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SHORT COMMUNICATION

First Detection of *Toxoplasma gondii* DNA in a Wild Bat from Colombia

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Abstract

Introduction *Toxoplasma gondii* infections have been reported for many warm-blooded animals around the world including chiropterans. However, in Colombia, the country that holds the highest taxonomic richness of this order of mammals in the Neotropics, up to date there are no reports of *T. gondii* in bats (*Carollia brevicauda*).

Purpose The objective of the present study was to detect *T. gondii* DNA from internal bat organs from Quindío, Colombia.

Results We report the first detection of *T. gondii* DNA from internal bat organs in the department of Quindio, Central Andes of Colombia. Out of three silky short tail bat (*Carollia brevicauda*) specimens collected at the natural reserve "La Montaña del Ocaso", organs were recovered (lungs, liver, heart, kidneys, small and large intestine) and tested for *T. gondii* through PCR for B1 sequence, with 1/3 (33.3%) positive result for the presence of *T. gondii* DNA in bat kidney tissues.

Conclusion Taking into consideration the high diversity of bat species in Colombia, and the complexity of the ecological and functional relationships that these organisms establish in the ecosystems they inhabit, we discuss on the urgent need for more detailed research and surveys for *Toxoplansma* in bats and other mammalian wild species.

Keywords *Toxoplasma* · Bat · PCR · Colombia

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with a worldwide distribution and the capacity to infect a wide variety of warm-blooded animals and humans through the infections of oocysts in the environment, by the consumption of tissue bradyzoites in infected intermediate hosts, or by congenital transmission [1]. *T. gondii* infections have been reported for many warm-blooded animals around the globe, but have been scarcely reported in bats, and several attempts failed to detect *T. gondii* in brown bat (*Eptesicus fuscus*), red bat (*Lasiurus borealis*) and evening bat (*Nycticeius humeralis*) by Sabin-Feldman and MAT-t techniques [2, 3].

Bats are considered an important natural reservoir of many zoonotic viruses, [4] carrying a wide range of pathogens and potentially disseminating them among wild and urban areas [5], including the new coronavirus SARS-CoV-2 (actually generating a pandemic, producing more than 1.773.000 infected people and 111.600 deaths in 185 countries and territories [6]), and presumably bats serve as reservoirs hosts for its progenitor [7]. The first report of *T. gondii* in bat species occurred in 1965 by Galuzo and collaborators through parasite isolation in two insectivorous bats, *Noctalus noctula* and *Vespertilio serotinus*, in Kazakhstan [8]. Later, in South America, the first report dates from 1969 in Brazil, obtained through the isolation of *T. gondii* parasite in four bats by bioassay in mice [9]. Toxoplasmosis has been reported in captive flying-foxes (*Pteropus conspicillatus* and *P. scapulatus*), being the first symptomatic cases of these diseases in a wild species [10]. The first case of

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T. gondii isolation and genotyping by sequencing of SAG1 gene occurred in 2013 [4], raising the interest of researchers of detecting *T. gondii* in bats and trying to describe prevalences, parasite acquisition and infection source, distribution and genetic relationships. Prevalences between 6.1–21.6% (Table 1) have been reported, being the most prevalent genotype related to the clonal type [11–15].

Despite its fundamental importance as a scientific repository of biological information, potentially useful for health surveillance purposes, little work has been conducted on this issue within mammals' scientific collections in Colombia. Further, research activities of the Collection of Mammals of the University of Quindío (CMUQ), Colombia, include bat collections in every natural region of the country. As part of the museology protocols established at the CMUQ, internal organs (lungs, liver, heart, kidneys, small and large intestine) are preserved along with information on voucher specimen's taxonomy and ecology. So, we took advantage from the creation of the Centro de Estudios de Alta Montaña (Center for Highland Studies, CEAM), an initiative in which 27 research groups from different disciplines converge, to start a transdisciplinary research on health surveillance of wild vectors of *T. gondii* at the Central Andes. Therefore, the objective of the present study was to detect *T. gondii* DNA from internal bat organs from Quindio, Colombia.

Methodology

Fieldwork

Bat sampling was conducted at the natural reserve "La Montaña del Ocaso", located at the Laurel locality, south of Quimbaya, Quindío, Colombia (4°34′08″N, 75°51′03″O), at 970 m above sea level administered by the University of Quindío. Sampling was carried under permission of the "Corporación Autónoma Regional del Quindío".

Mist nets were open between 6:00 pm and 2:00 am, and three specimens of silky short tail bats *Carollia brevicauda* (Chiroptera: Phyllostomidae) were captured. Specimens corresponded to adult females, without showing symptoms of any type of infection or that they were famine. Bats were euthanized with an overdose of lidocaine. Bats were preserved as skin and skull specimens and deposited at the "Colección de Mamíferos de la Universidad del Quindío (CMUQ)".

Preparation of Samples and DNA Extraction

Lungs, heart, liver, kidneys, stomach, small and large intestine from each captured and euthanized bat were extracted. The organs were stored in 2 ml Eppendorf tubes with 0,9% saline solution and they were stored at − 20 °C until used. Approximately 50 mg from each organ were cut and placed in a new 2 ml Eppendorf tube with 1 ml of cell lysis solution (Promega). We added 50 µl of proteinase K (20 mg/ml-Invitrogen) and incubated the sample at 65 °C for 2–3 h in shaker, and the Wizard Genomic DNA Purification kit was used according to the manufacturer.

Toxoplasma gondii DNA Detection by Nested PCR

To detect *T. gondii* DNA, we used conventional nested PCR as described previously [16–18], amplifying a 97-bp fragment of the B1 gene (GenBank accession number AF179871) from *T. gondii* described first by Burge and collaborators in 1989, because its sensitivity and specificity [19]. We used 1.5% agarose gel electrophoresis to analyze PCR products, which were defined as positive or negative. For PCR reactions, the positive control was DNA from the *T. gondii* control RH strain, and negative control was distilled water in the presence of primers. The PCR experiments were done in triplicate. PCR products were purified using the ammonium acetate protocol [20], and later sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). DNA sequences were edited and aligned with a B1 reference sequence (AF179871.1) and the RH positive control using

Table 1 DNA *T. gondii* prevalence by nested PCR techniques in other countries.

Country	% (*n*/*N*)	Bats species	References
China	6.1% (38/626)	*Plecotus auritus, Murina leucogaster, Myotis ricketti, Melomys leucogaster, Myotis chinensis, Hipposideros larvatus, Hipposideros armiger, Hipposideros pomona, Cynopterus sphinx, Rhinolophus ferrumequinum, Rousettus leschenaultii*	[12]
United Kingdom	10.4% (8/77)	*Pipistrellus pipistrellus, Pipistrellus pygmaeus*	[11]
Myanmar	29.3% (161/559)	*Miniopterus fuliginosus, Rhinolophus ferrumequinum, Myotis chinensis, Hipposideros armiger, Megaderma lyra*	[14]
Brazil	21.6%(11/51)	*Artibeus lituratus, Myotis nigricans, Uroderma bilobatum, Sturnira lilium, Carollia perspicillata, Lichonycteris degener, Glossophaga soricina, Centronycteris maximiliani*	[13]
Mexico	11.6% (8/69)	*Artibeus jamaicensis, Glossophaga soricina, Chirodemra villosum*	[15]

Chromas 1.51 (https://www.technely-sium.com.au/chromas.html) and BioEdit 7.0.5.2 [21].

Results and Discussion

In the present study, out of three silky short-tailed bat specimens collected in the wild, the kidney tissue from one individual (063 collection number) resulted in B1 sequence PCR positive for *T. gondii*, and it was confirmed by triplicate (Fig. 1), representing 33.3% (1/3) of the samples. We used PCR to detect *T. gondii* because it is sensitive enough to detect low quantities of parasites and are accessible for routine analyses [14]. Although we have a low number of samples, it could represent a high *T. gondii* DNA percentage in bats by PCR in contrast different studies realized until today (Table 1). The positive sample was confirmed to be from *T. gondii* DNA through sequencing and alignment (Fig. 2) and blast with our positive control (B1 sequence from *T. gondii* DNA of RH strain) and a reference sequence reported in GenBank with accession number AF179871 [19]. The few differences presented in alignment should be interpreted with precaution because it is not necessarily a different genotype or strain from *T. gondii*. To obtain the circulation of specific genotypes, one needs multi-locus analysis, using different genes like ROP18 [22], with a good amount and quality of DNA [16].

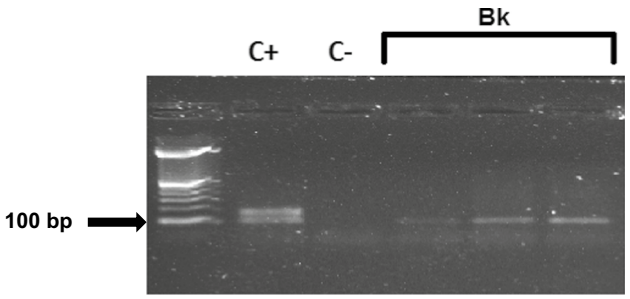

Fig. 1 Electrophoresis in 1.5% agarose gel, showing one positive sample performed in triplicate with a 100 bp band in the expected length. Bk: Bat kidneys; the positive control was *T. gondii* DNA RH strain (C+), while negative control was distilled water in the presence of primers (C−)

The individual that tested positive for *T. gondii* in this study had no symptoms of infection at the moment of its capture. Infected bats by *T. gondii* remain most of the times without symptoms like hindlimb paralysis, respiratory distress, panting and anorexia [4, 10], acting as vectors and transmitting the infection to other animals. Although little is known on the behavioral changes associated with toxoplasmosis for most bat species, individuals on the ground or exposed out of their refugia may represent a potential public health threat [23]. The warm and humid environments, like the one from the natural reserve in which we captured the positive bat individual, are more suitable for the survival of *T. gondii* oocysts [24].

Routes of infection of *T. gondii* in bats remain broadly unknown [25], and potential hypotheses explaining the infection can change according to roosting behavior and diet of said species. *Carollia brevicauda* is abundant in premontane and montane Andean forests, in which it feeds from a wide variety of fruit resources [26]. It is possible that the food resource acted as the source of parasite acquisition, which could had been in contact with contaminated water, and the low dietary selectivity of *C. brevicauda* [27] reinforces this possibility. Another possibility can be given by a direct contact with contaminated water. Bats could drink frequently from ponds and other water sources, which could be potentially contaminated with *T. gondii* oocysts. Some reports in Quindío have confirmed the presence of DNA *T. gondii* in water samples before plants treatment, with a frequency of 76,9% (10/13) [16]. *T. gondii* infection in insectivorous and frugivorous bats also suggests that the caves where bats live are contaminated with *T. gondii* oocysts [28].

The warm and humid environments, like the one from the natural reserve in which we captured the positive bat individual, are more suitable for survival of *T. gondii* oocysts [24]. Across *Carollia brevicauda* geographic distribution

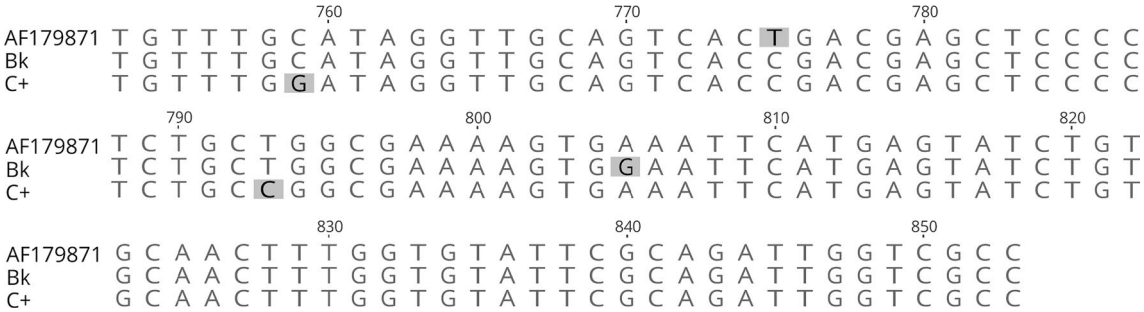

Fig. 2 Alignment of partial B1 sequences from *Toxoplasma gondii*, including a reference sequence (AF179871, 2214 bp), the positive sample (Bk) and a positive control (C+). Both Bk and C+ sequences aligned with the reference sequence from position 753–853

(from Panama to Southern Bolivia and Brasil), the species presents a wide array of plant items in its diet, which significantly overlaps those found in some congeneric species [29], as well as in other phyllostomid bat species such as *Sturnira erythromos* and *S. ludovici* [26]. The overlap in diets and the possibility of food items as a source of infection likely exposed other frugivorous bats to *T. gondii* acquisition.

Bats are one of the second most abundant, widely distributed and diverse group of mammals in the Neotropics, and the first in Colombia [30]. They may represent an interesting group to evaluate highly parasite transmission [31]. Furthermore, some bat species are considered staples in the diet of several species of wild felids, as well as domestic cats, which can disseminate oocysts into the environment. Previous studies have shown that the rate of *T. gondii* infection in felids is related to predator–prey relationships, and therefore dependent on the availability prey species [32]. Detailed understanding of how a virus, parasite or bacteria can infect different species, including humans, so productively will help in the prevention of future zoonotic events.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval The sampling permissions were granted for the "Corporación Autónoma Regional del Quindío" administered by the University of Quindío. The rules for research with non-commercial animals (ANLA) were taken into account.

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