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Persistent *Borrelia* Infection in Chronic Lyme Disease: A Review of the Medical Literature

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Abstract

Chronic Lyme disease (CLD) remains a controversial illness. The controversy is based on a profound disagreement over the existence of persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, and the ability of this persistent infection to cause chronic symptoms in patients who are untreated or undertreated for the spirochetal disease. Based on a review of the medical literature, we identified 56 studies confirming *B. burgdorferi* persistence. In this article, we summarize evidence from animal models and human studies that support persistent spirochetal infection as the cause of CLD. Specifically, direct and functional testing using culture, histology and xenodiagnosis has shown viable organisms following antibiotic therapy, and the potential role of cysts (L-forms) and biofilms in this process is examined. Future studies are required to investigate the mechanism of persistent infection in CLD.

Keywords

Lyme Disease, *Borrelia burgdorferi*, Cysts, Biofilms, Animal Models, Persistence, Chronic Lyme Disease, Post-Treatment Lyme Disease

1. Introduction

More than 500,000 new cases of Lyme disease are diagnosed each year in the USA, making it the most common vector-borne disease of North America [1] [2]. If the acute spirochetal infection is not adequately addressed due to variable symptoms, poor diagnostic test sensitivity and a lack of clinical biomarkers, patients may develop chronic Lyme disease (CLD), which remains a controversial illness [3]. At the heart of this controversy lies a profound disagreement over the existence of persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, and the ability of this persistent infection to cause chronic symptoms in patients who are un-

treated or undertreated for the spirochetal disease. CLD constitutes a significant health care burden, costing the US healthcare system nearly 1.3 billion dollars annually [4].

CLD is a multisystem illness with diverse musculoskeletal, neuropsychiatric and/or cardiovascular manifestations [3]. The disease is associated with pathogenic members of the *Borrelia* spirochete complex often in combination with other tickborne disease (TBD) pathogens. To qualify for the diagnosis of CLD, patients must have Lyme-compatible symptoms and signs that are either consistently or variably present for six or more months. Two subcategories of CLD include untreated chronic Lyme disease (CLD-U) and chronic Lyme disease following a limited course of antibiotic treatment (CLD-T), as defined elsewhere [3].

Although some earlier infectious disease articles maintain that there is no "credible scientific evidence" for persistent infection with *B. burgdorferi* following 2 - 4 weeks of antibiotic therapy [5], a number of animal and human studies provide evidence for persistent infection as a cause of chronic symptoms in Lyme disease patients, thereby contradicting the earlier infectious disease point of view [1] [6] (**Table A1** and **Table A2**). Alternative explanations for persistent symptoms in CLD include infection-induced immune dysfunction, inflammation due to persistent bacteria, bacterial "debris" and genetic and other mechanisms [7].

Unfortunately, the CLD controversy often results in misdiagnosis, inadequate treatment, and medical gaslighting that contribute to patient suffering and persistent infection [6] [8]. The failure to recognize persistent infection as a cause of CLD has a detrimental health impact on patients and society because these patients are often denied antibiotic treatment that may restore their health. These patients have worse quality of life than many other chronic disease conditions, including diabetes, multiple sclerosis, congestive heart failure, and arthritis [6].

This literature review presents the evidence for persistent infection with *B. burgdorferi* and provides a resource for academics and clinicians who require further validation for CLD.

2. Methods

We conducted a review of the medical literature to identify studies demonstrating persistent *Borrelia* infection in both animal models and humans. Two data bases (PubMed and Google Scholar) were searched using key words such as *Borrelia*, *B. burgdorferi*, Lyme disease, chronic, persistent, and infection. The literature was reviewed and categorized based on whether the subjects were animal or human and subcategories were created based on animal type. Animal studies were included if direct or functional testing techniques for *B. burgdorferi* were used, including culture, histology, xenodiagnosis, polymerase chain reaction (PCR) and/or RNA in-situ hybridization. Antibiotic therapy was not a requirement for this group, and length and sites of infection were noted. Human studies were only included if direct detection of *Borrelia* sequences or organisms was noted using culture, histology, xenodiagnosis, PCR and/or fluorescent in situ hybridization

(FISH) following antibiotic treatment.

3. Results

We identified 24 animal studies (13 rodent, 2 canine, 7 monkey, and 2 horse) and 32 human studies supporting persistence of *Borrelia* infection (Table A1 and Table A2). *Borrelia* was identified in various sample sites from 60 days to 46 months following infection. Persistent detection of *Borrelia* sequences following antibiotic treatment was found in 13 of 24 animal studies and 31 of 32 human studies. In 10 animal studies and 25 human studies viable spirochetes were demonstrated by culture, histology and/or xenodiagnosis following antibiotic treatment. The primary methods used to identify *Borrelia* in the animal and human studies included culture (13 animal, 15 human), histology (18 animal, 11 human), xenodiagnosis (4 animal, 1 human), and PCR (15 animal, 12 human). Additional modes of detection can be found in Table A1 and Table A2. Detection of *Borrelia* organisms was described in 21 (88%) animal studies and 26 (81%) human studies. The remaining studies only used PCR for molecular detection of spirochete sequences.

4. Discussion

After performing a literature review, we identified 56 studies showing that *B. burgdorferi* was detectable in longterm culture (Table A1 and Table A2). Viable spirochetes were identified by culture, histology and/or xenodiagnosis following antibiotic treatment in 10 animal studies and 25 human studies, demonstrating that live organisms can persist following this treatment. These findings contradict the earlier contention that the Lyme spirochete cannot survive antibiotics. Further evidence for evasion of the immune response and antibiotic therapy is described below.

A monkey study by Embers *et al.* published in 2012 provides the best animal evidence for persistent infection as a mechanism for CLD [9]. The study was conceived as an animal counterpart to the human trial by Klempner *et al.* that was published in 2001 [10], and the monkeys were treated with a regimen of intravenous ceftriaxone followed by oral doxycycline that was identical to the protocol used in the human trial. The results of this study showed that three-quarters of the monkeys failed treatment, and these animals had evidence of persistent infection in various tissues at necropsy using culture, immunofluorescence and PCR techniques [9]. Equally important, the study showed that 25% of treated monkeys cleared their infection, thereby demonstrating antibiotic efficacy in some animals. This finding contradicts the negative treatment results reported by Klempner *et al.* in humans to support the conclusion that antibiotics are not effective in treating patients with persistent Lyme disease symptoms [11]. In short, Embers was able to demonstrate persistence using an invasive approach (necropsy) that could not be used in human clinical trials [9] [12].

In another study, Bockenstedt *et al.* presented a mouse model of *B. burgdorferi* infection that on the surface appears to contradict the monkey study [13]. Follow-

ing infection, the mice were treated with subcutaneous ceftriaxone or doxycycline administered in drinking water. The authors arrive at the conclusion that non-infectious spirochetal "debris" gets deposited around the joints of these mice, and instead of being cleared by the reticuloendothelial system this "debris" is responsible for persistent inflammation in mouse tissues [13]. The "debris", which contained both DNA and protein particles, could not be cultured, transmitted to other mice via ear transplants or to ticks that were allowed to feed on the mice (xenodiagnosis).

This novel hypothesis of non-infectious persistence of *B. burgdorferi* "debris" including the presence of DNA contradicts previous experimental results. For example, Malawista *et al.* showed that *B. burgdorferi* DNA is rapidly cleared from culture-negative ear and bladder tissues of mice following prompt antibiotic treatment [14], and Lazarus *et al.* demonstrated that DNA from dead spirochetes is routinely cleared from mouse skin within several hours [15]. The "debris" hypothesis fails to explain persistence of viable spirochetes in culture, histology and xenodiagnosis experiments following antibiotic therapy. Furthermore, the study methods of Bockenstedt *et al.* may have been insufficient to rule out persistent spirochetal forms of *B. burgdorferi*, since ear transplants are often negative following antibiotic treatment, and using an insufficient number of animals for xenodiagnosis may fail to demonstrate transmissible infection [16] [17]. Of greater importance, there appear to be two alternative mechanisms of *B. burgdorferi* persistence that merit consideration in these mice: the persistence of cysts (L-forms) and the inability to detect spirochetes in biofilms.

In a commentary on the mouse study, Alan Barbour proposed the alternative hypothesis that cell-wall deficient cysts (L-forms) may be responsible for *B. burgdorferi* persistence in these animals [18]. He noted that these cystic structures, which Bockenstedt *et al.* observed in their infected animals, have been described as a persister mechanism employed by many bacteria, including *B. burgdorferi* [19]-[27]. Bockenstedt *et al.* claim that these are not true cysts because they form too fast, appearing in minutes rather than hours or days. However, Brorson and Brorson have demonstrated that cysts of *B. burgdorferi* may develop in minutes under appropriate culture conditions [28]. Thus the observation of Bockenstedt *et al.* supports *B. burgdorferi* cyst formation in their mouse model, and this cyst formation appears to be a better explanation for spirochetal persistence compared to the "debris" that the authors postulate.

As noted above, the methods employed by Bockenstedt *et al.* may not have been sufficient to exclude other persistent spirochetal forms such as cysts (L-forms) in their animals. Persistent viable organisms also may have been hidden in biofilms, the adherent polysaccharide-based matrices that protect bacteria against the host immune system and antibiotic therapy [1]. Biofilms of *B. burgdorferi* have been demonstrated in vitro by Sapi *et al.* [29]. These biofilms may take the form of "debris" on intravital microscopy, and they may contain organisms that are non-cultivable but still viable and prone to reactivation [29]-[31]. Biofilms of *B. burgdorferi* would also be consistent with the "amber hypothesis" proposed as a

mechanism of persistent Lyme disease symptoms due to "introduction into the joint space of non-viable spirochetes or spirochetal debris enmeshed in a host-derived fibrinous or collagenous matrix" [32]. Like the "debris" hypothesis, the "amber" hypothesis fails to explain live *Borrelia* persistence after antibiotic therapy. Persister spirochetes in biofilms could explain the experimental results of Bockenstedt *et al.* and would offer a more plausible explanation than the "debris" and "amber" hypotheses for the reasons outlined above.

Recently McClune *et al.* presented evidence that *B. burgdorferi* peptidoglycan (PG) can persist in synovial fluid after murine infection and may cause symptoms compatible with CLD [33]. McCausland *et al.* demonstrated unusual properties of the spirochete-derived PG that allow it to persist in mouse liver for weeks [34]. Although these observations may support the "debris" hypothesis of CLD, they do not rule out persistent *B. burgdorferi* infection in cysts (L-forms) and biofilms. Further work is needed to examine the relationship between PG-induced inflammation and persistent spirochete infection in CLD.

Like most aspects of Lyme disease, the role of cysts (L-forms) and biofilms in persistent *B. burgdorferi* infection has been controversial [1] [30] [35]. These spirochetal forms are resistant to common antibiotics due to reduced metabolic activity or protective matrices. Whether CLD arises from persisting spirochetal forms hidden in biofilms (as suggested by the monkey studies of Embers *et al.* and the work of Sapi *et al.*) or from cell wall-deficient cysts (L-forms) of *B. burgdorferi* (as suggested by the mouse study observations of Bockenstedt *et al.* and the interpretation of Barbour), persisting forms of bacteria require treatment. To date the treatment options for these bacterial persisters are extremely limited, but their recognition dictates a more aggressive approach to eradication of Lyme disease using combination antibiotic therapy modeled on treatment regimens for tuberculosis and HIV disease [2]. The fact that *B. burgdorferi* shares cyst (L-form) properties, biofilm configurations and resistance genes with pathogenic mycobacteria supports the need for this therapeutic approach [36] [37]. It remains to be seen which forms of *B. burgdorferi* are the true culprits in CLD and which treatments are most efficacious in clearing infection from patients [38]-[48].

5. Strengths and Limitations

The strengths of this review lie in the demonstration of longterm *Borrelia* infection in animal models and persistent infection following antibiotic therapy in animals and humans. Although various detection techniques were used, the culture, histology and xenodiagnosis testing identified viable spirochetes that persisted for long periods in animal models and survived antibiotics in animal and human cases. Although conventional PCR is a useful detection method, it can only detect fragments of the spirochete, leaving the door open for the "debris" hypothesis. Overall, this review confirms that *Borrelia* can persist despite treatment, but the exact mechanism for this observation remains to be determined. Although this study was not a systematic review of the literature, we sought to include as many studies as possible

that demonstrate viable *Borrelia* persistence through direct detection techniques following antibiotic therapy. Future more robust reviews are required to strengthen the level of evidence for the conclusions drawn from this study.

6. Conclusion

In this article, we summarize evidence from animal models and human studies that support persistent spirochetal infection as the cause of CLD. Specifically, direct and functional testing using culture, histology and xenodiagnosis has shown viable organisms following antibiotic therapy, and the role of cysts (L-forms) and biofilms in this process is highlighted. Determining the mechanism behind *Borrelia* persistence may hold the key to development of targeted treatments for CLD.

Authors' Contributions

Raphael B. Stricker, Melissa C. Fesler and Lorraine Johnson meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). All authors made substantial contributions to the conception, design and revisions of the current article and were involved in the analysis and interpretation of data. All authors have approved the final version.

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Conflicts of Interest

The authors have no conflicts to declare.

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Appendix

Table A1. Evidence for persistent infection in animal models of Lyme disease*.

Study/Year/Reference	Animal Origin	Persistence of *B. burgdorferi* Shown by	*B. burgdorferi* Detection**	Sample Source
1. Rodents				
Preac-Mursic *et al.*, 1990[1]	Gerbils	Culture, Histology	6 months	Joints, Skin Spleen
Duray & Johnson, 1986[2]	Hamsters	Culture, Histology	9 months	Spleen, Kidney Eye
Goodman *et al.*, 1991[3]	Hamsters	Culture, Histology	3 months	Heart, Bladder
Schmitz *et al.*, 1991[4]	Hamsters	Culture, Histology	16 months	Synovium, Spleen
Moody *et al.*, 1990[5]	Rats	Culture, Histology	12 months	Spleen, Kidney Joints
Sonnesyn *et al.*, 1994[6]	Guinea Pigs	Culture, Histology	16 weeks	Bladder, Heart Spleen, Joints Muscles
Malawista *et al.*, 1994[7]	Mice	Culture, PCR	60 days†	Ear, Bladder
Moody *et al.*, 1994[8]	Mice	Histology	90 days†	Joints, Heart
Bockenstedt *et al.*, 2002[9]	Mice	PCR Xenodiagnosis	12 weeks†	Joints, Bladder
Hodzic *et al.*, 2008[10]	Mice	PCR, Histology, Xenodiagnosis	12 weeks†	Joints, Heart
Yrjänäinen *et al.*, 2010[11]	Mice	PCR	30 weeks†	Joints
Barthold *et al.*, 2010[12]	Mice	PCR, Histology, Xenodiagnosis	12 weeks†	Joints, Heart Muscle
Bockenstedt *et al.*, 2012[13]	Mice	PCR, Histology	12 weeks†	Joints
2. Dogs				
Straubinger *et al.*, 1997[14]	Dogs	PCR, Histology	3 - 6 months†	Skin, LN Joints
Straubinger, 2000[15]	Dogs	PCR	500 days†	Skin, Muscle Joints
3. Monkeys				
Roberts *et al.*, 1995[16]	Monkeys	Culture, PCR, Histology	6 months	Joints, Nerve
Roberts *et al.*, 1998[17]	Monkeys	Culture, PCR, Histology	46 months	Nerve
Pachner *et al.*, 2001[18]	Monkeys	Culture, PCR, Histology,	3 months	Brain, Nerve, Heart
Cadavid *et al.*, 2004[19]	Monkeys	Culture, PCR, Histology	32 months	Heart
Miller *et al.*, 2005[20]	Monkeys	PCR	3 months	Brain, Nerve, Heart, Muscle, Skin, Bladder

Continued

Embers *et al.*, 2012[21]	Monkeys	Culture, Histology, PCR, Xenodiagnosis	6 - 12 months†	Skin, Heart Bladder Joints Tendon, Spleen
Crossland *et al.*, 2018[22]	Monkeys	Histology, RNA ISH	5 - 13 months†	Nervous System, Heart, Muscle, Synovium
4. Horses				
Chang *et al.*, 2005[23]	Ponies	Culture	5 months†	LN, Joints, Muscle
Imai *et al.*, 2011[24]	Horses	Histology, PCR	1 - 4 years†	Brain, Nerve

*PCR, polymerase chain reaction; LN, lymph node; ISH, in situ hybridization, **Time from initial infection to final positive testing point. †Detectable *B. burgdorferi* following antibiotic treatment.

Table A1 References

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Table A2. Evidence for persistent human infection following treatment of Lyme disease*†.

Study/Year/Reference	Study Origin	Persistence of *B. burgdorferi* Shown by	Sample Source
Weber *et al.*, 1988[1]	Europe	Histology	Brain, liver (Autopsy)**
Schmidli *et al.*, 1988[2]	Europe	Culture	Synovial Fluid
Cimmino *et al.*, 1989[3]	Europe	Histology	Spleen
Preac-Mursic *et al.*, 1989[4]	Europe	Culture	Skin Bx, CSF
Pfister *et al.*, 1991[5]	Europe	Culture	CSF
Strle *et al.*, 1993[6]	Europe	Culture	Skin Bx
Preac-Mursic *et al.*, 1993[7]	Europe	Culture	Iris Bx
Haupl *et al.*, 1993[8]	Europe	Culture	Ligament Bx
Strle *et al.*, 1996[9]	Europe	Culture	Skin Bx
Preac-Mursic *et al.*, 1996[10]	Europe	Culture	Skin Bx, CSF
Oksi *et al.*, 1996[11]	Europe	Culture	CSF
		PCR	Brain Bx
		PCR	Brain (Autopsy)
Priem *et al.*, 1998[12]	Europe	PCR	Synovial Bx/Fluid
Oksi *et al.*, 1999[13]	Europe	Culture, PCR	Blood
Breier *et al.*, 2001[14]	Europe	Culture	Skin Bx
Hunfeld *et al.*, 2005[15]	Europe	Culture	Skin Bx
Svecova *et al.*, 2008[16]	Europe	PCR	Blood
Hudson *et al.*, 1998[17]	Australia	Culture, PCR	Skin Bx
Steere *et al.*, 1988[18]	USA	Histology	Synovial Bx
Kirsch *et al.*, 1988[19]	USA	Histology	LN (Autopsy)
Liegner *et al.*, 1993[20]	USA	Histology	Skin Bx
		PCR	Blood
Battafarano *et al.*, 1993[21]	USA	Histology, PCR	Synovial Bx/Fluid
Chancellor *et al.*, 1993[22]	USA	Histology	Bladder Bx
Nocton *et al.*, 1994[23]	USA	PCR	Synovial Fluid
Shadick *et al.*, 1994[24]	USA	Histology	Brain (Autopsy)
Masters *et al.*, 1994[25]	USA	Culture	Blood
Lawrence *et al.*, 1995[26]	USA	PCR	CSF
Bayer *et al.*, 1996[27]	USA	PCR	Urine
Nocton *et al.*, 1996[28]	USA	PCR	CSF
Marques *et al.*, 2014[29]	USA	Xenodiagnosis	Tick***
Middelveen *et al.*, 2018[30]	USA	Culture, Histology	Blood, Genital
		PCR	Secretions, Skin
Sapi *et al.*, 2019[31]	USA	PCR, Histology, FISH, Confocal microscopy	Liver, Heart, Kidney, Brain (Autopsy)
Bransfield *et al.*, 2024[32]	USA	Histology, FISH	Pancreas, Heart, Brain (Autopsy)

†Adapted from Stricker RB, Johnson L. Lyme disease: the next decade. *Infect Drug Resist.* 2011; 4:1-9. *Except for case of Weber *et al.* (see below), all patients received a minimum of 10 days of antibiotic therapy. PCR, polymerase chain reaction; Bx, biopsy; CSF, cerebrospinal fluid; LN, lymph node. FISH, fluorescent in-situ hybridization; **Mother treated with antibiotics for one week during pregnancy; newborn died; ****B. burgdorferi* DNA recovered from ticks fed on human Lyme patients.

Table A2 References

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