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Rickettsia typhi IN RODENTS AND *R. felis* IN FLEAS IN YUCATÁN AS A POSSIBLE CAUSAL AGENT OF UNDEFINED FEBRILE CASES

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SUMMARY

Rickettsia typhi is the causal agent of murine typhus; a worldwide zoonotic and vector-borne infectious disease, commonly associated with the presence of domestic and wild rodents. Human cases of murine typhus in the state of Yucatán are frequent. However, there is no evidence of the presence of *Rickettsia typhi* in mammals or vectors in Yucatán. The presence of *Rickettsia* in rodents and their ectoparasites was evaluated in a small municipality of Yucatán using the conventional polymerase chain reaction technique and sequencing. The study only identified the presence of *Rickettsia typhi* in blood samples obtained from *Rattus rattus* and it reported, for the first time, the presence of *R. felis* in the flea *Polygenis odiosus* collected from *Ototylomys phyllotis* rodent. Additionally, *Rickettsia felis* was detected in the ectoparasite *Ctenocephalides felis* fleas parasitizing the wild rodent *Peromyscus yucatanicus*. This study's results contributed to a better knowledge of *Rickettsia* epidemiology in Yucatán.

KEYWORDS: *Rickettsia typhi;* Murine typhus; Rodents.

INTRODUCTION

Rickettsia typhi is the causal agent of murine typhus; a worldwide zoonotic infectious disease. The clinical manifestations in humans are commonly nonspecific and include fever, headache, chills and rashes[7].

The classical biological cycle of *Rickettsia typhi* involves rodent species like *Rattus rattus* and *Rattus norvegicus* as hosts and the oriental rat flea *Xenopsylla cheopsis* as a vector. However, *Rickettsia typhi* has been reported by molecular methods in other species like *Apodemus agrarius*, opossums and fleas (*Ctenocephalides felis, Leptopsylla segnis, Ctenophthalmus congeneroides* and *Rhadinopsylla insolita*)[3,5,12]. Rodent diversity in Yucatán, Mexico includes species belonging to the Muridae family: *Mus musculus, Peromyscus yucatanicus, Ototylomys phyllotis*, *Reithrodontomys gracilis*, *Sigmodon hispidus*, *Rattus rattus* and *Oligoryzomys fulvescens*, and the Heteromyidae family: *Heteromys gaumeri*. *Peromyscus yucatanicus* and *Heteromys gaumeri* are considered endemic rodent species in the Yucatán Peninsula[6,11].

Human cases of murine typhus in the state of Yucatán have been reported since 2009, with single infections and family clusters in places that have been associated with rodent presence[8,21]. The people in the municipalities of Yucatán mostly work in agriculture and livestock with close proximity to vegetation, domestic, peridomestic and wild animals around their houses, which are conditions that could favor a vector-borne disease transmission, such as rickettsiosis, ehrlichiosis or leptospirosis.

In the absence of any evidence for the presence of *Rickettsia typhi* in any host or vector in Yucatán, aside from the increment of human cases of murine typhus in the past five years, this study evaluates the presence of *Rickettsia typhi* in rodents and their ectoparasites in a small municipality of Yucatán, where nonspecific febrile illnesses are often reported, and the presence of rodents is associated with most of the cases.

MATERIALS AND METHODS

Study area: This study was conducted in the municipality of Oxkutzcab, in the state of Yucatán, Mexico (20° 18' 10" N, 89° 25' 6" W). There are no previous reports of rickettsiosis infection or detection of *Rickettsia* in hosts and/or vectors in this municipality.

Rodent and ectoparasite collection: From February to July 2012, 16 patients living in Oxkutzcab, Yucatán, were visited. These patients were previously diagnosed in 2011 with nonspecific febrile illnesses and with a negative dengue test, but the presence of rodents inside and/or outside their house was noted in their clinical history. The houses of each patient were visited and additional visits were made to houses in the surrounding areas. In this study, 3 x 3.5 x 9" Large Folding Aluminum traps (H.B. Sherman Traps, Inc.) were used to collect rodents. Traps were placed inside and outside the house in kitchens, basements, bedrooms and terraces, as well as in places where people reported the presence of rodents. Traps were placed for between three and five consecutive

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days. Trapped rodents were inspected to collect ectoparasites and blood samples. Rodents that survived the blood sample collection after inspection were released on the outskirts of the town. Ectoparasites collected were stored in 1.5 mL tubes and maintained at -70 °C until needed. Collected rodents and ectoparasites were identified by one of the authors using identification keys[1,10,18].

Samples were analyzed individually using whole blood or the entire ectoparasite. DNA was purified using DNA blood and tissue kit (QIAGEN, Valencia, CA). PCR amplicons were purified using Qiagen Gel Extraction Kit (QIAGEN, Valencia, CA); according to the manufacturer's instructions with a final elution volume of 100 µL.

PCR and sequencing: Conventional Polymerase Chain Reaction (PCR) technique was selected to amplify DNA fragments from two different rickettsial genes: 17 kDa lipoprotein and rickettsial outer membrane protein B (*ompB*). A 434 bp fragment from the rickettsial gene 17 kDa was obtained using primers Fw1: 5´-GCTCTTGCAACTTCTATGTT-3´ and Rv2: 5´-CATTGTTCGTCAGGTTGGCG-3´) [22] and a 990 - 999 bp fragment of the rickettsial gene *ompB* using primers ompB330(1) fw (5'-ATGGCTTCAAAAACCAAATTTTCTAA-3') and ompB330(1) Rv (5'- AGCTCTACCTGCTCCATTATCTGTACC-3')[15]. PCR reaction was performed using Platinum® *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and using a Multigene Thermalcycler (Labnet International, Inc). Ten microliters of each PCR product underwent electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and were examined in a UV transilluminator.

PCR products were sequenced by the PRISM Big Dye Terminator Cycle 3.1 Sequencing (Applied Biosystems, Foster City, CA) method. The sequenced products were purified with an ABI XTerminator kit and separated on a 3130*xl* genetic analyzer from Applied Biosystems. Sequence similarities were determined using the BLAST search engine from the National Center for Biotechnology Information (NCBI) website.

RESULTS

Rodent and ectoparasite collection: Forty-two rodents (n = 42) from three different species belonging to the Muridae family were collected: *Rattus rattus* (n = 32), *Peromyscus yucatanicus* (considered endemic in the state of Yucatán[20]) (n = 6), and *Ototylomys phyllotis* (n = 4).

Six *Polygenis odiosus* (*P. odiosus*) were obtained from fleas of the Rhopalopsyllidae family in two *O. phyllotis* of the four collected, and four *Ctenocephalides felis* fleas were obtained from one *P. yucatanicus* out of the six rodents collected (Table 1). No ectoparasites were obtained from any *R. rattus* collected.

PCR and sequencing: PCR amplification was achieved in 11 samples: eight from *R. rattus* blood samples, one from *a C. felis* flea collected from *P. yucatanicus*, and two from a *P. odiosus* flea collected from *Ototylomys phyllotis*. All amplicons were sequenced.

The 17 kDa gene fragment sequence, obtained from whole blood from *R. rattus* (GenBank accession no. KF241855.1), was 100% identical to *Rickettsia typhi* isolated from human blood in Yucatán (GenBank accession no. JX198507.1), *Rickettsia typhi* str. B9991CWPP (GenBank accession no. CP003398.1), *Rickettsia typhi* identified from *Ctenophthalmus congeneroides* in rodents in Korea (GenBank accession no. EU532435.1), and *Rickettsia typhi* str. in Wilmington (GenBank accession no. AE017197.1).

Table 1
Positive rodents and fleas tested for *Rickettsia* spp.

Rodent species	Rodents tested	Fleas tested	
	Rodent Positive (total tested)	*C. felis* Positive (total tested)	*P. odiousus* Positive (total tested)
R. rattus	8 (32)	--	--
O. phyllotis	0 (4)	--	2 (6)
P. yucatanicus	0 (6)	1 (4)	--

Sequences obtained from an *ompB* gene fragment obtained from blood sample of *R. rattus* (GenBank accession no. KF241858.1) were 99% identical to *Rickettsia typhi* str. B9991CWPP (GenBank accession no. CP003398.1), *Rickettsia typhi* str. TH1527 (GenBank accession no. CP003397.1), *Rickettsia typhi* str. in Wilmington (AE017197.1).

The 17 kDa gene fragment sequences obtained from *C. felis* (GenBank accession no. KF241853.1) and *P. odiosus* (GenBank accession no. KF241854.1), collected from *P. yucatanicus* and *O. phyllotis* rodents respectively, were 100% identical to *Rickettsia felis* identified in *Carios capensis* ticks in the United States (GenBank accession no. DQ102709), *Rickettsia felis* URRWXCal2 (GenBank accession no. CP000053), *Rickettsia* sp. Hf187 identified in ticks in Japan (GenBank accession no. AB114813); 99% identity to *Rickettsia felis* identified in *Pulex echidnophagoides* from opossums in Yucatán, Mexico (GenBank accession no. GU447234.1) and *Rickettsia rickettsii* identified in *Rhipicephalus sanguineus* ticks in Yucatán (GenBank accession no. KC713872.1).

Sequences obtained from an *ompB* gene fragment from *C. felis* (GenBank accession no. KF241856.1) and *P. odiosus* (GenBank accession no. KF241857.1) collected from *P. yucatanicus* and *O. phyllotis* rodents respectively, were 99% identical to *Rickettsia felis* URRWXCal2 (GenBank accession no. CP000053.1), *Rickettsia felis* identified in the insect pest *Liposcelis bostrychophila* (GenBank accession no. GQ385243.1), and 94% identical to Candidatus *Rickettsia hoogstraalii* (GenBank accession no. EF629536.1).

DISCUSSION

Small mammals are key components in the process of succession and regeneration of tropical forests and rainforests because they play an important role in predation and the dispersion of seeds[17]. The presence of rodent diversity in Yucatán, Mexico in domestic areas is, in most cases, due to the constant expansion of residential areas which encroaches upon wildlife ecosystems, thereby favoring the presence of vector-borne diseases, such as leishmaniasis[4,19], hantavirus[16] or rickettsiosis[12].

In this study, the presence of *Rickettsia felis* was identified in *Polygenis odiosus* fleas as a potential new vector of *R. felis*. This flea species is widely distributed and considered endemic in the Yucatán peninsula, with high preference to *O. phyllotis* which could possibly be a host of *R. felis*[9]. The presence of *R. felis* in *C. felis* collected from *P. yucatanicus* suggests the possible role of this rodent as a new reservoir of *R. felis*. This hypothesis is supported by documented evidence that demonstrates that other species of *Peromyscus* are involved in the ecology of Rickettsiae[13,14]. There is a need for further studies to focus on the identification of the presence of *Rickettsia* spp. in *P. yucatanicus*, in order to confirm it. The high presence of *R. typhi* in blood samples from *R. rattus* confirms its presence in the state of Yucatán and supports the importance of rodent control, as rodents are a disease host.

One of the considerations of these results is the need for comprehensive work with government authorities and the community to develop rodent control strategies in small communities, where living conditions favor the presence of rodent species, due mainly to poverty, agriculture and food habits (corn is the main food source), in order to prevent human cases of this, in the eyes of the authors, neglected febrile disease.

RESUMEN

Rickettsia typhi y *R. felis* en roedores y sus pulgas en Yucatán como posible agente causal de casos febriles indefinidos

Rickettsia typhi es el agente causal del tifo murino; una enfermedad zoonótica transmitida por vector mundialmente distribuida, comúnmente asociada con la presencia de roedores domésticos y silvestres. Los casos humanos de tifo murino en el Estado de Yucatán son frecuentes. Sin embargo, no existe evidencia de la presencia de *Rickettsia typhi* en mamíferos o vectores en Yucatán. En la búsqueda de vectores y reservorios de *Rickettsia typhi*, evaluamos la presencia de bacterias del género *Rickettsia* en roedores y sus ectoparásitos de un pequeño municipio del estado de Yucatán por medio de técnicas de PCR convencional y secuenciación de ADN. Se identificó la presencia de *Rickettsia typhi* en muestras de sangre obtenidas de *Rattus rattus* y reportamos por primera vez la presencia de *Rickettsia felis* en la pulga *Polygenis odiosus* colectado de *Ototylomys phyllotis*. Complementariamente, *Rickettsia felis* fue detectado en la pulga *Ctenocephalides felis* parasitando al roedor *Peromyscus yucatanicus.* No se identificó especie de *Rickettsia* en las muestras de sangre de *O. phyllotis* y *P. yucatanicus* analizados. Nuestros resultados contribuyen también en el conocimiento de ciclo de vida biológico del género *Rickettsia.*

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