

## Direct transmission of *Trypanosoma cruzi* between individuals of *Rhodnius prolixus* Stal\*

by

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It is a little known fact that triatomid bugs infested with *Trypanosoma cruzi* may infect "clean" bugs when they are kept together in the same container. Some authors (1, 2, 3) explain this by cannibalistic and coprophagous habits of the bugs. RYCKMAN (4) described the "act of theft of blood" with the term kleptohemodeipnonism.

In the epidemiological studies of South American human trypanosomes, the triatomid-trypanosomal index (T. - T. index) is a widely used criterion. The ratio of non-infected to infected triatomids from any given survey area gives the T. - T. index. Usually the bugs collected in one house are placed in a container and later examined in the laboratory. It is obvious that when bugs infected with trypanosomes are able to infect clean bugs during the time they are kept in the same container, the calculated index might not be correct. Such a direct transmission of trypanosomes between bugs may also occur under natural conditions, and might be a means of greatly increasing the percentage of infection of invertebrate hosts in nature.

Besides serological methods, it is an accepted fact that the method of xenodiagnosis gives excellent results for the diagnosis of Chagas disease. In this last method, laboratory-reared uninfected bugs are allowed to take a blood meal on men or animals suspected to be infected. After several weeks the bugs are examined for the presence of flagellates. In many places in tropical America the necessary bug material is regularly built up by using eggs, deposited by bugs collected in the field. As no transovarial transmission of trypanosomes to the

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eggs seems to exist, this is a safe method, even if the mother-bug is infected with *T. cruzi*. However, the deposited eggs must be regularly removed from the containers.

For several reasons this procedure may not be followed, and thus the first stage nymphs may become infected with trypanosomes from infected adults, if such direct transmission exists. If such offspring are used in the xenodiagnosis procedures, people may become infected instead of diagnosed.

In the light of these problems some experiments were done to get a better idea of the possibility and frequency of direct transmission of trypanosomes between different stages of bugs.

## MATERIALS AND METHODS

All observations were done on *Rhodinus prolixus*. It is the most common triatomid bug of Colombia and the only vector of importance for *T. cruzi* and *T. rangeli* in that country. It is also widely used for xenodiagnosis and its life cycle is considerably shorter than that of the other locally available bugs.

Bugs naturally infected with *T. cruzi* and *T. rangeli* were collected from houses in central Colombia and kept together with clean laboratory-reared bugs. Third and fourth instar nymphs and adult bugs infected with *T. cruzi* and *T. rangeli* were placed together with different stages of clean *R. prolixus*. One to five infected individuals were kept with 5 to 10 uninfected ones. The infected bugs were marked by removing the distal part of the leg and the hemolymph was examined for the presence of *T. rangeli*. The infected and uninfected bugs were fed separately every two weeks on trypanosome-free blood, and the feces of the original uninfected bugs examined soon after feeding.

Two months after the start of the experiment the originally uninfected bugs were sacrificed and the intestinal contents, mid-gut and hind-gut, hemolymph and salivary glands examined for the presence of flagellates. A mixture of this material was inoculated into various culture media and into C.F.W. mice.

A total of one thousand uninfected specimens was used. Of these 200 were first stage nymphs, 200 second stage, 200 third stage, 200 fourth stage and 200 adult bugs.

In another experiment 300 clean second, third or fourth instar bugs were placed with marked infected third and fourth stage bugs for two months. During this time the clean bugs were starved while the marked infected ones were fed regularly.

Two more experiments were done to test direct and indirect fecal transmission. In the first case, 100 clean third stage bugs were starved for 6 weeks and then offered eosin-stained bug feces. To test the latter possibility, the abdomens of 100 clean individuals in different stages, were exposed to bug feces or fluid culture media containing flagellate forms of *T. cruzi* and *T. rangeli*, for periods of 1 to 5 hours.

## RESULTS

Of the 1000 originally uninfected individuals 23 acquired *T. cruzi* infection when kept with infected bugs for a period of at least one month. All infections were detected between the 32nd and 61st day after exposure, with the majority between the 46th and 61st day. Six of the 200 originally clean first stage nymphs harbored *T. cruzi* when examined after they had reached their second or third stage. Of the 200 originally clean second stage nymphs 9 were positive when examined in their third or fourth stage. Of the 200 originally clean third stage bugs 5 harbored *T. cruzi* when sacrificed. Of the fourth stage bugs 3 showed *T. cruzi* flagellates in the intestinal tract after two months. None of the originally clean adult bugs acquired the infection. In one of the preceding experiments it was observed that one infected third stage bug was able to transmit the trypanosome to two originally clean third stage bugs within a period of 45 days.

When nymphal *R. prolixus* were partially fed once a week, and so prevented from molting, none of the uninfected bugs acquired trypanosomes from infected individuals maintained in the same container. However in the experiment, where the originally uninfected bugs were starved but the infected ones fed regularly, 10 % of the originally uninfected ones acquired *T. cruzi*. Among the clean bugs 8 out of 86 second stage, 12 out of 76 third stage and 10 out of 83 fourth stage nymphs had acquired the infection within 2 months; 55 of the bugs died before the experiment was finished, but all of these were negative.

In all secondarily infected bugs, critical stages were found in the rectal ampul and occasionally some metacyclic trypanosomal forms. The presence of *T. cruzi* was confirmed by inoculation of C.F.W. mice and by culture methods. No direct transmission of *T. rangeli* between individuals of *R. prolixus* was detected.

In the experiment where eosin-stained bug feces were offered to starved bugs, the eosin was present in 2 out of 100 bugs by the next day.

None of the 100 bugs, which had their abdomen exposed to bug feces or fluid culture media containing flagellate forms of *T. cruzi* and *T. rangeli*, became infected during the follow-up period of two months.

## DISCUSSION AND CONCLUSIONS

It is not understood why *T. rangeli* could not be transmitted between the bugs. The negative findings may be due to the relatively small number of observations. It is hard to believe that *T. rangeli* flagellates cannot be acquired by the act of feeding on other individuals. To suck the blood meal of another bug, the proboscis must penetrate body areas containing hemolymph and so should be able to pick up *T. rangeli* flagellates.

The mechanism of transmission of *T. cruzi* between the bugs is probably by ingestion of fecal deposits containing the flagellates and by feeding on each other. Various authors (1, 3) observed that the bugs may feed on each other's

hemolymph. Although I have no evidence for hemolymph ingestion, my observation is that hungry bugs not uncommonly suck the blood meal of an engorged mate. Cases were observed in which an engorged bug was practically emptied by another individual. Apparently such hemophagous attacks do not kill the victim.

These preliminary investigations lead to the conclusion that direct transmission of *T. cruzi* between individuals of *R. prolixus* is not uncommon, and more frequent when the non-infected bugs are hungry.

### SUMMARY

Experiments were done to test the possibility and frequency of direct transmission of trypanosomes between different stages of *Rhodnius prolixus*. It was found that direct transmission of *Trypanosoma cruzi* between individuals of *Rhodnius prolixus* is not uncommon, and more frequent when the non-infected bugs are hungry. The mechanism of transmission is probably by taking a blood meal from other engorged individuals and by ingestion of feces from infected bugs. No direct transmission of *Trypanosoma rangeli* between bugs was observed.

### RESUMEN

Se hicieron experimentos para probar la posibilidad y frecuencia de la transmisión directa de tripanosomas entre diferentes estadios de *Rhodnius prolixus*. Se encontró que la transmisión directa de *Trypanosoma cruzi* entre individuos de *R. prolixus* es común, y más frecuente cuando los triatóminos no infectados están hambrientos. La transmisión se hace probablemente por la introducción de la proboscis en el abdomen de otros individuos llenos de sangre y por la ingestión de heces de triatóminos infectados. Ningún caso de transmisión directa de *Trypanosoma rangeli* entre *R. prolixus* fue observado.

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