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BRIEF COMMUNICATION

NEW WILDLIFE HOSTS OF *Leptospira interrogans* IN CAMPECHE, MEXICO

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SUMMARY

Leptospira interrogans has been identified to cause leptospirosis, a widespread zoonotic disease that has been identified in domestic and wild animals. This work analyzed kidneys from two species of wild rodents from the state of Campeche, Mexico. Analyses were made by PCR using specific primers for detection of *Leptospira interrogans* DNA. The rodent species that tested positive were *Heteromys gaumeri* and *Ototylomys phyllotis*, both of which are new hosts for the bacteria in Southeastern Mexico. These records provide new insights into the disease's transmission that should be studied carefully in order to identify other potential host species, including humans, which are at risk of becoming infected if they are in contact with infected wildlife.

KEYWORDS: Wildlife hosts; *Leptospira interrogans*; Campeche; Mexico.

Several species of the genus *Leptospira* cause leptospirosis, a zoonosis of urban distribution[3,6]. Wild and domestic mammals (160 species) have been identified as hosts for these bacteria worldwide[2,6]. *Leptospira interrogans* has mainly been identified in domestic mammals because they have direct contact with humans[4,5,6]. However, in Neotropical areas, such as Panama[21], the Peruvian Amazon[6,8] and the city of São Paulo[16], some wild mammals (bats, carnivores, marsupials and rodents) have been identified as hosts of *L. interrogans*.

In Mexico, records of wildlife hosts for *L. interrogans* are scarce and widely scattered across different states (e.g. *Didelphis virginianus* in Yucatán[25] [Southeastern Mexico], *Odocoileus virginianus* in Coahuila[9] [Northern Mexico] and *Zalophus californianus* in the Gulf of California[1,18]). A study carried out in Cozumel, Quintana Roo, identified a 21.5% seroprevalence of *Oryzomys couesi cozumelae*[24]. In Tamaulipas, Northeastern Mexico, five species of wild rodents (*Baiomys musculus*, *Liomys irroratus*, *Oryzomys alfaroi*, *Peromyscus leucopus* and *Sigmodon hispidus*) tested positive for different serovars of *L. interrogans* by Microscopic Agglutination Technique (MAT)[22]. However, there are no records of wildlife hosts reported in Campeche, and in the Yucatan Peninsula only one species of rodent has been previously reported[24]. For this reason, the aim of this paper is to report two new species of wild rodents that are hosts of *L. interrogans* in Calakmul, Campeche, Mexico.

Ten rodents were collected (collection permit FAUT-0170) on August 17th, 2013 from the Yaax'che camp, Calakmul, Campeche, Mexico (located 43 km SSE from the archeological zone of Calakmul, 18° 29' 14'' N, 89° 53' 57'' W). These specimens were killed in compliance with the guidelines of the American Society of Mammalogy for the Use of Wildlife Mammals in Research[17]. All specimens were identified and deposited at the Museo de Zoología "Alfonso L. Herrera" in the Facultad de Ciencias (MZFC) of the Universidad Nacional Autónoma de México.

For the identification of *Leptospira* DNA in these rodents, one kidney was aseptically collected and deposited in 70% ethanol. A portion of 25 mg of kidney tissue was processed for DNA extraction using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's specifications (using the Purification of Total DNA from Animal Tissues Protocol). After extractions were done, a multiplex PCR was performed using primer sets G1/G2 (specific for the detection of pathogenic leptospires) and B64I/B64II (specific for *Leptospira kirschneri*) with expected products of 285 bp and 563 bp, respectively[19]. Additionally, the positive samples were analyzed using specific primers for the identification of pathogenic leptospira species[23]. The reaction mixture consisted of 12.5 µL of GoTaq® Green Master Mix, 2X of Promega Corporation (Madison, WI, USA), using a pair of primers, Intergroup A fwd and Intergroup A rev (100 ng each), 6.5 µL nuclease-free water and 200 ng DNA in a final volume of 25 µL.

In order to minimize cross-contamination and to avoid false positive results, a negative control (i.e. reaction mix without DNA) and a positive

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control (i.e. reaction mix and *L. interrogans* serovar Pomona DNA) were both included. Each PCR reaction was performed in triplicate.

The PCR products were analyzed by electrophoresis on 1.5% agarose gels, using a 100 bp molecular weight marker (Nucleic Acid Markers, LMW DNA Ladder of BioLabs) in 1X TAE buffer. Gels were stained with SYTO® 60 nucleic acid stain (Invitrogen by Life Technologies CA, USA) and visualized using an ODYSSEY CLx Imaging System (LICOR Biosciences).

Two rodents collected at Yaax'che camp, Calakmul, Campeche, Mexico that tested positive using the G1/G2 primers were identified as *Heteromys gaumeri* (temporary catalog RAVGA014) and *Ototylomys phyllotis* (temporary catalog RAVGA013).

These tests were confirmed positive with primers of Intergroup A designed by REITSTETTER[23], which specifically amplify a segment of 396 bp of *L. interrogans* DNA. *Leptospira kischneri* was not detected in any of the samples analyzed, and the DNA of *L. interrogans* was not found in any of the negative controls (Fig. 1).

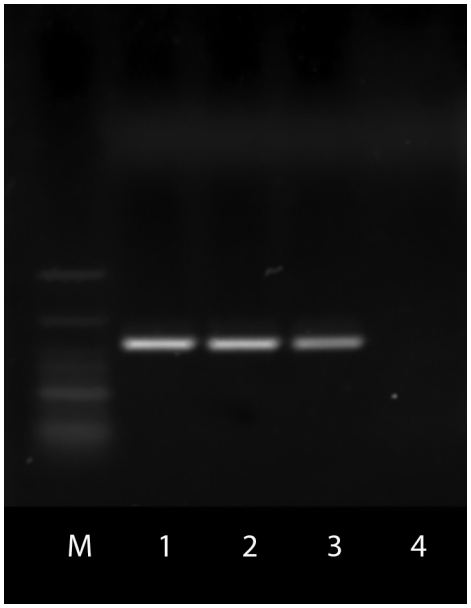

Fig. 1 - Agarose-gel electrophoresis of the single PCR products amplified with primers of Intergroup A designed by REITSTETTER[23]. M: 100 bp DNA marker ladder; Lane 1: *Heteromys gaumeri* (RAVGA014); Lane 2: *Ototylomys phyllotis* (RAVGA013); PCR Controls; Lane 3: Positive control (396 bp, *L. interrogans* serovar Pomona DNA); Lane 4: Negative control (without DNA).

Climate affects the timing and intensity of outbreaks of infectious diseases[14,15]. It has been stated by several authors[3,6,20,26] that adverse climatic events, such as hurricanes and floods, are related to the timing and intensity of *Leptospira* outbreaks. In the case of the present study, the presence of two tropical storms that occurred before and after the specimen's collection[12,13], allowed for speculation regarding the study's findings of *L. interrogans*.

This is the first work that identifies *Heteromys gaumeri* and *Ototylomys phyllotis* as new hosts for *L. interrogans*, by using the set of primers designed by REITSTETTER[23] to identify pathological samples. Moreover, the study area in which the specimens were collected corresponds to a new locality in Mexico, where the presence of the bacteria had not been previously reported. The presence of *L. interrogans* in wild rodents from the same locality should be studied carefully in order to identify the possibility of other species and particularly humans of this area being infected. The author's suggestion is based on previous studies made on domestic animals and humans. In the case of domestic animals (bovines, pigs and dogs) a study revealed a general positivity of 30.5%[10], while a more recent study showed a general positivity of 21.3% registered in dogs of Campeche city[7]. Particularly in the case of human leptospirosis, incidence varied from 0.7-2.2/100,000 inhabitants, with a general seroprevalence of 14.2%[11,25].

Since extreme weather events have been reported to promote the presence of *Leptospira* outbreaks[6], it is essential to further analyze potential reservoirs of several pathogenic species of *Leptospira* in order to identify the dynamics of the transmission between wild mammals and peri-urban human populations, in order to reduce the risks of a potential leptospirosis outbreak in vulnerable groups such as biologists, national and foreign campers and tourists that visit the study area.

RESUMEN

Nuevos huéspedes silvestres de *Leptospira interrogans* en Campeche, México

Lepstospira interrogans ha sido identificada como uno de los agentes causantes de la leptospirosis, una zoonosis ampliamente distribuida, la cual se ha identificado en numerosos animales domésticos y silvestres. En este trabajo se analizaron los riñones de dos especies de roedores silvestres procedentes del estado de Campeche, México mediante la técnica de PCR con iniciadores específicos para la detección de DNA de *Leptospira interrogans*. Las especies de roedores que resultaron positivas corresponden a *Heteromys gaumeri* y *Ototylomys phyllotis*, ambas representan nuevos registros de huéspedes para la bacteria en el sureste de México. Estos nuevos huéspedes deberán ser estudiados cuidadosamente con el fin de determinar la posibilidad de que otras especies de animales, y en particular los humanos, entren en contacto con el patógeno presente en animales silvestres.

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