**Scientific Note**

*Rickettsia rickettsii* and *Rickettsia felis* infection in *Rhipicephalus sanguineus* ticks and *Ctenocephalides felis* fleas co-existing in a small city in Yucatan, Mexico

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Rickettsiosis is an important febrile illness affecting the population of Yucatan State in Mexico, and is often confused with dengue fever. Since 1999, four *Rickettsia* species have been reported affecting humans in Yucatan: *Rickettsia felis* (Zavala-Velazquez et al. 2000), *Rickettsia akari* (Zavala-Castro et al. 2009a), *Rickettsia rickettsii* (Zavala-Castro et al. 2008), and *Rickettsia typhi* (Zavala-Castro et al. 2009b). In all cases, the presence of arthropods, rodents, or opossums were reported. In most of these cases, *R. typhi* and *R. felis* were involved, with two to five cases reported per month in our laboratory. However, this number can be subjective because it only includes patients who have been referred by physicians who have knowledge of the disease.

In the Americas, Rocky Mountain spotted fever rickettsioses, transmitted by *R. rickettsii*, are mainly reported in the United States, Brazil, Panama, and Mexico (Chen and Sexton 2008), Costa Rica (Arguello et al. 2012), and Colombia (Hidalgo et al. 2011), where poverty, presence of children, and close contact with domestic animals are involved. Despite the human cases of rickettsioses diagnosed in Yucatan state, Mexico, only *Ctenocephalides felis* fleas has been identified as the vector of *R. felis* (Pérez-Osorio et al. 2008, Zavala-Velazquez et al. 2002).

From September, 2010, to June, 2011, 16 patients from Oxxutzcab municipality were diagnosed with rickettsiosis in our laboratory. There was no *Rickettsia* species identification due to sample integrity. We visited houses of patients from January, 2012, to June, 2012, to collect ectoparasites and identify the possible causal agent of rickettsiosis. Oxxutzcab municipality (20° 18′ 10″ N, 89° 25′ 6″ W) is an agricultural center focused mainly on the production of tropical citrus fruits, exposing the workers to wild animals and their arthropods. The living and cultural conditions of the population are very similar to some tropical areas in Central America. Houses have a backyard that is often inhabited by synanthropic mammals and domestic animals living in close contact with their inhabitants, increasing the transmission of vector-borne diseases, such rickettsial infections.

Ticks and fleas that were parasitizing dogs were collected and free-living arthropods were captured by flagging and by inspecting the walls and rocks surrounding the houses. Collected arthropods were stored in 1.5 ml tubes and maintained at -70° C. Tick and fleas species collected were determined using existing keys (Acosta and Morrone 2003, Faccioli 2011, Ponce Ulloa and Llorente Bousquets 1993). Samples were analyzed individually. DNA was purified following the instructions of a DNA blood and tissue kit (QIAGEN, Valencia, CA).

Screening for identification of *Rickettsia* species was accomplished by a conventional PCR assay, amplifying three different DNA fragment genes from *gltA*, 17kDa, and *ompB* genes: a 380-bp fragment of the rickettsial gene *gltA*, using primers RpCS.877fw (5′-GGGCGGCTGTCACGGCG-GG-3′) and RpCS.1258rv (5′-ATTGCAAAAAATCATGTGAACA-3′) (Regnery et al. 1991), a 434-bp fragment of the rickettsial gene 17 kDa using primers Fw1: 5′-GCTCTTTCGCACTTCTATGT-3′, and Rv2: 5′-CATTTTGTCGTCAGGTTGGCG-3′) (Webb et al. 1990) and 990–999-bp fragment of the rickettsial gene *ompB* using primers ompB330(1)fw (5′-GGCGTCTCAAACCAATTCTAA-3′) and ompB330(1)rv (5′-AGCTCTACTGTCCTATTATCTG-TACC-3′) (Peniche-Lara et al. 2013). The PCR reaction was performed using Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions in a Multigene Thermalcycler (Labnet International, Inc). The PCR products were electrophoresed on a 1.5% agarose gel at 100 V for 60 min and visualized using a UV transilluminator. PCR amplicons were purified using the Qiagen Gel Extraction Kit (QIAGEN, Valencia, CA), according to the manufacturer’s instructions. Purified PCR products were sequenced using an Applied Biosystems 3130xl genetic analyzer. Sequences similarities were detected using the BLAST search engine from the National Center for Biotechnology Information (NCBI) website.

A total of 28 dogs was screened for arthropod collection. No arthropods were obtained from free-living ticks or flea species. A total of 106 *Rhipicephalus sanguineus* adult ticks (73 males and 33 females) and 65 *Ctenocephalides felis* fleas were collected. No ticks or flea species were collected in one house; in four houses we only collected tick species, and in three houses we only collected flea species. Eight houses had both arthropods (Table 1). We identified *Rickettsia* species in six houses out of 16 visited. Two houses harbored only *R. rickettsii*; two houses only identified *R. felis* species and two houses had both *Rickettsia* species presence (Table 1). Positive arthropods included 16 male adult ticks (73 total), 12 female adult ticks (33 total), and 13 fleas (65 total).

Fragments obtained from *gltA*, 17 kDa, and *ompB* genes from tick samples (GeneBank accession No. JX198506, KC107823, and KF056800, respectively) had 99-99.8%