

ORIGINAL ARTICLE

INFECTION BY *Rickettsia felis* IN OPOSSUMS (*Didelphis* sp.) FROM YUCATAN, MEXICO

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SUMMARY

*Rickettsia felis* is an emergent pathogen and the causative agent of a typhus-like rickettsiosis in the Americas. Its transmission cycle involves fleas as biological vectors (mainly *Ctenocephalides felis*) and multiple domestic and synanthropic mammal hosts. Nonetheless, the role of mammals in the cycle of *R. felis* is not well understood and many efforts are ongoing in different countries of America to clarify it. The present study describes for the first time in Mexico the infection of two species of opossum (*Didelphis virginiana* and *D. marsupialis*) by *R. felis*. A diagnosis was carried out from blood samples by molecular methods through the *gltA* and 17 kDa genes and sequence determination. Eighty-seven opossum samples were analyzed and 28 were found to be infected (32.1%) from five out of the six studied localities of Yucatan. These findings enable recognition of the potential epidemiological implications for public health of the presence of infected synanthropic *Didelphis* in households.

**KEYWORDS:** *Rickettsia felis*; *gltA*; 17 kDa; Transmission cycle; *Didelphis marsupialis*; *Didelphis virginiana*; Yucatan.

INTRODUCTION

*Rickettsia felis* is an emergent pathogen that infects humans and is the causative agent of the probably underestimated flea-borne spotted fever<sup>1,2</sup>. The general transmission cycle of *R. felis* has been described as a vector-borne disease and fleas acquire the bacteria mainly by vertical infection and apparently less frequently by horizontal infection through other fleas or infected vertebrate hosts<sup>3</sup>.

The agent *Rickettsia felis* has been reported in different countries around the world to infect its biological vector, the cat flea *Ctenocephalides felis*, four domestic or synanthropic hosts (cats, dogs, rats and opossums), and humans<sup>2,4,5,6,7</sup>. In Mexico, Yucatan is the only region where human cases have been reported<sup>7,8</sup>, mainly from rural and suburban localities where people are in closer contact with sylvan and synanthropic mammals and their ectoparasites<sup>7,9</sup>. The most common wild synanthropes in Yucatan are opossums (*Didelphis* spp.) and rodents, which can potentially harbor flea-borne zoonotic pathogens and carry them to domestic and peridomestic areas since they are frequently infested with fleas<sup>10,11</sup>. Records of *C. felis* collected in *Didelphis virginiana*, cats, and dogs are common<sup>1,3,12,13</sup>. Opossums have been found infected with *R. felis* in some countries of the Americas but not in Mexico<sup>4,5,6</sup>.

The aim of the present study is to report, for the first time in Mexico, the finding of this rickettsial infection in opossums captured in six

different localities in Yucatan, which were assessed by systematic and occasional field trips monitoring of the presence of relevant zoonotic pathogens to public health in the peninsula.

MATERIALS AND METHODS

**Study area and opossum trapping.** Opossums were captured from six localities: *Kikil*, *Cholul* (July to November 2005), *Dzidzilche*, *Dzitya*, *Molas* (July to December 2011), and *Buctzotz* (July to December 2012) in the State of Yucatan (Fig. 1, see Table 1 for geographical coordinates). The climate is predominantly tropical sub-humid, with a rainy season from June to November, and a dry season from December to May. The vegetation of the region is predominantly tropical deciduous forest but human activities have modified extensive areas for urban, agriculture, farming, and livestock practices<sup>8</sup>.

The capture of opossums was carried out using Tomahawk Live Traps® with tropical fruit as bait; in the peridomiciliar area, one trap was placed in the backyard of households. The trapping period in the peridomicile varied from one to three consecutive nights. Trapping in the sylvatic areas was performed only in *Kikil* and *Dzidzilche* because in these two localities a wider study on opossum ecology was performed (nonetheless, all captured animals were included in this report). Methods consisted of a grid of 4 x 4 traps (each separated by 100 m), which was established in wild habitats located 1 to 5 km away from the communities.



**Fig. 1** - Geographic distributions of localities where opossum captures were carried out in the Yucatan.

In the sylvatic areas, the trapping period consisted of three consecutive nights.

**Blood sampling.** Blood was collected from the lateral vein of the opossum's tail, preserved in 3.8% sodium citrate (anticoagulant) and maintained at 4 °C for transportation to the laboratory where it was preserved at -20 °C until analysis. All opossums were carefully manipulated and subsequently released in accordance with the protocols of Sikes & Gannon<sup>14</sup> and the guidelines established in NOM-062-ZOO-1999<sup>15</sup>.

**Rickettsia diagnosis by PCR.** DNA extraction was performed using a QIAamp DNA kit (QIAGEN® Valencia, CA) in accordance with the manufacturer's instructions. A single-step polymerase chain reaction (PCR) was used to detect *Rickettsia* DNA.

The single-step PCR amplification was performed using genus-specific primers for the rickettsial 17 kDa protein gene (5'-GCTCTTGCAACTTCTATGTT-3' and 5'-CATTGTTTCGTCAGG TTGGCG-3') generating an amplification product of 480 bp and the citrate synthase gene (*gltA*) (5'-GGGGGCCTGCTCACGGCGG-3' and 5'-ATTGCAAAAAGTACAGTGAACA-3') generating an amplification product of 384 bp as indicated in the guidelines for molecular detection (PCR and sequencing) of tick-borne rickettsiosis from the *Red Iberoamericana para la Investigación y Control de Enfermedades Rickettsiales* (RIICER)<sup>16</sup>. In all PCR assays, we used sterile water in one reaction as a negative control and a *Rickettsia conorii* DNA sample (*Rickettsia* species with no presence in America) as a positive control. All PCR reactions were carried in a Gene Amp PCR System 2400 Thermal Perkin Elmer® thermal cycler. Negative samples to the 17 kDa gene single-step PCR were tested with a 17 kDa gene nested PCR using the forward primer 5'-CATTACTTGGTTCTCAATTCGG-3' and reverse primer 5'-GTTTTATTAGTGT TTTACGTAACC-3' (232 bp), using the same amplification parameters of the single-step 17 kDa gene PCR.

The *gltA* and the 17 kDa gene PCR products were cloned into the TOPO TA pCR 2.1-TOPO vector (Invitrogen®, Carlsbad, CA, USA). Two clones from the same cloning reaction were sequenced three times each using a Perkin Elmer® ABI Prism 377 automated sequencer. Primer sequences were removed before comparison to sequences from the GenBank database using the BLAST 2.0 software available at the National Center for Biotechnology Information (NCBI) site.

## RESULTS

A total of 87 opossums were captured in the different localities. From those, 85 *D. virginiana* blood samples were taken from rural and suburban peridomestic and tested: 10 from *Molas*, 7 from *Dzidzilche*, 32 from *Buctzotz*, 8 from *Dzitya*, 2 from *Kikil*, and 26 from *Cholul*; only two opossums were captured in sylvatic quadrants, one from *Kikil* (identified as *D. marsupialis*) and one *D. virginiana* from *Dzidzilche*.

**Table 1**

Locality, species identity, and ecotope of *Didelphis* opossums captured/infected (+) and percentage of individuals (%) infected by *Rickettsia felis* diagnosed by PCR of blood samples tested with the *gltA* and 17 kDa genes

Locality	Geographic Coordinates	Opossum species	Ecotope	Captured/+ (%)
Kikil	21°11'32.47"N, 88°10'6.17"W	<i>D. marsupialis</i>	Sylvatic	1/1 (100)
		<i>D. virginiana</i>	Peridomestic	2/2 (100)
Dzitya	21°3'1.96"N, 89°40'39.30"W	<i>D. virginiana</i>	Peridomestic	8/0
Cholul	21°2'24.98"N, 89°33'19.31"W	<i>D. virginiana</i>	Peridomestic	26/11 (42.3)
Molas	20°49'0.15"N, 89°37'52.78"W	<i>D. virginiana</i>	Peridomestic	10/3 (30)
Buctzotz	21°12'8.17"N, 88°47'36.23"W	<i>D. virginiana</i>	Peridomestic	32/9 (28.1)
Dzidzilche	21°9'8.75"N, 89°41'24.39"W	<i>D. virginiana</i>	Peridomestic	7/2 (28.5)
		<i>D. virginiana</i>	Sylvatic	1/0
Total				87/28 (32.1)

A blood sample from an opossum was considered infected by *Rickettsia* when both PCR reactions (17 kDa and *gltA*) yielded positive results. The PCR procedure showed *Rickettsia* DNA identification in at least one opossum from each locality except for those from *Dzitya*. In all the positive samples, the *Rickettsia* DNA sequences obtained (GeneBank accession number: KR709306 and KR709307, respectively) showed 99% similarity with the *gltA* and the 17 kDa sequence of the *R. felis* strain Marseille-URRWFxCa2 (Table 1).

## DISCUSSION

The opossums *D. virginiana* have been implicated in the zoonotic transmission of *R. typhi* and *R. felis* in the United States<sup>4,17</sup>, and in Yucatan previous studies have reported their role as natural hosts in the peridomestic transmission cycle of other zoonotic pathogens<sup>10,18,19,20</sup>. As far as we know, there has been no previous report of the presence of Rickettsial infection in *D. marsupialis* from Mexico. Yucatan State represents a sympatric region in the geographic distribution of *D. marsupialis* and *D. virginiana* in which both species clearly show different ecological requirements, with *D. virginiana* being more closely associated with human peridomesticity than *D. marsupialis*, both in rural and suburban households<sup>10</sup>.

Currently, there is no clear consensus about the role of mammals in *R. felis* transmission dynamics, since infection by *Rickettsia* sp. in these natural hosts is characterized by a very short rickettsemia<sup>1,21</sup>. As a consequence, the probability of capturing hosts with rickettsial infection is very low in nature, which makes it difficult to understand their role in the transmission cycle. Some studies have suggested that opossums may act as an important link in the horizontal transmission as a source of *R. felis* to uninfected vectors or as a source of vertically infected vectors that can transmit the pathogen to other mammalian hosts and humans<sup>22</sup>, in case domestic or companion animals and synanthropic opossums could maintain the infection dynamics of *R. felis* in Yucatan. Both are frequently infested by *C. felis*, the main vector and true reservoir of *R. felis*<sup>1,3,12,13</sup>, due to the frequent presence of peridomestic opossums, mainly *D. virginiana*, in the backyards of human households.

In the case of *D. marsupialis*, the transmission scenario in Northern Yucatan would seem to be more restricted to a sylvatic habitat. However, its role as a source of *Rickettsia* for fleas in human households in the Southern area of Yucatan State could be enhanced because in that region their populations are more abundant and predominant than those of *D. virginiana*<sup>11</sup>.

The main implication of opossums infected by *R. felis* lies in the very close association of these marsupials with human dwellings<sup>11</sup>, allowing them to potentially contribute to the transmission cycle of this vector-borne zoonotic pathogen, as suggested in a previous study<sup>13</sup>.

Despite the fact that the results of this study cannot be used to characterize the population of opossums in the entire Yucatan peninsula, the detection of two species of opossum infected by *R. felis* demonstrates the need to conduct epidemiological studies focused on this marsupial as a sentinel of the presence of *Rickettsia* in the peridomesticity of human dwellings.

Additionally, the first finding of infected *D. marsupialis* is very

interesting, because this species is more restricted to sylvatic areas than peridomesticity in Yucatan and could represent an important link between these ecotopes. These findings reveal, for the first time in Mexico, the potential role of opossums in the ecology of *Rickettsia* transmission and their utility as sentinels of this pathogen in human dwellings.

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