ANNALES DE PARASITOLOGIE
HUMAINE ET COMPARÉE

Tome 57 1982 N° 2

Annales de Parasitologie (Paris)
1982, t. 57, n° 2, pp. 111-119.

MÉMOIRES ORIGINAUX

MORPHOLOGICAL AND BIOLOGICAL FEATURES OF *TRYPANOSOMA (HERPETOSOMA) MARIAE* MELLO, 1978 IN EXPERIMENTALLY INFECTED *CALOMYS CALLOSUS*[1]

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SUMMARY. In this paper some observations on the biology of *Trypanosoma (Herpatosoma) mariae*, Mello, 1978 are presented. The experiments were carried out in *Calomys callosus* (Rodentia), reservoir of this parasite.
The prepatent period varied from 6.6-10.5 days and the patent infection varied from 38-141 days. Amastigote forms in division were seen mainly among the cellular spaces of the spleen. Only trypomastigote forms were seen in the blood stream. Measurements of these forms showed that the total lentgh varied with the course of the infection.

Caractères morphologiques et biologiques de *Trypanosoma (Herpatosoma) mariae*, Mella. 1978, chez son hôte habituel, *Calomys callosus*, infesté expérimentalement.

RÉSUMÉ. Dans ce travail, sont présentées des observations sur la biologie du *Trypanosoma (Herpetosoma) mariae* Mello, 1978, dans son hôte normal *Calomys callosus* (Rodentia).
Les résultats obtenus ont démontré que la période prépatente varie de 6,6 à 10,5 jours et la puissance de l'infection est restée entre 38-141 jours.
Les formes amastigotes en divisions ont été trouvées parmi les espaces cellulaires de la rate, alors que dans le sang périphérique on n'a trouvé que les formes trypomastigotes.
Plusieurs mesures ont été prises pendant les jours qui ont suivi les inoculations. Les résultats de ces mesures ont démontré des variations en rapport avec l'évolution de l'infection.

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1. This work was supported by a grant of National Research Council of Brazil (SIP/08-032-CNPq).

Accepté le 15 septembre 1981.

Introduction

Trypanosoma (Herpetosoma) mariae, first described by Mello (1978), is a specific parasite of *Calomys callosus*, a cricetid rodent of wide distribution in Brazil.

This trypanosome is little known. Seven rodent species have been shown to be resistant to experimental infection with this parasite: *Oryzomys eliurus, O. subflavus, Zygodontomys lasiurus, Cercomys cunicularius, Cavia aperea, Mus musculus, Rattus norvegicus.* Also it has been shown experimentally that fleas are probably the vectors of *T. mariae* (Mello, 1978).

In the current paper some complementary biological features of *T. mariae* are described, such as: prepatent period, parasitamia levels, distribution of reproductive forms and morphological variations of the blood forms during the " adult phase " and " reproductive phase ".

Material and methods

1 — The strain studied

The *T. mariae* strain used was the original one described by Mello (1978).

Experiments were begun one year after the first isolation of *T. mariae.* This strain was maintained by serial intraperitoneal inoculation in *C. callosus*, bred in the laboratory. (Mello, 1978.)

2 — Inocula

All inoculations were made intraperitoneally using citrated blood obtained from the tail of infected *C. callosus.*

Blood parasites in fresh preparations were counted at 500x by the method of Brener (1961), and numbers expressed per $5mm^3$. The inocula, used contained either 370, 1,850 or 3,850 trypomastigotes.

3 — Evolution of the infection

Three groups of six *C. callosus, (Table I)* 1.5 to 2 months old were used. The animals' tail blood was examined daily begining 72 hours from the inoculation, to determine the prepatent period. Counts of circulating parasites were made on alternate days, until parasites were no longer seen in the blood.

TABLE I. — Prepatent period in *Calomys callosus* experimental infected with *Trypanosoma (Herpetosoma) mariae.*

Nº parasites inoculated	prepatent period (days) mean	range
370	10.5	9 — 12
1,850	7.3	7 — 13
3,850	6.6	6 — 7

4 — SEARCH FOR REPRODUCTIVE FORMS

29 *C. callosus*, aged 1.5 to 2 months, were inoculated with 1,850 trypomastigotes. The animals were killed after inoculation at the following times: 2, 3, 5, 7, 8, 12, 13, 15, 17, 20, 22, 24, 30, 38 and 72 hours (a animal at each time) 6, 18, 40, 48 and 120 hours (2 animals each) 96 hours (3 mice). From all of them, pieces of heart, brain, kidney, lung, spleen and liver were taken at necropsy, fixed in 10% formalin and stained with haematoxylin and eosin. Impression smears were made, fixed with methanol and stained with Giemsa-May Grünwald. Fresh blood samples were also examined from all killed infected animals.

5 — MORPHOLOGY OF THE BLOOD CIRCULATING FORMS

Morphological studies were made from the blood of one animal inoculated with 1,850 parasites. 20 trypomastigote forms were measured on different days after the inoculation as shown in *table II*.

The following measurements were made: the distance from the posterior end to kinetoplast (PK); the distance from the kinetoplast to the center of the nucleus (KN); the distance from the centre of the nucleus to the anterior end (NA); the distance from the posterior end of the body to the end of the free flagellum i.e., total lentgh (TL), and free flagellum (FF).

Means and their standard deviations were calculated for each group of measurements.

Results

1 — EVOLUTION OF THE INFECTION

Duration of the prepatent period is shown at *table I*. There was a relation between the number of parasite inoculated and the lentgh of the prepatent period. The mean duration of patent infection in all animals inoculated, was 49.9 (± 7, 8)

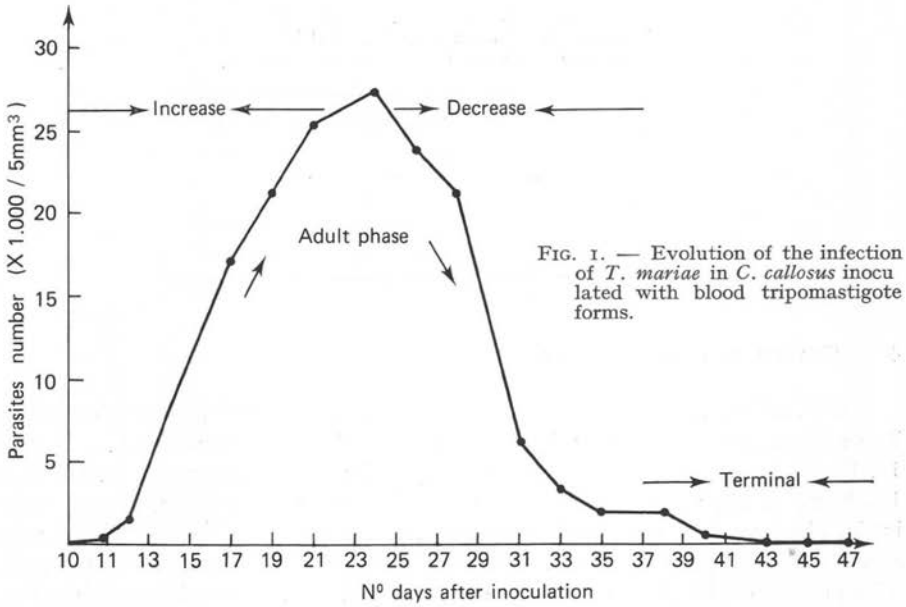

FIG. 1. — Evolution of the infection of *T. mariae* in *C. callosus* inoculated with blood tripomastigote forms.

TABLE II. — Parasitaemia average during the course of infection of *trypanosoma (Herpetosoma) mariae* in six *Callomys callosus* inoculated with 370 trypomastigote forms.

Days after infection	nº of parasites/5mm³
10	0
12	1.346
14	8.866
17	16.128
19	22.481
21	25.907
24	27.302
26	24.100
28	21.270
31	6.825
33	3.058
35	2.104
38	2.175
40	579
43	4
45	6
47	2
50	0
52	1
54	0
57	0
59	0
61	0
64	0

days. The range was 38 (animal inoculated with 3,850 parasites) to 141 days (animal inoculated with 1,850 parasites).

Table II and *figure 1* show the intensity and duration of the parasitamia, as well as the phases of infection, in a group of *C. callosus* inoculated with 370 trypomastigotes.

The parasite population attained its peak at the 24th day after infection (27,302/5mm^3). The average duration of patent parasitaemia was 57 days.

In animal groups inoculated with 1,850 and 3,850 parasites the course of the infection was very irregular making meaningless the determination of parasitamia mean values. The duration of patent infection of the above groups were as follow: 47, 54, 57, 58, 59, 141 days and 29, 40, 42, 45, 59 (twice) days, respectively.

2 — Reproductive forms

In *C. callosus* killed 2, 3 and 5 hours after the infection, trypomastigote forms were found in the blood circulation. However no parasites were found in any organ smear examined from these animals. It is likely that the trypomastigotes were those originally inoculated.

In one animal killed 13 hours after inoculation, masses of parasites with numerous nuclei were found in the spleen (*fig. 2*).

Similar pockets of amastigote forms were also seen, mainly in the spleen and a lower number in the liver, of the animals killed after 15 *(fig. 3 a-c)*, 17, 18, 22, 36, 38 *(fig. 3 d, e)*, 40, 48 *(fig. 3 f, g)* and 72 hours. All these masses were seen among the cellular spaces where some amastigotes were lying free *(fig. 3 h, i*-15 hours; *fig. 3 j, l,* 40-48 hours).

Only the animal killed 20 hours after infection failed to show amastigotes. One animal killed at 120 hours after infection showed trypomastigote forms without a visible flagellum in the spleen *(fig. 3, m-r);* the same animal showed broad trypomastigotes in the lung *(fig. 3, s-u)*.

It was also possible to find trypomastigote forms with marked vacuolation in the spleen and liver of the animals killed after 36, 38, and 48 hours, but not in the blood stream. The same aspect was observed in the animals killed 72 ans 120 jours after inoculation.

The amastigotes and the vacuolated trypomastigotes were very fragile and usually fragmented during the preparation of the slide.

Parasites were not found in any tissue section.

3 — Measurement of bloodstream forms

Table III shows the means and standard deviations of measurements of bloodstream forms during the course of infection.

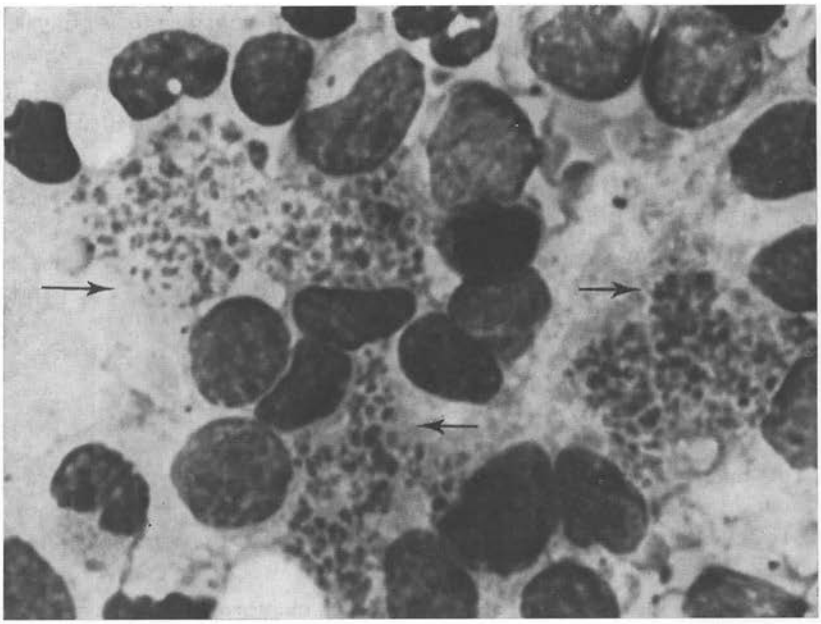

FIG. 2. — Photomicrograph of reproductive forms of *Trypanosoma (Herpetosoma) mariae* from spleen tissue of an *Calomys calolsus* killed after 13 hours of experimental infection (impression smear stained with Giemsa — × 1,200).

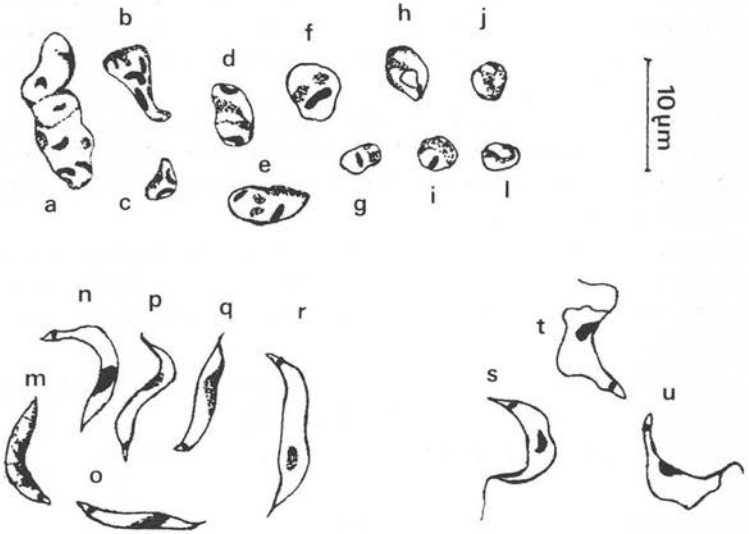

FIG. 3. — Drawings of *Trypanosoma (Herpetosoma) mariae*, obtained from impression smears of spleen, liver and lungs tissues of experimentally infected *Calomys callosus:* a-c, h, i (15 hours - spleen); d-e (38 hours - liver); f-g, j, l (40-48 hours - spleen); m-r (120 hours - spleen); s-u (120 hours - lungs).

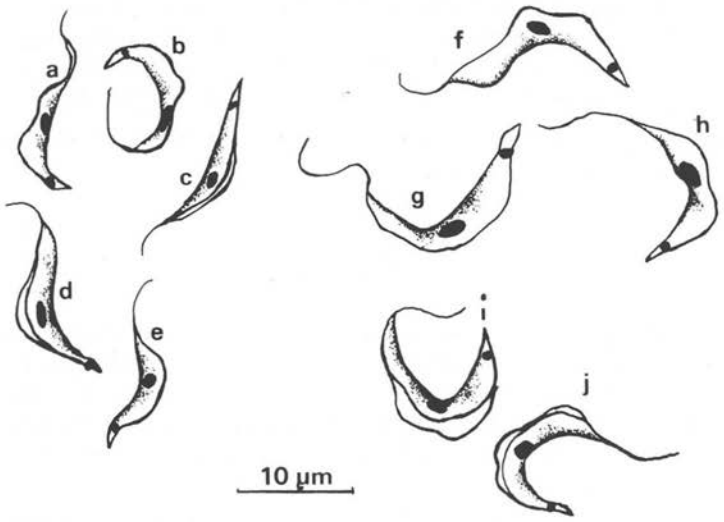

FIG. 4. — Trypomastigote forms from the bloodstream, on the 9th (a-e) and 35th (f-j) days after inoculation.

TABLE III. — Mean dimensions of *Trypanosoma (Herpetosoma) mariae*. (in µm), from bloodstream of experimentally infected *Calomys callosus* with.

Measurements		Days After Infection														
		9	10	11	13	15	17	19	21	26	28	33	35	37	40	44
TL	M	18.5	18.8	19.2	19.6	18.9	20.5	23.8	27.6	26.9	26.4	26.3	27.7	26.6	26.1	26.4
	SD	0.8	0.6	0.6	1.1	0.6	0.7	1.2	1.0	1.8	1.4	1.3	0.9	0.9	0.9	0.9
FF	M	3.9	4.2	5.0	5.2	5.5	4.9	8.4	8.8	7.4	9.2	8.1	7.3	8.1	8.2	6.2
	SD	0.6	0.5	0.6	0.7	0.5	0.6	0.7	0.9	1.3	1.5	0.9	0.5	0.6	0.6	0.8
NA	M	4.9	5.1	4.8	4.2	4.1	5.4	6.7	9.6	8.6	8.5	8.8	9.5	8.3	8.0	8.9
	SD	0.7	0.6	0.8	0.6	0.4	0.6	0.6	0.5	0.7	0.8	0.7	1.0	0.5	0.6	0.9
KN	M	6.1	5.9	5.9	6.1	6.4	7.1	7.6	10.1	9.8	8.8	8.3	8.4	8.7	8.4	8.7
	SD	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.9	0.6	0.7	0.6	0.5	0.5	0.7	0.9
PK	M	1.6	1.5	1.5	1.6	1.6	1.7	1.7	2.2	1.9	1.9	2.0	1.9	1.8	1.9	1.9
	SD	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.2	0.2

TL altered during the evolution of infection. From the 9th day to 17th day after inoculation the mean varied from 18.5 μm to 20.5 μm; on the 19th day it was 23.8 μm; and from the 21st to 51st days it varied from 27.6 μm to 25.3 μm. Also, the mean FF, NA, KN and PK, followed a similar pattern during this time.

Figure 4 (a-j) ilustrates trypomastigote forms from the bloodstream on the 9th and 35th days after inoculation.

Discussion

The duration of infection and the prepatent period of *T. mariae* inoculated in *C. callosus*, were similar to the other trypanosomes of the subgenus *Herpetosoma* (Hoare, 1972). Only in the groups of *C. callosus* inoculated with 1,850 and 3,850 trypanosomes were the highest number of parasites found in the bloodstream. The course and duration of the patent infection were probably related both to number of parasites and/or individual host response.

The course of infection of *T. mariae* in a group of *C. callosus* inoculated with 370 parasites *(fig. 1)* was characterized by a rapid rise of the initial parasitamia (increase) followed by a gradual decline to a low level (decrease) towards the end of the infection (terminal), as show by Hilton and Mahrt (1972) in *T. otospermophili.*

The site of development of *T. mariae* was probably confined to the visceral capillaries and inter-cellular spaces of spleen and liver. Although the steps of the multiplication process have not been completely clarified it is believed that *T. mariae* reproduces by multiple fission in amastigote forms like *T. zapi* (Davis, 1952), *T. microti* (Molyneux, 1969) and *T. evotomys kudickei* (Molyneux, 1976) (*fig. 2* and *fig. 3, a-u*).

The trypomastigote forms without flagella *(fig. 3, m-r)* suggest a transitional stage from which adults develop. Probably these forms were developed during transformation of the amastigotes.

T. mariae never exhibits dividing forms in the bloodstream. This confirms the observation of Molyneux (1976), that species of *Herpetosoma* subgenus which reproduce in the amastigote stage never divide in the peripheral blood. The variations of the size of bloodstream forms could reflect the reproduction occuring in the tissues. *Table III* shows a great variation in total lentgh (18.5 μm-26.6 μm) related to the stage of infection. These results indicate that the most accurate measurement can only be obtained after 21 days of infection when TL has become uniform.

Davis (1952) studied *T. zapi* concluded that within a single population of trypanosomes, the inherent variability of total length and the other measurements " is so great these that measurements could not be used to distinguish clearly between populations of *lewisi*-like trypanosomes derived from different hosts ". Hilton and Mahrt (1972) studing the size and variation of various strains of *T. otospermophili*, concluded that the intensity and the course of infection were dependent on the host.

Molyneux (1969) studing *T. microti* considered that the " adult phase " was the stage of the infection in which parasitamia was stable or declining and the " reproductive phase " the stage in which the number of parasites in the blood was increasing. *Figure 1* shows that " adult phase " of *T. mariae* began, approximately, at the 21st day after infection. The " reproductive phase " of this trypanosome may be long, since the prepatent period varied form 9-12 days *(table I)* when *C. callosus* was inoculated with 370 blood parasites. The long " reproductive phase " ,as pointed out by Molyneux (1969), explains the difficulty in finding reproductive forms.

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