

HHS Public Access

Author manuscript

Zoonoses Public Health. Author manuscript; available in PMC 2020 February 26.

Published in final edited form as: Zoonoses Public Health. 2019 September ; 66(6): 579–586. doi:10.1111/zph.12588.

Evaluating the risk of tick-borne relapsing fever among occupational cavers—Austin, TX, 2017

Stefanie B. Campbell1, **Anna Klioueva**2, **Jeff Taylor**2, **Christina Nelson**1, **Suzanne Tomasi**3, **Adam Replogle**1, **Natalie Kwit**1, **Christopher Sexton**1, **Amy Schwartz**1, **Alison Hinckley**¹

¹Centers for Disease Control and Prevention, Fort Collins, Colorado

²Austin Public Health, Austin, Texas

³Centers for Disease Control and Prevention, Morgantown, West Virginia

Abstract

Tick-borne relapsing fever (TBRF) is a potentially serious spirochetal infection caused by certain species of *Borrelia* and acquired through the bite of *Ornithodoros* ticks. In 2017, Austin Public Health, Austin, TX, identified five cases of febrile illness among employees who worked in caves. A cross-sectional serosurvey and interview were conducted for 44 employees at eight organizations that conduct cave-related work. Antibodies against TBRF-causing Borrelia were detected in the serum of five participants, four of whom reported recent illness. Seropositive employees entered significantly more caves (Median 25 [SD: 15] versus Median 4 [SD: 16], $p =$ 0.04) than seronegative employees. Six caves were entered more frequently by seropositive employees posing a potentially high risk. Several of these caves were in public use areas and were opened for tours. Education of area healthcare providers about TBRF and prevention recommendations for cavers and the public are advised.

Keywords

Borrelia; Borrelia hermsii; Borrelia turicatae; TBRF; TX

1 | INTRODUCTION

Tick-borne relapsing fever (TBRF) in humans follows infection with one of several Borrelia bacteria species. These bacteria can be trans-mitted rapidly to humans through the bite of Ornithodoros tick species. Ornithodoros ticks feed quickly and then detach and scatter; therefore, a victim might be unaware of the tick bite (Boyle, Wilder, Lawrence, & Lopez, 2014). TBRF is characterized by episodes of fever, headache, myalgia, arthralgia and chills (Dworkin, Schwan, Anderson, & Borchardt, 2008). The initial fever will last approximately 3 days, followed by 7 days without fever, followed by another 3 days of fever. Without antibiotic treatment, this process can repeat several times (Davis, Vincent, & Lynch, 2002;

Correspondence: Stefanie B. Campbell, Centers for Disease Control and Prevention, Fort Collins, CO. ybe0@cdc.gov. CONFLICT OF INTEREST

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC.

Dworkin et al., 2008). In the United States, most TBRF cases are attributed to infection with Borrelia hermsii with patients commonly exposed in mountainous areas of western states, often following stays in seasonally used cabins (Dworkin, Shoemaker, Fritz, Dowell, & Anderson, 2002). A total of 504 TBRF cases were reported from twelve western states during 1990–2011; 18 of these were thought to have acquired their infection in Texas (Forrester, Kjemtrup, et al., 2015).

In Texas, Ornithodoros turicata transmits the TBRF causative agent, Borrelia turicatae (Davis, 1943; Donaldson et al., 2016; Rawlings, 1995). The B. turicatae lifecycle is maintained in this region by small and medium-sized animal hosts (Armstrong et al., 2018). Since 1930, human cases of TBRF in TX have occurred sporadically with activity in caves suggested as a risk factor (Dworkin et al., 2008; Forrester, Kjemtrup, et al., 2015; Rawlings, 1995; Weller & Graham, 1930; Wilder et al., 2015). Weller and Graham (1930) described inspection of a cave outside of Austin, TX, in which "the floor was alive with ticks." Other specific risk factors for TBRF in Texas have not been described.

In 2017, Austin Public Health (TX) identified an increase in reports of TBRF-like illness. In February, 11 people became ill after attending a workshop near Austin (Bissett et al., 2018). Attendees spent time in wooded areas and near wildlife, but none reported entering a cave. Blood samples from four patients were tested, and all had evidence of Borrelia infection, however, not specific for relapsing fever agents. In June and July, five people became ill after entering Austin area caves. Serum from three of the five was tested at CDC in Fort Collins, and two had antibodies to relapsing fever group borreliae.

Austin is located in Travis County and adjacent to Hays and Williamson Counties. The three counties encompass 2,791 square miles in southern central Texas (United States Census Bureau, 2010). Within these three counties, there are 1,338 documented caves, of the 5,600 in Texas (Texas Speleological Society, 2016). Near Austin, a number of organizations employ people who enter caves or assess karst features for various occupation-related duties. State and local government organizations employ park rangers, biologists, surveyors, mammalogists, geologists, environmental conservationists and natural resource specialists. Private consulting companies employ people with similar titles and most often focus on performing karst surveys. Occupational cavers usually enter multiple caves in their duty area during all seasons and can spend hours in each cave.

For this investigation, Austin Public health worked with CDC to: (a) determine the frequency, duration and types of cave exposures among occupational cavers; (b) document the frequency of seropositivity for TBRF, occurrence of illness consistent with TBRF, and the clinical spectrum of illnesses experienced by occupational cavers; and (c) identify any differences in exposure, risk factors, or preventive strategies between seronegative and seropositive occupational cavers.

2 | METHODS

2.1 | Inclusion criteria of organizations and employees

A local recreational caving group was consulted prior to the investigation. They provided information on government and private organizations in Hays, Travis and Williamson counties, TX, that employed at least one person who entered caves for their job. In addition, they identified a list of caves that are open to the public and/or perceived to be caves of risk for TBRF. Caves of risk for TBRF included ones where the tick vector had been previously seen or ones that people entered and then became ill a few days later. Organizations were contacted, and employees were invited to participate. All identified government organizations in the inclusion area of Travis, Williamson and Hays counties that employed at least one occupational caver participated. Of the ten identified private organizations, four agreed to participate. In total, eight government and private organizations participated and 46 employees were enrolled. A point of contact for each organization received an email 1 week prior to the study with information describing the purpose and logistics of the investigation.

2.2 | Data collection

We developed a questionnaire to collect demographic information, employment history, cave exposure, work-related activities in caves, use of protective measures in caves, and illness history. Participating employees who provided informed consent were interviewed in person during October 9 to 13, 2017. To capture cave exposure, employees were asked to name and rank their top 10 most frequently entered caves in the past 12 months. We asked about the duration of time (in hours) that employees spent in their top 10 most frequently visited caves.

2.3 | Serological testing

Serum samples were collected from participants at the time of interview. The CDC laboratory in Fort Collins, CO performed serology on the samples using two-tier testing (enzyme immunoassay and Western immunoblot) in which antibody reactivity to B. hermsii antigens was assessed (Fritz et al., 2004; Trevejo et al., 1998). Samples having positive results for both assays were interpreted as evidence of previous infection. TBRF serologic tests at CDC utilize *B. hermsii* antigen since the majority of cases in the United States are caused by B . hermsii, and it has been shown that TBRF caused by B . turicatae is detectable by the current tests (Christensen et al., 2017).

2.4 | PCR and DNA sequencing

To confirm that utilizing B . hermsii reagents to screen for antibodies to B . turicatae was appropriate, DNA sequencing was performed on a 627-bp fragment of the $glpQ$ gene PCRamplified from cultures of two patients in the study that were previously diagnosed with TBRF. DNA was extracted from 200 μl of EDTA-treated whole blood from each patient and 100 μl of the cultured spirochetes using a QIACube (Qiagen) with a QIAamp DNA Mini kit utilizing the blood and body fluid protocol in a final elution of 100 μl. PCR targeting the Borrelia relapsing fever group glpQ gene was performed. PCR primer sequences $(5^{\prime}-3^{\prime})$

were as follows: RFglpQ-F GGAGCTGACTACCTAGAAC and RFglpQ-R GGGTATCCAAGGTCCAATTCC. Reactions were made with illustra puReTaq Ready-To-Go PCR Beads (GE Healthcare) and contained 400 nM each primer, 2 μl DNA and nucleasefree water to a final volume of 25 μl. PCR controls consisted of a DNA extraction buffer negative control (PBS), a PCR negative control (molecular grade H_2O) and a PCR positive control (B. hermsii DNA). PCR was performed using a MJ Mini Thermal Cycler (Bio-Rad). Cycling conditions were 94 \degree C for 3 min, and 30 cycles of 94 \degree C for 1 min, 55 \degree C for 1 min, 72°C for 1 min, followed by 72°C for 10 min. PCR amplicon sizes (expected size of 627 bp) were verified using E-Gel 2% aga-rose gels (Invitrogen). For sequencing of PCR products, amplicons were purified using the QIAquick PCR purification kit (Qiagen) and quantified with a Nanodrop 8000 (ThermoFisher). Each amplicon was sequenced $4x$ each direction with the glpQ PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Reaction conditions included 8 μl of reaction mix, 0.33 nM PCR primer, 10 ng amplicon and nuclease-free water to 20 μl. Sequencing reactions were performed on the MJ Mini at 96°C for 1 min and 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Reactions were purified with Centri-Sep spin columns (Applied Biosystems) and sequencing performed on a 3130xl Genetic Analyzer (Applied Biosystems). Sequence files were assembled with Seq Builder Pro (DNAStar), and contig files with trimmed ends (556 bp) were aligned to *Borrelia* sequences from GenBank using MegAlign (DNAStar). Clustal W slow-accurate pairwise alignment was performed to determine sequence identity.

2.5 | Statistical analysis

Data were entered into an Epi Info 7.1.5.0 database. Statistical analyses were conducted using SAS 9.4. For this analysis, employees who tested seropositive were compared to the referent group of seronegative employees. Odds ratios were generated for dichotomous variables, and the Fisher's exact test was used to compare frequencies between seropositive and seronegative employees. The Wilcoxon rank-sum test was used to evaluate associations between seropositive and seronegative employees with p values $\langle 0.05 \rangle$ considered statistically significant. This investigation underwent ethical and regulatory review following standardized processes at the National Center for Emerging and Zoonotic Infectious Diseases, and in accordance with CDC policy. It was determined to be a non-research public health response, and therefore was not subject to formal IRB review requirements, although informed consent was obtained from all participants, in keeping with accepted ethical standards.

3 | RESULTS

Questionnaires were completed, and serum samples were collected from 44 employees; median age of respondents was 40 years (range: 21–73), and 27/44 (61%) were male (Table 1). Nearly all (42/44; 95%) employees entered caves for their occupation at some point in the past. Many employees (33/44; 75%) entered caves in Hays, Travis or Williamson Counties in the past 12 months (Table 1). Overall, employees reported entering a median of 4.5 (range: 0–50) different caves in the past 12 months (Table 1). Employees spent an average of 2.2 (range: 0.1–12) hours in their 10 most frequently entered caves. More than half of employees (26/44; 59%) reported seeing small or medium-sized animals in the caves.

The most common animal types seen were porcupines (21), raccoons (15) and mice (13). Among all employees, five of 44 were identified in the serosurvey with two-tier positive reactivity to B. hermsii antigens on EIA and immunoblot $(5/44; 11\%)$. Two of the five seropositive respondents were previously diagnosed with TBRF, with B. turicatae culture confirmation performed at CDC 3 months prior to this investigation. These employees were interviewed and gave a second blood sample. Comparison of g/pQ sequences from both the blood samples and the recovered cultures with publicly available relapsing fever group Borrelia species in NCBI demonstrated they shared 100% identity to B. turicatae (Table 2). Both confirmed cases attributed to B . turicatae infection were seropositive using B . hermsii antigen, suggesting that B. hermsii reagents can be used to screen for antibodies to B. turicatae.

3.1 | Cave exposure patterns among seropositive employees

Among the five seropositive employees were three biologists and two park rangers. All five employees reported cave exposure in the inclusion area within the previous 12 months. Seropositive employees entered significantly more caves than seronegative employees (Median 25 vs. Median 4; $p = 0.04$; Table 3). There were no other significant differences in exposures among the seropositive and seronegative employees. In addition to entering Texas caves, one person who was seropositive reported entering caves in New Mexico and another seropositive person reported entering caves in Germany and Mexico. Of all caves entered by employees, six were identified as being significantly more visited by seropositive than seronegative employees (Figure 1).

There was no difference between seronegative and seropositive employees with respect to any protective measures (Table 4). Among employees that did not use permethrin, seventeen said the primary reason was that it was harmful to the cave ecosystem (17/37; 45.9%; Table 5). Of all employees that did not use repellents, ten said the primary reason was that it was harmful to the cave ecosystem (10/22; 45%; Table 5).

3.2 | Illness patterns among seropositive employees

Of the five seropositive employees, four reported illness, during the past year, consistent with TBRF for which they sought medical care. Among these, three were diagnosed with TBRF by a healthcare provider. One of the four seropositive employees who sought medical care was possibly misdiagnosed with typhoid fever in the previous 12 months. The fifth person who was seropositive reported no history of illness consistent with TBRF.

Among the four seropositive that reported TBRF-like illness, all reported experiencing fever, with a temperature range of 103.5–105 F. Only one person experienced a relapsing fever consisting of a single relapse, lasting 6 days, with 5 days between fevers. In addition, all felt fatigue and experienced sweating, chills and a loss of appetite. Three out of four employees reported at least one of the following symptoms: vivid dreams, hallucinations, sensitivity to light, aching, headache, confusion, tingling or weakness of extremities, vomiting and nausea. Half of the employees who reported illness said they had a rash (Table 6); only one person each reported having irregular heart rate, neck pain or sore throat. All four of the seropositive employees with symptoms were treated with doxycy-cline. Three of the four

seropositive employees who were treated reported experiencing additional symptoms, including worsening fever, lethargy, dizziness and others, after starting treatment. These symptoms were consistent with a Jarisch–Herxheimer reaction.

4 | DISCUSSION

This investigation provides additional information in support of the association between TBRF and cave exposure. Of the 44 enrolled, five persons had evidence of infection with relapsing fever Borrelia, all of which entered caves in the previous 12 months. Entering a greater number of caves was significantly associated with seropositivity. By entering more caves, employees could have increased opportunity for exposure to the vector O. turicata. Providing cave tours was a marginally significant finding, and it is possible that providing cave tours is a surrogate for frequency of cave visits. If employees were repeatedly providing tours in caves and these caves had infected O. turicata ticks, then this could increase the employee's opportunity for exposure and risk of infection.

Seropositive employees entered six specific caves more frequently than seronegative employees. Five of the six caves are located in southwest Austin, and three were previously identified by the local caving group as potentially high-risk caves. Interestingly, the location of the workshop where 11 people became ill with febrile illness in early 2017 is located within ten miles of the five suspect Austin area caves. In response to the findings of this investigation, the City of Austin performed tick sampling on three separate occasions in three of the six caves and found no soft ticks. They chose these three caves, from the six identified as potentially risky, because they are primarily used for educational tours. The lack of soft-bodied ticks identified in these caves is reassuring, but continued monitoring for Borrelia-infected ticks is essential to evaluate the identified caves in this area of Austin to determine the risk assessment, compared to less frequently visited caves.

There is no information on the rate of subclinical infection with B. turicatae in the literature. One seropositive individual did not report symptoms of TBRF or ever being diagnosed with TBRF. It is possible that this person experienced mild symptoms that they associated with another type of illness or that they were misdiagnosed. In addition, because serological testing is usually performed during or shortly after acute illness, there is limited information on how long a person will have serologic reactivity for TBRF utilizing the recommended two-tier testing. The person who reported no symptoms may have experienced illness >12 months ago, which was outside of the timeframe of this investigation. The two employees who were diagnosed with TBRF 3 months prior to this investigation had positive serology results in this study. This was a meaningful finding and provided evidence that serology can be a useful diagnostic tool several months post-infection.

The seropositive individual with no clinical signs and the employee diagnosed with a different infectious disease in this investigation suggest that there could be other unrecognized or misdiagnosed cases of TBRF in Texas. It is possible that a background rate of infection exists among people living in Texas who have not been tested or diagnosed with TBRF. In 2016, Texas reported 71 cases of Lyme disease. Lyme disease is not endemic to Texas, and while many of these cases likely involved travel to high-incidence areas, it is also

possible that some of these were unrecognized cases of TBRF (Forrester, Brett, et al., 2015). Previous reports indicate cross-reacting antibodies to *Borrelia burgdorferi* among people and animals confirmed with Borrelia-causing relapsing fever (Esteve-Gasent, Snell, Adetunji, & Piccione, 2017; Rath, Rogler, Schonberg, Pohle, & Fehrenbach, 1992). Immunoblot results have shown reactivity to specific antigens shared by both pathogens (Rath et al., 1992). In areas where Lyme disease is not endemic and the patient has not travelled to a highincidence area, serologic results for Lyme borreliosis should be interpreted cautiously (Forrester, Brett, et al., 2015).

In this population, signs and symptoms were consistent with previous case reports of relapsing fever (including fever, chills, headache, sensitivity to light, confusion, myalgia, arthralgia, vomiting and nausea) (Dworkin et al., 2008). In addition, three employees reported having vivid dreams, hallucinations and tingling or weakness of extremities. These symptoms have not been reported previously among patients infected with B . hermsii, and only infrequently among patients with B. turicatae (Rawlings, 1995). This is supportive of the idea that B. turicatae is more neurotropic than other relapsing fever Borrelia (Cadavid $\&$ Barbour, 1998; Sethi, Sondey, Bai, Kim, & Cadavid, 2006). It is not surprising that three of the four employees who were ill did not experience relapsing fevers as a result of the promptness of initiating treatment with doxycycline after initial symptoms. Three employees experienced ongoing or additional symptoms after starting treatment with doxycycline which could be consistent with a Jarisch–Herxheimer reaction. This would be consistent with TBRF literature, where over 50% of cases experience a Jarisch–Herxheimer reaction upon initiation of antibiotic treatment (Dworkin et al., 2008).

There were several limitations in this investigation. The organizations that were invited to participate were identified by word of mouth from members of the caving community near Austin, TX. It is possible that some organizations were not identified or invited to participate. It is also possible that there are differences among participating organizations or among employees that chose to participate as compared to the ones that did not participate. In addition, we recruited people that had cave exposure in the previous 12 months, so there was no control group without cave exposure for comparison. Another consideration is that in the south and southwest states where B. turicatae occurs, infection is not always associated with cave exposure. TBRF can also be acquired by entering other environments inhabited by infected ticks such as crawl spaces and underneath houses. So, while we believe the five seropositive employees in this investigation likely were exposed to the relapsing fever bacteria by a tick bite in caves, we cannot rule out other potential exposure locations. Lastly, a diagnostic limitation is that serology cannot differentiate between species of relapsing fever Borrelia, so we cannot definitively know that all of the seropositives were due to B. turicatae. Since relapsing fever *Borrelia* are geographically specific, with *B. turicatae* predominantly identified from Texas cases, and since speciation was performed on two of the five seropositive specimens, we believe it is likely that seropositivity was due to B . turicatae infection.

In this investigation, there were no significant differences with respect to any protective measures. The cave environment also presents challenges to making prevention recommendations. Employees emphasized the need to be mobile throughout the caves

without clothing or personal protective equipment catching on the cave wall or floor, or impeding movement. Some of the employees included in this investigation enter caves to conduct animal and in-vertebrate surveys. These employees play a role in preserving and protecting the caves' ecosystems; thus, removing host animals that ticks parasitize is not a viable option to preventing human illnesses. In addition, although use of chemical acaricides is the most common recommendation for tick control, studies have suggested that chemical acaricides would cause a disruption to the cave ecosystem (Diaz-Martin, Manzano-Roman, Obolo-Mvoulouga, Oleaga, & Perez-Sanchez, 2015). Similarly, many employees believed that using permethrin on their clothes or repellents on their skin would be harmful to the cave ecosystem. Small and medium-sized animals are known hosts in the TBRF lifecycle. Employees who work in caves should be aware that the presence of animals or their nests may indicate an increased risk for the Ornithodoros tick.

As an uncommon disease with a range of clinical symptoms, TBRF can be difficult to identify (Christensen et al., 2017). To improve healthcare provider recognition and knowledge, key TBRF information was summarized and published in a Texas medical society journal (Campbell, Huang, Kirkpatrick, Klioueva, & Taylor, 2018). Providing education to cavers in Texas could similarly allow for awareness of tick presence and recognition of disease. Cavers can serve as their own health advocates and discuss treatment options with their healthcare provider. Additional studies on the incidence of TBRF in Texas and ecology of the vector O. turicata are warranted.

ACKNOWLEDGEMENTS

The authors would like to thank Jeannine Petersen and Martin Schriefer for their assistance with laboratory testing and interpretation.

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Impacts

- **•** The findings of this investigation support the hypoth-esized association between tick-borne relapsing fever (TBRF) and cave exposure. Occupational cavers who entered a greater number of caves were significantly more likely to have serologic evidence of past exposure to TBRF bacteria.
- **•** Certain caves appeared to be riskier than others for exposure to TBRF bacteria. Six caves were found to be higher risk in this investigation, five of which are located in southwest Austin. Monitoring this part of Austin through tick sampling and additional ecologic assessment is needed.
- **•** The cave environment presents challenges to standard tick bite prevention practices because of its sensitive ecosystem that could be harmed by chemical acaricides. People entering caves should be aware of TBRF and utilize alternative protective measures, such as wearing long clothing.

Legend

- Downtown \star
- Cave $\ddot{}$

Figure 1.

Location of caves, within Austin city limits, most frequently visited by employees seropositive ($n = 5$) for antibodies against TBRF-causing Borrelia–Austin, Texas, 2017. See figure attached. One cave found to be frequently visited by seropositive employees is not shown on this map due to its location outside of the defined investigation area

Demographic characteristics of employees surveyed from eight organizations that employ occupational cavers $(n = 44)$ —Austin, Texas, 2017

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Sequence comparison of 556 bp region of the glpQ gene of Borrelia turicatae from two patients—Austin, Texas, 2017 Sequence comparison of 556 bp region of the *glpQ* gene of *Borrelia turicatae* from two patients—Austin, Texas, 2017

Cave exposure and activities performed while working in caves for the previous 12 months among employees seropositive $(n = 5)$ and seronegative $(n = 1)$ $n = 5$) and seronegative (Cave exposure and activities performed while working in caves for the previous 12 months among employees seropositive (39) for antibodies against TBRF-causing Borrelia-Austin, Texas, 2017 39) for antibodies against TBRF-causing Borrelia—Austin, Texas, 2017

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TABLE 4

Comparison of protective measures used between employees seropositive $(n = 5)$ and seronegative $(n = 39)$ for antibodies against TBRF-causing Borrelia $n = 39$) for antibodies against TBRF-causing Borrelia $n = 5$) and seronegative (Comparison of protective measures used between employees seropositive (-Austin, Texas, 2017 —Austin, Texas, 2017

Primary reason for not using permethrin ($n = 37$) or repellents ($n = 22$) among employees—Austin, Texas, 2017

Clinical signs among employees seropositive ($n = 5$) for antibodies against TBRF-causing *Borrelia*—Austin, Texas, 2017

