Emerging Tickborne Bacteria in Cattle from Colombia

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Ehrlichia minasensis is a new pathogenic bacterial species that infects cattle, and *Borrelia theileri* causes bovine borreliosis. We detected *E. minasensis* and *B. theileri* DNA in cattle from southwestern Colombia by using PCR. *E. minasensis* and *B. theileri* should be considered potential etiologies of febrile syndrome in cattle from Colombia.

hrlichia spp. are tickborne obligate intracellular L bacteria and comprise different pathogenic species that affect both veterinary and public health (1). Ehrlichia minasensis was first detected in cattle and deer in Canada and later in cattle and Rhipicephalus microplus ticks from Brazil (2-4). Infected cattle manifest signs that include fever, lethargy, depression, and anorexia (3,4). Borrelia theileri belongs to the relapsing fever group of borreliae and causes bovine borreliosis, which is a mild febrile disease associated with lethargy, hemoglobinuria, and anemia (5). This spirochete is transmitted by Rhipicephalus (formerly Boophilus) sp. ticks and has been documented in Africa, Europe, Oceania, and South America (5,6). To our knowledge, E. minasensis or B. theileri infections have not been reported in cattle from Colombia.

During September and October 2017, we collected blood samples from 30 bovids with tick parasitism in El Tambo and Santander de Quilichao municipalities, Cauca department, Colombia (Appendix Figure, https://wwwnc.cdc.gov/EID/ article/28/10/22-0657-App1.pdf). We extracted DNA from blood using the QIAGEN DNeasy Blood

& Tissue Kit (https://www.qiagen.com), according to the manufacturer's instructions. We verified DNA quality using PCR amplification of the vertebrate cytochrome B gene CYTB. We subsequently performed PCR to detect dsb and trp36 genes for Ehrlichia spp.; flaB and 16S rRNA genes for Borrelia spp.; and rpoB, msp4, and msp1a genes for Anaplasma spp. (Appendix Table). DNA samples that produced strong PCR bands underwent sequencing on an Applied Biosystems 3130/3130xl Genetic Analyzer (Thermo Fisher Scientific, https://www. thermofisher.com). The *msp1a* PCR products were poor quality and did not undergo sequencing. We aligned sequences using GeneStudio (GeneStudio, Inc., https://genestudio-pro.software.informer. com) and performed multiple sequence alignments using the EMBL-European Bioinformatics Institute tools MUSCLE (for Ehrlichia spp.) and ClustalW (for Borrelia and Anaplasma spp.) (https://www. clustal.org). Phylogenetic analyses were performed with MEGA X software (https://www.megasoftware.net). We generated phylogenetic trees for *dsb*, flaB, and 16S rRNA genes and Trp36 protein using the maximum-likelihood estimation method. All procedures were approved by the Pontificia Universidad Javeriana Ethics Committee in Colombia.

We detected *CYTB* in all samples. We detected the *dsb* gene in 10% (3/30) and *trp36* in 20% (6/30) of samples. The *flaB* gene was detected in 13.3% (4/30), and the 16S rRNA gene was detected in 10% (3/30) of samples. The *rpoB* gene was amplified in 90% (27/30), *msp4* was amplified in 83.3% (25/30), and *msp1a* was amplified in 83.3% (25/30) of samples. We performed phylogenetic analyses of 3 sequences for dsb (GenBank accession nos. ON209405-7), 6 sequences for Trp36 protein (inferred from GenBank accession nos. OL513405-10), 3 sequences for the 16S rRNA gene (GenBank accession nos. ON112216-8), 4 sequences for *flaB* (GenBank accession nos. ON135431-4); 6 sequences for rpoB (GenBank accession nos. ON209412-7), and 4 sequences for msp4 (GenBank accession nos. ON209408-11).

Phylogenetic analyses showed that our *dsb* gene sequences clustered with *E. minasensis dsb* sequences from Brazil, Australia, and Colombia (Figure, panel A). The greater genetic diversity of Trp36 protein compared with *dsb* provided more detailed characterization of *Ehrlichia* sp. genotypes. Our Trp36 sequences clustered with 3 sequences from Brazil and a recently described *E. minasensis* strain from China isolated from *Haemaphysalis hystricis* ticks (Figure, panel B). The *flaB* and 16S rRNA genes clustered with *B. theileri* sequences (Figure, panels C, D). Bootstrap values were 86% for *flaB* and 72% for the 16S rRNA gene. The *flaB* sequences grouped with sequences from Argentina, Republic of the Congo,

Egypt, and Israel (Figure, panel C). The 16S rRNA gene sequences grouped independently but close to sequences from Egypt and the United States (Figure,



Figure. Phylogenetic analysis of emerging tickborne bacteria in cattle from Colombia. Phylogenetic trees are shown for *dsb* genes (A), Trp36 proteins (amino acid sequences) (B), *flaB* genes (C), and 16S rRNA genes (D). We performed PCR on blood samples from cattle in El Tambo and Santander de Quilichao, Cauca department, Colombia to detect *dsb* and *trp36* genes for *Ehrlichia* sp.; *flaB* and 16S rRNA genes for *Borrelia* sp.; and *rpoB*, *msp4*, and *msp1a* genes for *Anaplasma* sp. We generated phylogenetic trees using the maximum-likelihood method and Tamura 3-parameter model with a gamma distribution parameter of 0.28 to compare evolutionary relationships between our sequences and publicly available sequences from Genbank (accession numbers indicated on trees). We applied bootstrap tests using 1,000 replicates; bootstrap values are shown at key nodes. The •, •, and \blacktriangle symbols represent the differential clustering of sequences obtained in this study. Scale bars indicate nucleotide substitutions per site.

panel D). We confirmed *A. marginale* using identity analysis (100% identical to GenBank sequences; accession nos. CP023731, CP006846, CP001079, CP000030, and AF428086) of *rpoB* and *msp4* genes. Co-infection with *E. minasensis* and *A. marginale* was confirmed in 6 animals from El Tambo. Co-infection with *B. theileri* and *A. marginale* was documented in 1 animal from El Tambo and 1 animal from Santander de Quilichao.

These results showed the simultaneous circulation of E. minasensis, B. theileri, and A. marginale in bovids from Cauca department, Colombia. In Latin America, E. minasensis has been identified in Brazil (7) and Colombia (found in *R. microplus* ticks) (8), and B. theileri has been found in Argentina (6), Mexico (9), and Brazil (10). The R. microplus tick is likely the main vector for both pathogens in these regions and has been confirmed by molecular detection of E. minasensis in tick specimens collected from the same animals (H.C. Martínez-Diaz, unpub. data) and various reports in Latin America for B. theileri (9,10). Despite the lack of clinical signs in these animals, tickborne infections caused by these pathogenic bacteria often occur as subclinical infections or with intermittent clinical manifestations. E. minasensis and B. theileri infections, either separately or as co-infections, may be more frequent than previously recognized and should be considered potential etiologies of febrile syndrome in cattle from this and other regions of Colombia.

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Appendix

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Target gene	Primer sets	Sequence (5'-3')	temperature (°C)	References
CYTB*	Cvt bF	CCCCTCAGAATGATATTTGTCCTCA	60	(1)
	Cvt bR	CCATCCAACATCTCAGCATGATGAAA		(.)
<i>dsb</i> (heminested)	Dsb-330	GATGATGTCTGAAGATATGAAACAAAT	52	(2,3)
	(first reaction)			
	Dsb-380	ATTTTTAGRGATTTTCCAATACTTGG		
	(second reaction)			
	Dsb-720	CTATTTTACTTCTTAAAGTTGATAWATC		
	(both reactions)			
<i>trp36</i> (heminested)	TRP36-F2	TTTAAAACAAAATTAACACACTA	45	(4,5)
	(first reaction)			
	TRP36-DF (second	CACACTAAAATGTATAATAAAGC		
	reaction)			
	TRP36-R1	AAGATTAACTTAATACTCAATATTACT		
	(both reactions)			
<i>flaB</i> (nested)	FlaLL	ACATATTCAGATGCAGACAGAGGT	55	(6)
	(first reaction)			
	FlaRL	GCAATCATAGCCATTGCAGATTGT		
	(first reaction)			
	FlaLS	AACAGCTGAAGAGCTTGGAATG	55	
	(second reaction)			
	FlaRS	CTTTGATCACTTATCATTCTAATAGC		
	(second reaction)			
16S rRNA (nested)	B1	CAGTGCGTCTTAAGCATGC	56	(7)
	(first reaction)			
	B8	CCTTAAATACCTTCCTCCC		
	(first reaction)			
	B3	GCAGCTAAGAATCTTCCGCAATGG	59	
	(second reaction)			
	B6	CAACCATGCAGCACCTGTATAT		
	(second reaction)			
rpoB	Ana-rpoBF	GCTGTTCCTAGGCTYTCTTACGCGA	55	(8)
	Ana-rpoBR	AATCRAGCCAVGAGCCCCTRTAWGG		
msp4	MSP45	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	60	(9)
	MSP43	CCGGATCCTTAGCTGAACAGGAATCTTGC		
msp1a	MSP1aP	GCATTACAACGCAACGCTTGAG	68	(9)
	MSP1a3	GCTTTACGCCGCCGCCTGCGCC		

*Primer set was developed to detect cytochrome B genes in multiple vertebrate species, including cattle. Primer names were not provided in the original publication.



Appendix Figure. Maps showing locations where emerging tickborne bacteria *Ehrlichia minasensis* and *Borrelia theileri* were found in cattle from El Tambo and Santander de Quilichao municipalities in Cauca department, Colombia. We collected blood samples from cattle and performed PCR analysis to detect different genes specific for *Ehrlichia minasensis*, *Borrelia theileri*, and *Anaplasma* sp. A) Blue area indicates Cauca department within Colombia. B) Green areas indicate El Tambo and Santander de Quilichao municipalities within Cauca department, Colombia.

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