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Typhus Group Rickettsiosis, Brazilian Amazon

Appendix

Collection and Identification of Ticks in Amazon Forest Area Visited by the Febrile Patient

On October 2019, we collected host-questing ticks by flagging the vegetation in the patient-visited areas in the Saracá-Taquera National Forest, Porto Trombetas District, Brazilian Amazon. In addition, we collected ticks from a fresh carcass of an agouti (*Dasyprocta leporina*) that was found in the area. Collected ticks were transported alive to the laboratory, where they were identified to species according to the procedure of Dantas-Torres et al. (*1*). A total of 170 ticks were identified in 7 species, including 6 *Amblyomma* spp. (Appendix Table).

For molecular detection of rickettsiae, we processed ticks individually (adults and few nymphs) or in pools of 2 to 10 ticks (most of the nymphs) by using the guanidine isothiocyanate phenol technique (*2*) and tested by using PCR and primers CS-78 and CS-323 targeting a 401-bp fragment of the citrate synthase gene, which is common in all representatives of the genus *Rickettsia* (*3*). Samples yielding expected size amplicons were subsequently tested with primers Rr190.70p and Rr190.701n, targeting a 631-bp fragment of the rickettsial 190-kDa outer membrane protein gene, which is present only in members of the spotted fever group (*4*). A negative control tube containing ultrapure water and a positive control tube containing DNA of *Rickettsia vini* were included in each PCR run (*5*). Obtained amplicons were treated with ExoSap (US Biochemicals, https://www.thermofisher.com) and DNA-sequenced in an ABI automated sequencer and model ABI 3500 Genetic Analyzer (Applied Biosystems/Thermo Fisher Scientific, https://www.thermofisher.com) with the same primers used for PCR. Acquired sequences were assembled with Geneious R9 software (https://www.geneious.com) and subjected to blast analyses (https://www.ncbi.nlm.nih.gov) to infer the closest similarities available in GenBank.

The 170 ticks were tested in 50 samples (16 individual adults, 4 individual nymphs and 30 nymphal pools), from which only 17 samples of *A. cajennense* sensu stricto yielded amplicons by both the citrate synthase gene and 190-kDa outer membrane protein gene PCRs. PCR products from these 17 samples yielded DNA sequences that showed 100% identity with *R. amblyommatis* (GenBank accession no. CP012420).

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Appendix Table. Ticks collected in study of typhus group rickettsiosis, Brazilian Amazon*

Tick species	Source	No. specimens collected	No. samples tested	No. samples yielding rickettsial DNA (% infection)
Amblyomma cajennense sensu stricto	Vegetation	9 adults	9	5 (55)
	Vegetation	100 nymphs	11 pools	11 (11)†
	Agouti	10 nymphs	1 pool	1 (10)†
Amblyomma coelebs	Vegetation	9 nymphs	7 pools	0
Amblyomma naponense	Vegetation	1 adult	1	0
	Vegetation	7 nymphs	3 pools	0
Amblyomma oblongoguttatum	Vegetation	3 adults, 2 nymphs	5	0
Amblyomma pacae	Agouti	2 nymphs	1 pool	0
Amblyomma scalpturatum	Vegetation	3 adults	3	0
	Vegetation	22 nymphs	7 pools	0
Haemaphysalis juxtakochi	Vegetation	2 nymphs	2	0
Total	NA	170	50	17

*NA, not applicable.
†% infection, in this instance, refers to minimal infection rate (≥1 infected tick in each pool): no. infected pools/total no. tested ticks x 100.