Lack of Convincing Evidence that *Borrelia burgdorferi* Infection Causes Either Alzheimer's Disease or Lewy Body Dementia

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Summary: We investigated whether there is credible evidence that Lyme disease may cause Alzheimer's disease or Lewy body dementia. We conclude that no convincing evidence exists that Lyme disease is a cause of either Alzheimer's disease or Lewy body dementia.

Abstract

The role that microorganisms might have in the development of Alzheimer's disease is a topic of considerable interest. In this article we discuss whether there is credible evidence that Lyme disease is a cause of Alzheimer's disease and critically review a recent publication claiming that *Borrelia burgdorferi* sensu stricto infection, the primary cause of Lyme disease in the United States, may cause Lewy body dementia. We conclude that no convincing evidence exists that Lyme disease is a cause of either Alzheimer's disease or Lewy body dementia.

Keywords: Dementia; Lyme disease; *Borrelia burgdorferi*; Alzheimer's disease; Lewy body dementia

Alzheimer's Disease

Alzheimer's disease occurs as a result of progressive neuronal death within the brain parenchyma, resulting in characteristic clinical phenotypes. The clinical spectrum of Alzheimer's disease includes subjective cognitive decline, mild cognitive impairment, and dementia [1]. Dementia from Alzheimer's disease is progressive, with brain atrophy as a prominent feature. Initially, there is difficulty with short-term memory, followed by language problems, mood swings, behavioral changes, self-neglect, apathy, and problems with orientation, including easily getting lost. Delusions and hallucinations are also common.The speed of progression can vary, but is usually relatively rapid with death occurring within 3-9 years after diagnosis [2].

An infectious cause of Alzheimer's disease has been hypothesized. Supportive evidence, but of questionable validity or generalizability, exists for a variety of microbes including Herpes simplex virus type 1, other herpes viruses, *Chlamydophila pneumoniae*, *Porphyromonas gingivalis*, *Treponema denticola*, other *Treponema spp*., *Helicobacter pylori*, *Propionibacterium acnes*, *Burkholderia spp*. and fungi [3-10]. Some studies have suggested that the cause is polymicrobial, with multiple microorganisms present in brain tissue simultaneously [5]. Microorganisms within the oral microbiome have been of special interest because of the reported association between chronic periodontitis and Alzheimer's disease [11]. However, causality has not been established for this association [11].

Of particular relevance to this article, a few studies have suggested that *Borrelia burgdorferi* sensu lato may cause Alzheimer's disease based on detection of this microorganism in tissue samples from patients with Alzheimer's disease [6,12,13]. However, multiple other studies have been unable to confirm this association [5,9,14-21]. In one of these studies [17], co-authored by one of us (CSP), it was shown that, in neuropathologically

confirmed cases of Alzheimer's disease, *B. burgdorferi* sensu stricto could not be cultured using standard microbiologic methods (using Barbour Stoenner Kelly medium), nor were borrelial antigens present in extracts of brain tissue based on Western blot analyses. In another study [15], also conducted by a co-author (AM), PCR targeting the 16S gene of *Borrelia* species was used to examine specimens from the frontal, temporal, and occipital lobes of 15 patients with Alzheimer's disease and 15 age- and sex-matched controls. No patients with Alzheimer's disease and no controls were positive for *Borrelia* species in the brain (15).

In addition, questions as to the validity of studies purporting to demonstrate the presence of *B. burgdorferi* sensu lato in patients with Alzheimer's disease should have been raised based on the comment in one of the reported studies that *B. burgdorferi* sensu lato was detected by dark field microscopy in 14 blood samples (100%) of the 14 patients evaluated, and could be recovered by culture from 4 (80%) of the 5 blood samples that were cultured [13], despite the infrequency with which spirochetes can be found in blood samples by either culture or polymerase chain reaction testing (PCR) even in untreated patients with early Lyme disease [22]. In addition, 2 separate studies have challenged this association based on the lack of an appropriate geographic correspondence between fatal cases of Alzheimer's disease and that of Lyme disease in the United States [21,23]. Moreover, a prospective, 6-year follow-up cohort study of 689 retired farmers 65 years of age or older in France showed no increased risk of dementia in participants who were seropositive for antibodies to *B. burgdorferi* sensu lato [19]. Also, a study comparing 2067 patients with Lyme neuroborreliosis in Denmark with a gender- and age-matched cohort from the general population (n = 20,670) found that patients with Lyme neuroborreliosis had no long-term increased risks of dementia or Alzheimer's disease, Parkinson's disease, motor neuron diseases, or epilepsy [20].

A factor that also impedes the hypothesis that Lyme disease may cause Alzheimer's disease is the lack of a suitable animal-infection model. Animal models of *B. burgdorferi* sensu lato infections have yet to be developed for studying the pathologic changes occurring in Alzheimer's disease. Interestingly, representative animal models for primary syphilis (in the rabbit and guinea pig) [24,25], and for extracutaneous Lyme disease (in the mouse, rat and non-human primates, among others) [26,27], have existed for many years and have provided key insights into the infectivity, disease manifestations, immune mechanisms and treatment options for both infections.

Lewy Body Dementia

Lewy body dementia is the third most common type of dementia after Alzheimer's disease and vascular dementia [28]. Lewy body dementia is characterized by fluctuations in cognitive function, sometimes also with fluctuations of alertness and attention. Patients with Lewy body dementia are easily distracted and can appear to be "zoning out" at times. Impaired job performance is a common early sign, and patients with Lewy body dementia have problems with multitasking. Sleep disorders are common. Lewy body dementia can progress faster than Alzheimer's disease and is felt to have a worse prognosis than Alzheimer's disease [29].

Lewy Body Dementia: Response to Case Report

A recently published case report has raised the question of whether *B. burgdorferi* sensu stricto might be responsible for causing Lewy body dementia [30]. This concern was raised, despite the fact that the patient reported had been appropriately treated for early Lyme disease with the antibiotic doxycycline, which is also highly effective for treatment of Lyme neuroborreliosis [31], and that there was a multi-year delay following treatment of early Lyme disease before development of cognitive decline, at which point the cerebrospinal fluid

examination was normal, and the patient was seronegative for antibodies to *B. burgdorferi* sensu stricto based on 2-tier testing (Table 1) [30,31]. A positive serologic test to diagnose Lyme disease requires both a positive first-tier assay plus a positive second-tier test [31]. In addition, intensive retreatment with various antibiotics did not resolve the dementia. Also, there was no significant difference in the rate of detection of *B. burgdorferi* sensu stricto on postmortem tissues by PCR between the case patient and the 7 controls from Macedonia that were studied (1/1 vs 1/7, p = 0.25, using the Fisher's exact test) [30]. In addition, the PCR positivity could not be confirmed by the 23S rRNA probe used.

The nested PCR technique used in that study [30] began with an assay targeting the 16S−23S internal transcribed spacer (ITS) region of the ribosomal DNA of *B. burgdorferi* sensu stricto. The nested PCR assay used, however, had 55 amplification cycles for both PCRs, much above the recommended limit of no more than 45 cycles, as nonspecific PCR bands often begin to appear at cycles >40. Because two rounds of amplification are employed, nested PCR testing is generally more prone to false positive results unless strict protocols for quality controls are applied. These controls apply not only to nucleic acid extractions but also to the PCR procedure as well. Another limitation of the PCR testing was restricting the testing to only a single gene target; PCR positivity of more than a single gene target would be expected, and if found, would provide greater credibility in establishing the presence of *B. burgdorferi* sensu stricto infection in the tissues evaluated [32]. Moreover, there was also a positive PCR result in 1 case of the 7 human tissue controls [30]. Instead of attributing this finding to technical problems with the assay, and thereby casting doubt on the validity of all of the results, the authors considered the positive result in the controls to represent true infection. In addition, no PCR testing was even done to detect the species of Lyme borrelia that are most common in Europe, despite using neural tissue samples of individuals from Macedonia as the control group.

The authors provided the sequences generated through the nested PCR both for the patient with Lewy body dementia (2 sequences, 1 from the amygdala and 1 from the spinal cord) and for the PCR positive control (1 sequence from an inferior parietal lobule) [30]. Although the authors provided a BLAST (basic local alignment search tool) analysis, there are several issues that need to be addressed in regard to this analysis. Although the authors accurately state that the second round PCR generated a 195 nucleotide product, this product included the very long primer sequences used; primer sequences, however, should be excluded from BLAST analyses. Therefore, the PCR product that actually should be analyzed would only be 133 nucleotides in length.

More importantly, the sequencing results provided in the publication [30] contain only sequences generated with the forward primer, and inexplicably, the sequences were not curated properly. As a result, sequencing errors at both the 5' and the 3' ends of each provided sequence were included in the analysis. First, the authors did not trim the 5' ends of the sequences. When the three sequences are aligned to each other, as well as to other ITS regions of *B. burgdorferi*, it is clear that approximately 35 nucleotides at the 5' end harbor multiple sequencing errors, introduced immediately after the end of the 5' primer sequence. This is a common occurrence that is typically rectified by analysis of the reverse sequence that is obtained with the reverse primer, which unfortunately was not performed in this study [30]. Therefore, this approximately 35 nucleotide region needs to be excluded from any BLAST analysis. Secondly, approximately 30-50% of each sequence at the 3' end represents spurious "junk" sequence that is often generated when other PCR products are present in the sample during dideoxy sequencing. For the patient with Lewy body dementia, this consisted of 137 nucleotides out of 298 nucleotides of the provided spinal cord sequence, and 84 nucleotides out of the 240 nucleotides provided for the amygdala sequence. For the control,

this consisted of 147 nucleotides out of the 306 nucleotides provided for the inferior parietal lobule sequence.

When the errors at both ends are excluded and the remaining sequences are curated properly and analyzed by BLAST, the remaining 92 nucleotide fragments are 100% identical to 99 other ITS *B. burgdorferi* sequences in GenBank. This includes the *B. burgdorferi* B31 sequence, which was the strain used as a positive control for the assay. Thus, the authors cannot claim that the sequence generated in their study is unique, nor can they be certain that it was not the result of contamination.

Additionally, it would be highly unexpected to find *B. burgdorferi* sensu stricto in a control from Macedonia, as most Lyme disease cases in Europe are caused by *B. afzelii* or *B. garinii* [33]. It would be highly unusual that a *B. burgdorferi* sensu stricto strain, similar to that found in the patient with Lewy body dementia and to the positive control *B. burgdorferi* B31, would be the cause of neural "infection" in the Macedonian controls, unless they had previously spent time in North America.

The authors then performed immunofluorescence using a primary rabbit polyclonal anti–*B. burgdorferi* antibody [30]. No further information was provided, and the reference cited by the authors does not provide any specific information regarding the characteristics of the antibody used. Immunofluorescence was performed only on tissues that tested positive by the nested PCR of the case patient and of the one control, and was not evaluated by an examiner blinded as to the clinical status of the cases being investigated. Blinding is important as immunofluorescence testing tends to be highly subjective and poorly reproducible [32]. In addition, it has been suggested that use of only a single antibody against a particular microorganism may result in misleading observations [32]. The authors report that one *Borrelia* spirochete was identified in tissue from the spinal cord of the patient with Lewy body dementia. RNA *in-situ* hybridization with a probe against *B. burgdorferi* 23S

rRNA transcript, however, was negative in the tissues. In addition, the image provided does not resemble the classic spiral shape of this microorganism [30]. Therefore, we conclude that it is most likely that this finding does not prove the presence of *B. burgdorferi* sensu stricto in the tissue sample evaluated and that the positive results of the nested PCR assays in both the patient and the one control are likely to reflect laboratory contamination.

Dementia from Lyme Disease

Can Lyme disease cause objective cognitive dysfunction or dementia? Cognitive complaints, such as concentration or memory disturbances, are common in patients with Lyme disease and in patients with residual subjective symptoms after treatment for Lyme disease; the latter is often referred to as post-treament Lyme disease symptoms or syndrome (PTLDS). For example, in a prospective study of 52 patients with untreated early Lyme disease who had the characteristic erythema migrans skin lesion, 11 (21.2%) reported that they had difficulty concentrating or memory problems, compared with 5 (4.8%) of 104 matched controls at the baseline visit, p=0.004 [34]. Objective evidence of cognitive dysfunction, however, is typically not found when neurocognitive testing is performed for either patients with early Lyme disease or those with PTLDS [35,36].

Anecdotal evidence, however, does suggest that Lyme disease may rarely cause dementia. Dementia-like syndromes from Lyme disease occur as a consequence of the very rare late neurologic manifestation of Lyme disease referred to as chronic progressing meningoencephalomyelitis (also referred to as chronic encephalomyelitis). Like other cases of chronic progressing meningoencephalomyelitis due to Lyme disease, dementia occurring in the context of Lyme disease is seen predominantly in Europe, since the species of Lyme borrelia that are more neurotropic exist in this geographic area [37]. The best documented cases of dementia from Lyme disease have had clinical features that resemble normal pressure hydrocephalus, and have had clearly abnormal cerebrospinal fluid parameters with a

lymphocytic pleocytosis and an elevated protein level, in conjunction with a positive test for intrathecal antibody production against *B. burgdorferi* sensu lato [38-40]. Of note, these patients' dementia progressed over 6-12 months, more rapidly than in Alzheimer's disease. Further evidence supporting the premise that active infection with *B. burgdorferi* sensu lato was the cause of these patients' dementia was the clear and sustained response to a 14-28 day course of antibiotic therapy [40]. Thus, the few reported cases of dementia-like syndromes from Lyme disease are clinically very different from the Lewy body dementia case attributed to Lyme disease by Gadila et al [30].

Conclusion

In conclusion, there is no convincing evidence that Lyme disease is a cause of either Alzheimer's disease or Lewy body dementia.

NOTES

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Table 1. Concerns Regarding a Case Report [30] Implying that Lyme Disease Despite Antibiotic Treatment with Doxycycline Caused Lewy Body Dementia

Topic	**Comment**
1. Patient said to have had a well-documented erythema migrans skin lesion	No picture or other information provided
2. Patient had a 40^0C fever	High fevers are very atypical for Lyme disease.
3. Speculation that the patient also had Lyme meningitis	No CSF examination performed and the patient was treated with doxycycline, which is an effective therapy for Lyme meningitis.
4. No acute phase serology done	Only a convalescent phase serologic test was performed.
5. 4 years later developed cognitive difficulties	2-tier serologic testing at that point was negative, since only 1 of the 2 tests performed was seroreactive, but both are required to be reactive.
6. Then treated with 60 days of IV ceftriaxone	60 days is a longer duration of treatment than is recommended for neurologic Lyme disease.
7. "60%" improvement in cognition	How was this documented? No proof that this was due to the antibiotic. In addition, it was transient.
8. Then treated with amoxicillin x 6 months followed by minocycline	Unestablished therapy and no benefit seen.
9. When CSF was eventually obtained, inappropriate testing was performed to detect intrathecal production of anti-borrelia antibodies; there was no pleocytosis and no elevated protein level in CSF	
10. Autopsy specimen of case patient was compared with autopsy specimens of 7 deceased Macedonians	The limited medical history available for the controls did not provide evidence for a prior history of Lyme disease. However, given that Lyme disease occurs in Macedonia, why would these cases be a satisfactory control group?
11. No significant difference in the rate of detection of *B. burgdorferi* sensu stricto between the case patient and	1/1 vs 1/7, p = 0.25, using the Fisher's Exact Test

the 7 controls from Macedonia	
12. Unconventional nested PCR testing used on tissues, each stage of the PCR testing used 55 cycles	55 cycles exceeds the recommended approach, as it increases the likelihood of developing nonspecific PCR bands. A nested PCR approach increases the risk of false positive results.
13. Only a single gene target for the PCR testing	PCR positivity of multiple, highly polymorphic gene targets should be used to conclude that DNA of *B. burgdorferi* sensu lato was present in the tissues and should be a minimum standard to conclude that DNA of *B. burgdorferi* sensu lato was present in the tissues sampled.
14. Properly curated sequences from the PCR products generated for the patient with Lewy body dementia and the control from Macedonia were identical to *B. burgdorferi* strain B31, the strain which was used as the positive control in the study	Therefore, laboratory contamination cannot be excluded. In addition, it would be unexpected that the positive control from Macedonia would also be infected with a borrelial strain resembling B31, since the majority of Lyme disease cases in Europe are caused *B. afzelii* or *B. garinii*.

IV = Intravenous; CSF = Cerebrospinal fluid; PCR = polymerase chain reaction