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Short Report: Temporal Fluctuation of Infection with Different *Trypanosoma cruzi* Genotypes in the Wild Rodent *Octodon degus*

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Abstract. We identified and followed-up for two years *Octodon degus* rodents infected with *Trypanosoma cruzi* genotypes by using xenodiagnosis with two vector species (*Mepraia spinolai* and *Triatoma infestans*), polymerase chain reaction DNA-based detection of insect dejections, Southern blot analysis, and minicircle hybridization with genotype-specific probes. Results show temporal fluctuations of infection with four parasite lineages (TCI, TCII, TCV, and TCVI) in one co-infected *O. degus*. Results are discussed in the context of parasitemia level and infection control in mammal hosts.

Chagas disease is a vector-borne zoonosis caused by the protozoa *Trypanosoma cruzi*. This taxon had been described as composed of two lineages (TCI and TCII) and five subgroups (IIa–IIe), but a recent study reported six lineages or discrete typing units (DTUs) (*T. cruzi* I–VI).[1] These lineages are defined as sets of stocks that are genetically more related to each other than to any other stock and are identifiable by common genetic molecular and immunologic markers.[2]

Trypanosoma cruzi populations circulate in nature in multiple *T. cruzi* genotypes that coexist in different hosts, including *Octodon degus* rodents.[3–5] After a short acute or primary infection, the mammal host sustains subclinical infections, which are microscopically undetectable in peripheral blood during the undetermined and chronic phases. Conversely, parasitemia in those phases is detected only by polymerase chain reaction (PCR). The classic parasitologic diagnostic method for Chagas disease xenodiagnosis, which can amplify *T. cruzi* after feeding on infected hosts.[6] Although xenodiagnosis is specific, it lacks sensitivity and is limited to high levels of parasitemia.[7] The epidemiology of Chagas disease and clinical symptoms are associated with the infective *T. cruzi* genotypes.[8] Therefore, would be useful to know the dynamics of these genotypes.

In the present study, we assess the occurrence of temporal fluctuations of *T. cruzi* DTUs in peripheral blood of two naturally infected wild reservoir specimens of *O. degus* by using a combination of two diagnosis methods: 1) xenodiagnosis with domestic and sylvatic vectors (*Triatoma infectans* and *Mepraia spinolai*), respectively, and 2) PCR DNA-based detection specific for minicircles and hybridization analyses with *T. cruzi* genotype-specific probes.

Ten nymphs (stages II and III) of each vector species were allowed to feed simultaneously on anesthetized *O. degus* rodents for 30 minutes or until engorgement on the rodent (mean ± SD weight of ingested blood = 0.2 ± 0.05 mg). After 30 days, feces and intestinal contents of the triatomines were observed under a light microscope. The minimal theoretical parasitemia detected under these conditions is approximately 5 parasites/mL (1 parasite/0.2 mL). However, because several but not all insects (2–5) were parasite positive by visual examination, the estimated parasitemia would be > 10–25 parasites/mL.

After microscopic inspection, the intestinal contents of each vector species pool was collected and PCR was performed as reported.[9] Amplicons were subjected to electrophoresis on an agarose gel and transferred to nylon membranes. Copies of these membranes were hybridized separately with each probe under high stringency conditions.[3] Construction of genotype-specific probes was performed as described.[10] Different *T. cruzi* clones were used as templates to generate DNA probes to determine parasite genotypes. The probes were P^{32}-labeled.[4]

A total of 35 *O. degus* were captured at the field and analyzed. Overall, only two *O. degus* showed infection with both vector species and six were positive only for *M. spinolai*.[9] The two *O. degus* samples positive for both vector species were subjected to serial xenodiagnosis to determine the genotype of the *T. cruzi* population circulating at different times: time 0, one year, two years, and two and a half years. Results for *O. degus* sample 5, which was infected with a one genotype (TCI), are shown in Figure 1. This result was confirmed with both vector species at different times. Results obtained with *O. degus* sample 8 showed mixed infection with DTUs TCI, TCII, and TCVI at time zero for *M. spinolai*, but only TCII for *T. infestans*. However, one year later, both vectors showed mixed infections with lineages TCI and TCV. After two years, both vectors contained only genotype TCII. After two and a half years, vectors were still infected with TCII.

Trypanosoma cruzi colonizes several tissues and evades the immune response by a concomitant low parasitemia level not detectable by several diagnosis methods.[11] Parasites circulate as mixed infections. This finding is common for *T. cruzi* because several mammals and vectors are infected with more than one *T. cruzi* genotype,[4,5] which results in recombination and hybrid genotypes.[8]

We report that infection of rodents can show temporal fluctuations with different *T. cruzi* genotypes, which is probably the result of fluctuation of relative proportions of parasite loads of different genotypes in peripheral blood. We detected infections in this *O. degus* with at least three of the four *T. cruzi* genotypes during the complete follow-up (xenodiagnosis at time 0). Two genotypes (TCII and TCVI) disappeared, and another one (TCV) appeared one year later. During the second year, only one genotype (TCII) was detected and maintained. A different scenario was detected for *O. degus* sample 5, which showed infection with only TCI during the entire sampling period.

In this study, we preferentially detected genotype TCII in both vector species. This genotype was likely circulating at high parasitemia levels in *O. degus* sample 18 because experimental infections in *T. infestans* with different *T. cruzi* DTUs indicated that genotype TCII is transmitted at a low rate; genotype TCI

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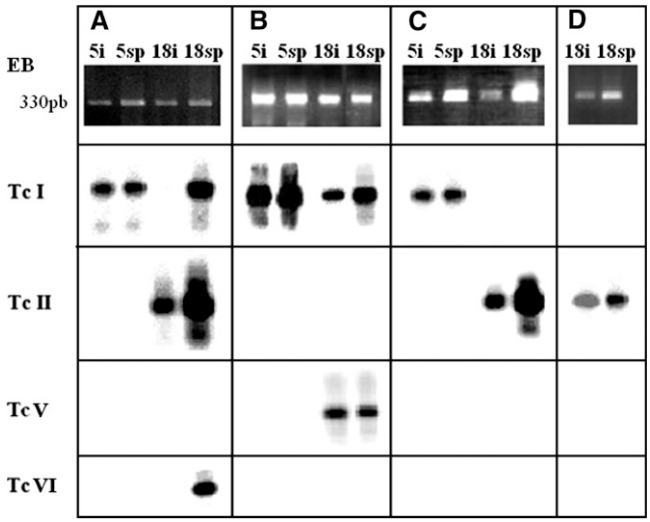

FIGURE 1. Hybridization patterns of xenodiagnosis samples from *Mepraia spinolai* (sp) and *Triatoma infestans* (i) and staining with ethidium bromide (EB) and Southern blot analyses with specific probes (TCI, TCII, TCV, and TCVI) for xenodiagnosis samples at **A**, time 0 (i.e., immediately after capture); **B**, one year later, **C**, two years later, and **D**, two and a half years later. Numbers 5 and 18 correspond to identification numbers for *Octodon degus* rodents.

is transmitted at a high rate.[12] Our results for *T. cruzi* genotypes in these two animals are consistent with local prevalence in the study area.[4] Recent studies of *T. cruzi* genotypes circulating in the wild vector in this disease-endemic area showed that TCI and TCII are the most prevalent genotypes.[5]

We suggest that both rodent species showed moderate or high levels of parasitemia. We used xenodiagnosis with two triatomine species because insect vectors amplify *T. cruzi* in the midgut, which enables easy detection. Our results indicate fluctuation in specific genotype infections in a *T. cruzi*-infected sylvatic rodent.

The temporal fluctuation of the four *T. cruzi* genotypes could be explained by at least two hyptheses that are not mutually exclusive. First, colonization of different tissues with *T. cruzi* described in patients and experimentally infected animals with organ damage[11,13] releases *T. cruzi* into the vascular system; these parasites then colonize other tissues. Second, infection is controlled by the immune system. Both processes might reach an equilibrium and explain the low parasitemia levels observed in immunocompetent patients in the chronic phase of Chagas disease. Future parasitologic studies of molecular pathogenesis may be necessary to understand the mechanisms underlying infection control in naturally infected hosts.

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