RESEARCH NOTE

Infection of *Triatoma guasayana, Triatoma sordida* and *Triatoma infestans* by *Trypanosoma cruzi* from a Naturally Infected Opossum

Nicolás Schweigmann+, Silvia Pietrokovsky, Osvaldo Conti, Mariana Escosteguy, Victoria Bottazzi, Nora Solarz, Cristina Wisnivesky-Colli

Unidad de Ecología de Reservorios y Vectores de Parásitos, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Pabellón II, 4º Piso, Ciudad Universitaria, 1428 Nuñez, Buenos Aires, Argentina

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The province of Santiago del Estero, Argentina, is located in a highly endemic area of Chagas disease. *Triatoma infestans* is the main domiciliated vector, whereas the triatomine species *Triatoma guasayana* and *Triatoma sordida* live in wild biotopes such as bromeliads, dry cactacea and fallen logs (C Wisnivesky-Colli 1994 *Talleres Venezuela 3*: 83-89). Between 1984 and 1987, we studied the *Trypanosoma cruzi* infection of wild mammals in the forest that surrounded a rural village (C Wisnivesky-Colli et al. 1992 *Trans R Soc Trop Med Hyg 86:* 38-41). Among all the mammals examined, two skunks (*Conepatus chinga*), one ferret (*Galictis cuja*) and 23 opossums (*Didelphis albiventris*) were found infected. In the same study area, N Schweigmann (1994 Doctoral Thesis University of Buenos Aires) found that *D. albiventris* showed prevalence annual rates that ranged between 29 to 50%, the highest prevalence recorded among the local wild mammals. Therefore, like in the rest of America, opossums arose as the major wild reservoir host of the parasite. However, less than 1% of the triatomines collected in wild biotopes from Santiago del Estero showed the flagellate in their feces (C Wisnivesky-Colli et al. 1993 *Mem Inst Oswaldo Cruz* (Suppl.): 266) and this finding made us wonder about the actual capacity of *T. guasayana* and *T. sordida* to acquire *T. cruzi* infection.

In this paper we compared the proportion of *T. guasayana*, *T. sordida* and *T. infestans* from Argentina that showed parasites in their feces after being fed on a naturally infected opossum.

We had already observed that the infection rate of third *T. infestans* nymphs used in the xenodiagnosis of wild mammals was higher than those reached by *T. guasayana* and *T. sordida* (unpublished). A brief explanation of this finding is that third *T. infestans* nymphs would ingest a larger number of parasites because their blood meal is twice the amount of *T. guasayana* and *T. sordida* (S Pietrokovsky et al. 1996 *Mem Inst Oswaldo Cruz 91*: 241-242). To study if the blood meal size determines the proportion of infected triatomines from the three species, in the current experiment we used third instar nymphs of *T. infestans* and fifth instar nymphs of *T. guasayana* and *T. sordida*, because the amount of blood ingested by fifth instar nymphs is larger than that of third instar nymphs. Insects had been reared in the Insectary of the Servicio Nacional de Chagas, Córdoba, and fasted for 20 days before the experiment trial.

A naturally infected *D. albiventris* adult male (1,900 g) was used as parasites source. The *T. cruzi* isolate circulating among local opossums was characterized previously (Wisnivesky-Colli *loc. cit.*). Parasites showed an electrophoretic zymogram pattern that was classified as Z10, closely related to zymodemes Z2 and Z12 isolated from humans in Argentina. The naturally infected opossum had shown the capacity to infect 78% (14/18) of the third *T. infestans* nymphs used in a xenodiagnosis carried out 60 days before. After the marsupial was anesthetized with a mixture of Acedan^{R} (Holliday-Scott) and ketamine, *T. infestans*, *T. guasayana* and *T. sordida* (80 insects per species) were placed to feed on him simultaneously during 1 hr to avoid differences in the host parasitemia level and to even out the nutritional status of insects. After the infective meal, insects were allowed to feed on chicken every 20 days. Triatomine feces were examined microscopically (x 400) at days 30, 45, 60 and 75 after the infective meal to check *T. cruzi* presence. In every occasion we set apart the positive insects, recording their number and the date

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+Corresponding author. Fax: +54-1-373.6102
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they were found infected. We used the test for multiple proportions (JL Fleiss 1981 *Statistical methods for rates and proportions*, 2nd ed., New York) to compare the cumulative percentages of infections of the three species at each examination time.

A mortality of 1 *T. infestans*, 13 *T. sordida* and 8 *T. guasayana* was recorded during the first month. On day 30, one sample of each species was examined; from day 45 on, all the insects were examined, except for those that had previously been found infected. Table shows that *T. infestans* reached the highest cumulative percentages of infection throughout the experiment ($p < 0.001$). At the day 30 the three species showed statistical differences, being *T. sordida* the less infected (13.9%). However, from day 45 on, no significant differences between the percentages of the wild triatomine species were observed.

The susceptibility of triatomines to *T. cruzi* is affected by insects species and stage, the number of parasites ingested by the bug, the parasite strain and the interaction between the vector and the parasite (ES Garcia & P Azambuja 1991 *Parasitol Today 7:* 240-244). Results suggest that the infection outcome of *T. infestans*, *T. guasayana* and *T. sordida* exposed to the *T. cruzi* opossum isolate is not determined by the blood meal size because the blood intake of *T. guasayana* and *T. sordida* fifth nymphs is respectively five-fold and three-fold larger than that recorded for *T. infestans* third nymphs (Pietrokovsky *loc. cit.*). The better perfomance showed by third instar nymphs of *T. infestans* could be due to a more suitable inner environment than that of fifth instar nymphs of *T. guasayana* and *T. sordida* for the development of the *T. cruzi* opossum isolate. Several intrinsic factors related to the vector species, such as hemolitic factors and anti-epimastigote lectins, have been described as affecting the development of certain *T. cruzi* strains over others (Garcia & Azambuja *loc. cit.*). Therefore, *T. infestans* third instar nymphs would be the best vectors to detect eventual wild hosts infections.

On the other hand, we found that the prevalence found in the field does not reflect the potential susceptibility of *T. guasayana* and *T. sordida* to the parasite. A factor that should be studied is the posibility that both wild species lose the *T. cruzi* infection after long periods of time. Moreover, a comprehensive analysis of the variables involved in the natural transmission cycle should be needed. The triatomine abundance and their distribution pattern in the wild biotopes, as well as the moving activity and resting behavior of infected mammals near or within the triatomine location sites, could affect the probability of encounter between the bugs and their hosts in the field.

TABLE

Infection of *Triatoma infestans, T. sordida* and *T. guasayana* by *Trypanosoma cruzi* after an infective meal on opossum

Examination days [a]	Cumulative frequencies of infection (%)[b]		
	T. infestans	*T. sordida*	*T. guasayana*
30	16/21 (76.2)	6/43 (13.9)	5/12 (41.6)
45	60/79 (75.9)	23/67 (34.3)	27/72 (37.5)
60	66/79 (83.5)	39/67 (58.2)	38/72 (52.8)
75	-	40/67 (59.7)	38/72 (52.8)

a: after the infective meal; b: insects with *T. cruzi* / total no. of insects (percentage); -: not examined.