

RESEARCH NOTE

Massive Experimental Inoculation of *Trypanosoma rangeli* (Kinetoplastida, Trypanosomatidae) in *Rhodnius neglectus* Lent, 1954 (Hemiptera, Reduviidae) and *Mus musculus*

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The extra-intestinal development of *Trypanosoma rangeli* Tejera, 1920, in triatomines was studied by several authors and nowadays it is well known that this flagellate is able not only to develop in the hemolymph of the vector but also to invade its salivary glands being transmitted by the bite [R Zeledón 1965 *Rev Biol Trop (Costa Rica)* 13: 157-159, EJ Tobie 1965 *J Parasitol* 51: 837-841, MS Grewal 1970 *Res Bull (NS) Panjab Univ* 20: 449-480]. The infection can be transmitted both by the contaminative and inoculative methods, although this last one seems more efficient.

The development of *T. rangeli* in the mammal host was accompanied in experimentally infected animals, specially rodents. In mice or rats, the parasites appear in the blood some hours after inoculation with trypomastigotes (from culture me-

dium or salivary glands of triatomines), but in some cases only one or two weeks after, representing a period of incubation (H Groot 1952 *Am J Trop Med Hyg* 1: 585-592). The patent period in mammals is variable; in mice and rats experimentally infected it may last from three to seven months and eventually up to a year (EJ Tobie 1961 *Exp Parasitol* 11: 1-9). In humans with natural infection it persists from three to 18 months (F Pifano 1954 *Arch Venez Patol Trop* 2: 89-120).

Rhodnius negectus Lent, 1954 were found naturally infected by *T. rangeli* in Brazil (L Diotaiuti et al. 1992 *Mem Inst Oswaldo Cruz* 87: 451). A study on experimental vectors of *T. rangeli* showed that among eight triatomine species tested, *R. neglectus* and *Triatoma infestans* (Klug, 1834) showed statistically higher rates of infection (A D' Alessandro 1972 *J Med Ent* 9: 187-195). Since it is well known that *T. rangeli* loses its capacity to develop in the insect and the vertebrate host after prolonged *in vitro* cultivation, the aim of our study was to verify the establishment of infection in *R. neglectus* by intra-celomic inoculation and in albino swiss mice, *Mus musculus* by intra-peritoneal inoculation with high concentrations of the Brazilian strain of *T. rangeli*, SC-58.

R. neglectus were from a colony maintained at the Departamento de Biologia of the Instituto Oswaldo Cruz (IOC) obtained from the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, IOC. They were maintained at room temperature (22°C to 28.5°C and 70 ± 5% of relative humidity). The 5th instar nymphs were separated from the colony five days after molting.

The strain of *T. rangeli* used was SC-58, isolated by M Steindel et al. (1991 *Mem Inst Oswaldo Cruz* 86: 73-79) from a wild rodent, *Echymys dasythrix* (Hensel, 1872), by blood culture and by *in vivo* xenodiagnosis using nymphs of *Panstronglylus megistus* (Burmeister, 1835) and of *T. infestans*. The strain was maintained in biphasic culture medium, blood agar and LIT (liver infusion tryptose), supplemented with 10% of fetal bovine serum, at room temperature and the passages were made every two weeks.

The triatomines had one leg cut off to obtain one drop of hemolymph and the abdomen compressed to obtain fresh posterior midgut contents. They were killed by freezing at 0°C, and the salivary glands were drawn out with tweezers and macerated. The midgut contents were also obtained by tearing the posterior midgut. The parasites, when found, were fixed with methanol (3 min), stained with Giemsa (10 min) and washed tap water.

In the first experiment, the triatomines were inoculated intra-celomically with 0.1 ml of culture

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medium containing parasites: 5th instar nymphs (n=26) inoculated with 135.5×10^6 parasites per ml of culture medium and dissected on the 10th, 20th, 30th, 40th, 50th, 60th, and 70th day post-inoculation (p.i.). The triatomines were dissected also after the imaginal ecdysis. Those that died from natural causes were dissected on the 28th, 33rd, 36th, 43rd day p.i. After the inoculation, the triatomines were fed weekly on uninfected swiss mice until repletion.

In the second experiment, two adult male swiss albino mice Balb-c (*M. musculus*) were inoculated intra-peritoneally with 1 ml of culture medium containing 62.0×10^6 parasites per ml. From the 3rd to the 60th day post-inoculation (p.i.), three 5th instar nymphs of *R. neglectus* were fed once, until repletion, on the swiss mice (3rd, 8th, 13th, 20th, 25th, 30th, 45th and 60th days p.i.) (a total of 24 nymphs) and dissected on the 15th days after the xenodiagnosis. One week after the infective meal, the nymphs were fed once on non-infected mice.

Thirty one percent of the 26 inoculated triatomines were infected. On the 28th day p.i. the parasites were seen free in the hemolymph. On the 33rd day they were free and also seen adhered to the hemocytes. On the 36th day, one 5th instar nymph presented heavy hemolymph infection (more than 10 parasites per field), with some parasites showing signs of division. On the 50th day, only one 5th instar was infected, presenting a low number of parasites (less than 2 parasites per field). On the 60th day only female was infected (after imaginal ecdysis), and it was the only triatomine of this group to present salivary gland infection (extremely low number of parasites) (Fig. 1). None of them presented midgut infection.

One hundred percent of the nymphs used for xenodiagnosis presented intestinal infection 15 days after feeding on mice on the 20th day after inoculation. Fig. 2 shows the percentages of infection of the nymphs used for xenodiagnosis. None of them presented hemolymph or salivary gland infection.

Some authors state that the development of *T. rangeli* in the hemolymph and salivary glands, involving the inoculative method of transmission, is the only essential part of its cycle in the invertebrate, while its intestinal phase is less important (CA Hoare 1972 *The Trypanosomes of Mammals*, Blackwell Scientific Publications, Oxford and Edinburgh, 749 pp.). Opposed to a constant occurrence of intestinal infection, *T. rangeli* does not always invade the hemocoel.

In our study, the 5th instar nymphs were inoculated with a high concentration of parasites per ml of culture medium. The percentage of

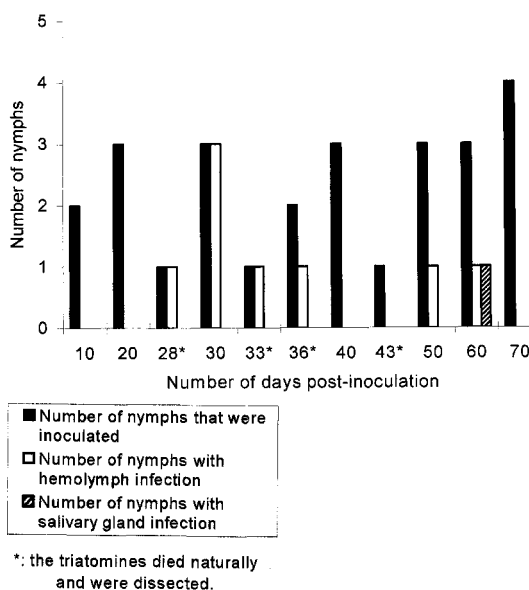


Fig. 1: number of nymphs and adults of *Rhodnius neglectus* that presented infection by *Trypanosoma rangeli*, strain SC-58, after intra-celomic inoculation.

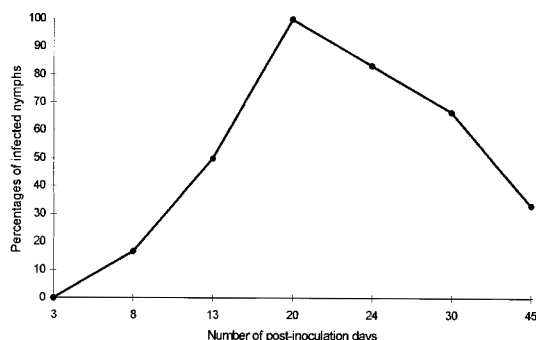


Fig. 2: percentages of 5th instar nymphs of *Rhodnius neglectus* that presented intestinal infection after xenodiagnosis on swiss mice inoculated intra-peritoneally with *Trypanosoma rangeli*, strain SC-58.

triatomines that presented hemolymph infection was high, however, only one triatomine presented salivary gland infection (one female after the imaginal ecdysis), 32 days after the emergence of hemolymph infection. The number of parasites was usually low. Thus, our results suggest that the concentration of parasites in the inoculum does not have an influence on the establishment of the infection, only on its duration. JO Coutinho and V Nussenzweig (1952 *Fol Clin Biol* 18: 181-188) inoculated intra-celomically *T. vitticeps*, *T. infestans*, *R. prolixus* and *P. megistus* with one Venezuelan strain of *T. rangeli* and observed that the flagellates develop exclusively in the hemolymph when inoculated. MJ Soares (1982 *J*

Parasitol 29: 529) inoculated intracelomically *R. prolixus* with a Colombian strain of *T. rangeli*, and observed free parasites only in the hemolymph. Our results are in accordance with these authors.

G Rosa et al. (1995 *Mem Inst Oswaldo Cruz* 90 (Suppl): 299) made intra-femur inoculation of four triatomines species (including *R. neglectus*) with three strains of *T. rangeli* (including SC-58 from Brazil). The Brazilian strain presented low rates of hemolymph and salivary gland infection for *R. neglectus* (less than 10% of the inoculated triatomines). The Brazilian strain (SC-58) that was used by Rosa et al. (1995 *loc. cit.*), was the same that we used in our study, and their result for *R. neglectus* are different from ours since we obtained 31% of infection, probably due to the via of inoculation.

Tobie (1961 *loc. cit.*) infected *R. prolixus* with several Venezuelan strains of *T. rangeli* by intracelomic inoculation and observed that the flagelates were destroyed 24 hr after the invasion of the hemocoel, before they could reach the salivary glands. Zeledón (1965 *loc. cit.*) did not succeed in infecting *R. prolixus* by intra-celomic inoculation with a Panamanian strain.

In our study, the parasites were not completely eliminated from the hemocoel of the inoculated triatomines, but, in most cases, they were unable to invade the salivary glands.

According to Hoare (1972 *loc. cit.*), when the rodent was inoculated with infective forms isolated from salivary glands of triatomines, the parasites could be found in the blood from 10 to 14 hr after the inoculation. This author also reported that the blood parasitaemia, usually low, increased from the 4th day forward and could last months, but it could

also become latent.

Even though we used LIT as medium for the inoculum, it did not seem to have any influence on the growth of the parasites in the vertebrate host, since the parasitaemia of the mice inoculated was very low. EJ Tobie (1964 *J Parasitol* 50: 593-598) reported that two days after inoculation, the parasites could be found in the blood of the rodents. According to this author, it could be due to the strain of *T. rangeli* used (Venezuelan), and of the rodent. These results differ from ours since the swiss mice inoculated intra-peritoneally with *T. rangeli*, strain SC-58, developed latent infection, detected only by xenodiagnosis.

Our results suggest that *T. rangeli* strain SC-58, may recover its infectivity after massive inoculation in the vertebrate host, although the infection was only detected in the blood of swiss mice by xenodiagnosis. It is also suggested that, even though *R. neglectus* is not considered the natural invertebrate host of the strain of *T. rangeli* used in these experiments despite the fact of having been found naturally infected (Diotaiuti et al. 1992 *loc. cit.*), the infectivity of the strain SC-58 of the protozoa is partially recovered after massive inoculation in this species of triatomine, even though only one insect presented salivary gland infection. Further studies are needed to establish the number of passages through vertebrate and invertebrate hosts that are necessary for complete recovery of the infectivity of *T. rangeli* in the laboratory.

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