

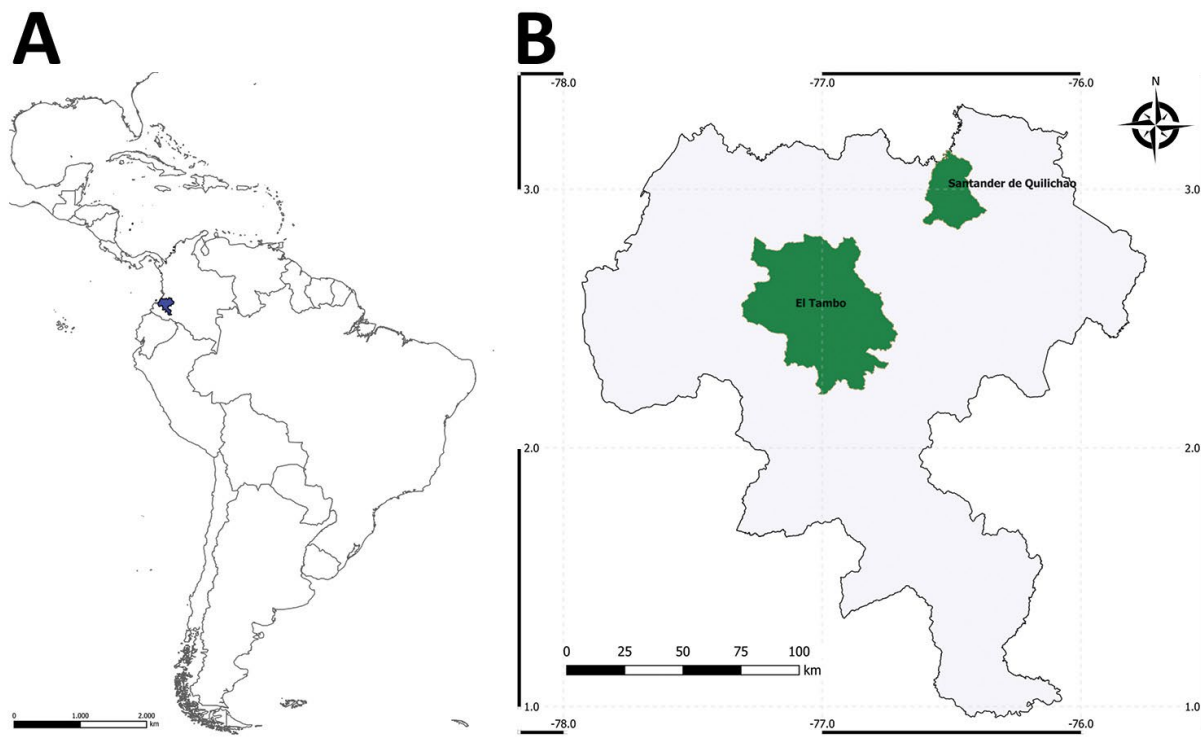
Emerging Tickborne Bacteria in Cattle from Colombia

Appendix

Appendix Table. List of PCR primers used to identify emerging tickborne bacteria in cattle from Colombia

Target gene	Primer sets	Sequence (5'-3')	Annealing temperature (°C)	References
<i>CYTb*</i>	Cyt bF	CCCCTCAGAATGATATTTGTCCTCA	60	(1)
	Cyt 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 reaction)	CACACTAAAATGTATAATAAAGC		
	(second reaction)			
	TRP36-R1	AAGATTAECTTAATACTCAATATTACT		
	(both reactions)			
<i>flaB</i> (nested)	FlaLL	ACATATTCAGATGCAGACAGAGGT	55	(6)
	(first reaction)			
	FlaRL	GCAATCATAGCCATTGCAGATTGT		
	(first reaction)		55	
	FlaLS	AACAGCTGAAGAGCTTGGAATG		
	(second reaction)			
	FlaRS	CTTTGATCACTTATCATTCTAATAGC		
	(second reaction)			
16S rRNA (nested)	B1	CAGTGCCTTAAGCATGC	56	(7)
	(first reaction)			
	B8	CCTTAAATACCTTCTCC	59	
	(first reaction)			
	B3	GCAGCTAAGAATCTCCGCAATGG		
	(second reaction)			
	B6	CAACCATGCAGCACCTGTATAT		
	(second reaction)			
<i>rpoB</i>	Ana-rpoBF	GCTGTTCTAGGCTYTCTTACGCGA	55	(8)
	Ana-rpoBR	AATCRAGCCAVGAGCCCCTRTAWGG		
<i>msp4</i>	MSP45	GGGAGCTCCTATGAATTACAGAGAATTGTTTAC	60	(9)
	MSP43	CCGGATCCTTAGCTGAACAGGAATCTTGC		
<i>msp1a</i>	MSP1aP	GCATTACAACGCAACGCTTGAG	68	(9)
	MSP1a3	GCTTACGCCGCCCTGCGCC		

*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.

References

1. Boakye DA, Tang J, Truc P, Merriweather A, Unnasch TR. Identification of bloodmeals in haematophagous *Diptera* by cytochrome B heteroduplex analysis. *Med Vet Entomol.* 1999;13:282–7. [PubMed https://doi.org/10.1046/j.1365-2915.1999.00193.x](https://doi.org/10.1046/j.1365-2915.1999.00193.x)
2. Doyle CK, Labruna MB, Breitschwerdt EB, Tang YW, Corstvet RE, Hegarty BC, et al. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. *J Mol Diagn.* 2005;7:504–10. [PubMed https://doi.org/10.1016/S1525-1578\(10\)60581-8](https://doi.org/10.1016/S1525-1578(10)60581-8)
3. Almeida AP, Souza TD, Marcili A, Labruna MB. Novel *Ehrlichia* and *Hepatozoon* agents infecting the crab-eating fox (*Cerdocyon thous*) in southeastern Brazil. *J Med Entomol.* 2013;50:640–6. [PubMed https://doi.org/10.1603/ME12272](https://doi.org/10.1603/ME12272)

4. Aguiar DM, Zhang X, Melo AL, Pacheco TA, Meneses AM, Zanutto MS, et al. Genetic diversity of *Ehrlichia canis* in Brazil. *Vet Microbiol.* 2013;164:315–21. [PubMed](#)
<https://doi.org/10.1016/j.vetmic.2013.02.015>
5. Aguiar DM, Ziliani TF, Zhang X, Melo AL, Braga IA, Witter R, et al. A novel *Ehrlichia* genotype strain distinguished by the *TRP36* gene naturally infects cattle in Brazil and causes clinical manifestations associated with ehrlichiosis. *Ticks Tick Borne Dis.* 2014;5:537–44. [PubMed](#)
<https://doi.org/10.1016/j.ttbdis.2014.03.010>
6. Moore VA 4th, Varela AS, Yabsley MJ, Davidson WR, Little SE. Detection of *Borrelia lonestari*, putative agent of southern tick-associated rash illness, in white-tailed deer (*Odocoileus virginianus*) from the southeastern United States. *J Clin Microbiol.* 2003;41:424–7. [PubMed](#)
<https://doi.org/10.1128/JCM.41.1.424-427.2003>
7. Takhampunya R, Thaloengsok S, Tippayachai B, Promsathaporn S, Leepitakrat S, Gross K, et al. Retrospective survey of *Borrelia* spp. from rodents and ticks in Thailand. *J Med Entomol.* 2021;58:1331–44. [PubMed](#) <https://doi.org/10.1093/jme/tjaa279>
8. Dahmani M, Davoust B, Rousseau F, Raoult D, Fenollar F, Mediannikov O. Natural Anaplasmataceae infection in *Rhipicephalus bursa* ticks collected from sheep in the French Basque Country. *Ticks Tick Borne Dis.* 2017;8:18–24. [PubMed](#) <https://doi.org/10.1016/j.ttbdis.2016.09.009>
9. de la Fuente J, Van Den Bussche RA, Kocan KM. Molecular phylogeny and biogeography of North American isolates of *Anaplasma marginale* (Rickettsiaceae: Ehrlichieae). *Vet Parasitol.* 2001;97:65–76. [PubMed](#) [https://doi.org/10.1016/S0304-4017\(01\)00378-8](https://doi.org/10.1016/S0304-4017(01)00378-8)