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Studies that Report Unexpected Positive Blood Cultures for Lyme Borrelia-- Are They Valid?

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Abstract

Positive blood cultures for Lyme borrelia have been well documented in untreated patients with early Lyme disease. In this report we review the validity of three studies that reported the recovery of *Borrelia burgdorferi* sensu lato from the blood of a high proportion of patients for whom no evidence was presented, and no claim was made, that the patients had untreated early Lyme disease. In two of the studies the patients had been treated extensively with antibiotics for Lyme disease before the cultures were obtained. Critical evaluation of the three reports suggests that they are invalid. Indeed, two subsequently published studies could not reproduce the results of one of the reports. In a published analysis of another of the reports, investigators from the Centers for Disease Control and Prevention concluded that the cultures were likely to have been contaminated. When the biologic plausibility of recovering borrelia from blood is extremely low, the level of scientific rigor required of a study that claims a positive result should be particularly high.

Keywords

Lyme disease; *Borrelia burgdorferi*; Spirochetemia; Blood cultures; Post-treatment symptoms

Lyme disease is the most common tick-borne illness in North America [1,2]. Most patients with erythema migrans, the most frequent clinical manifestation, recover fully after a 10-14 day course of antibiotic therapy [3,4]. Approximately 10% of patients will have continued subjective symptoms, such as fatigue and/or joint pain, for six months or more after diagnosis and treatment despite resolution of the erythema migrans skin lesion [3-5]. Systematic studies have not demonstrated that viable spirochetes persist in these patients or that there is a meaningful benefit from intensive retreatment with antibiotics [6]. Additional

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research on the pathogenesis and most appropriate management of these symptoms is needed.

Similar symptoms are also common in persons in the general population who never had Lyme disease, regardless of whether they reside in a geographic area that is endemic for Lyme disease [3,5,7,8]. Not infrequently, such patients are labeled as having “chronic Lyme disease” and are treated with long-courses of antibiotic therapy [5]. In addition to never having had an objective clinical manifestation of Lyme disease, such patients typically are seronegative for antibodies to *Borrelia burgdorferi* sensu stricto by Centers for Disease Control and Prevention (CDC)-defined interpretative criteria and United States Food and Drug Administration-approved laboratory tests [3,5].

Those who believe that “chronic Lyme disease” is a real entity have made extensive efforts to try to document the existence of viable Lyme borrelia in these patients. Among the approaches used have been attempts to cultivate *B. burgdorferi* sensu stricto from the blood of these patients, despite the fact that such individuals have often already been treated extensively with antibiotics for Lyme disease and despite evidence from other studies that spirochetemia in patients with Lyme disease is usually detectable only at the very earliest stages of untreated infection [9]. Studies that successfully cultivated *B. burgdorferi* sensu stricto from patients with early Lyme disease used specialized media (modified Barbour Stoenner-Kelly [BSK] media) and incubated cultures for up to 3 months [10,11]. Also, although serum has been shown to be inferior to plasma as a source of culture material in patients with early Lyme disease, and despite the fact that large volumes of plasma (≥ 9 mL) are usually required to reliably cultivate *B. burgdorferi* sensu stricto from untreated patients with erythema migrans [10,11], a number of studies have reported a high rate of recovery of spirochetes from blood of patients, either from cultures of serum exclusively [12] or from cultures of small quantities of plasma [13,14]. In some of these studies spirochetemia was found in seronegative patients with long-standing, nonspecific symptoms who had already been treated with a prolonged course, or courses, of antibiotics directed towards *B. burgdorferi* sensu stricto infection [13,14]. The purpose of this manuscript is to review the validity of three of these studies (Table 1).

The first study, published in 1998, reported growth of *B. burgdorferi* sensu stricto from the serum of 91% of 47 patients previously treated for chronic Lyme disease of whom > 90% were seronegative for *B. burgdorferi* sensu stricto antibodies by two-tier testing [13]. The medium used was unconventional and specifically included Detroit tap water. The findings could not be reproduced [15,16], and the novel medium employed was actually shown to be bactericidal for *B. burgdorferi* sensu stricto in vitro [15,16], documenting that this report of culturing *B. burgdorferi* sensu stricto from the blood of such patients was not valid.

Another study reported positive culture results on serum specimens from 94% of 72 patients using a novel, two-step culture technique that incorporated collagen-coated slides in the second step [12]. All patients in this study were stated to be two-tier seropositive for antibodies to *B. burgdorferi* sensu stricto, but no information was provided about either the clinical characteristics of the patients or whether the patients had previously been treated with antibiotic therapy directed to Lyme disease. The authors did state that none of the

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patients had received antibiotic therapy within the 4 weeks prior to collection of the blood samples. Investigators from the CDC analyzed genetic sequencing data of the isolates said to be recovered using this technique and found that most were not *B. burgdorferi* sensu stricto [17,18], making the results highly implausible, as infection with Lyme borrelia species other than sensu stricto has not been documented in North America, aside from a very small number of infections due to *B. mayonii* [19]. The findings of this study were most consistent with laboratory contamination [17,18].

The most recent study claimed that at least three (13%) of 24 patients who lived in non-Lyme disease endemic areas of the United States had a positive serum or plasma culture using the modified Kelly-Pettenkofer (MKP) medium, but none was positive using BSK-H medium [14]. Two had positive blood cultures for *B. burgdorferi* sensu stricto and one had a positive blood culture for *B. bissettii*, a species of Lyme borrelia not established as a cause of infection in North America. All of these three cultures had been inoculated with just 1 mL of plasma that had been stored at 4°C for an imprecisely defined period of time of at least 5 days and perhaps as long as 48 days prior to inoculation. All three patients had non-specific symptoms, were seronegative for antibodies to *B. burgdorferi* sensu stricto, and had been treated extensively with antibiotics effective against Lyme borrelia before the blood specimen for culture was obtained. The two patients with positive cultures for *B. burgdorferi* sensu stricto were a married couple, both of whom had received a 9-month course of treatment with doxycycline. The patient with a positive culture for *B. bissettii* apparently still had doxycycline in blood at or around the end of a 40-day course of doxycycline when the blood sample for culture was obtained. This information alone raises concern about the validity of the assertion that the patient's blood culture was positive for borrelia, particularly since no evidence was provided that the isolate was resistant to doxycycline. The authors' laboratory had previously isolated and investigated numerous strains of *B. burgdorferi* sensu stricto, as well strains of *B. bissettii*-like spirochetes, from non-human sources [20-22], raising the question of whether the current findings could have been attributable to laboratory contamination. In a separate publication, the authors did state that, based on multilocus sequence typing, the *B. bissettii* isolate appeared unique in their experience [23]. However, the analysis [23] demonstrated that the "*B. bissettii*" isolate was actually more closely related genetically to *Borrelia carolinensis* strains sw21 and sw22 [20] (isolated by the authors from cotton mice and *Ixodes minor*, respectively) than to the type strain of *B. bissettii*. Direct polymerase chain reaction (PCR) testing of the patients' blood samples was not performed prior to inoculation into culture, and blood cultures were not repeated to verify the positive results.

Is it plausible that MKP medium, which is closely related in composition to BSK-H medium, as well as to BSK-II medium, might have superior sensitivity to BSK-H medium for recovery of Lyme borrelia from blood? Although two European studies found a greater yield from culture of biopsy specimens from erythema migrans skin lesions when inoculated into MKP medium compared with BSK-H medium [24,25], another study found no difference in the yield of cultures using MKP medium versus BSK-II medium [26]. No study has systematically compared the sensitivity of MKP medium with that of either BSK-H or BSK-II for recovery of Lyme borrelia from blood samples. If such a study is conducted, it should first be demonstrated that the media can support growth in vitro of reference strains

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of *Borrelia* species, with fewer than 10 organisms per inoculum (preferably a single bacterial cell), since it is well-known that different sources and lots of bovine serum albumin used to prepare media differ in their ability to support growth of borrelia [27].

In conclusion, all of the reviewed reports that claimed to have had patients with positive blood cultures for Lyme borrelia, including two studies that employed novel techniques for which no clear rationale exists, are unconvincing [12-18]. The finding of positive blood cultures in patients with chronic non-specific symptoms, in seronegative patients from non-endemic areas for Lyme disease, or in patients previously treated with antibiotics for the illness that prompted performance of the blood culture (an illness referred to as Lyme disease by the study authors), are likely to be invalid. When the biologic plausibility of cultivating borrelia from the blood is extremely low, it should raise the bar for the level of scientific rigor acceptable for publication of a study that reports positive results. Ideally, studies that claim to have recovered borrelia from the blood of patients with a very low probability of spirochetemia should have their findings confirmed by independent laboratories prior to publication. In addition, the results should be supported by repeating the blood cultures in positive cases, as well as by direct PCR testing, preferably by a quantitative PCR, of blood specimens before they are inoculated into culture [28-30]. Specimens from control subjects also should be tested simultaneously, and the investigators should be blinded to the sources of the blood specimens.

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Highlights

- Positive blood cultures have been documented in untreated early Lyme disease.
- Studies that reported positive blood cultures later in infection were reviewed.
- Critical evaluation of three such reports raises concerns over their validity.
- Low biologic plausibility increases the need for a high level of scientific rigor.

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Table 1

Features of Studies Demonstrating Unexpected Positive Blood Cultures for Lyme Borrelia

Reference	Year	Material Cultured	Culture Method	Findings	Why Findings Unexpected	Aspects of Study that Raise Concerns on Validity	Comment
Phillips et al [13]	1998	Plasma	"MPM"medium used	43 (91%) of 47 patients with "chronic Lyme disease" had a positive blood culture. Positive blood cultures were confirmed using both polyclonal and monoclonal antibody to Lyme borrelia, as well as by both electron microscopy and immunoelectron microscopy.	All patients had previously been treated with antibiotics and most (>90%) were seronegative for antibody to Lyme borrelia by two-tier testing. Indeed, all patients had previously received ≥ 6 weeks of IV ceftriaxone or another third generation cephalosporin. The 91% rate of positive blood cultures far exceeded the rate reported for untreated patients with early Lyme disease [10,11].	Used an unconventional culture medium that included Detroit tap water. Study results could not be replicated by other investigators. The novel culture medium used was shown to be bactericidal against strains of *Borrelia burgdorferi* sensu stricto inoculated into it [15,16].	Study did not present evidence that patients ever actually had Lyme disease.
Sapi et al [12]	2013	Serum	Modified BSK-H medium with rifampin using a 2-stage culture method that included collagen-coated slides.	68 (94%) of 72 patients had a positive blood culture. Positive blood cultures were confirmed using both polyclonal and monoclonal antibody to Lyme borrelia, as well as by PCR and by sequencing of PCR products.	The 94% rate of positive blood cultures far exceeded that reported for untreated patients with early Lyme disease [10,11].	Genetic sequencing data suggested that most of the isolates were not *Borrelia burgdorferi* sensu stricto, consistent with laboratory contamination. No data provided on the patients' clinical manifestations.	No corroboration of blood culture success by an independent laboratory. Unclear how many of the patients had previously been treated for Lyme disease, but some, if not all, may have been treated previously, although not within the prior four weeks before the blood sample was collected.
Rudenko et al [14]	2016	Culture of plasma or serum with three positive cultures from plasma.	Modified Kelly-Pettenkofer (MKP) medium and BSK-H medium; unclear how long after blood collection it was cultured, but was at least 5 days and possibly as long as 48 days.	At least 3 (13%) of 24 patients with non-specific symptoms had a positive blood culture for borrelia, two for *Borrelia burgdorferi* sensu stricto and one for a *Borrelia bissettii*-like strain. Positive blood cultures were confirmed using transmission electron microscopy, scanning electron microscopy, PCR, sequencing of PCR products, sequence analysis and multilocus sequence analysis.	Lyme disease not endemic in the States in which the patients resided. *Borrelia bissettii* not established to cause human infection in the United States.	No confirmatory testing of blood specimens by PCR before culture and no repeat blood cultures performed in patients with positive cultures. All patients had been previously treated for Lyme disease before cultures done. One patient had doxycycline still present in blood at time of culture and after completion of a 40 day treatment course. Doxycycline sensitivity of borrelial isolates not evaluated.	Investigators had previously isolated many strains of both *Borrelia burgdorferi* sensu stricto and *Borrelia bissettii* from non-human sources, raising concern that there could have been laboratory contamination. Apparently isolates could not be sub-cultured successfully in BSK-H medium.

PCR= Polymerase chain reaction

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