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Developmental stages of *Trypanosoma cruzi*-like flagellates in *Cavernicola pilosa*

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Abstract: The developmental stages of *Trypanosoma cruzi* ssp., found in the intestinal tract of *Cavernicola pilosa*, are described and measurements given for nine life stages. The frequencies of the various stages in foregut, midgut and hindgut of the triatomines are provided; parasites were rare in the foregut and metatrypomastigotes were seen only in the mid- and hindguts. All adult bugs examined harboured intestinal infections of *T. cruzi*-like flagellates, large clumps of amastigotes were frequently observed in the midgut. The faeces of *C. pilosa*, containing metacyclic trypomastigotes, did not produce patent parasitaemia when inoculated into mice. Inoculated mice were not protected against subsequent challenge infections with the highly virulent Tulahuen stock of *T. c. cruzi*. The blood of bats also failed to produce parasitaemia when inoculated into mice, nor were the mice protected against subsequent challenges with *T. c. cruzi*. Although the developmental stages described were very similar to those of *T. c. cruzi* it is presumed that they were stages of *T. c. marinkellei* because of their failure to infect mice and *Rhodnius prolixus*, and their failure to protect inoculated mice against challenge with *T. c. cruzi*.

The role of bats infected with *Trypanosoma cruzi*-like flagellates in the epidemiology of human Chagas' disease is still uncertain. Under laboratory conditions several triatomine species are capable of transmitting *T.c. cruzi* to and from bats (Marinkelle, 1976) but information on the suspected natural vector, *Cavernicola pilosa* is scarce (Hoare, 1972). *Cavernicola pilosa* is a triatomine bug intimately associated with bats. It has been reported from Brazil, Colombia, Panama and Venezuela (Lent and Jurberg, 1969; Marinkelle, 1972). Dias *et al.* (1942) and Marinkelle (1967) reported a high incidence of *T. cruzi*-like flagellates in the intestine of these triatomines, but their detailed morphology has not been described.

Two subspecies of *T. cruzi* have been reported from neotropical bats (Baker *et al.*, 1978) and *C. pilosa* is suspected of transmitting both. The main purpose of this paper is to describe the morphological characteristics of the various flagellate stages found in the intestine of *C. pilosa*.

MATERIAL AND METHODS

Cavernicola pilosa bugs were collected in a small cave near the town of Honda in Central Colombia. These triatomines are photophobic and when disturbed crawl very rapidly into cracks in the cave walls. By quickly scraping the cave wall with a piece of paper 130 adults, 16 nymphs and 24 larvae were deposited in a large plastic bag. In the laboratory the intestinal contents of the larvae and nymphs were examined in saline solution for flagellates. Haemolymph obtained from legs of 30 adults was examined. The bugs were subsequently dissected and the contents of the salivary glands, malpighian tubes, foregut, midgut and hindgut were separately examined under the light microscope. When flagellates were present the preparations were washed with saline, air dried, fixed with methyl alcohol and stained with Giemsa. The frequencies of amastigotes, sphaeromastigotes, epimastigotes and trypomastigotes in the three sections of the intestine were studied by counting at random 2005 flagellates in each of 20 adult *C. pilosa*. Seven hundred forms of different developmental stages of the flagellates were drawn with the aid of a camera lucida and measurements taken from the drawings.

Pools of intestinal contents containing flagellates from 30 *C. pilosa* were injected intraperitoneally (i.p.) into 30 male albino mice

(20 ± 2 g each). Blood from the tail of the mice was examined for trypomastigotes four times at weekly intervals. Fifteen mice were killed one month after inoculation and impression smears of liver, spleen, heart, lung and skeletal muscles examined for amastigotes. The other 15 mice were challenged with approximately (10^5) haematozoic trypomastigotes of *T.c. cruzi* Tulahuen stock. The challenged mice were observed for 30 days after the second inoculation and the day of death recorded (D'Alessandro, 1963).

Eighty bats, 22 *Desmodus rotundus,* 20 *Artibeus jamaicensis,* 18 *Carollia perspicillata* and 20 *Glossophaga soricina* were caught in the same cave and examined for trypanosomes by standard techniques. These techniques included examination of fresh blood obtained by cardiac puncture, Giemsa-stained blood films, blood cultures in diphasic blood agar NNN media, and inoculation (i.p.) of mice with positive cultures for detection of patent parasitaemia or protection against a subsequent challenge infection with *T. c. cruzi* Tulahuen stock (D'Alessandro, 1963). For 10 of the *A. jamaicensis,* blood concentration methods (Deane and Kirchner, 1962) were applied; xenodiagnosis was performed on eight others using six laboratory reared fourth-instar larvae of *Rhodnius prolixus* per bat.

RESULTS

All adult *Cavernicola pilosa,* six nymphs and one larva, presented intestinal infestation with flagellates. The haemolymph, salivary glands and malpighian tubes were not infested. Few forms were found in the foregut, the majority of these were triangular or short sausage-shaped trypomastigotes with the nucleus and kinetoplast widely separated and the flagellum alongside the periphery of the body (Figs. 1-2). Less frequent were rounded or pear-shaped trypomastigotes, sometimes with a single or double loop of the flagellum protruding from the cell (Figs. 3-6) and least frequent were small sphaeromastigotes (Table 1, Fig.10).

In preparations made from the midgut, large clumps of hundreds of amastigotes with poorly defined walls, were frequently observed. Single amastigotes (Figs. 7-9), sphaeromastigotes (Figs. 7-9), sphaeromastigotes (Figs. 10-12) and pear-shaped epimastigotes (Figs. 13-18) were sometimes found (Table 1). Some of these

TABLE 1

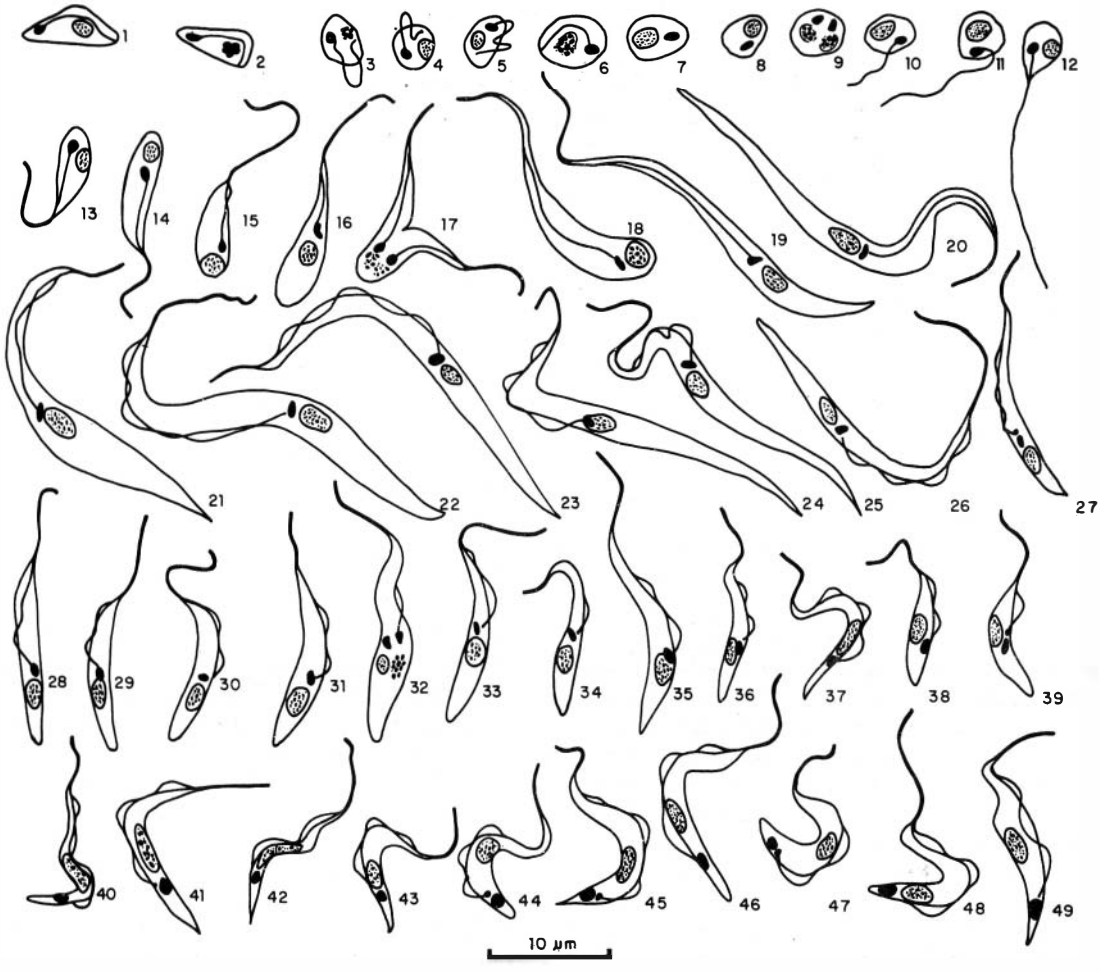
Frequency of different developmental stages of Trypanosoma cruzi *ssp. in the intestine of 20* Cavernicola pilosa

Number of stages counted	Figs. No.	Foregut 100	Midgut 20,000	Hindgut 20,000
Flagellate stage				
Short sausage-shaped trypomastigotes	1- 2	81%	–	1%
Round trypomastigotes	3- 6	12%	1%	1%
Amastigotes	7- 9	–	4%	1%
Large clumps of amastigotes	–	–	Frequent	–
Sphaeromastigotes	10-12	7%	6%	2%
Pear-shaped epimastigotes	13-18	–	10%	6%
Ibidem but in division	17	–	2%	–
Large elongated epimastigotes	19-25	–	10%	3%
Short spindle-shaped epimastigotes	27-36	–	68%	66%
Ibidem but in division	32	–	2%	2%
Early metacyclic trypomastigotes	37-39	–	2%	3%
Metacyclic trypomastigotes	40-49	–	3%	15%

forms were in the process of binary fission (Figs. 9,17). The most frequent stages were large elongated epimastigotes (Figs. 19-25, Table 1). A few short spindle-shaped epimastigotes (Figs. 27-36) less than 33 µm in total length (Table 2) were seen, as well as a few other flagellate stages (Table 1). The short spindle-shaped epimastigotes were often present in rosettes of 20 to 35 cells. The preparations of the hindgut contents were similar to those from the midgut, but more trypomastigotes (Table 1, Figs. 37-49) were present. A small proportion of the trypomastigotes were early metatrypomastigotes with the kinetoplast close to the nucleus (Figs. 37-39). The late metatrypomastigotes, with a large subterminal kinetoplast, were usually stout but an occasional slender form with elongated nucleus was seen (Figs. 40-42). The nucleus of the trypomastigotes was usually posteriorly placed.

The morphological characteristics of the flagellates permitted an artificial differentation between nine different stages, for which the measurements are given in Table 2. The flagellate densities in midgut and hindgut were similar and were estimated to be approximately 5000 to 16000 in each of the two parts of the intestine of 20 bugs. Not included in these counts are the thousands of amastigotes found in the midgut, since estimates of the numbers were difficult to make.

Trypanosoma cruzi ssp. infections were found in only 12 of 20 *A. jamaicensis* bats. Trypomastigotes were present in the Giemsa-stained preparations from two bats, detected by blood concentration in 10 bats and found in cultures of the blood of 12 bats. No parasitaemia was produced in mice during the



Figs. 1-49. Line drawings of flagellate stages of *Trypanosoma cruzi* ssp. found in the intestine of the bat bug, *Cavernicola pilosa*, Figs. 1-2. Short sausage-shape of triangular trypomastigotes, Figs. 3-6. Rounded trypomastigotes, Figs. 7-9. Amastigotes, Figs. 10-12. Sphaeromastigotes. Figs. 13-18. Pear-shaped epimastigotes, Fig.17, Dividing epimastigote. Figs. 19-25. Large elongated epimastigotes. Fig.26. Transitional form, Figs. 27-36. Short spindle-shaped epimastigotes, Fig. 32. Dividing epimastigote. Figs. 37-39. Early metacyclic trypomastigotes, Figs. 40-49. Late metacyclic trypomastigotes.

30 days following the inoculation of cultured flagellates.

None of the mice inoculated with the intestinal contents of infected *C. pilosa* demonstrated parasitaemia over a period of 30 days. No amastigotes were found in the impression smears of the organs of the inoculated mice. All mice challenged with the lethal stock of *T. c. cruzi* died between the 10th and 12th day after inoculation.

None of the *R. prolixus* used in xenodiagnosis on the bats demonstrated infestation with intestinal flagellates when examined after 1,2 and 3 months.

DISCUSSION

Although bats may harbour five different morphologically similar species or subspecies of *Schizotrypanum* (*T. cruzi cruzi, T. c. marinkellei, T. vespertilionis, T. dionisii dionisii* and *T. d. breve*), only two subspecies of *T. cruzi* have been previously demonstrated by biochemical methods to occur in neotropical bats. All *Schizotrypanum* isolates from the 12 *A. jamaicensis* bats inhabiting the cave are presumably *T. cruzi marinkellei* since inoculated cultures did not protect mice against subsequent challenge infection with the lethal

TABLE 2

Measurements in µm of flagellate stages of Trypanosoma cruzi *ssp. in the intestine of* Cavernicola pilosa

Flagellate stage	Number	PK	KN	PN	NA	A	F	W	TL	NI	KI
Short sausage-shaped trypomastigotes	50	1	3	4-6	–	1-2	5-10	2-3.5	6-12	–	1.3-2.0
Round trypomastigotes	50	–	1-2	–	–	1-2	5-11	2	4	–	–
Amastigotes	50	–	1-2	–	–	1-2	–	2-5	4-6	–	–
Sphaeromastigotes	50	–	1-2	–	–	1-2	1-18	2-5	4-6	–	–
Pear-shaped epimastigotes	100	5-20	1-2	0.5-5	4-21	1-2	1-14	1-3	7-25	0.02-1.2	0.25-4.8
Large elongated epimastigotes	100	11-20	1-2	7.2-20	11-25	1.5-3	4-12	1.5-3	33-50	0.3-1.9	3.6-9.8
Short spindle-shaped epimastigotes	100	4-10	1-3	3-8	7-14	1-3	1-8	1.5-2	11-27	0.2-1.0	1.0-7.9
Early meta-trypomastigotes	100	3-4	0.5-1.5	4-5.5	4.5-9	2-3	3-7.5	1.5-2	15-20	0.4-1.2	2.6-2.9
Metatrypomastigotes	100	1-4.5	1-5	2-8.5	6-11	2-4.5	2.5-10	1-3	17-26	0.2-1.4	0.4-8.4

*PK= distance from posterior end to kinetoplast; KN from kinetoplast to middle of nucleus; PN= from posterior end to middle of nucleus; NA= from middle of nucleus to anterior end; N=length of nucleus; F=length of free flagellum; W=maximum width; TL= total length; NI (nuclear index)= PN/NA; KI (kinetoplastic index) = PN/KN.

stock of *T. c. cruzi*. If some of the bats had harboured the nominate subspecies of *T. cruzi*, the challenged mice would have survived more than 16 days after the challenge infection (Kagan and Norman, 1961; Baker *et al.*, 1978). Morever the mice did not demonstrate parasitaemia after inoculation of the trypanosome cultures and the xenodiagnoses failed to demonstrate the presence of flagellates. The trypanosomes found in artificial culture media were also identical with those of *T. cruzi* but different from *T. dionisii* (Baker *et al.*, 1978; Baker and Miles, 1979). These criteria are sufficient to indicate that *T. c. marinkellei* was present in the examined bats. Unfortunately it was not possible to verify the identification by the biochemical methods used by Baker *et al.* (1978), since these techniques were not available at the time of this study. The fact that the nominal subspecies of *T. cruzi* could not be found in the 80 bats examined does not exclude its presence in *C. pilosa* which may have fed on other bats. In fact it is most likely that *T. c. cruzi* is also capable of infecting *C. pilosa* since in previous studies amastigotes were found in mice inoculated with the faeces of cavernicolid bugs caught in a hollow tree (Marinkelle, 1966). For this reason it cannot be established whether or not some of the developmental stages in the *C. pilosa* belonged to *T.c. cruzi*. Nevertheless, the intestinal flagellates of *C. pilosa* reported in this study did not produce parasitaemia in mice and did not protect the mice againt challenge infection. Therefore it seems most likely that the stages described are those of *T. c. marinkellei*.

Shape and size of the developmental stages were identical with those of *T. c. cruzi* (Petana, 1971; Hoare, 1972), although no reports could be found of the existence of large clumps of amastigotes in triatomines infected with *T.c. cruzi*. The observed frequencies of the various stages of flagellates in the different parts of the intestine are similar to those of *T. c. cruzi* developing in *Triatoma dimidiata* (Petana, 1971). Although only *T. cruzi*-like flagellates were observed in the present study. *T. rangeli*-like flagellates have also been found in cavernicolid bugs (Marinkelle, 1967).

Since metacyclic trypomastigotes were encountered only in mid- and hindgut, bats must become infected with the trypanosome by contamination with the faeces or by ingestion of the vector.

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RESUMEN

Se describen los estadios de desarrollo de *Trypanosoma cruzi* ssp., encontrados en el tracto intestinal de *Cavernicola pilosa*, así como las mediciones de nueve estadios de este

flagelado. Se presentan las frecuencias de los diferentes estadios en el intestino anterior, intestino medio e intestino posterior de los triatomineos, encontrándose muy baja frecuencia en el intestino anterior mientras que los metatripomastigotes fueron hallados solamente en el intestino medio y posterior. Todos los 130 insectos examinados albergaban infecciones intestinales con flagelados parecidos a *T. cruzi.* Se observaron frecuentemente grandes aglomeraciones de amastigotes en el intestino medio de los insectos. Las heces de *C. pilosa* que contenían tripomastigotes metacíclicos, no produjeron parasitaemia patente al ser inoculadas en ratones. Estos ratones inoculados no demostraron protección contra una subsecuente inoculación con la virulenta cepa Tulahuen de *T. c. cruzi.* La sangre de los murciélagos no produjo parasitemia al inocularla en ratones y tampoco los protegió contra la subsecuente infección con *T. c. cruzi.* Aunque los estadios de desarrollo descritos son bastante similares a los de *T. c. cruzi,* se presume que corresponden a estadios de *T. c. marinkellei,* dada su incapacidad de infectar tanto ratones como *Rhodnius prolixus,* así como la ausencia de protección en los ratones inoculados posteriormente con *T. c. cruzi.*

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