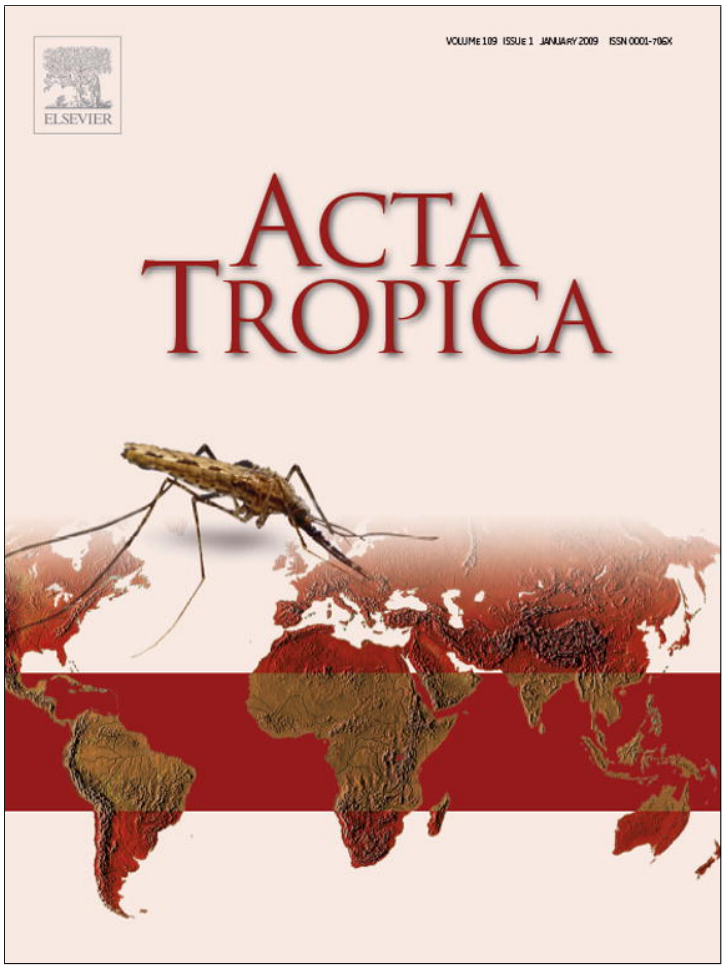

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Short communication

Trypanosoma cruzi congenital transmission in wild bats

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ABSTRACT

Trypanosoma cruzi congenital transmission in wild bats (*Molossus molossus*), associated with infected *Rhodnius prolixus* in a natural habitat from a rural locality in western Venezuela, is reported. *T. cruzi* blood circulating trypomastigotes in a pregnant bat were detected by parasitological methods. Polymerase chain reaction (PCR) assays carried out in samples from the heart and the fetus of the same infected female, revealed the presence of *T. cruzi*-specific DNA in both of the tissues, demonstrating transmission of the infection from the mother to the offspring. Eighty percent of the captured bats and 100% of the examined fetuses from pregnant specimens were shown to be infected by *T. cruzi*, indicating that *M. molossus* is a very susceptible species for this parasite, and that *T. cruzi* congenital transmission is a common phenomenon in nature. To our knowledge, this seems to be the first report on congenital *T. cruzi* transmission in wild bats in Venezuela. The circulation of *T. cruzi* lineage I in the study area was demonstrated by typing the isolates from bats and triatomine bugs captured in the same habitat. The potential epidemiological implication of these findings in areas where Chagas disease is endemic is discussed.

The potential epidemiological significance of bats as possible reservoir hosts for *Trypanosoma cruzi*, due to the frequency of infection in nature, has been previously remarked (Marinkelle, 1982). Common species of neotropical bats including those of the genera *Artibeus*, *Noctilio*, *Mormoops*, *Natalus*, *Pteronotus*, *Myiotis*, *Carollia*, *Desmodus*, *Glossophaga*, *Phyllostomus* and *Molossus*, among others, have been reported to be susceptible to *T. cruzi* infection both under natural and experimental conditions (Marinkelle, 1982; Thomas et al., 2007). However, information on how natural infection is attained and spread is mostly lacking. Due to the relevance of *T. cruzi*, the etiological agent of Chagas disease (a triatomine borne infection) in most Latin-American countries, where the parasite infects 16–18 million people from endemic areas, the finding of the wild infected bats–triatomine bugs association in natural habitats is by itself a matter of concern. This association may be considered as an additional risk factor in the maintenance of *T. cruzi* as a source for infection to humans. Congenital transmission of *T. cruzi* in man and experimental animals is a well known phenomenon (Bittencourt, 1976; Delgado and Santos-Bush, 1978; Eberhard and D'Alessandro, 1982; Torrico et al., 2004; Moreno et al., 2003). Recently, polymerase chain reaction (PCR) as diagnostic tool for congenital Chagas disease has proven to have a higher sensitivity than classical parasitological methods in blood samples taken at birth (Diez et al., 2008). In the present paper we report the presence of *T. cruzi*-infected bats and triatomine bugs association at the same habitat in a rural locality of Barinas state in western Venezuela, where Chagas disease is endemic. In addition, parasitological and molecular (PCR) methods revealed blood circulating and tissue parasites both in infected pregnant specimens and their fetuses, demonstrating *T. cruzi* congenital transmission in wild bats. These findings shed some light into how bats may be involved in the epidemiology of Chagas disease in endemic areas.

The study area in the present work was a farm at the village of Mata-Rala located at 200 m a.s.l., between 70°30′ and 71°00′W, and 8°30′ and 9°00′N in the state of Barinas in western Venezuela, where Chagas disease is endemic. A palm tree (*Acrocomia* sp.), located 30 m apart from the farm main house, was dissected out looking for triatomine bugs, insects reported by the farm workers as visiting their dwellings during nights. Fifth instar nymphs and adults of *R. prolixus* were collected during dissection of the palm tree. In addition, from a group of bats living on the palm tree (Fig. 1), we were able to capture five of them while the rest flied away. The collected bugs and bats were placed into flasks and transported to be examined in our laboratory at the Faculty of Sciences, Universidad de Los Andes, Mérida, Venezuela. Two of the bats (1♀ and 1♂) died during the trip back to the laboratory, but the bugs arrived alive. The wild bugs were dissected out and abundant flagellates were detected in the rectal ampulla in one of the specimens. A sample from the rectum was placed on a glass slide, fixed with methanol and stained with Giemsa stain, which revealed metacyclic-forms morphologically similar to *T. cruzi* (Fig. 2). Flagellates were collected and used to inoculate young mice. Once the

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Fig. 1. Specimen of *Molossus molossus* on a dissected palm tree (*Acrocomia* sp.).

mice exhibited parasitemia, as shown by microscopic observations, blood circulating parasites were collected by cardiopuncture and placed into NNN culture medium tubes to grow a culture that was then used for genetic typing of *T. cruzi* isolates by a specific PCR assay.

The collected bats (4 pregnant females and 1 male) were identified taking into consideration the morphological characteristics of the specimens, which matched well with those described by Linares (1998) for *M. molossus*. The five specimens are kept at the vertebrate collection of Universidad de Los Andes (CVULA) Mérida-Venezuela, and their catalogue numbers are from CVULA-I-8520 to 8524.

The three survivor bats were sampled by cardiopuncture. Each bat was sampled taking a fresh blood sample for microscopic observations on glass slides, and another sample for hemoculture using NNN culture medium. Observations on fresh blood samples revealed negative results in all cases. However, hemoculture showed positive results in one of the pregnant bats (I-8522). The positive hemoculture was transferred and used to produce an isolate mass culture to be characterized and typed by molecular methods. To look for *T. cruzi* tissue forms or part of its genome, in all the pregnant bats the hearts and the fetuses were taken out to be processed for molecular (PCR) assay. A similar process was performed for the heart of the male bat.

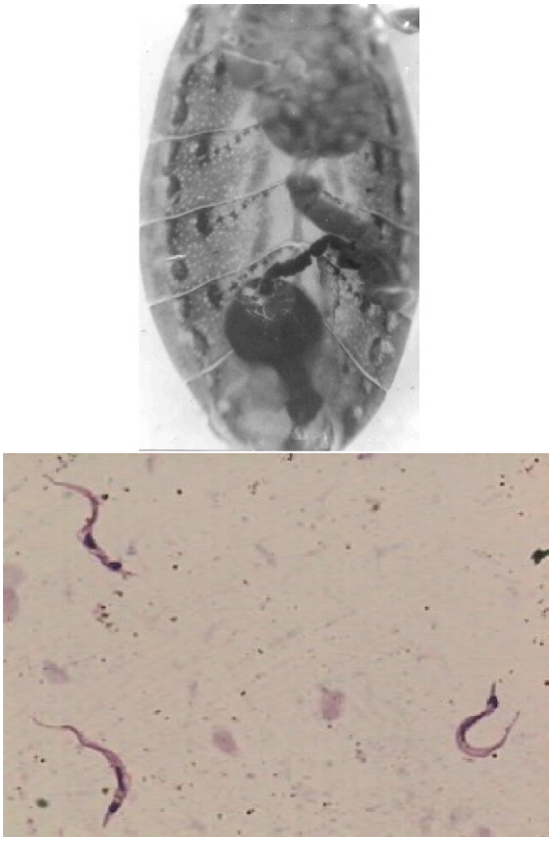

Fig. 2. Metacyclic infective forms of *Trypanosoma cruzi* detected in the alimentary canal of *Rhodnius prolixus* captured in a palm tree associated with infected bats.

Two *T. cruzi* isolates were obtained during the present work. One from a natural infected *R. prolixus*, named as IRHO/Ve/06/CHMR-06, and another from an infected specimen of *M. molossus* (MMOL/Ve/06/Bat-06). For molecular characterization, the DNA from the two cultured *T. cruzi* isolates was extracted by the classical phenol–chloroform method. The two isolates were typed through a specific PCR amplification using primers D71 (5′-AAG GTG CGT CGA CAG TGT GG-3′) and D72 (5′-TTT TCA GAA TGG CCG AAC AGT-3′) based on the divergent domain of the 24Sα RNA gene as described by Souto et al. (1996), and the pool of primers TC (5′-CCC CCC TCC CAG GCC ACA CTG-3′), TC1 (5′-GTG TCC GCC ACC TCC TTC GGG CC-3′), and TC2 (5′-CCT GCA GGC ACA CGT GTG TGT G-3′) for the amplification of an intergenic region of the mini-exon gene as described by Souto et al. (1996). The aforementioned primers generated, for both of the isolates, 110 bp and 350 bp DNA bands for ribosomal and mini-exon genes, respectively, corresponding to *T. cruzi* lineage I. The PCR amplified products from both reactions were separated by electrophoresis in 3% agarose gels and stained with ethidium bromide (Fig. 3).

The presence of the parasite in samples from the hearts of the infected female bats and their respective fetuses was confirmed with a PCR specific for *T. cruzi* DNA (Sturm et al., 1989; Guhl et al., 2002). This assay (S35-5′-AAA TAA TGT ACG GGT GAG ATG CAT GA-3′; S36-5′GGG TTC GAT TGG GGT TGG TGT-3′) amplified a fragment of 330 bp from kDNA minicircle variable regions of the parasite, which made possible to detect a portion of the *T. cruzi* genome both in the pregnant females and their offspring, demonstrating congenital transmission in this species of bat. Fig. 4 shows amplification products with the expected sizes both in hearts and fetuses of the same female bats. A similar methodology used for parasite in the heart of the male bat showed negative results.

As far as we know, this seems to be the first report demonstrating congenital *T. cruzi* transmission in bats using molecular methods. In addition, the isolation and molecular typing of living *T. cruzi* directly from wild bats, is described, also showing its

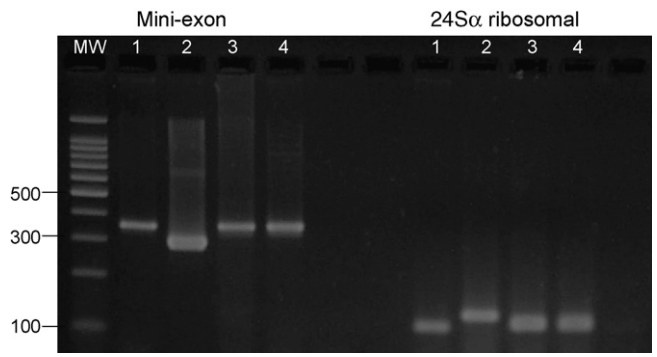

Fig. 3. *Trypanosoma cruzi* typing using agarose gel (3%) electrophoresis of PCR products generated by amplification of the mini-exon gene and the 24Sα-rDNA sequences from Venezuelan bat and triatomine bug isolates. DNA from the reference strains G (lane 1) and Y (lane 2) for *T. cruzi* I and *T. cruzi* II, respectively were used as positive control. MW: DNA Molecular ladder. (3) IRHO/Ve/06/CHMR-06 and (4) MMOL/Ve/06/Bat-06.

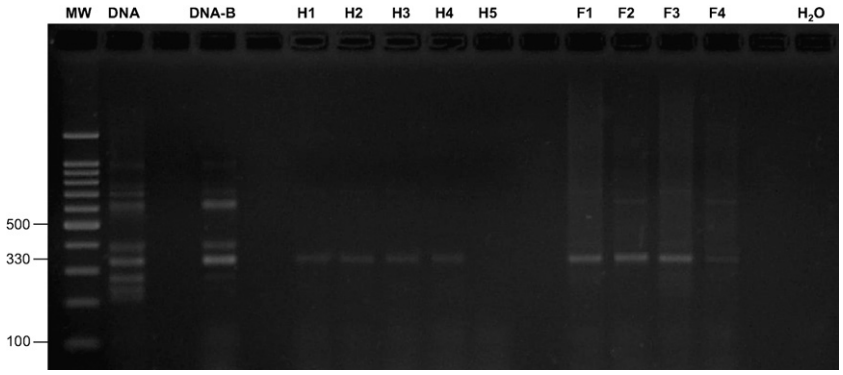

Fig. 4. *Trypanosoma cruzi* specific PCR assay on heart and fetus specimens from infected wild bats. Ethidium bromide-stained gel showing PCR amplification products. Lanes MW: DNA Molecular ladder. DNA: *T. cruzi* positive control. DNA-B: DNA sample of the isolate MMOL/Ve/06/Bat-06. H1–H4: specimens from heart of pregnant bats. H5: specimen from heart of a male bat. F1–F4: fetuses from infected females. Each F sample (F1–F4) corresponding to the same female identified as H (H1–H4). H_2O: reaction control.

association with similar parasites present in the insect vector that shares the same habitat in nature.

It is interesting to note that being *M. molossus* an aerial insectivore bat that forage in open areas (Linares, 1998), 4 out of 5 (80%) captured specimens were infected with *T. cruzi*, which suggests the high susceptibility of this bat to the infection by this parasite. This fact indicates that they may become infected with *T. cruzi* either by contamination with metacyclic-forms defecated by infected triatomine bugs during feeding on them, or through the ingestion of infected bugs as experimentally demonstrated by previous workers (Thomas et al., 2007). However, what appears to be more impressive is the fact that 100% of fetuses from infected pregnant bats were positive to *T. cruzi* infection, suggesting that congenital transmission is a common phenomenon in this species of bat in the wild. These findings add evidence to consider bats as an additional risk factor in the maintenance of the sylvatic cycle of *T. cruzi* in endemic areas. This seems to be a fact of potential epidemiological importance taking into consideration that bats are perhaps the more abundant mammals in the new world tropics and some species, as demonstrated here with *M. molossus*, are excellent hosts of *T. cruzi*, with which have possibly had an ancient association (Marinkelle, 1976).

The genetic typing carried out in this study made it possible to identify *T. cruzi* I in infected bats and *R. prolixus*, associated in the same habitat, which suggests the circulation of this lineage in this particular endemic area for Chagas disease in western Venezuela. This fact corroborates previous suggestions that *T. cruzi* I is widespread in mammalian hosts within sylvatic cycle, and add evidence to consider bats as reservoir for this lineage of the parasite, taking into consideration the effectiveness of congenital transmission and its high susceptibility to *T. cruzi* infection.

Finally, in addition to the aforementioned facts, the findings reported here may have potential epidemiological implications in the region due to the presence of *T. cruzi* I, a lineage considered to be predominant in human population causing Chagas disease in Venezuela, Colombia and the Brazilian Amazonia, where different clinical forms of the disease have been reported, ranging from asymptomatic to fatal both in the acute and chronic phases (Añez et al., 2004; Montilla et al., 2002; Coura et al., 2002; Teixeira et al., 2006).

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