps in 1991.

Various signs and symptoms, cutaneous, neurologic, rheumatologic or psychiatric, linked to a tick-bite, were first observed and described at the end of the 19th century. During decades, the link between these various clinical presentations, observed by various specialists, was not made. Therefore, a unique disease was not suspected and this infection did not have a name. Acrodermatitis chronica atrophicans was first described in 1883 by Alfred Buchwald. This syndrome was later named Pick-Herxheimer syndrome. Arvid Afzelius first discovered erythema migrans in 1909. In 1922, Garin and Bujadoux reported the first case of meningoradiculitis in the *Journal de Médecine de Lyon*. The responsibility of an infectious agent transmitted by ticks was suspected (3). This clinical neurological form was confirmed by Bannwarth in 1941 (Garin-Bujadoux-Bannwarth syndrome). It was in the United States, in Lyme, a town in Connecticut, in 1975, that an epidemic of juvenile arthritis occurred. In 1982, Willy Burgdorfer, an American entomologist and bacteriologist born in Basel, Switzerland in 1925, discovered the cause of the disease: a spirochete named «*Borrelia burgdorferi* » in his honour (4).

Lyme disease is mainly transmitted by tick bites, with an increased risk of contamination if the tick has been attached for a long time and is saturated with blood. Other modes of transmission are also possible, particularly perinatal and sexual (5, 6).

Three phases of the disease are classically described:

The primary phase is represented by erythema migrans. It is a red patch that spreads progressively, with a typical concentric appearance in the shape of a cockade or ring, the "bull-eye" rash. However, erythema migrans is inconsistent and may be absent or not visible (if it is located on a hairy scalp, for example) in around half of the cases (7). At this stage, the patient should be checked for other symptoms (e.g. fatigue, joint pain), which would suggest an immediately disseminated form.

The secondary phase is the consequence of an acute attack following dissemination of *Borrelia* via the blood, which occurs at a later stage. Examples include heart attacks, multiple erythema migrans, borrelial lymphocytoma (a protruding skin lesion 1 to 2 cm in diameter, reddish-purple and usually located on the earlobe, nipple or scrotum), arthritis or meningo-radiculitis.

The tertiary phase corresponds to a chronic course of the disease. This phase includes acrodermatitis chronica atrophicans, also known as Pick-Herxheimer syndrome (parchment-like atrophic skin), chronic arthritis, neurological disorders and various other manifestations. The existence of a chronic form, although abundantly supported in the literature, as we shall see, manifests itself through extremely varied symptoms which can affect all organs; it is called « Post-treatment Lyme Disease Syndrome » (PTLDS) by scientists who recognize the presence of long-term somatic manifestations, but refuse to admit the existence of a real chronicity of the disease. Rebman et al. give a good description of the range of symptoms that can be observed (8). Lyme disease is currently nicknamed, as was tertiary syphilis, "the great imitator" (note that both diseases are caused by a spirochete) and can therefore be confused with many illnesses. Many conditions could be secondary to Lyme disease or another infection. It should be remembered that lupus can give a false syphilis serology. Lyme disease can cause auto-immunity and could be the trigger for so-called systemic diseases such as systemic lupus erythematosus, dermatomyositis, local scleroderma and systemic sclerosis (9, 10). Antibodies to myelin have indeed been detected in

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some patients with Lyme borreliosis (11, 12). The French High Authority for Health (Haute Autorité de Santé, HAS) adopted a new entity in 2018, the SPPT (syndrome polymorphe persistant après possible piqûre de tique) or polymorphic persistent syndrome after a possible tick bite, which is defined by:

- a possible tick bite;
- the clinical triad associating several times a week, for more than 6 months: a polyalgic syndrome (musculoskeletal and/or neuropathic pain and/or headaches), persistent fatigue with reduced physical capacity, and cognitive complaints (concentration and/or attention problems, memory problems, slowness of thought);
- with or without a history of erythema migrans. This triad may be associated with polyorganic functional signs.

This entity is very similar to fibromyalgia, chronic fatigue syndrome and post-treatment Lyme disease syndrome (PTLDS). The difference between SPPT and PTLDS is that the diagnosis of Lyme disease has not to be proven and patients may not have received antibiotic treatment.

Lyme disease is the subject of a great deal of controversy, mainly concerning the reliability of diagnostic tests and the existence of a chronic form, which we will discuss below (13).

Finally, it should be remembered that, although Lyme disease itself is an infection due to *Borrelia* spp., PTLDS is often associated with poly-infections with several kinds of micro-organisms, bacteria, parasites, viruses and even fungi. These co-infections may be transmitted by ticks or may have other modes of transmission. Their possible role in the triggering or the maintenance of a chronic disease is not yet well established. The possible role of “hidden infections” or “occult infections” (les “infections inapparentes”) in the genesis of chronic inflammatory or degenerative diseases has been described in 1933 by Charles Nicolle, who was director of the Pasteur Institute in Tunis and who won the Nobel prize in 1928 (14). The concept of “occult infections” has been taken up by Willy Burgdorfer himself in 1954 (15). The term “crypto-infections” is now proposed (Fourth European Conference on Crypto-infections, Compiègne, France, October 4-5, 2024).

Epidemiology

In France, according to data from Santé publique France, the incidence of the disease has increased almost tenfold in 10 years: more than 55,000 new cases in 2016 according to the sentinel network, which underestimates the incidence because statistics are based on serology results and do not take into account most chronic forms. This has also been observed in the United States. In England, the incidence of facial paralysis linked to Lyme disease has increased by 42% in four years, while in southern Poland, the incidence of the disease increased 35-fold between 1998 and 2014 (16, 17). The annual incidence rate of Lyme borreliosis in France was estimated at 91 cases per 100,000 inhabitants (60,033 estimated cases) in 2020, compared with 76 cases per 100,000 inhabitants (50,133 estimated cases) in 2019. Since 2009, it has fluctuated between a low of 41 in 2011 and a high of 104 in 2018 (18).

Diagnostic tests

Today, it is customary and recommended to document an infection whenever possible. *Borrelia* is difficult to observe and culture. For this reason, diagnosis is usually made (except at the erythema migrans stage, when diagnosis is clinical) on the basis of serology using the “two-tiered testing” system: an ELISA method followed, if positive, by a Western blot for confirmation. Antibodies are looked for in blood and sometimes in cerebrospinal fluid. However, in 2011, the American Centers for Disease Control and Prevention (CDC) acknowledged the existence of seronegative cases of Lyme disease and modified their reporting criteria. Thus, a physician can declare as “probable Lyme disease” a case for which other differential diagnosis have been searched and excluded and if the patient condition has improved after an empirical antibiotic treatment recommended for Lyme disease. In France, Eldin et al., while concluding that serology is a highly sensitive method (at a later stage than erythema migrans), warn against interpreting the test in isolation. The authors recommend correlating serology with the clinical context: it is necessary to assess the pre-test probability of the disease, as this probability has a direct impact on the positive and negative predictive values (19).

Serology is an opaque method that can be negative in the presence of genuine Lyme disease (20-22), for a number of reasons that we will now develop. Serology is an indirect method based on the detection of antibodies. Antibodies may be absent or undetected for various reasons: (a) antibody levels may be a function of the patient's immune status, which is variable, and *Borrelia* can induce relative immunosuppression (23-25), (b) due to recent antibiotic therapy (c) because antibodies may be sequestered in immune complexes and therefore not detected (26, 27), (d) the bacterium may be quiescent, in low numbers, localized in sanctuaries (fibrous tissues, intracellular localization) and therefore not or no longer stimulating the immune system (28, 29), (e) not all *Borrelia* strains are taken into account in these tests which, without any evidence, mention testing for antibodies matching with the whole *Borrelia burgdorferi sensu lato* complex. *Borrelia* can evade the immune system by various mechanisms (30, 31). Above all, the test has been calibrated a priori to never find more than 5% of patients with

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a positive test, as stated in full in the article by Assous et al. (32). *« The European Concerted Action on Lyme Borreliosis (EUCALB) recommends testing at least 100 negative controls from the normal population in the same geographical area, and checking that no more than 5% of these controls are positive at the chosen threshold."* We do not know where these 5% come from (under these conditions, an epidemic can in no way be detected), and we don't know what the reference test is. The antibody detection threshold is therefore arbitrary (Figure 2).

The European Centre for Disease Prevention and Control (ECDC) reported a low sensitivity of the enzyme immunoassay/ immunoblot of 0.77 (95% CI: 0.67-0.85) for the diagnosis of neuroborreliosis, and warned that serology results should be interpreted with caution (33). A comprehensive analysis of all published data on the subject by two British researchers, Cook and Puri, published at the end of 2016 demonstrated that the detection capacity of serology is less than 60% (34). Another article published in the *Journal of Clinical Microbiology* explains, as already highlighted in a report by the ECDC published in April 2016, that it is not possible to properly calibrate serologies for Lyme disease. Indeed, to calibrate tests properly, you need to be able to determine a diseased population and a non-diseased population. The problem with this disease is to define and detect the diseased population (35). So, let's take a look at how these tests were put together. When we look at the validated ELISA kits, we see that all the kits validated by the manufacturers have been compared with previously validated kits. Moreover, the clinical

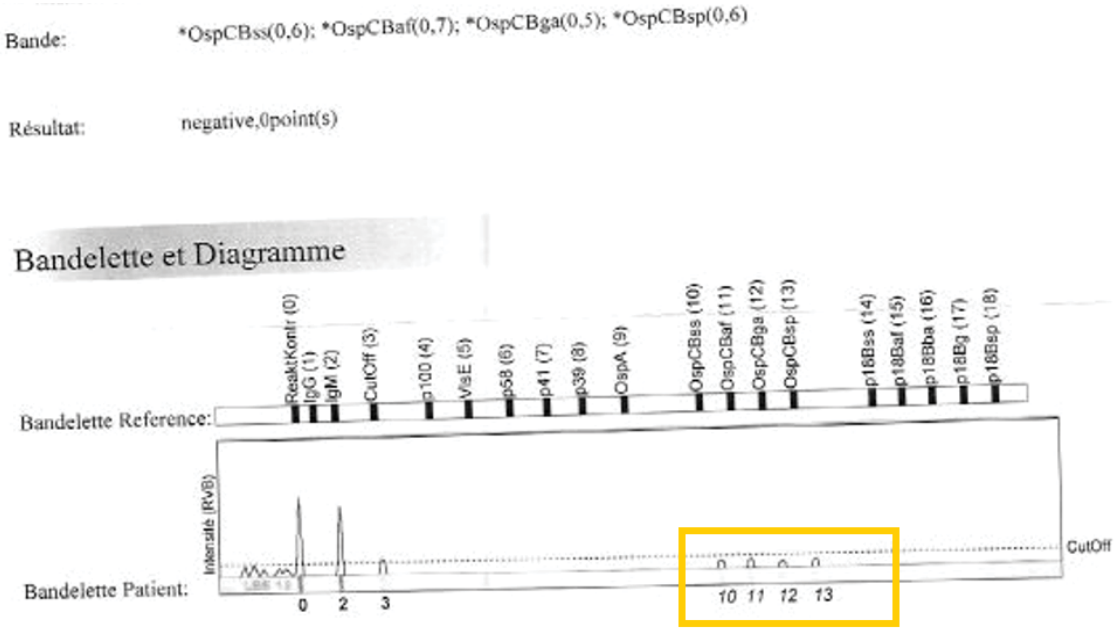

Figure 1: Presence of multiple antibodies (yellow box) below the arbitrary threshold (dotted line) in a patient with Lyme disease.

pictures chosen by all manufacturers are erythema migrans and acrodermatitis chronica atrophicans; these expressions of Lyme disease are just one of the many manifestations of Lyme disease. In 1989, the MarDx *Borrelia burgdorferi* EIA IgG + IgM kit, had its diagnostic performance calibrated by comparison with an "in-house" kit manufactured by a "referent" laboratory. However, neither the name of the kit nor that of the "referent" laboratory is mentioned in the instructions. The development of these tests is completely opaque. What's more, as already mentioned, the MarDx *Borrelia burgdorferi* EIA IgG + IgM kit is indisputably a serological test based on the antigens of *Borrelia burgdorferi sensu stricto*, discovered by Willy Burgdorfer in the early 80s in the USA, and because of the way it is constructed, would only recognize the antigens of *Borrelia burgdorferi sensu stricto.* However, experts around the world continue to say, against the published evidence, that Lyme serology is highly sensitive and that it can detect all the different species, mainly of the *B. burgdorferi sensu lato* complex. There is no evidence to support such statement. Moreover, we saw above that many species of *Borrelia*, pathogenic for humans, are not belonging to the *B. burgdorferi sensu lato* complex. Surprisingly, no diagnostic test was developed for them. We could confirm that Lyme serology is not able to diagnose *Borrelia miyamotoi* infection (36-38). In France, as in many countries, it is forbidden to perform a Western blot serological test as a first-line diagnostic. This test is only performed if the ELISA test is positive or doubtful. It's a pity not to be able to

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carry out the Western blot test systematically, as it is more sensitive (39). What's more, it allows to see all antibodies, including those below the arbitrary detection threshold (Figure 2). Another controversial point is the fact that many experts say that the presence of IgM antibodies is a marker of primary infection only. This is usually the case in infectious diseases. However, it has been demonstrated that *Borrelia* can interfere with plasmocytes, empedding the switch from IgM production to IgG production. Thus, in many patients suffering from chronic Lyme disease, IgM may be present on the long term and are a good marker of chronicity (40-43).

In France, the 2014 report by the High Council for Public Health (Haut Conseil de la Santé Publique, HCSP) had already highlighted the poor quality of French ELISA and Western blot tests: (Table 1) variable and imprecise reagent composition, cross-reactions, mode of cut-off establishment, incomplete or non-existent performance studies, lack of comparison between reagents (44). For patients suspected of suffering from Lyme disease and having a negative serology, an empiric antibiotic treatment was recommended: a true diagnostic test based on the patient's response to treatment. As noted above, the US CDC in 2011 had already asked US doctors to declare as probable Lyme disease cases of patients with negative serology who had improved on an empiric course of antibiotics. All these limitations were also taken into account in June 2018 in France by the High Authority for Health (HAS): in 2018, an empiric antibiotic therapy was recommended for seronegative cases (45). The official US Department of Health and Human Services task force report to the US Congress on 14 November 2018 fully confirmed that current diagnostic tests are unreliable and that co-infections associated with Lyme disease, due to other pathogens transmitted by tick bites, are not investigated (46).

Benoît Jaulhac, Director of the National Reference Center for borreliosis (Centre National de Référence, CNR) in Strasbourg, France, who has long defended the reliability of tests, finally acknowledged in a published article that no biological diagnostic test is perfect (19). In 2007, Benoît Jaulhac was co-author of European recommendations for neuroborreliosis (47). It is stipulated in their guidelines that pragmatic diagnostic criteria, in particular the response to an empiric antibiotic treatment used as a diagnostic test, are relevant for diagnosing neuroborreliosis in case of negative serology. It is surprising that this diagnostic strategy was not recommended for other clinical forms of the disease. We believe that PCR tests enabling direct identification of the bacterium's genetic material are of interest, a point already made by Bil-Lula et al. (48). However, many PCR tests for *Borrelia* spp. do not detect *Borrelia hermsii* or *Borrelia miyamotoi*.

We performed PCR studies looking for *Borrelia* and various co-infections (bacterial, viral, parasitic and fungal), on four media, called matrices (venous blood, capillary blood, urine and saliva), with two samples drawn two days apart. The results showed the presence of numerous bacteria, viruses, parasites and *Candida*, with different results depending on the day of sampling (49, 50). In the Bil-Lula study, 3% of negative IgM ELISA results, 2.8% of negative IgM Line blot results, 3.1% and 2.7% of negative IgG ELISA and IgG Line blot results, respectively, were PCR positive (48). In one of our studies, of the 9 patients tested positive for *Borrelia* by PCR, only one had positive *Borrelia* serology (IgG). This is further evidence of the poor efficiency of Lyme serology (22). Perhaps, for patients with chronic Lyme disease, sampling once a day for a week on all four matrices would enable optimal mapping of co-infections. If a nycthemeral cycle is thought to exist, it could be of value to collect samples at different times of the day, as observed for filariae, which can be diurnal or nocturnal. *Borrelia* can be found in different environments (51-53). It is also conceivable to perform PCR in different media, on deep biopsies, lymph node cytopunctures, synovial fluid and cerebrospinal fluid. The use of drugs capable of lysing biofilms could perhaps improve PCR performance (54). ELISPOT (enzyme-linked immunospot) measures the specific response of T-lymphocytes to *Borrelia* (or other infectious agents) and could be of interest, showing the active nature of the infection (55). These methods are still poorly evaluated. Nanotrap® particles for the detection of *Borrelia* outer surface protein A in urine can be a highly sensitive technique (56). Despite all these published data, some experts continue to argue that research in the field is no longer useful since serological tests are perfect!

Some of them say that it is not useful to develop diagnostic tests for *Borrelia* not belonging to the *Borrelia burgdorferi sensu lato* complex, arguing that these species are rare or not really pathogenic. But no studies support this statement. In France, the National Reference Center for Borreliosis says that *Borrelia miyamotoi* is not a significant pathogen in France. However, we published the largest series of *Borrelia miyamotoi* infection isolated by PCR in 43 French patients suffering from chronic Lyme-like disease (36). We could observe that erythema migrans was rarely observed in these patients and, as mentioned above, Lyme serology was not reliable. Many of the patients infected with *Borrelia miyamotoi* presented with signs of thermic dysregulation, such as flushes, chills, episodes of sweat or fever, resembling signs of relapsing fevers. Thus, our observation is in accordance with the fact that *Borrelia miyamotoi*, on a genetic point of view, is intermediate between the Lyme *Borrelia* species and the relapsing fever *Borrelia* species.

Other methods for detecting the various micro-organisms need to be devised and developed, such as light microscopy

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of "live" (i.e. unfixed) fresh blood, using various combined techniques: phase contrast, darkfield and Köhler illumination. However, it should be noted that the presence of spirochetes is often observed in healthy subjects.

The search for these crypto-infections remains difficult at present. For the time being, empiric treatment remains essential, targeting *Borrelia* (doxycycline, ceftriaxone, macrolides) and piroplasms (atovaquone - proguanil, and azithromycin, for example) as a second-line treatment. Many cases of Lyme disease therefore go undiagnosed, especially as this disease is a great imitator.

The Chronicity Controversy

The existence of the chronic form of Lyme disease is controversial and the subject of much debate. The 2006 Consensus Conference in France limits antibiotic treatment to 3 weeks (57), even though *Borrelia* has been shown to persist. Most doctors believe that Lyme disease is cured after 3 weeks of antibiotic treatment, and that the symptoms observed thereafter are either dysimmune or psychological. We do not deny the possibility of immune system abnormalities, in particular *Borrelia* -induced autoimmunity (8,9,58). Some systemic diseases could have an infectious cause and then evolve on their own. In fact, the role of persistent infection in the persistence of symptoms has never been studied correctly, as no patient studies have evaluated the effect of sufficiently long antibiotic therapy. We do know, however, that the combination of doxycycline and hydroxychloroquine can be particularly effective in patients with rheumatoid arthritis. Hydroxychloroquine is an immunomodulator, has anti-inflammatory properties and, above all, is a powerful anti-infectious agent, which also potentiates the action of some antibiotics (such as doxycycline) by alkalinizing the phagolysosome (59-64). Tetracyclines alone are also effective (65). It is important to note that hydroxychloroquine is effective, per se, on *Borrelia*. Although some scientists might explain the efficacy of hydroxychloroquine by its intrinsic anti-inflammatory properties, its anti-infectious action is, in our view, the main mechanism, since inflammation disappears with the causative micro-organism. At present, one of the officially recommended treatments is anti-TNF or immunosuppressive agents that are effective on symptoms, but dangerous in the long term, as they can promote infections and are potentially carcinogenic (66).

Although autoimmune phenomena do occur, the persistence of the bacterium despite a 3-week course of antibiotics probably explains the chronic symptoms of Lyme disease. The persistence of *Borrelia* is supported by numerous books (67) and articles in the scientific literature on humans and animals. We list a large number of them so that it is no longer possible to deny it. The autopsy of a patient suffering from Lyme disease, treated for several years with antibiotics, showed the presence of *Borrelia* (68). The existence of persistent forms of Lyme disease is well known, in fact accepted and studied in the literature. These forms could be treated with pulsed ceftriaxone (69). Samples taken from patients correctly treated according to current recommendations show the presence of the bacterium by PCR and even by culture (70-74). After antibiotic treatment, the synovial membrane may still contain spirochetes: although PCR was negative in synovial fluid and urine, it confirmed the persistence of *Borrelia* in the synovial membrane of four previously treated Lyme arthritis patients (74). Battafarano et al. described the case of a patient with chronic septic Lyme arthritis of the knee lasting for seven years, despite multiple antibiotic trials and synovectomies. The presence of *Borrelia* was documented in synovia and synovial fluid (75). Bayer et al. reported the presence of *Borrelia burgdorferi* DNA on a series of 97 PCR-positive patients who had been treated with antibiotics for long periods and had symptoms of chronic Lyme disease (76). Feng et al. also demonstrate the persistence of *Borrelia* despite treatment, and advocate the evaluation of antibiotic combinations (77, 78). Weber et al. found *Borrelia* in a newborn whose mother had been treated with amoxicillin (79). Many other articles describe cases of *Borrelia* persistence (70-74, 80-97). The assertion that positive PCR tests do not mean that the bacterium is persisting alive in the body, due to the so-called persistence of nucleic acid (DNA) fragments, is totally false. In fact, following the work of Nobel Prize winner Jules Hoffmann, it has been demonstrated that "naked" DNA, which may have escaped from a dead bacterium, is rapidly destroyed by enzymes in the tissues of mammals, including humans, and therefore cannot be detected by PCR. Prof. Christmann's "fossil DNA" theory, which suggests that a bacterium present in the body's blood or tissues weeks or years ago is no longer present, but has nevertheless left a "memory" of its passage leading to a positive PCR, is antiscience. In fact, it's worth remembering that bacteria can be found in culture even after antibiotic treatment (70-74). The bacteria can evade the immune system (30-31), induce relative immunosuppression (23-25), and take refuge intracellularly (98), or in poorly vascularized sanctuaries that are less accessible to the immune system (as they are less irrigated by the bloodstream), such as fibrous tissue, e.g. tendons (99).

Borrelia spp. have two intrinsic mechanisms which enable them to remain unaffected by antibiotic treatments: (Table 1)

(a) Biofilms: these are "shells" of extracellular material under which the bacterium protects itself, either alone or in association with other micro-organisms (68, 100-103). Most antibiotics do not penetrate this shell and are therefore ineffective. *Borrelia* biofilms have been observed in vitro and in vivo, including in borrelian lymphocytoma (104), and

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in the brains of Alzheimer's patients, correlating with the development of amyloid plaques (105-110).

(b) Round forms: the spiral form of *Borrelia* can transform, especially under hostile conditions, into atypical, non-spiral forms: "round bodies" or wall-less L-form spheroplast variants (spherical cells of *Borrelia burgdorferi* with a flexible cell envelope containing numerous flagella) (111-118). These forms are thought to be more capable of long-term survival, particularly in the presence of beta-lactam antibiotics. The transition from spiral to round forms, and vice versa, has already been filmed and published (116). Round forms can give rise to spirochetes: their pathogenic potential is therefore certain. There are also "blebs" morphotypes characterized by the formation of outer membrane vesicles on the *Borrelia* surface (113), which can also appear on the surface of cells. These blebs, expressing *Borrelia* antigens at the surface of the infected cell, could trigger autoimmune reactions.

Candida spp. may have a symbiotic relationship with *Borrelia*, promoting persistence (119). This is a well-founded hypothesis which we have formulated. We recently demonstrated that *Candida* could be isolated by PCR in the blood or other matrices from PTLDS patients (Multi-matrix real time *Candida* PCR in 108 patients with polymorphic signs submitted for publication). These results deserve to be confirmed. We recall that *Candida* can produce mycotoxins whose deleterious actions simulate a true Lyme disease (120, 121). Fluconazole, an antifungal agent, has already been used successfully in neuroborreliosis, either because it is also active against *Borrelia*, or because these patients had associated candidiasis (122). Cooperation between bacteria and fungi has been described and may play a role in the chronicity of the disease (123). Parasites such as helminths may also contain *Borrelia*, which would explain the Jarisch-Herxheimer reactions observed when flubendazole is used in patients with Lyme disease (124). In 2023, the existence of chronic symptoms due to Lyme disease has been recognized and published by the CDC in Atlanta (125). As we have just seen, the persistence of *Borrelia* is an indisputable scientific fact. If *Borrelia* persists, chronicity is a logical consequence. If a micro-organism that has induced a disease is able to induce a relapse, it means that it is still present and capable of multiplying. Patients considered cured, because they have become asymptomatic and remain so after several years, are very probably still carrying *Borrelia*, but only in a quiescent form. *Borrelia burgdorferi* is, among the bacteria which are pathogenic for humans or animals, the bacterium which contains the higher number of function genes, allowing it to adapt to variations of environment and to hostile conditions, such as immune attacks, change in pH of the medium, antibiotic challenge, etc.

The evidence of persistence is obvious in the literature, with many observations of patients alternating between remission and relapse when antibiotics are started and stopped (126), or of patients maintained in remission with low-dose maintenance antibiotic therapy (127). It is astonishing that despite the abundant bibliography on this subject, the French speaking infectious disease society (Société de Pathologie Infectieuse de Langue Française, SPILF) and other learned societies in France and other nations persist in denying the existence of the chronic form of Lyme disease, or at least are unwilling to look into it. Furthermore, they do not propose high-level studies to put an end to this controversy. The result is that thousands of patients go untreated for a potentially partially or totally curable disease, and suffer for years. Patients are then not listened to and considered to have a psychiatric or psychosomatic illness (128). We can recall here what the discoverer of *Borrelia burgdorferi*, Willy Burgdorfer himself, said in an interview, "*that the recommendations of the Infectious Diseases Society of America (IDSA), (on which the obsolete French recommendations of 2006 were based), were shameful, that the money had been going for over thirty years to the same people who found nothing, and that Lyme serology should be reviewed by researchers who don't publish results before they've done their research!*"

Co-infections

Ticks can be poly-infected and therefore transmit numerous infectious agents. In the study by Moutailler et al, of the 267 female ticks analysed individually, almost half (45%) were infected with at least one pathogen. Of these, the most common agents were *Borrelia* spp. 21.7% in total, including *Borrelia burgdorferi sensu stricto* (5.6%), *Borrelia afzelii* (9.4%), *Borrelia garinii* (10.8%), *Borrelia valaisiana* (6.0%) and *Borrelia spielmanii* (2.2%). The most common micro-organisms were *Bartonella henselae* (17.6%) and *Rickettsia* of the spotted fever group (16.8%), mainly *Rickettsia helvetica*, followed by *Borrelia miyamotoi* (3.0%), *Anaplasma phagocytophilum* (2.6%), Candidatus *Neoehrlichia mikurensis* (1.4%) and *Babesia divergens* (0.37%). Moreover, 9% of ticks carried DNA from two pathogenic species, 6.7% carried DNA from three pathogens, 1.9% carried DNA from four pathogens and 0.75% carried DNA from five different pathogens (129). We have conducted several studies looking for a variety of bacteria, parasites, viruses and *Candida* in patients with SPPT or suspected chronic Lyme disease (49, 50, Multi-matrix real time *Candida* PCR in 108 patients with polymorphic signs, submitted for publication). In one of these studies, 108 patients were included (50). A total of 864 samples were analysed on day 0 and day 3, from venous blood, capillary blood, urine and saliva (Figure 2). The most frequently found micro-organisms were *Mycoplasma spp*. followed by *Rickettsia spp*. and *Theileria spp*. It is interesting to note that *Theileria* are piroplasm parasites well known

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in veterinary medicine but unknown, before our study, in human medicine. We can only find what we are looking for. Collaboration with veterinaries should be encouraged since humans and animals share the same environment. In 2016, M. Vayssier-Taussat et al. reported in patients suffering from

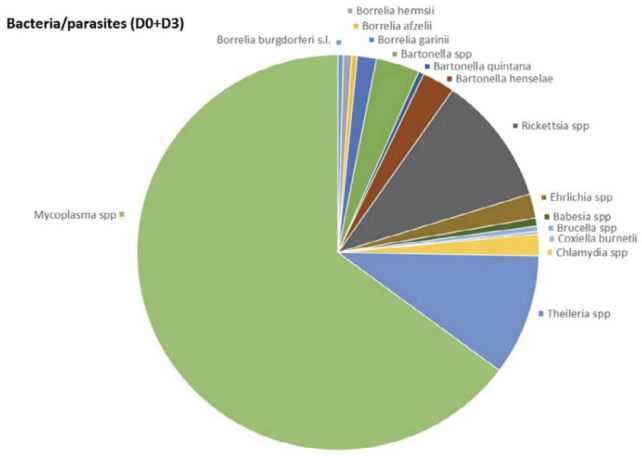

Figure 2a: Bacteria and parasites: PCR results at D0 + D3

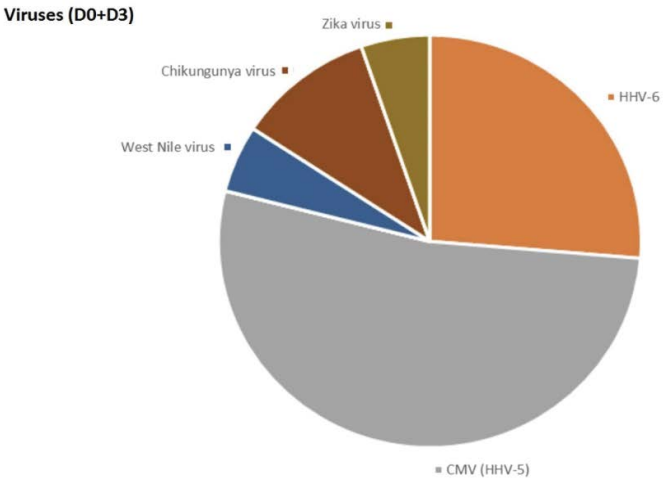

Figure 2b: Virus: PCR results at D0 + D3

Figure 2: Mas M, Lacout A, Véronique Perronne V, Lequette Y, Gadiolet Y, Rambeaud B, Trouillas P, Franck M, Perronne C. Multi-Matrix Real Time PCR in 108 Patients with Polymorphic Signs Suggestive of Fibromyalgia or Related to A Tick Bite. Archives of Microbiology and Immunology. 7 (2023): 250-270 (Reference 50).

a Lyme-like syndrome, the isolation of several species of *Bartonella*, well known in animals, but previously unknown in humans (130). In our study, the bacteria most frequently found were *Mycoplasma* spp. followed by *Rickettsia* spp. and *Theileria* spp. Fourteen PCRs (5 at D0 and 9 at D3) in 10 patients (9.3%) were positive for *Borrelia.* Thirty PCRs (18 at D0 and 12 at D3) in 22 patients (20.4%) were positive for *Bartonella* (50).

Infectious agents were more often found in capillary blood than in venous blood, which may be explained by local flow or temperature conditions (131). Patients were frequently polyinfected. Among the 108 patients included in our study (50), no micro-organisms were found in 5 patients (4.6%), a single micro-organism in 32 patients (29.6%), two different micro-organisms in 33 patients (30.6%), three different micro-organisms in 25 patients (23.1%), four different micro-organisms in 10 patients (9.3%), five or more different micro-organisms in 3 patients (2.8%). Infections with the piroplasm *Babesia* may be more common than previously thought. In one study, seropositive IgG for at least one *Babesia* spp. were significantly more frequent in *Borrelia*-seropositive individuals (16.3%) than in the healthy control group (2.5%) (132). One of our retrospective series, the primary aim of which was to evaluate *Babesia* infections, showed 41% of positive serology (22). Patients who have undergone splenectomy may have very acute parasitemic episodes which are rapidly fatal. Apart from these acute cases, persistent infection seems to be frequent but very rarely diagnosed (133). Patients suffering from babesiosis often complain of chills, sensations of heat and cold, profuse sweating, sometimes feverishness and shortness of breath; arthralgias and myalgias have also been described (134, 135). Babesiosis can also relapse, often in immunocompromised patients (136-138). Martinot et al. admit to being unaware of this pathology, which they consider to be rare and difficult to diagnose (139).

In the United States, the teams of Maggi, Moyazeni and Breitschwert have shown a high prevalence of *Bartonella* spp. in fibromyalgia, chronic fatigue and PTLDS (post-treatment Lyme disease syndrome), which are similar to or indistinguishable from SPPT (140, 141). In short, these co-infections should be investigated and treated in patients presenting with an illness suggestive of chronic Lyme disease. The absence of specific treatment may obviously result in therapeutic failure, and doctors may falsely conclude that the patient's Lyme disease is not improving with antibiotics.

Crypto-Infections, A New Paradigm

Talking about “Lyme disease” is undoubtedly simplistic, given the multiple co-infections and different genetic backgrounds in which these diseases develop. What these infections have in common is a torpid course, and a difficult diagnosis that often goes undiagnosed. As noted above, Charles Nicolle showed great interest in the concept of “hidden infections” (les infections inapparentes”), such as typhus, syphilis and *Borrelia recurrentis* relapsing fever (14). The subtle game played by the deniers of chronic Lyme disease is to limit Lyme disease to *Borrelia burgdorferi sensu lato*. This is why we now refer to these hidden infections as

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crypto-infections (67, 142). It could be that many diseases, whether idiopathic, degenerative such as Alzheimer's disease (105-111), or autoimmune (8-12), are the consequence of these infections, especially as infectious agents can sometimes trigger autoimmunity. Autism may sometimes have a relationship with infections and the intestinal microbiota, which could explain why some children see their illness improve with anti-infective treatments (143-149). The association between *Streptococcus* A and obsessive disorders is well known in PANDAS syndrome. Caution is advised, but the cases observed and the pathophysiological mechanisms argue in favour of carrying out scientific studies.

Doxycycline may be effective in rheumatoid arthritis (65) and inflammatory degenerative disc disease, suggesting a hidden infectious cause (150, 151). Crohn's disease may in fact be caused by an atypical mycobacterium, and anti-infective treatments with rifabutin and macrolides may be effective (152). In fact, the so-called “disappearance” of infectious diseases thanks to hygiene, vaccination and anti-infective therapies, mainly concerns the apparent forms of infection. Research in infectious diseases should now focus on crypto-infections that seem to play a major role in chronic diseases. This is a new paradigm that could lead to many progresses in the field.

Treatments

Let's talk briefly about a preventive treatment following a tick bite. There are differing opinions, with some advocating antibiotic treatment as soon as the tick is saturated with blood, i.e. spherical, which means that it has been attached long enough to have taken a blood meal and transmitted the infection. Unfortunately, given the frequency of tick bites, this would mean a great deal of treatment. Some have recommended a systematic treatment for children, pregnant women and immunosuppressed persons. It is accepted, that the site of the bite should be monitored for a long time to check for the appearance of erythema migrans. However as soon as 1986, Willy Burgdorfer warned with colleagues that erythema migrans was observed in less than 50% of cases of disseminated Lyme disease (7). Morerover It should be noted that other infectious agents do not give rise to cutaneous signs and that transmission of a piroplasm, for example, will in any case go unnoticed. Any erythema migrans should be rapidly treated with antibiotic for two weeks.

With regard to the indication for treatment, we have seen that diagnostic tests are unreliable and can in no way rule out Lyme disease. Furthermore, co-infections are not usually investigated. At present, an empiric anti-infectious treatment should therefore be required in all cases of chronic Lyme disease or fibromyalgia, for a minimum duration of one month. The antibiotics usually used for Lyme disease are tetracyclines, macrolides and penicillins. Ceftriaxone is the drug of choice for severe neurological disease, but other antibiotics can also be effective in these cases. Hydroxychloroquine is often added at a low dose (100 to 200 mg per day), because it has its own anti-infectious effect on *Borrelia* and alkalinises the phagolysosome, thereby potentiating the action of the combined antibiotics. As mentioned above, it is likely that the anti-inflammatory effect of hydroxychloroquine is essentially due to its anti-infectious effect (59-64).

There is some controversy about the duration of treatment. Most doctors believe that the disease is cured after three weeks of antibiotic treatment. Doctors who are in denial about the chronic form of the disease tell their patients that they are cured at the end of the three-week course of antibiotics. However, it is well established in the medical literature that a significant proportion of treated patients still show signs of progression. Doctors then consider the disease to be psychosomatic. We have mentioned the persistence of *Borrelia*, explained by the various biological mechanisms. In our opinion, treatment should be continued as long as the patient's symptoms diminish, until a plateau is reached. Treatment can therefore be lengthy, lasting several weeks, or several months for the oldest more severe cases. Everytime we asked for funding to set a randomized clinical trial to confirm the high efficacy of a combination of doxycycline and hydroxychloroquine for at least four months, the demand was rejected with the pretext that it is not useful to make a study on the chronic form of the disease which, officially, does not exist! In addition, relapses, which can occur more or less rapidly when treatment is stopped, need to be treated quickly. Doctors specialized in the management of chronic Lyme disease are well aware of these frequent events. We published several clinical case reports of severely disabled patients who eventually went into remission (126, 153). This experience is shared by doctors around the world who treat cases of chronic Lyme disease, with improvement/cure rates approaching 80 % (154). It should be added that co-infections also need to be taken into account, and patients with associated piroplasmosis need to be treated with specific drugs, such as the combination of atovaquone-proguanil and azithromycin. We have published the case of a 36-year-old woman, immunocompetent, non-splenectomised, presenting for several years with a polymorphic persistent syndrome with major asthenia, neurological and cognitive disorders (concentration and memory disorders) and polymorphic somatic signs: joint, muscle and neurological pain, night sweats, chills, etc. *Babesia* serology and PCR were positive. After an initial exacerbation of the symptoms, a combined treatment was spectacularly effective. The patient has been in complete remission for several years (133). The presence of candidiasis must also be taken into account, especially as these fungi can secrete mycotoxins that induce symptoms

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similar to those of chronic Lyme disease or SPPT (120, 121). We have observed cases of improvement with fluconazole, as already published by Schardt et al (122). Other anti-infective treatments deserve to be evaluated, such as disulfiram, dapsone or nitazoxanide (NTZ), which have anti-bacterial, particularly anti-*Borrelia*, anti-parasitic and anti-viral effects (155-158) and could inhibit the formation of biofilms (159).

Controversy also stems from a published study that showed no efficacy (160). However, this study is of mediocre quality because (a) it is too short; (b) it does not take into account the possibility of co-infections; (c) it does not take into account the Jarisch-Herxheimer reactions linked to the destruction of bacteria and the release of toxins by them, reactions which have been well recognized in the treatment of syphilis in the past. For chronic Lyme disease, these exacerbations induced by treatment may be prolonged and may fluctuate with alternance of worsenings and improvements of symptoms. These exacerbations may last several days, weeks or even months. After 3 or 4 weeks, the 'classic' duration of treatment, it is often difficult to observe sustainable improvement. For experienced physicians, these exacerbations confirm that the treatment is effective. Many physicians, not accustomed to the treatment of chronic Lyme disease, consider these exacerbations as failures of treatment and stop it, looking for another diagnosis, usually a psychiatric disorder. That's why many patients, experiencing a transitory worsening and on the road to improvement or recovery, are rejected by the medical system. The Jarisch-Herxheimer reaction is thought to be mediated by TNF (161, 162). This explains why anti-TNF drugs can be effective in certain autoimmune diseases which may in fact be secondary to crypto-infections, including Lyme disease. Another older study seems to have been designed to fail, as Bransfield et al. state in a response letter (163, 164). Indeed, in addition to other criticisms that may have been made, the authors of the study excluded patients with a positive PCR for *Borrelia burgdorferi* in plasma or cerebrospinal fluid: 'Patients with a positive polymerase-chain-reaction (PCR) test for *Borrelia burgdorferi* DNA in plasma or cerebrospinal fluid at base line were also excluded.'! Bransfield et al. answered: 'Why was a positive PCR test for *B. burgdorferi* a formal criterion for exclusion from a study designed to shed light on the controversy surrounding chronic Lyme disease? If there is a consensus that PCR positivity constitutes laboratory confirmation of active infection, and if patients with a positive result were excluded from this placebo-controlled study on ethical grounds, this point should have been highlighted' (164). Donta criticises these studies for being too short: 'The study did not answer the question of whether better results would have resulted from a longer duration of treatment with intravenous ceftriaxone or oral doxycycline, or from treatment with different antibiotics for the same or a longer period.' (165). In the past, there was no need for randomized studies to prove the efficacy of penicillin in pneumococcal acute lobar frank pneumonia, for example. When a rate of cure is high, there is no need for a placebo. For chronic Lyme disease, the efficacy of a long course of treatment has already been demonstrated (154). The choice of compound is also important. Tinidazole and metronidazole are reputed to be more effective against these forms of the disease than the other antibiotics usually used (penicillins, cephalosporins and doxycycline, for example) (166-167). Pulsed treatment could also be of interest, perhaps by allowing *Borrelia*, during the no treatment period, to re-transform into antibiotic-sensitive spirochetes (69). In theory, the drugs should also penetrate intracellularly, crossing the blood-brain barrier at a sufficiently high dosage (168, 169). Co-infections must be investigated and treated with appropriate anti-infective drugs.

In our practice, we observed that relapses should be treated as quickly as possible with the drugs that have been effective. Remission is then almost always faster (126). Vitamin D supplementation, which plays a role in immunity, and which may have an anti-infective efficacy, as shown for tuberculosis, could be useful (170). We advocate a study that is (a) sufficiently long, (b) takes account of possible co-infections, and (c) includes regular assessment and scoring of all the patient's symptoms and general condition, both during treatment and when it is stopped. It should be borne in mind that this would be a complex study, because the disease is not homogeneous due to the presence of possible co-infections, and the population is also not homogeneous. As for most chronic maladies, multiple factors may influence the course of the disease: genetic background, eating habits, physical exercise, stress, exposure to toxic products, endocrine disruptors, heavy metals, autoimmunity, immunosuppression, etc. The scientific controversy surrounding Lyme disease, which we have touched on in this article, is explained in the book "*La Vérité sur la Maladie de Lyme"* (67), translated in English ("*Crypto-infections"*. Hammersmith 2021) where an abundant bibliography can be found.

Who benefits from denial?

In June 2019, Kris Newby is publishing a book entitled "*Bitten"*, the secret history of Lyme Disease and biological weapons, in which she said to have interviewed Willy Burgdorfer, the discoverer of the disease's causative agent (171). Kris Newby explains that she watched a recording of Willy Burgdorfer, who died in 2014, in which he recounts working on biological weapons for the US army during the Cold War at Rocky Mountain Laboratories in Hamilton, Montana. His tasks included breeding fleas, ticks, mosquitoes and other blood-sucking insects or arthropods and infecting them with pathogens capable of transmitting disease to humans. As Willy Burgdorfer, before his death, gave part of

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his lab archives to Kris Newby, all what she wrote is proven. A few weeks after the publication of her book, the Chamber of Representatives voted in July 2019 a resolution asking for an investigative commission on the Pentagone activity in this field. Unfortunately, a senior official of the Ministry of Defense blocked the project.

If we look at history, a Nazi veterinary researcher, Erich Traub, worked in Germany during World War II to develop bioweapons. He studied on human prisoners several vector borne diseases, including *Borrelia*. Arrested by the Soviets in his laboratory on Riems Island, he was later exfiltrated to the USA in 1949, as part of the US government's Operation Paperclip. The goal of this program was to exploit the German scientific knowledge in the context of the Cold War leading to rivalry with the Soviet Union. Erich Traub had to help developing the "Plum Island animal disease center". This center was located on Plum Island, some sixteen kilometers from the town of Lyme (Connecticut). This is what Kris Newby's book says: "Shortly before his death, Willy was filmed saying that he thought the epidemic of tick-borne diseases that had broken out around Lyme, Connecticut, had been caused by the spread of biological weapons. It was a stunning admission, but it could explain why Lyme disease is so difficult to diagnose and treat, and why the epidemic is spreading so far and so fast." We learn from Willy Burgdorfer's archives that in the Lyme area, at the very beginning of the epidemic, were observed many cases of infection with a newly man-made *Rickettsia, Rickettsia helvetica*, initially described in the archives as "l'agent Suisse" ("the Swiss agent"). Willy Burgdorfer could write his notes in French. This newly described bacterium was in fact made from a recombination of genes from Swiss *Rickettsia* and *Rickettsia* from the Rocky Mountains. Many cases of babesiosis were also reported in the region at that time. By reading these archives, we discover that the so-called Lyme epidemic, due to a single *Borrelia* species, was in fact a mixture of crypto-infections. Despite the denial of authorities, it has been proven that the army sprayed ticks marked with carbon 14 on: Montpelier (Vermont) in August 1966, September 1967, August 1968 and 1969, Newport News (Virginia) in September 1967 and August 1967, Mill Canyon (Utah) in August 1966 and July 1967. These elements raise two questions. Is there a connection with the growing epidemic of Lyme disease (ticks and micro-organisms possibly modified, more resistant and with increased infectious potential)? Do these military experiments explain the omerta on Lyme disease, given the potentially considerable responsibilities of the scientists and the states involved? (Appendix 2 of Kris Newby's book Bitten) (171, 172). There are also financial implications. Chronic Lyme disease can be expensive (173, 174). Conversely, we need to think about the fact that a cured Lyme disease patient (with inexpensive treatments) is a patient lost for expensive drugs, such as anti-TNF, which could be prescribed for the rest of his life. Indeed, as we have seen, this disease can mimic many other conditions, notably autoimmune ones. It is clear that, on a financial point of view, pharmaceutical companies do not favor the recognition of chronic Lyme disease.

Conclusion

Lyme disease is more complex than is generally accepted, because the *Borrelia* cause very different clinical pictures depending on the genetic background of the patients affected, and because it is frequently associated with other co-infections, bacterial, parasitic, viral or fungal. The association with fungi, particularly *Candida* spp., should be taken into account, as fungi can produce mycotoxins and could cooperate with *Borrelia.* There is scientific evidence that tests are unreliable, particularly serology, and that the chronic form of the disease exists, due to bacterial persistence. The mechanisms of persistence have been observed and published extensively. Lyme disease can simulate or provoke a large number of diseases, particularly autoimmune diseases. The consequence of denying these scientific realities is that hundreds of thousands of patients are left to wander, suffering from debilitating symptoms and untreated, despite the fact that appropriate anti-infective treatment over a long enough period of time leads to remission in the majority of cases.

Table 1

***Borrelia* persistence capacity**
Biofilms
Round or L-shaped forms
Sanctuarization (fibrous tissue, intracellular, possibly inside parasites)
Ability to evade the immune system
Cooperation with fungi such as Candida spp.?
Lyme serology
Poor calibration and quality of reagents
Opaque preparation
No precise reference diagnosis for test development
Arbitrary positivity threshold (less than 5% of tests must be positive)
More sensitive Western blot test prohibited in France in first intention
Test developed from *Borrelia burgdorferi* sensu stricto
Sequestration of antibodies in immune complexes
Relative immunosuppression of patients (fewer antibodies)
Quiescence of *Borrelia* which do not stimulate antibody production

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