Genotyping revealed the *S. hematobium–S.bovis* hybrid parasite described in previous outbreaks, suggesting ongoing transmission rather than reintroduction. The parasite's emergence in another river cannot be explained by the persistence of infected snails (*9*) but could be explained by reseeding of the river by a mammalian host.

Animal reservoirs have been discussed as a possible explanation for ongoing transmission; however, evidence of a major role is lacking. No infection has been detected in livestock in the region, and the only infected animals found were 2 rats (*10*). Even if we cannot rule out the influence of an undetected animal reservoir (e.g., *Ovis aries musimon,* wild sheep native to Corsica, have never been tested), the most likely explanation is that 1 or several infected persons continue to infest the water.

In summary, this case highlights that transmission of schistosomiasis in Corsica is ongoing and is no longer restricted to the Cavu River. The parasite appears to be of the same strain detected previously on the island. The infection was acquired at a frequented tourist site, suggesting that more persons might have been infected. Further screening of residents and tourists is urgently needed.

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# Relapsing Fever Group Borreliae in Human-Biting Soft Ticks, Brazil

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We conducted a molecular survey for *Borrelia* spp. in *Ornithodoros* ticks previously reported as biting humans. We collected specimens in natural ecosystems and inside human dwellings in 6 states in Brazil. Phylogenetic analyses unveiled the occurrence of 4 putatively new species of relapsing fever group borreliae.

Tick-borne relapsing fever (TBRF) is a vectorborne disease caused by spirochetes of the genus *Borrelia* that thrive in enzootic cycles and are transmitted mainly by soft ticks of the genus *Ornithodoros* (*1*). Humans bitten by infected ticks can become ill and present a typical recurrent febrile syndrome (*1*). In the New World, research on TBRF persists mainly in North America, where *Borrelia turicatae*, *B. parkeri*, and *B. hermsii* infect humans (*1*). Meanwhile, the knowledge on relapsing fever spirochetes in South America has remained comparatively incomplete. In Brazil, *Ornithodoros brasiliensis*, *O. fonsecai, O. mimon*, *O. rietcorreai*, and *O. rostratus* ticks have been reported to parasitize humans (*2*,*3*), yet their role as vectors of *Borrelia* spp. is unknown. Recently, in Brazil, *B. venezuelensis*, the agent of South American TBRF during the first half of the 20th century, was isolated from the anthropophilic tick *O. rudis* (*4*). This finding highlighted the occurrence of pathogenic relapsing fever group borreliae (RFGB) and called attention to study human-biting *Ornithodoros* ticks as possible vectors of these microorganisms.

During December 2018–October 2019, we conducted collections of soft ticks in the Brazilian states of Ceará (CE), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS), Maranhão (MA), and Rondônia (RO) (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/1/20-0349-App1.pdf). Collections in MS were implemented using dry ice as an attractor; in CE, GO, MA, and RO, we collected soft ticks inside caves, abandoned nests or between rocks in rural areas. In MT, specimens were collected on the walls of an inhabited house in an urban area. Collections of ticks were authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio permits 65137-1 and 36413-1).

A total of 665 specimens (236 males, 145 females, 284 nymphs) belonging to 8 species of the genus *Ornithodoros* were submitted to individual or pooled DNA extractions (Appendix Table 1). We screened extractions with a *Borrelia*-specific real-time PCR with primers Bor16S3F and Bor16S3R and probe Bor16S3P, using 2 μL of genomic DNA, to amplify a fragment of the 16S rRNA gene (*5*). Samples with cycle threshold values <32 were tested with a battery of PCRs targeting the 16S rRNA and the *flab* and *glpQ* borrelial genes.

Four species of ticks were positive by *Borrelia*-specific real-time PCR. We generated sequences of *Borrelia* 16S rRNA, *flaB*, and *glpQ* genes for these specimens (Appendix Table 1). Two haplotypes of 16S rRNA gene were sequenced from each of the 2 positive *O. mimon* ticks, and the obtained sequences of *flaB* and *glpQ* were identical for both specimens. One haplotype for each gene was obtained for *O. hasei* and the *Ornithodoros* sp. ticks from CE, and only a 16S rDNA sequence was obtained from *O. rietcorreai* ticks (Appendix Table 2). With high support values, Bayesian phylogenetic analyses showed that the *Borrelia* spp. characterized from *O. mimon*, *O. rietcorreai*, and the *Ornithodoros* sp. ticks from CE form a monophyletic clade related to RFGB occurring in the Old World. In turn, the *Borrelia* sp. harbored by *O. hasei* ticks clustered within New World RFGB (Figure). These results add further evidence that Old and New World RFGB do not necessarily have defined geographic distributions but rather correspond to arbitrary groups.

Five species of *Ornithodoros* ticks have been reported to parasitize humans in Brazil (*2*,*3*). We have added 2 more species to this list, as *O. hasei* and the *Ornithodoros* sp. ticks from CE avidly bit us during collections in the field (data not shown). Although with low prevalence, these 2 species, together with *O. mimon* and *O. rietocorreai*, harbored DNA of putatively new *Borrelia* spp. phylogenetically related to the relapsing fever group. The implications of these new spirochetes as human pathogens are still unknown. *O. mimon* and *O. rietcorreai* ticks are associated with human parasitism in urban and rural dwellings in Brazil (*2*,*3*), so vector roles of both species should not be overlooked.

TBRF courses with febrile episodes and should be considered as a differential diagnosis within the spectrum of diseases that cause an undifferentiated febrile syndrome (UFS) (*6*). Although specific data are vague for the states where tick collections were performed in this study, UFS is common in Brazil; mosquitoborne viruses and malaria are the main etiologic agents (*7*,*8*). Nevertheless, febrile illnesses still remain underdiagnosed in a substantial proportion of the cases in the country (*7*,*8*). The results of this study are a contribution to the knowledge of RFGB in human-biting *Ornithodoros* ticks, and stress the

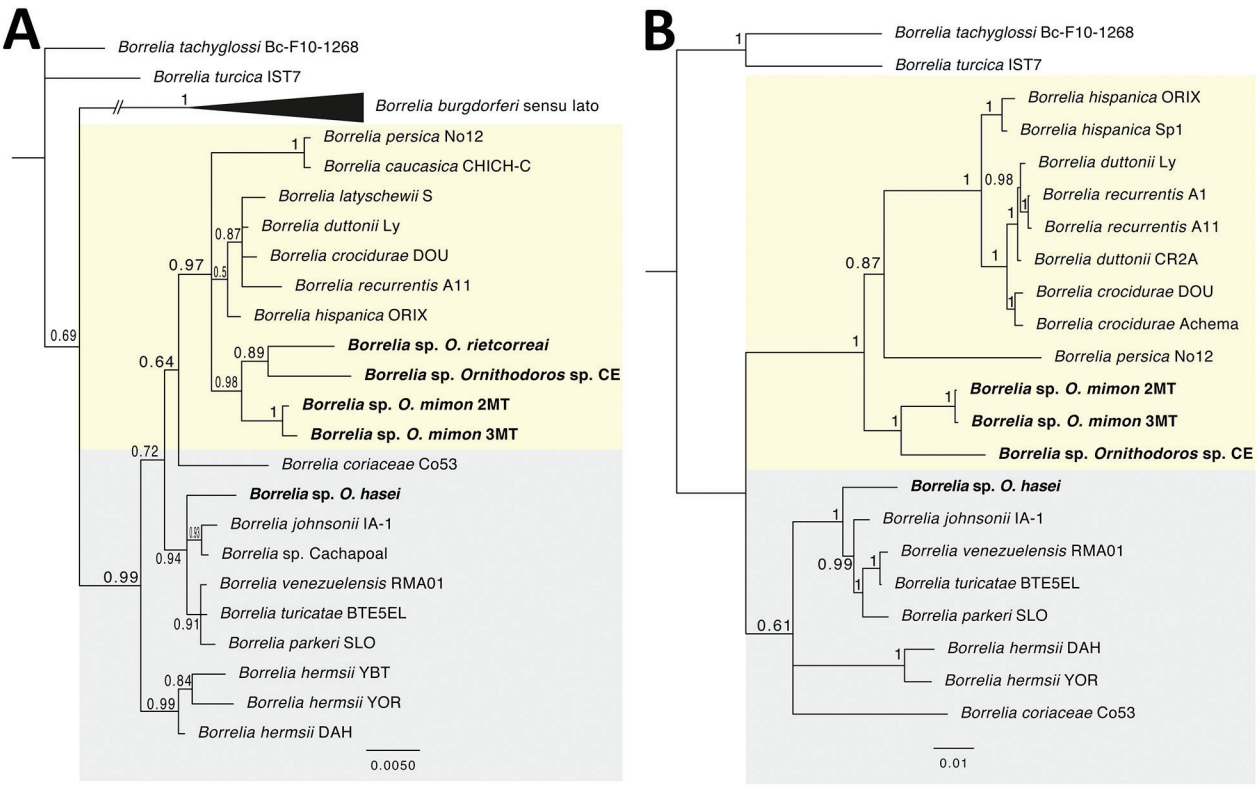


**Figure.** Bayesian phylogenetic trees inferred for the *Borrelia* spp. characterized in study of relapsing fever group borreliae in human-biting soft ticks, Brazil. A) Ambiguous alignments of single 16S rRNA gene (1,274 bp); B) concatenated 16S rRNA-*flaB-glpQ* genes (2,435 bp). Bold indicates borreliae from this study. Trees are drawn to scale. Four independent Markov chain runs for 1,000,000 metropolis-coupled MCMC generations were implemented for the analyses, sampling a tree every 100th generation. The first 25% of the trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability values. Both trees were inferred using the Hasegawa-Kishino-Yano model with gamma distribution. Numbers above or below tree branches represent Bayesian posterior probabilities. Light yellow and gray backgrounds denote Old World and New World relapsing fever group *Borrelia* spp., respectively. Scale bar indicates nucleotide substitutions per site.

investigation of TBRF as a possible cause of UFS in Brazil. It is known that antibodies of patients exposed to RFGB infection cross-react in serologic tests for the diagnosis of Lyme borreliosis (*9*). This cross-reactivity is particularly relevant in Brazil because serologic evidence for an alleged Lyme-like disease in humans has been reiteratively published, yet refuted (*10*), and TBRF has not yet been considered as a possible cause of such disease.


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# Etiology of Severe Acute Respiratory Infections, Bangladesh, 2017

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In April 2017, surveillance detected a surge in severe acute respiratory infections (SARI) in Bangladesh. We collected specimens from SARI patients and asymptomatic controls for analysis with multipathogen diagnostic tests. Influenza A(H1N1)pdm09 was associated with the SARI epidemic, suggesting that introducing vaccines and empiric antiviral drugs could be beneficial.

In April 2017, the Institute of Epidemiology Disease Control and Research (IEDCR) in Bangladesh noted an 89% increase in severe acute respiratory infections (SARI) compared with April 2016 through the National Influenza Surveillance Bangladesh (NISB) at 10 tertiary-care hospitals. During April 10–June 21, 2017, we conducted a case–control study to ascertain the cause of the outbreak and its associated risk factors.

We defined a SARI case as acute respiratory illness in a patient within 10 days of onset, with history of fever and cough, and requiring hospitalization (*1*). We sought to enroll all adults ≥18 years of age who were admitted to NISB hospitals with SARI. Staff screened patients for eligibility, obtained written informed consent, surveyed participants about demographics, and took combined nasal and throat swab samples. Patients who died in hospital wards before enrollment were ineligible. Within 2 days of case-patient enrollment, staff enrolled 2 asymptomatic controls, identified by convenience from the same hospitals' outpatient clinics, surveyed them, and took combined nasal and throat swab samples. Patients who had fever or respiratory symptoms in the previous 14 days were ineligible to serve as controls. Swab

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# Relapsing Fever Group Borreliae in Human-Biting Soft Ticks, Brazil

## Appendix

## Supplemental Methods

Collected ticks were morphologically identified following original descriptions and redescriptions of South American species (*1*–*5*), and by comparisons with specimens deposited in the tick collection Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva, São Paulo, Brazil. DNA extraction was performed using a phenol chloroform-based protocol (*6*). Successful extractions were confirmed by amplifying tick mitochondrial 16S rRNA gene with primers 16S+1 and 16S–1 in all the samples (*7*). Amplicons of this locus were sequenced only for soft ticks collected in new localities for the country, confirming their morphological identities by a phylogenetic analysis (data not shown). Amplicons of expected size for tick mitochondrial 16S rRNA gene and *Borrelia* 16S rRNA, *flab*, and *glpQ* genes were treated with Illustra ExoproStar (GE Healthcare, https://www.gehealthcare.com) and sequenced using an ABI 3500 genetic analyzer with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, https://www.thermofisher.com). Sequences were assembled and analyzed using Geneious R9 (*8*). Quality values (Q) for base calls were scaled between Q20 (error probability of 1 in 100) and Q40 (error probability of 1 in 10,000). Scores <Q20 in the 5′ and 3′ ends of each sequence were automatically trimmed. Values of quality, coverage, and length are shown in Appendix Table 2. BLASTn analyses were performed to infer most identical sequences in GenBank (https://blast.ncbi.nlm.nih.gov/Blast). Bayesian phylogenetic trees (http://mrbayes.csit.fsu.edu/) were inferred for single (*Borrelia* 16S rRNA) and concatenated (*Borrelia* 16S rRNA-*flaB*-*glpQ*) gene alignments constructed with MAFFT (https://mafft.cbrc.jp/alignment/server).

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**Appendix Table 1.** Results of molecular survey for *Borrelia* spp. in 8 *Ornithodoros* species from different parts of Brazil

| Species | Site of collection | Locality/state | No. tested specimens (N, M, F) | Pools/individual ticks | Real-time PCR positive samples (%) | GenBank accession no.,16S | GenBank accession no.,*flaB* | GenBank accession no.,*glpQ* |
|---|---|---|---|---|---|---|---|---|
| *O. rudis* | Abandoned bird nests in hollow palm trees | Araguapaz/GO | 82 (15N, 35M, 32F) | Pools: 15N; 17M; 18M; 15F; 17F | 0 | None | None | None |
| *O. mimon** | Abandoned bird nests in hollow palm trees | Formosa da Serra Negra/MA | 22 (11N, 7M, 4F) | Pools: 11N; 7M; 4F | 0 | None | None | None |
| | House | Cuiabá/MT | 19 (8N, 9M, 2F) | Individual ticks | 1F, 1N (10.5) | MT013211, MT013212 | MT076262 | MT076265 |
| *O. hasei** | Cave | Jericoacoara National Park/CE | 129 (48N, 51M, 20F) | Pools: 25N, 23N; 25M; 26M; 20F | 23N pool (0.05†) | MT013210 | MT076261 | MT076264 |
| *O. rietcorreai** | Between rocks | Serra das Almas Natural Reserve/CE | 111 (75N, 23M, 13F) | Pools: 40N; 35N; 23M; 13F | 13F pool (0.1†) | MT013213 | None | None |
| *Ornithodoros* sp.* | Between rocks | Serra das Almas Natural Reserve/CE | 39 (6N, 27M, 6F) | Pools: 6N; 27M; 6F | 6F pool (0.2†) | MT013214 | MT076263 | MT076266 |
| *O. rostratus* | Sandy soil | Fazenda São José/MS | 171 (121N, 40M, 10F) | Individual ticks | 0 | None | None | None |
| *O. marinkellei* | Cave | Porto Velho/RO | 68 (22M, 46F) | Individual ticks | 0 | None | None | None |
| *O. fonsecai** | Cave | Furna do Araticum/CE | 34 (22M, 12F) | Individual ticks | 0 | None | None | None |

*Tick with mitochondrial 16S rDNA sequence generated in this study (GenBank accession nos. MT021429, MT021430, MT021431, MT021432, MT021433, MT021434, MT021435). F, female; M, male; N, nymph.

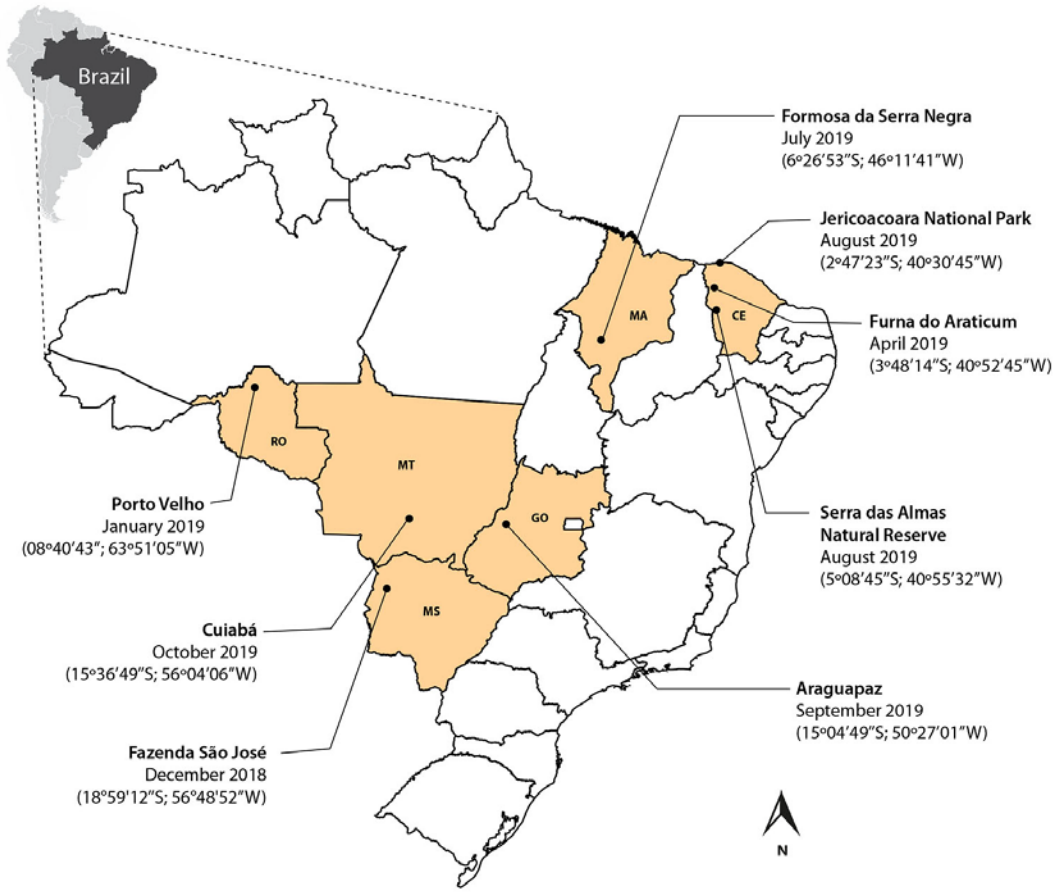
†Minimal infection rate.

The following primers (forward/reverse) were used for PCR (‡) and sequencing (§) *Borrelia* genes: 16S rRNA gene: FD3/T50‡§, 16s-1/16s-2§, Rec4/Rec9§ (*9*); *flaB* gene: FLA LL/FLA LS‡§ (*10*); *glpQ* gene: glpQ F+1/ Rev-2‡§, Rev-1/2glpQ F-1§ (*9*).

**Appendix Table 2.** Coverage (mean ± standard deviation), quality values (% of base calls matching Q20, Q30, and Q40 values, discounting trimmed bases), and length (base pairs, bp) for the sequences of *Borrelia* 16S rRNA, *flab*, and *glpQ* genes obtained in this study

| Tick species/*Borrelia* sp. | Gene | Coverage | Quality | | | Trimmed length |
|---|---|---|---|---|---|---|
| | | | Q20 | Q30 | Q40 | |
| *Ornithodoros mimon* | | | | | | |
| *Borrelia* sp. Omi2MT | 16S rRNA* | 2.7 ± 0.8 | 92.9 | 90.6 | 87.6 | 1,342 bp |
| | flaB | 5.5 ± 1.2 | 93.2 | 90.3 | 88.1 | 504 bp |
| | glpQ | 3.3 ± 0.6 | 96.7 | 93.5 | 85.5 | 447 bp |
| *Borrelia* sp. Omi3MT | 16S rRNA* | 2.8 ± 0.8 | 94.1 | 92.4 | 89.1 | 1,342 bp |
| | flaB | 5.5 ± 1.2 | 93.2 | 90.3 | 88.1 | 504 bp |
| | glpQ | 3.3 ± 0.6 | 96.7 | 93.5 | 85.5 | 447 bp |
| *Ornithodoros rietcorreai* | | | | | | |
| *Borrelia* sp. OrietCE | 16S rRNA | 2.8 ± 0.6 | 94.3 | 92.4 | 90.5 | 1,326 bp |
| *Ornithodoros hasei* | | | | | | |
| *Borrelia* sp. JericoCE | 16S rRNA | 2.1 ± 0.9 | 95.5 | 90.8 | 85.9 | 1,345 bp |
| | flaB | 3.1 ± 1.2 | 92.5 | 90.1 | 88.9 | 674 bp |
| | glpQ | 2.9 ± 0.8 | 94.3 | 92.3 | 86.3 | 715 bp |
| *Ornithodoros* sp. CE | | | | | | |
| *Borrelia* sp. Tabajara CE | 16S rRNA | | | | | |
| | flaB | 2.2 ± 1.1 | 98.6 | 96.8 | 95.5 | 614 bp |
| | glpQ | 3.9 ± 0.8 | 96.4 | 94.7 | 92.4 | 598 bp |

*Haplotypes for 16S rRNA gene of *Borrelia* sp. Omi2MT and *Borrelia* sp. Omi3MT differed in a single nucleotide with unambiguous base calls in each sequence (1341/1342 bp, 99.93% of identity).




**Appendix Figure.** Map of Brazil showing the dates and localities where collection of ticks was performed. CE, Ceará State; GO, Goiás State; MA, Maranhão State; MS, Mato Grosso do Sul State; MT, Mato Grosso State; RO, Rondônia State.