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First molecular evidence of *Toxoplasma gondii* in opossums (*Didelphis virginiana*) from Yucatan, Mexico

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Abstract

Toxoplasma gondii is an obligate intracellular parasite recognized as a causal agent of toxoplasmosis; zoonotic disease endemic in many countries worldwide, including Mexico. Different species of animals participate in the wild cycle infection, including opossums of the species *Didelphis virginiana*. Thirteen *D. virginiana* were captured in Yucatan, Mexico. Detection of *T. gondii* was achieved by Polymerase Chain Reaction, which determined an infection of 76.9% (10/13) in brains. Positive amplicons were sequenced for analysis, this produced results similar to *T. gondii* with identity and coverage values of 98% and 96-100%, respectively. This study presents the first molecular evidence of the circulation of *T. gondii* in *D. virginiana* from Mexico.

Keywords: *Didelphis virginiana*, Mexico, *Toxoplasma gondii*, Yucatan.

Introduction

Toxoplasmosis is a zoonotic parasitic disease with a worldwide distribution that is produced by *Toxoplasma gondii*. This protozoan has the capacity to infect birds and mammals, including human beings (Hernández-Cortazar *et al*., 2015). Their definitive hosts are individuals of the family Felidae such as domestic (*Felis catus*) and wild cats, that expel oocysts in their feces with sporozoites, infective stage of the parasite (Dubey *et al*., 2009).

Approximately, 30% of the human population worldwide is chronically infected with *T. gondii*. This stage of the disease is characterized by the persistence of the parasite in the tissues of the affected organism, especially brain and heart. The most common sources of human infection are ingestion of tissue cysts in undercooked meat or of food or water contaminated with oocysts shed by felids and transplacental transmission (Pomares *et al*., 2011).

Toxoplasmosis presents cycles of domestic and wild transmission. In the former, human beings, domestic cats and a wide variety of production animals are involved, for which reason it is well understood. This is not the case with the wild transmission, in which the synanthropic opossums participate along with numerous species of wild rodents (Dubey *et al*., 2009). In Mexico, human and animal toxoplasmosis has been reported in different regions; however, its rates of prevalence and incidence are higher in tropical zones where, given the prevailing environmental conditions such as high temperature and humidity, *T. gondii* can remain infective for long periods of time (Hernández-Cortazar *et al*., 2015).

Opossums of the species *Didelphis virginiana* are widely distributed on the Yucatan Peninsula. They are abundant in domestic and peridomestic environments and act as hosts or reservoirs of infectious agents capable of causing diseases in human beings (Parada-López *et al*., 2013). Different studies in the region have shown that *D. virginiana* may act as a carrier of some zoonotic pathogens such as *Salmonella entérica*, *Leptospira interrogans* (Ruiz-Piña *et al*., 2002), *Trypanosoma cruzi* (Parada-López *et al*., 2013) and *Rickettsia felis* (Panti-May *et al*., 2015). However, the role of *D. virginiana* in the transmission cycle of *T. gondii* has not been established, despite the fact that the opossums can act as a source of infection by consumption of definitive hosts and even by human beings who inhabit tropical forest and rural areas (Santos-Fita *et al*., 2012). For this reason, the objective of this study was to determine the circulation of *T. gondii* in *D. virginiana* captured in communities in Yucatan, Mexico.

Materials and Methods

As part of a study conducted to identify pathogenic agents in synanthropic rodents; juvenile specimens of *D. virginiana* were captured in "Sherman" type traps

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(7.5 x 9 x 23, HB 83 Sherman Traps Inc., USA) that had been set in the peridomestic environment of dwellings in two rural and one urban communities of Yucatan, Mexico (Fig. 1). The animals were captured with the permission of the Ministry of Environment and Natural Resources (*SEMARNAT*, by its Spanish acronym) of Mexico (Registry No. SGPA/DGVS/02528/13) and following the statutes of the American Society of Mammalogists (ASM).

Captured individuals were transported to the *Campus de Ciencias Biológicas y Agropecuarias-Universidad Autónoma de Yucaán* (*CCBA-UADY*), where morphometric measurements were recorded. The individuals were then euthanized with pentobarbital sodium (intraperitoneal injection; 390 mg/ml; Stoskopf *et al*., 1999). The cranial cavities were opened using scissors and forceps sterilized. The brain was divided into two hemispheres, one of which was fragmented in two parts. One of these parts was macerated with a previously sterilized mortar and pestle, and preserved in 500 µl of RNAlater® (Thermo Fisher Scientific Inc., USA). A subsample (100 µl) was taken and processed with the solution TRIzol® Reagent (Thermo Fisher Scientific Inc., USA) for DNA extraction.

Molecular detection was conducted using end-point PCR with the primers and methodology proposed by Boughattas *et al*. (2014). These primers amplify a fragment of 114 bp that repeats in tandem within the chromosome IX of *T. gondii*. All reactions included positive (total DNA of rodent brain [BALB/c] experimentally infected with *T. gondii*) and negative (sterile water) controls. Electrophoresis of the PCR products was conducted in 8% polyacrylamide gels dyed with silver nitrate (1.1M).

In order to purify the positive amplicons, another electrophoresis was conducted in 1% agarose gels dyed with ethidium bromide. The band at 114 bp was removed and purified with the Zymoclean™ Gel DNA Recovery Kit (Zymo Research Company™, USA), following the manufacturer´s instructions. Only six of the positive and purified products of PCR were sent for sequencing to the *Instituto de Biotecnología-Universidad Nacional Autónoma de México* (*IBT-UNAM*). The sequences obtained were compared using the Basic Local Alignment Search Tool (BLAST), of the National Institute of Health (NIH, USA; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

A total of 13 specimens of *D. virginiana* were captured. Of these, 76.9% (10/13) were captured in the urban community and the other 23.1% (3/13) in the rural communities. All the captured individuals were juveniles and presented a range of weights from 69.5 to 130.9g. The PCR test produced a percentage of positivity of 76.9% (10/13). Fig. 2 shows a representative gel with positive fragments of 114bp.

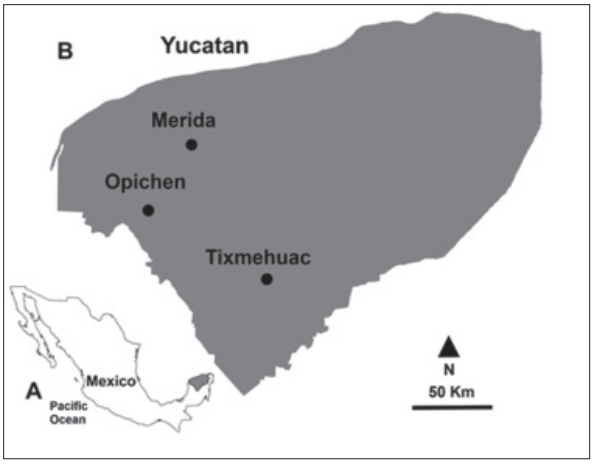

Fig. 1. Map of Mexico (A) showing the location of Yucatan (B) with the communities where the individuals of *Didelphis virginiana* were captured (black points).

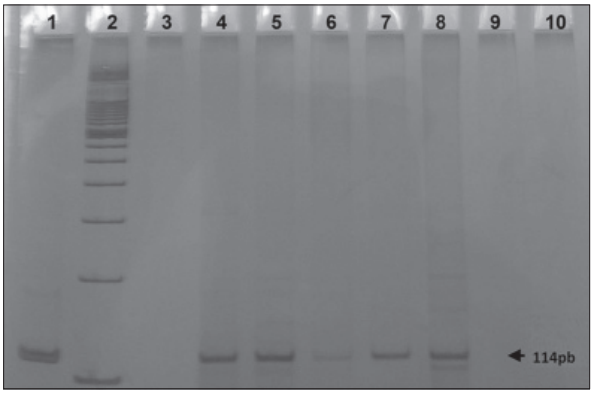

Fig. 2. Polyacrylamide gel 1%, showing the positive extractions: (1): Positive control; (2): Molecular weight ladder of 100 bp; (3): Empty lane; (4-8): Lanes with positive samples; (9-10): Empty lanes.

Similarly, Table 1 specifies the number of animals captured per community, the community type and its percentage of positivity according to the PCR test.

The purified and sequenced of positives products were known as: SJTDV1, SJTDV2, SJTDV3, SJTDV4, SJTDV5 and TIXDV1. Unfortunately, due to the size of our amplified fragment was not possible to get an access number in the GenBank®. Table 2 shows the sequence products and the percentages values of identity and coverage for each one. In all cases, the sequences were similar to *T. Gondii* of the VEG strain (access number LN714499.1).

Discussion

Information regarding the participation of *D. virginiana* in the infection cycle of *T. gondii* is scarce and limited (Mitchell *et al*., 2006). Two serological studies have been conducted to date in Mexico; one in Durango (Dubey *et al*., 2009) and the other in Mexico City (Suzán and Ceballos, 2005). The first reported a seroprevalence

Table 1. Number of individuals captured per community and their percentage of positivity in the PCR test.

Number of individuals captured	*Community*	*Type of community*	*Location*	*Positives/total (%)*
10	Mérida	Urban	20°58´04´´N, 89°37´18´´W	9/13 (69, 2)
2	Tixméhuac	Rural	20°13´48´´N, 89°06´15´´N	1/13 (7, 7)
1	Opichén	Rural	20°32´59´´N, 89°51´25´´W	0/13 (0)
Total: 13				10/13 (76, 9)

Table 2. Name and sequence of the positive products PCR and the values of percentages of identity and coverage obtained by BLAST analysis.

Name	*Sequence*	*Identity %*	*Coverage %*
SJTDV1	TAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCA	98	100
SJTDV2	TAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCA	98	100
SJTDV3	CATAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCA	98	100
SJTDV4	CATAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCA	98	98
SJTDV5	CATAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCA	98	98
TIXDV1	CATAGAGAGTACTAGAACGTCGCCGCTACTGC CCAGTTGTCATGCCATCGACGTAGACCCAA	98	96

of 16.6%, while the second reported a percentage of positive reactions of 10%. Similarly, serological studies conducted on same animal species in Iowa (Smith *et al*., 1992), Connecticut (Mitchell *et al*., 2006), Louisiana (Houk *et al*., 2010) and Georgia (Dubey *et al*., 2011) in the USA, have produced percentages of seropositivity of 2.9%, 29%, 27% and 33.3%, respectively.

The percentage of infection in our study (76.9%; 10/13), obtained via a molecular test, was very high. This percentage may be due to environmental factors presents in the region, especially temperature and relative humidity, which allow the survival of the parasite in the environment (Rendón-Franco *et al*., 2014). Likewise, the population characteristics of the opossums used in our study directly affects the result (Hernández-Cortazar *et al*., 2015). Another element that may influence the high percentage of infection found, is that most of the positive individuals were captured at the same site (Table 1), so sampling from different sites could produce a different percentage of infection.

The manner in which *D. virginiana* comes into contact with *T. gondii* is not clear, due to the fact that the diet of omnivorous animals is composed mainly of endemic fruits and insects, which are rarely contaminated with oocysts (Smith and Frenkel, 1995). Nevertheless, Castillo-Morales *et al*. (2012) report that Yucatan presents elevated environmental contamination with oocysts, for which reason different domestic and wild species can be infected. Similarly, Ortega-Pacheco *et al*. (2013) state that infection in animals may be acquired through sources of water, soil and even in the air; which could be related to the presence of *T. gondii* in juvenile specimens of *D. virginiana* and other wild or synanthropic species. However, for a fuller understanding of the participation of *D. virginiana* in the transmission cycle of toxoplasmosis, studies with experimental infections are necessary. These could also provide information about the pathogeny of *T. gondii* in this particular species.

The circulation of *T. gondii* in Yucatan has been widely documented in human beings (Jiménez-Coello *et al*., 2011) and domestic animals such as pigs, dogs and cats, in which high percentages of seroconversion have been found (Castillo-Morales *et al*., 2012; Jiménez-Coello *et al*., 2013; Ortega-Pacheco *et al*., 2013), demonstrating the wide distribution of the parasite. However, the participation of *D. virginiana* in the infectious cycle of toxoplasmosis has not been identified previously, for which reason our findings contribute to the understanding of the circulation of the pathogen in the region. To our knowledge, this is also the first report to use molecular techniques to

show circulation of *T. gondii* in *D. virginiana* captured in Mexico.

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Conflict of interest

The authors declares that there is no conflict of interest.

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