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# ***Brucella neotomae* Infection in Humans, Costa Rica**

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Several species of *Brucella* are known to be zoonotic, but *B. neotomae* infection has been thought to be limited to wood rats. In 2008 and 2011, however, *B. neotomae* was isolated from cerebrospinal fluid of 2 men with neurobrucellosis. The nonzoonotic status of *B. neotomae* should be reassessed.

Members of the genus *Brucella* are the infectious agents of brucellosis, a neglected disease responsible for economic losses resulting from abortion and low performance in production animals (1). The 4 species mainly responsible for this widespread bacterial zoonosis are *B. melitensis*, *B. abortus*, *B. suis*, and to a lesser extent *B. canis*. Underdiagnosis and limited awareness of the disease among healthcare practitioners is common in many countries (1).

*B. neotomae*, isolated in 1957 from wood rats (*Neotoma lepida*) in North America (2), has been considered nonzoonotic (3). It has been isolated from target organs of experimentally infected mice and guinea pigs (4,5). We report the isolation of *B. neotomae* from cerebrospinal fluid samples from 2 human patients with neurobrucellosis.

## **The Study**

In 2008, a *Brucella* sp. isolate was submitted to the Tropical Diseases Research Center at the Universidad de Costa

Rica. This isolate (denoted strain bneohCR2) was cultured from a cerebrospinal fluid sample obtained from a 64-year-old male patient at one of the main hospitals in San José, Costa Rica. In 2011, another isolate (denoted strain bneohCR1) was recovered from a cerebrospinal fluid sample from a 51-year-old male patient at a regional hospital in Costa Rica. As is common for other patients with brucellosis, the blood leukocyte count for each patient was almost within the reference range, and C-reactive protein level was within reference range. Both patients showed clinical signs compatible with neurobrucellosis (6), had positive Rose Bengal test results, and recovered after receiving 1 month of streptomycin (750 mg/d) and 3 months of doxycycline (100 mg/12 h).

Further bacteriologic analysis (7,8) confirmed that the isolates were a *Brucella* sp. (Table). Real-time PCR high-resolution melting analysis (9) confirmed genus designation but was inconclusive regarding species designation. Bruce-ladder multiplex PCR (10) and multiple loci variable number of tandem repeats–16-loci panel analysis (<http://mlva.u-psud.fr/brucella/>; Figure 1) indicated that the profiles for both DNA isolates corresponded to profiles for *B. neotomae*. Analysis of bneohCR2 by multiplex single-nucleotide polymorphism (SNP) primer extension assay (11) and by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of protein extracts (12) (online Technical Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/23/6/16-2018-Techapp1.pdf>) confirmed that the isolate was *B. neotomae*.

We performed whole-genome sequencing of bneohCR1 and bneohCR2 and resequencing of reference strain *B. neotomae* 5K33. Sequencing data were deposited at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena/>) under accession codes ERS1563929 (bneohCR1), ERS1563928 (bneohCR2), and ERS1624467 (SK33). To place the bneohCR1 and bneohCR2 in a phylogenetic context, publicly available reads from 51 *Brucella* whole-genome sequences (online Technical Appendix Table 2) were aligned and then mapped to *B. suis* 1330 by using SMALT version 0.5.8 (<ftp://ftp.sanger.ac.uk/pub/resources/software/smalt/>). Reads from bneohCR1 and bneohCR2 genomes mapped to 98.6% of the *B. suis* 1330 genome. SNPs were called from the alignment by use of Samtools (<http://samtools.sourceforge.net/>), and 34,307 variable sites across all isolates were extracted by using SNP sites (13). The resulting alignment of SNPs

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**Table.** Differential biochemical profile of *Brucella* isolates from 2 men with neurobrucellosis, Costa Rica, 2008 and 2011

Analysis	bneohCR1	bneohCR2
<b>Biochemical tests</b>		
Oxidase	-	-
Citrate utilization	-	-
Nitrate reduction	+	+
CO <sub>2</sub> required	-	-
H <sub>2</sub> S production	+	+
Urease activity, h	0–0.5+	0–0.5+
<b>Growth in presence of dyes</b>		
Thionin 20 µg/mL	-	-
<b>Basic fuchsin 20 µg/mL</b>		
A	+	+
M	-	-

was used for maximum-likelihood phylogenetic reconstruction by use of RAxML version 7.0.4 (<https://github.com/stamatak/standard-RAxML>). The generated phylogenetic tree confirmed that the bneohCR isolates clustered together with *B. neotomae* reference strain 5K33 (ENA accession no. JM5C01, assembly accession no. GCA\_00742255.1) (Figure 2). Isolates bneohCR1 and bneohCR2 differed from the reference genome by 174 and 160 SNPs, respectively. This number of SNPs is smaller than that between *B. abortus* 9–941 and *B. abortus* 2308 (214 SNPs), which are 2 well-recognized strains of the same *Brucella* species.

Analysis of 23 previously reported genomic islands or anomalous genomic loci (14) was performed for both bneohCR genomes. For this analysis, a “genomic-island pseudo-molecule” was constructed by concatenation of 23 genomic regions obtained from different *Brucella* genomes. BLAST

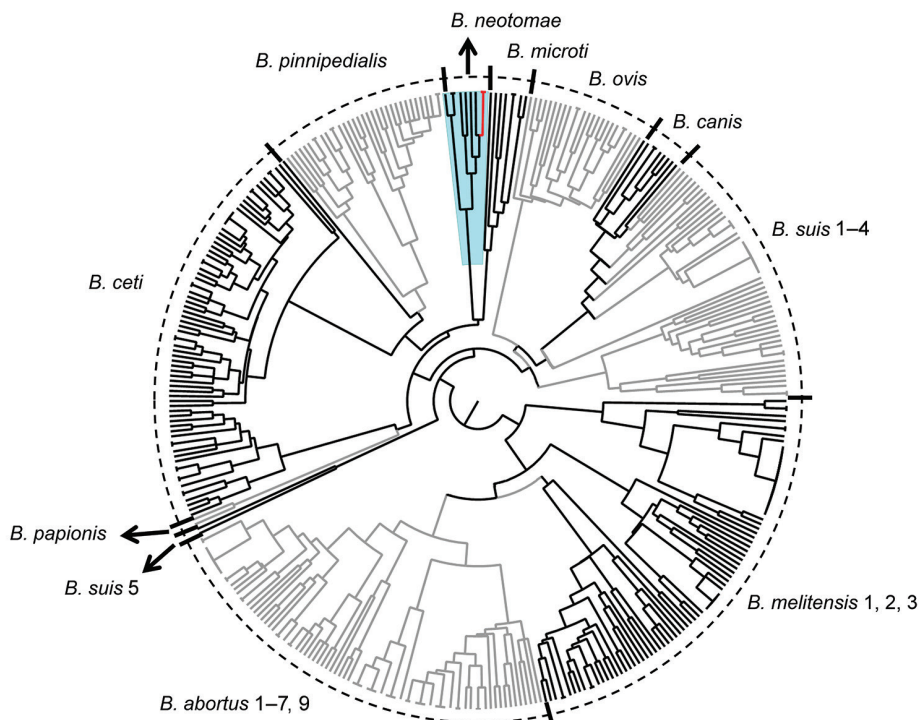
([https://github.com/sanger-pathogens/Farm\\_blast](https://github.com/sanger-pathogens/Farm_blast)) comparison of this pseudo-molecule and the bneohCR draft genomes, generated by assembly with Velvet (15), showed that the genomic loci known as 26.5 kb, 12 kb, and GI-6 that are absent in *B. neotomae* (14) are also absent in the queried genomes.

## Conclusions

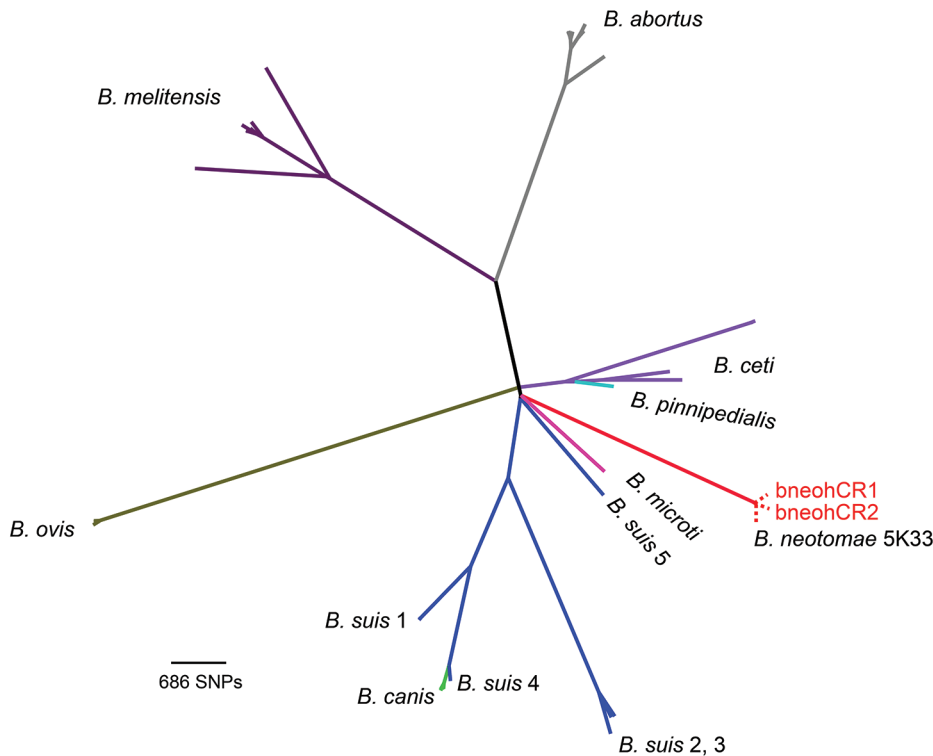
This report of *B. neotomae* as a cause of zoonotic disease raises questions about possible underrepresentation of reported cases. This study also has implications for brucellosis diagnosis. Specifically, the differences among *B. neotomae* and the other *Brucella* species at the biochemical level are subtle. The major difference between *B. neotomae* and *B. abortus*, the main cause of human brucellosis in Costa Rica, is the presence of oxidase activity in *B. abortus*, which is assessed subjectively (7,8). Because *B. neotomae* has not, until now, been considered zoonotic, some cases of brucellosis reported as being caused by atypical zoonotic classical *Brucella* might have been misdiagnosed cases of *B. neotomae* infection. The introduction of whole-genome sequencing into the clinical field will thus improve diagnosis.

A lack of epidemiologic information with regard to the 2 patients reported here precluded the investigation of exposure or contact with hosts known to harbor *B. neotomae*. Further studies are needed to establish which animals may act as reservoirs for *B. neotomae* in Costa Rica.

In summary, *B. neotomae* should be considered a zoonosis risk for infection in humans. Incorporation of molecular techniques for diagnosis will help resolve the



**Figure 1.** Dendrogram based on multiple locus variable number of tandem repeats–16-loci panel analysis of *Brucella* spp. (<http://mlva.u-psud.fr/brucella/>) and clinical isolates from human cerebrospinal fluid samples from 2 patients with brucellosis. The isolates bneohCR1 and bneohCR2 (red branches) showed a pattern consistent with previously reported profiles for *Brucella neotomae* (blue shading). Black, gray, and tic marks are used to differentiate between adjacent species. Arrows separate small neighboring clusters and indicate the *B. neotomae* cluster.



**Figure 2.** Phylogenetic tree based on 34,307 single-nucleotide polymorphisms (SNPs) found among 51 *Brucella* genome sequences. The clinical isolates bneohCR1 and bneohCR2 cluster with *B. neotomae* 5K33 and differ by 164 SNPs. A different color is used to represent each *Brucella* species. Dotted red lines denote the 3 *B. neotomae* isolates, which overlap at the tip of the branch because of the high identity among them.

*Brucella* genus homogeneity obtained when only biochemical assays are used.

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Miss Suárez-Esquivel is a PhD student at Programa de Doctorado en Ciencias at Universidad de Costa Rica, working at Programa de Investigación en Enfermedades Tropicales at Universidad Nacional. Her primary research interest is the evolution of *Brucella* species and their host specificity.

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