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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.

Lyme disease is the most common tick-borne illness in North America (Hinckley et al., 2014; Nelson et al., 2015). Most patients with erythema migrans, the most frequent clinical manifestation, recover fully after a 10–14-day course of antibiotic therapy (Cerar et al., 2010; Wormser et al., 2006). 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 (Cerar et al., 2010; Feder et al., 2007; Wormser et al., 2006). Systematic studies have not demonstrated that viable spirochetes persist in these patients or that there is a meaningful benefit from intensive retreatment with antibiotics (Klempner et al., 2013). Additional 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 (Blackwell and Clarke, 2013; Centers for Disease Control and Prevention, 2002; Feder et al., 2007; Wormser et al., 2006). Not infrequently, such patients are labeled as having "chronic Lyme disease" and are treated with long-courses of antibiotic therapy (Feder et al., 2007). 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 (Feder et al., 2007; Wormser et al., 2006).

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 (Nowakowski et al., 2009). 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 (Wormser et al., 2000, 2001). 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 (Wormser et al., 2000, 2001), a number of studies have reported a high rate of recovery of spirochetes from blood of patients, either from cultures of serum exclusively (Sapi et al., 2013) or from cultures of small quantities of plasma (Phillips et al., 1998; Rudenko et al., 2016). In some of these studies spirochetemia was found in seronegative patients with long-standing, non-specific

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symptoms who had already been treated with a prolonged course, or courses, of antibiotics directed towards *B. burgdorferi* sensu stricto infection (Phillips et al., 1998; Rudenko et al., 2016). 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 (Phillips et al., 1998). The medium used was unconventional and specifically included Detroit tap water. The findings could not be reproduced (Marques et al., 2000; Tilton et al., 2001), and the novel medium employed was actually shown to be bactericidal for *B. burgdorferi* sensu stricto in vitro (Marques et al., 2000; Tilton et al., 2001), 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 (Sapi et al., 2013). 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 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 (Johnson et al., 2014a, 2014b), 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* (Kingry et al., 2016). The findings of this study were most consistent with laboratory contamination (Johnson et al., 2014a, 2014b).

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 (Rudenko et al., 2016). Two had positive blood cultures for

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. (1998)	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 immuno-electron 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 (Wormser et al., 2000, 2001).	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 (Marques et al., 2000; Tilton et al., 2001).	Study did not present evidence that patients ever actually had Lyme disease.
Sapi et al. (2013)	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 (Wormser et al., 2000, 2001).	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. (2016)	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.