Am. J. Trop. Med. Hyg., 86(6), 2012, pp. 1054–1056
doi:10.4269/ajtmh.2012.11-0742

Short Report: *Rickettsia felis* in *Ctenocephalides felis* from Guatemala and Costa Rica

Adriana Troyo,* Danilo Álvarez, Lizeth Taylor, Gabriela Abdalla, Ólger Calderón-Arguedas, Maria L. Zambrano, Gregory A. Dasch, Kim Lindblade, Laya Hun, Marina E. Eremeeva, and Alejandra Estévez

Departamento de Parasitología, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica; Centro de Estudios en Salud, Instituto de Investigaciones, Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala; Departamento de Microbiología e Inmunología, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica; Rickettsial Zoonoses Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; International Emerging Infectious Diseases Program, Centers for Disease Control and Prevention, Atlanta, Georgia; Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, Georgia

Abstract. *Rickettsia felis* is an emerging human pathogen associated primarily with the cat flea *Ctenocephalides felis*. In this study, we investigated the presence of *Rickettsia felis* in *C. felis* from Guatemala and Costa Rica. *Ctenocephalides felis* were collected directly from dogs and cats, and analyzed by polymerase chain reaction for *Rickettsia*-specific fragments of 17-kDa protein, OmpA, and citrate synthase genes. *Rickettsia* DNA was detected in 64% (55 of 86) and 58% (47 of 81) of flea pools in Guatemala and Costa Rica, respectively. Sequencing of *glt*A fragments identified *R. felis* genotype URRWXCal$_2$ in samples from both countries, and genotype Rf2125 in Costa Rica. This is the first report of *R. felis* in Guatemala and of genotype Rf2125 in Costa Rica. The extensive presence of this pathogen in countries of Central America stresses the need for increased awareness and diagnosis.

Rickettsia felis is an emerging pathogen, which was first detected in the cat flea, *Ctenocephalides felis*. It was later associated with human disease manifesting with fever, headaches, myalgia, and occasionally rash.[1] Even though *R. felis* has also been detected in domestic and wild animals, their susceptibility to this bacterium and their role as reservoirs has not been well established.[2]

Rickettsia felis has a cosmopolitan distribution associated with fleas. In the Americas, human disease caused by *R. felis* has been described from the United States, Mexico, and Brazil.[2,3] Furthermore, DNA of *R. felis* has also been detected in fleas from Peru, Uruguay, Chile, Argentina, and more recently in the West Indies, Panamá, and Costa Rica.[2–7] There is no evidence yet of human disease caused by *R. felis* in Central America, although human exposure to pathogenic spotted fever group rickettsiae different from *R. rickettsii*, may occur in this region.[8,9] As part of an ongoing project to characterize rickettsial diseases in Guatemala and Costa Rica, we assessed the presence of *R. felis* in sites where cases of rickettsioses have been previously reported.

Entomological surveys were carried out at several locations in Guatemala and Costa Rica throughout 2009 and 2010, including wet and dry seasons. Collection sites in Guatemala were located in the Southeastern region, departments of Santa Rosa (14°16′N, 90°18′W) and Jutiapa (14°16′N, 89°53′W), an area suspected of a spotted fever outbreak in 2007 (Eremeeva ME, unpublished data). In Costa Rica, collections were performed at sites from the Caribbean slope of the country, where cases of Rocky Mountain spotted fever and uncharacterized spotted fevers have been documented,[10] specifically in the districts of Turrialba (9°54′N, 83°41′W), La Virgen (10°23′N, 84°08′W), Limón (9°59′N, 83°02′W), Cahuita (9°44′N, 82°50′W), Guápiles (10°13′N, 83°47′W), Guácimo (10°12′N, 83°41′W), and Jiménez (10°12′N, 83°44′W). Additional samples of *C. felis* from two locations in San José (9°55′N, 84°04′W), obtained by the laboratory of Medical Arthropodology (University of Costa Rica) through the general public as part of inquiries and identification services, were also analyzed.

At collection locations (households, farms, etc.) fleas were collected from household cats and dogs, and from opossums captured using live animal traps. *Ctenocephalides felis* were grouped into lots according to host species, collection site, and location.

For the preliminary detection of DNA of *Rickettsia* spp., pools of 1–10 *C. felis* from each lot were analyzed using nested and semi-nested polymerase chain reaction (PCR) assays targeting specific fragments of the 17-kDa protein and OmpA genes. Primers R17-122 and R17-500 were used for the primary PCR of *Rickettsia*-specific 17-kDa protein gene, and nested PCRs were performed using primers TZ15 and TZ16 or RP2 and RPID, which detect fragments specific for *Rickettsia* of the spotted fever group and typhus group, respectively.[11,12] For *omp*A, primers Rr190-70 and Rr190-701 were used in the first PCR, and Rr190-70 and Rr190-602 for the semi-nested PCR.[12] Detection of positive samples was further confirmed in samples from Guatemala using a TaqMan assay for the citrate synthase (*glt*A) gene that is species specific for detection of *R. typhi* and *R. felis*,[13] or by the *Rickettsia* spp. wide-range *gltA* assay using primers CS-78 and CS-323 in samples from Costa Rica.[14]

Three-hundred thirty-three *C. felis* were collected from two sites in Guatemala and grouped into 86 pools, including 73 flea pools from dogs and 13 from cats (Table 1). The DNA of *Rickettsia* was detected in 55 pools (64%), 54 from Jutiapa (78% collected from dogs and 22% from cats) and one pool from Santa Rosa collected from a dog.

In Costa Rica, a total 439 *C. felis* was collected from the different sites, all samples collected from dogs and cats (Table 1). Forty-seven pools (58%) contained *Rickettsia* DNA by positive PCR for at least two of the three genes analyzed, and positivity varied between sites. Forty-four of 74 pools from dogs (59%) and 3 of 7 pools from cats (43%) were positive. No *C. felis* was found on two *Didelphis marsupialis* and three *Philander opossum* captured in Cahuita, Guácimo, Limón, and Turrialba.

*Address correspondence to Adriana Troyo, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José 11501, Costa Rica. E-mail: adriana.troyo@ucr.ac.cr

TABLE 1

Rickettsia felis frequency and *glt*A genotypes in *Ctenocephalides felis* pools from different areas of Guatemala and Costa Rica

Country	Site	*C. felis* collected	Flea pools			Positive pools (%)	Samples sequenced	*R. felis* genotype
			Dogs	Cats	Total			
Guatemala	Jutiapa	269	57	13	70	54 (77)	22	URRWXCal$_2$
	Santa Rosa	64	16	0	16	1 (6)	1*	URRWXCal$_2$
	All sites	333	73	13	86	55 (65)	23	–
Costa Rica	Cahuita	96	11	1	12	6 (50)	6	Rf2125
	Guápiles/Jiménez/Guácimo	107	20	1	21	12 (57)	7	Rf2125
	La Virgen	71	11	3	14	3 (21)	2	Rf2125
	Limón	59	13	2	15	10 (67)	9	Rf2125
	Turrialba	73	14	0	14	13 (93)	11	Rf2125
	San José	33	5	0	5	3 (60)	3	URRWXCal$_2$
	All sites	416	74	7	81	47 (58)	38	–

**omp*A amplicon.

The DNA of *R. typhi* was not detected during this study. The presence of *R. felis* DNA in *C. felis* from Guatemala was confirmed by multiplex TaqMan *glt*A assay. Only one genetic type of *glt*A was found by sequencing of the TaqMan product, and it was the same as the URRWXCal$_2$ reference strain of *R. felis* from California (CP000053). In contrast, two genotypes were identified in fleas from Costa Rica after sequencing *glt*A amplicons of 38 of 47 positive samples (81%), Rf2125 (AF516333) and URRWXCal$_2$ of *R. felis* (Table 1). The *glt*A fragments were identical between the three sequences of *R. felis* URRWXCal$_2$ analyzed from Costa Rica, and similarity was 99.25% (399 of 402) with the sequence reported in GenBank (CP000053). The only Costa Rican fleas containing *R. felis* URRWXCal$_2$ were from dogs from the capital city, San José. Two different sequences were detected in *glt*A fragments of *R. felis* Rf2125 from positive samples of all other sites in Costa Rica, and they were both 99.25% (399 of 402) similar to the corresponding fragment of the sequence reported in GenBank (AF516333). GenBank accession nos. for fragments of *R. felis glt*A obtained in this study are JF523341 (Guatemala) and JN982948-JN982950 (Costa Rica).

To our knowledge, we describe the first detection of *R. felis* in *C. felis* from Guatemala. This common and widespread occurrence of *R. felis* in fleas in Costa Rica and Guatemala is similar to findings previously reported from other countries in Latin America.[15–17] For example, in a study that analyzed pools of *C. felis* from Iquitos, Peru, 71 of 74 pools contained *R. felis*.[17]

In both countries, *R. felis* was detected in *C. felis* from both dogs and cats, although more dogs were sampled. Furthermore, *R. felis* was detected frequently on fleas from dogs, suggesting this may be a relevant host in maintaining *C. felis* and possibly *R. felis* in the areas studied. Considering the close relationship with pet owners, dogs may indirectly pose a risk for human infection, because they may promote exposure by transporting the infected fleas to the resident environment. Although *Didelphis virginiana* opossums have been associated with a life cycle of *R. felis* in wild-caught *C. felis* in Texas and California,[18] *C. felis* were not found on the few opossums captured during this study. Therefore, additional trapping of opossums is needed to elucidate their role in circulation of *R. felis* in Costa Rica and Guatemala.

Human infection with *R. felis* has not been documented in Central America, although *R. felis* was reported recently in Panamá and Costa Rica.[5–7] There are previous reports of *R. felis* Rf2125 in Latin America and other countries, but as no human disease has yet been associated with this genotype, its pathogenic potential warrants further evaluation.[19,20] Moreover, this study shows the presence of at least two different genotypes of *R. felis*, including the pathogenic URRWXCal$_2$ strain, in regions of Central America. Whether either of these genetic types has an adaptive advantage in infecting fleas has not yet been evaluated.

Because *C. felis* was frequently found on cats and dogs in this study, and substantial numbers of fleas tested were infected with *R. felis*, humans may have a high probability of exposure to this pathogenic *Rickettsia*. Clinical signs and symptoms of *R. felis* infection are very similar to those of other rickettsioses and resemble other more commonly diagnosed tropical diseases, such as dengue and malaria.[3,21,22] Therefore, it is likely that infections caused by this pathogen are underestimated and misdiagnosed by the medical community throughout Central America. Because *R. felis* may cause severe illness in some individuals,[3,23] proper physician education, disease awareness, and adequate diagnosis are essential.

Received November 28, 2011. Accepted for publication March 4, 2012.

Acknowledgments: We thank David Morán, Luis Escobar, and Ramón Medrano from Guatemala, and Adrian Avendaño, Ivan Coronado, Luis Vargas, Greivin Rodriguez, Julio Rojas, and Gilberth Alvarado from Costa Rica, for technical support during entomological surveys. Jusara Ortiz, Carlos Mata, and Carlos Vargas are also acknowledged for technical support during PCR analyses in Costa Rica. We also thank Marcelo B. Labruna for confirmation of *Rickettsia* genotypes from Costa Rica, and Michael L. Levin and Marcelo Labruna for their helpful guidance and suggestions.

Financial support: This research was supported by NeTropica 9-N-2008, Universidad de Costa Rica 803-A8-127, and Cooperative Agreement No. UO1 GH000028 from the U.S. Centers for Disease Control and Prevention (CDC).

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services of the United States, or the institutions with which the authors are affiliated.

Authors' addresses: Adriana Troyo, Lizeth Taylor, Ólger Calderón-Arguedas, and Laya Hun, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica, E-mails: adriana.troyo@ucr.ac.cr, mayra.taylor@ucr.ac.cr, olger.calderon@ucr.ac.cr, and ruchlia.hun@ucr.ac.cr. Danilo Álvarez, Gabriela Abdalla, and Alejandra Estévez, Centro de Estudios

en Salud, Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala, E-mails: dalvarez@ces.uvg.edu.gt, gabdalla@ces.uvg.edu.gt, and alestevez@yahoo.com. Kim Lindblade, CDC Regional Office for Central America and Panama, Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala, E-mail: kil2@cdc.gov. Maria L. Zambrano, and Gregory A. Dasch, Rickettsial Zoonoses Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: mzambrano@cdc.gov and gdasch@cdc.gov. Marina E. Eremeeva, Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, E-mail: meremeeva@georgiasouthern.edu.

Reprint requests: Adriana Troyo, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José 11501, Costa Rica, Tel: (506) 2511-5430, Fax: (506) 2511 4360, E-mail: adriana.troyo@ucr.ac.cr.

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