Case Report: A Retrospective Serological Analysis Indicating Human Exposure to Tick-Borne Relapsing Fever Spirochetes in Texas

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Presentation of Case

In June 2013, a Caucasian male from Kerr county, Texas, with an extensive history of outdoor activity working with sheep, goats, and exotic game became ill, displaying fever, chills, uveitis, headache, retrobulbar pain, severe malaise, and weakness. Myalgia was centered on upper extremities, most notably the shoulders, arms, and hands, and by July 2013 the patient had experienced two febrile episodes (39°C). Numerous insect bites were reported, most notably on areas of the body that were in direct contact with the ground, but offending arthropods were not found. The patient was initially tested for Coxiella burnetti exposure, but repeated serological results demonstrated that phase I and phase II immunoglobulin G (IgG) antibody titers failed to increase. Antibiotic treatment was administered, and symptoms improved. A retrospective evaluation of the patient’s history and clinical summary led to suspicion of tick-borne relapsing fever borreliosis. The patient did not have a history of traveling outside of Texas prior to the onset of symptoms, and Borrelia turicatae was suspected as the causative agent. A serum sample was sent to us four months after antibiotic treatment, and we implemented a molecular approach to determine seroreactivity against two diagnostic antigens for relapsing fever spirochetes, recombinant glycerophosphodiester phosphodiesterase (rGlpQ) and Borrelia immunogenic protein A (rBipA).

Approach

Ethics Statement

Verbal and written informed consent was obtained from the patient to test the serum sample. The study was submitted to the Mississippi State University Institutional Review Board, protocol #13–369, and approved by designated review.

Protein Analysis, Production of Diagnostic Antigens, and Serological Testing

rGlpQ has been used to evaluate mammalian exposure to species distributed in the western United States and East and West Africa [1–4]. The gene is absent from other pathogenic
spirochetes, and Schwan and colleagues first characterized rGlpQ as an antigen that could discriminate between exposure to relapsing fever and Lyme-causing *Borrelia* [1]. Amino acid alignments of GlpQ using Vector NTI 11.5 software (Life Technologies, Foster City, California, US) indicated the *B. turicatae* homologue is highly conserved between New (*B. turicatae*, *Borrelia parkeri*, and *Borrelia hermsii*) and Old (*Borrelia recurrentis*) World species of relapsing fever spirochetes (Table 1).

To produce *B. turicatae* GlpQ as a recombinant fusion protein, the gene was amplified using genomic DNA from the 91E135 isolate with 5′-CACCATGAAATTAATTAAAACAAATTTATTAAT GCTTACAATGAATATTTTT-3′ and 5′-TTGTTTTACAAACTTCACTAC TGTATCA GTAAAATCTGTAAAT-3′ forward and reverse primers, respectively. The amplicon was cloned into the pET102 expression vector, and the absence of nucleotide errors was confirmed by sequencing analysis using Vector NTI Advanced 11.5 software (Life Technologies). rGlpQ was produced as a six-histidine and thioredoxin fusion protein and purified by immobilized metal affinity chromatography using HisTrap FF Crude columns precharged with Ni²⁺ (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania, US). Thioredoxin was removed by incubating the purified protein with 0.01 U of EKMax Enterokinase (Life Technologies), following the manufacturer’s protocol. The molecular mass of native GlpQ and rGlpQ are 40 and 45 kDa, respectively.

Previous studies reported the identification and antigenicity of BipA and indicated a homologue was absent outside of relapsing fever *Borrelia* [5,6]. BipA also shares 24%–76% amino acid identity between relapsing fever spirochete homologues (Table 2) and was demonstrated to differentiate between infections caused by *B. turicatae* and *B. hermsii* [5]. *B. turicatae* rBipA was produced using the pET102 expression system as previously described [5], and thioredoxin was left attached to maintain protein solubility. The molecular mass of native and rBipA are 60 and 75 kDa, respectively.

Protein lysates from 1 x 10⁷ spirochetes and 1 μg of rGlpQ and rBipA were electrophoretically separated and transferred to nitrocellulose membranes as previously described [5] using TGX gels, Mini-PROTEAN Tetra cell, and the Mini Trans Blot system (BioRad, Hercules, California, US). Immunoblots were probed with the patient’s serum sample at a 1:400 dilution, and Rec-Protein G-HRP (Life Technologies) diluted 1:4,000 was used as the secondary molecule. Serological reactivity to multiple proteins in the *B. turicatae* whole spirochete lysates, rGlpQ,

### Table 1. Percent amino acid identity of GlpQ between species of relapsing fever spirochete.

|               | *B. turicatae* | *B. parkeri* | *B. hermsii* | *B. recurrentis* |
|---------------|---------------|--------------|--------------|------------------|
| *B. turicatae*| -             | -            | -            | -                |
| *B. parkeri*  | 97            | -            | -            | -                |
| *B. hermsii*  | 87            | 87           | 80           | -                |
| *B. recurrentis* | 81          | 80           | 80           | -                |

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### Table 2. Percent amino acid identity of BipA between species of relapsing fever spirochete.

|               | *B. turicatae* | *B. parkeri* | *B. hermsii* | *B. recurrentis* |
|---------------|---------------|--------------|--------------|------------------|
| *B. turicatae*| -             | -            | -            | -                |
| *B. parkeri*  | 76            | -            | -            | -                |
| *B. hermsii*  | 36            | 34           | -            | -                |
| *B. recurrentis* | 25          | 24           | 26           | -                |

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and rBipA (Fig. 1A) was detected by chemiluminescence and indicated exposure to relapsing fever spirochetes. A serum sample from a subject living in a nonendemic region of the US without a history of exposure to relapsing fever spirochetes was used as a negative control (Fig. 1B). The nitrocellulose membrane was subsequently probed with a Monoclonal Anti-polyHistidine-Peroxidase antibody (Sigma-Aldrich, St. Louis, Missouri, US), indicating the presence of the recombinant proteins (Fig. 1C and D). Additionally, the patient’s antibody titers to rGlpQ and rBipA were over a 1:6,400 dilution.

**Case Discussion**

The ecological overlap and nonspecific symptoms caused by vector-borne bacterial, viral, and parasitic pathogens signifies the importance of utilizing improved molecular assays to accurately determine pathogen exposure. In this report, the patient’s extensive outdoor activity and nonspecific clinical manifestation made it difficult to initially determine the causative agent. Retrospective analysis of serum reactivity to rGlpQ and rBipA suggested likely exposure to *B. turicatae*. A potential limitation of BipA as a species-specific antigen is that *B. turicatae* and *B. parkeri* are closely related [7], and the homologues share 76% amino-acid identity. However, we are unaware of a report indicating the occurrence of *Ornithodoros parkeri*, the vector for *B. parkeri*, in Texas, as the ticks have been collected throughout the western and midwestern US [8,9], further causing us to suspect *B. turicatae*.

Accurate epidemiological studies for tick-borne relapsing fever borreliosis in Texas have been challenging because of nonspecific symptoms and limited molecular diagnostic assays. Human exposure has been evaluated retrospectively, by confirming spirochete colonization of the tick vector, *Ornithodoros turicata*, collected from presumed contact sites, which frequently included caves and manmade dugouts [10,11]. The disease has also been misdiagnosed as Lyme borreliosis because of similar neurological symptoms [10]. Further complicating a correct diagnosis was serological cross-reactivity with immunofluorescent assays (IFA) and enzyme-linked immunosorbent assays (ELISA), in which patient serum samples were assessed for reactivity to fixed *Borrelia burgdorferi* or total protein lysates, respectively [10]. Given the number of conserved antigens between *Borrelia* species and observed serological cross-
reactivity [12,13], IFA and ELISA may be misleading. Furthermore, Ixodid ticks that transmit *B. burgdorferi* and feed for 5–7 days were not found to be attached on the patients [10].

Transmission of *B. turicatae* from the tick and the ecology and feeding behavior of *O. turicata* indicate that outdoor enthusiasts, military ground personnel, and low-income families living in primitive housing conditions are at-risk populations [14,15]. The ticks complete their blood meal within 5–60 minutes and subsequently return to the cave crevice, nest, or den in which they cohabit with small mammals [10,16]. Consequently, *O. turicata* is rarely found on the host, and a full blood meal is not required for transmission and infection [14]. Moreover, mammalian hosts supporting the maintenance of *B. turicatae* in nature are not completely known. Schwan and colleagues isolated the spirochetes from symptomatic domestic dogs, while the tick vector has been collected in caves [10,17,18]. These findings suggest a role of wild canids and bats in *B. turicatae* maintenance. Our ecological studies in Texas utilizing rGlpQ and rBipA to determine small mammal exposure to *B. turicatae* indicate coyotes and rodents may maintain the pathogens (manuscript in preparation). As improved molecular assays are utilized to evaluate mammalian exposure to relapsing fever spirochetes, we will further define the ecology and human health burden in regions where the pathogens are overlooked.

### Key Learning Points

- Given nonspecific clinical symptoms, relapsing fever spirochetes are likely underdiagnosed.
- Serological responses to rGlpQ and rBipA can indicate exposure to relapsing fever spirochetes.
- Likely at-risk populations include outdoor enthusiasts, military ground personnel, and those living in primitive housing conditions.

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### References

1. Schwan TG, Schrumpf ME, Hinnebusch BJ, Anderson DE, Konkel ME (1996) GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. J Clin Microbiol 34: 2483–2492. PMID: 8880505
2. Porcella SF, Raffel SJ, Schrumpf ME, Schriefer ME, Dennis DT, et al. (2000) Serodiagnosis of louse-borne relapsing fever with glycerophosphodiester phosphodiesterase (GlpQ) from *Borrelia recurrentis*. J Clin Microbiol 38: 3561–3571. PMID: 11015364
3. Schwan TG, Anderson JM, Lopez JE, Fischer RJ, Raffel SJ, et al. (2012) Endemic foci of the tick-borne relapsing fever spirochete *Borrelia crocidurae* in Mali, West Africa, and the potential for human infection. PLoS Negl Trop Dis 6: e1924. doi: 10.1371/journal.pntd.0001924 PMID: 23209863
4. Schwan TG, Raffel SJ, Schrumpf ME, Webster LS, Marques AR, et al. (2009) Tick-borne relapsing fever and *Borrelia hermsii*, Los Angeles County, California, USA. Emerg Infect Dis 15: 1026–1031. doi: 10.3201/eid1507.090223 PMID: 19624916
5. Lopez JE, Wilder HK, Boyle W, Drumheller LB, Thornton JA, et al. (2013) Sequence analysis and serological responses against *Borrelia turicatae* BpA, a putative species-specific antigen. PLoS Negl Trop Dis 7: e2454. doi: 10.1371/journal.pntd.0002454 PMID: 24069498
6. Lopez JE, Schrumpf ME, Nagarajan V, Raffel SJ, McCoy BN, et al. (2010) A novel surface antigen of relapsing fever spirochetes can discriminate between relapsing fever and Lyme borreliosis. Clin Vaccine Immunol 17: 564–571. doi:10.1128/CVI.00518-09 PMID: 20147497
7. Schwan TG, Raffel SJ, Schrumpf ME, Policastro PF, Rawlings JA, et al. (2005) Phylogenetic analysis of the spirochetes Borrelia parkeri and Borrelia turicatae and the potential for tick-borne relapsing fever in Florida. J Clin Microbiol 43: 3851–3859. PMID: 16081922
8. Cooley RA, Kohls GM (1944) The Agarasidae of North America, Central America, and Cuba. 1–152 p.
9. Davis GE (1939) Ornithodoros parkeri: distribution and host data; spontaneous infection with relapsing fever spirochetes. Pub Health Rep 54: 1345–1349.
10. Rawlings JA (1995) An overview of tick-borne relapsing fever with emphasis on outbreaks in Texas. Tex Med 91: 56–59. PMID: 7778052
11. Davis H, Vincent JM, Lynch J (2002) Tick-borne relapsing fever caused by Borrelia turicatae. Pediatr Infect Dis J 21: 703–705. PMID: 12237608
12. Coleman JL, Benach JL (1992) Characterization of antigenic determinants of Borrelia burgdorferi shared by other bacteria. J Infect Dis 166: 658–666. PMID: 1372635
13. Magnarelli LA, Anderson JF, Johnson RC (1987) Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J Infect Dis 156: 183–187. PMID: 3298452
14. Boyle WK, Wilder HK, Lawrence AM, Lopez JE (2014) Transmission Dynamics of Borrelia turicatae from the Arthropod Vector. PLoS Negl Trop Dis 8: e2767. doi:10.1371/journal.pntd.0002767 PMID: 24699275
15. Davis GE (1941) Ornithodoros turicata: the males; feeding and copulation habits, fertility, span of life, and the transmission of relapsing fever spirochetes Pub Health Rep 56: 1799–1802.
16. Balashov YS (1972) Bloodsucking ticks (Ixodoidea)- vectors of diseases of man and animals. Misc Publ Entomol Soc Am 8: 161–376.
17. Whitney MS, Schwan TG, Sultemeier KB, McDonald PS, Brillhart MN (2007) Spirochetemia caused by Borrelia turicatae infection in 3 dogs in Texas. Vet Clin Pathol 36: 212–216. PMID: 17523100
18. Breitschwerdt EB, Nicholson WL, Kiehl AR, Steers C, Meuten DJ, et al. (1994) Natural infections with Borrelia spirochetes in two dogs from Florida. J Clin Microbiol 32: 352–357. PMID: 8150943