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No Evidence for Contamination of *Borrelia* Blood Cultures: a Review of Facts

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Fact checking of the recent report of Johnson et al. of the CDC (1) discloses errors of fact and misrepresentations in their critique of the work of Sapi et al. (2). The CDC authors have produced three 603-base nucleotide haplotypes (partial sequences for PyrG open reading frame gene equivalents) and deposited these in GenBank under the accession numbers KF170280.1 (allegedly derived from *Borrelia burgdorferi sensu lato* strain B023), KF170281.1 (allegedly derived from *B. burgdorferi sensu stricto* strain 297), and KF170282.1 (allegedly derived from *B. burgdorferi sensu lato* strain Fuji P1).

Assertions by Johnson et al. (1) that living borreliae from strains B023, Fuji P1, and 297 contaminated 41 of the Sapi et al. (2) blood culture isolates are not based on facts. BLASTn DNA match searches with GenBank *pyrG* sequences using KF170281.1 (*B. burgdorferi*) and KF170282.1 (*Borrelia garinii*) invalidate the conclusions of Johnson et al. (1). Further CDC misrepresentations to *Journal of Clinical Microbiology* readers include wording that *garinii*-type *Borrelia* human infections vectored by ticks in the Western Hemisphere have never been described. These statements are overturned by the published literature.

Among the overlooked references to support *B. garinii* in the Western Hemisphere are two cases of *Borrelia* lymphocytoma in patients from Mexico (3). *Borrelia* lymphocytoma is never due to U.S. strains of *burgdorferi*-type *Borrelia* but is linked to European epidermotropic *Borrelia afzelii* and *Borrelia garinii* strains and European *Borrelia burgdorferi* strains. Reports from Brazil implicate *Borrelia garinii* as an etiologic agent of Brazilian Lyme borreliosis (4, 5).

A case report from Wisconsin (6) is also overlooked.

Johnson et al. (1) allege specifically that there is DNA evidence of contamination of human blood isolates of borreliae (patient no. 1 to 20, 28 to 47, and 50 in Table 1 of reference 1). BLASTn interrogations using CDC interrogators KF170280.1, KF170281.1, and KF170282.1 show that only one of the Sapi et al. (2) human blood isolates (accession numbers beginning with "JX86") matches a CDC interrogator 100% (i.e., 603/603 bases), and that blood isolate is *Borrelia afzelii* (accession number JX867398.1), which matches CDC interrogator KF170280.1. There is no DNA support for contamination of any other Sapi et al. (2) blood culture isolates based on supercomputer BLASTn searches with the CDC interrogators KF170281.1 and KF170282.1. The reader is invited to engage the NCBI BLASTn supercomputer and to audit these results. To do so, on the BLAST home page (http://blast.ncbi.nlm.nih.gov/Blast.cgi), choose "nucleotide blast" as the program to run. Then, copy the CDC interrogators (e.g., "KF170281.1") one at a time into the search box, scroll to the bottom of the window, and click the "BLAST" button at the bottom of the screen. All DNA sequences with partial or complete identities will be presented in rank order. Scroll down past the color bars to find these detailed listings. Inspect the list of numbers in the far-right column (headed "Accession"). Sequences for Sapi et al. (2) blood isolates have accession numbers beginning with "JX86." DNA sequences in the list with accession numbers that do not begin with "JX86" will not be from Sapi et al. (2). The simplicity of this audit exercise is remarkable.

Johnson et al. (1) failed to comprehend that Sapi et al. utilized, in addition to *B. garinii*-type Fuji P1, five other *B. garinii* type strain *pyrG* sequences in their quality control procedures, specifically, DNA sequences AB555778.1, JF331269.1, JF331231.1, JF331248.1, and JF331265.1. In sum, what Johnson et al. (1) and the CDC have called into question is not the analytical methods of Sapi et al., which are sound. Rather, it is clear with fact checking that opportunities for correction of errors in the Johnson et al. (1) paper were missed. Assertions of contamination of patient specimens by Advanced Laboratory Services are not substantiated by BLASTn supercomputer interrogations with CDC DNA sequences as described above.

REFERENCES

1. **Johnson BJB, Pilgard MA, Russell TM.** 2014. Assessment of new culture method to detect *Borrelia* species in serum of Lyme disease patients. J. Clin. Microbiol. **52:**721–724. http://dx.doi.org/10.1128/JCM.01674-13.
2. **Sapi E, Pabbati N, Datar A, Davies EM, Ratelle A, Kuo BA.** 2013. Improved culture conditions for the growth and detection of *Borrelia* from human serum. Int. J. Med. Sci. **10:**362–376. http://dx.doi.org/10.7150/ijms.5698.
3. **Gordillo-Pérez G, Torres J, Solórzano-Santos F, de Martino S, Lipsker D, Velázquez E, Ramon G, Onofre M, Jaulhac B.** 2007. *Borrelia burgdorferi* infection and cutaneous Lyme disease, Mexico. Emerg. Infect. Dis. **13:**1556–1558. http://dx.doi.org/10.3201/eid1310.060630.
4. **Sulene P, Yoshinari NH, Médicis da Silveira A, Ferreira Bento R, Bonoldi V.** 2002. Serological reactivity to *Borrelia burgdorferi*, *Borrelia afzellii*, and *Borrelia garinii* antigens in patients afflicted by peripheral facial paralysis in Brazil. Otol. Neurotol. **23:**S33.
5. **Talhari S, Nunes de Souza Santos M, Talhari C, Carlos de Lima Ferreira L, Moreira Silva R, Jr, Zelger B, Massone C, Ribeiro-Rodrigues R.** 2010. *Borrelia burgdorferi* "sensu lato" in Brazil: occurrence confirmed by immunohistochemistry and focus floating microscopy. Acta Trop. **115:**200–204. http://dx.doi.org/10.1016/j.actatropica.2010.02.017.
6. **Finkel MF, Johnson RC.** 1990. *Borrelia* lymphocytoma: a possible North American case. Wis. Med. J. **1989:**683–686.

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