

NYCTHEMERAL VARIATION IN THE PARASITAEMIA OF *TRYPANOSOMA MINASENSE* IN NATURALLY INFECTED MARMOSETS OF THE GENUS *CALLITHRIX* (PRIMATES, CALLITHRICIDAE)

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SUMMARY

Nycthemeral parasitaemia of *Trypanosoma minasense* in marmosets of the species *Callithrix penicillata jordani* and *C. geoffroyi* was measured in two series of experiments: in one, the parasites were counted in the peripheral blood collected at 6:00, 12:00, 18:00 and 24:00 hours, and the density of the trypanosomes was seen to be usually much higher at sunset or midday than at sunrise or midnight. In the other group of experiments, in which the blood was taken every two hours starting also at 6:00, parasitaemia reached its peak at 16:00 hours, decreasing thereafter and remaining low during the night and morning. This indicates a circadian rhythm and its implications are discussed in the article.

INTRODUCTION

Trypanosoma minasense CHAGAS, 1908 is a very common haematozoon in marmosets of the genus *Callithrix*. Discovered by CHAGAS^{2, 3} in *Callithrix penicillata* from the State of Minas Gerais, Brazil, it was described immediately after by CARINI¹ in another species, *C. jacchus*, from the State of São Paulo and later recorded by other workers, in specimens of the first species of host from diverse areas. The senior Author of the present paper has found *T. minasense* in a large proportion of *Callithrix* from various regions of Brazil: *C. penicillata jordani* from the States of Goiás, Minas Gerais and Bahia, *C. geoffroyi* from the States of Minas Gerais and Espírito Santo, and *C. jacchus* from the States of Bahia, Sergipe, Alagoas and Ceará. The presence of this trypanosome in primates of other genera has been reported

several times and we intend to discuss such findings in another article.

T. minasense is typically a large and broad trypanosome with pointed extremities, a large vacuole in front and another behind the nucleus, which stands almost at the center and usually in a transverse position; the kinetoplast is very far from the posterior tip, more often closer to the nucleus, and the free flagellum is always present. It was placed by HOARE⁵ in the subgenus *Megatrypanum*. Only the blood forms are known. We have not been able to cultivate it, and attempts by other workers yielded inconsistent results. It has not been successfully inoculated in laboratory animals and its natural vectors have not been discovered. It was, indeed, our interest in finding its vectors that lead us to the experiments described in this arti-

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cle. We started in the beginning of 1971, with a series of trials to investigate the fate of *T. minasense* in various haematophagous insects. One of the difficulties early confronted was the usually very low parasitaemia in the marmosets. We tried, unsuccessfully, to raise it by cortisone, X-radiation or immunosuppression, and while doing this we had the opportunity to do repeated counts of the trypanosomes in the blood of the animals and soon noticed that there was a great variation in the parasitaemia apparently related to the hour of the day when

the blood was taken. This was later confirmed by the experiments mentioned below.

MATERIAL AND METHODS

From August, 1971 to March, 1973, a total of 30 experiments were carried out to measure the parasitaemia, at different hours of the day and night, in marmosets naturally infected with *T. minasense*. We used 15 Brazilian marmosets of the genus *Callithrix* Erxleben, 1777: 8 *C. penicillata jordani* Thomas, 1904 and 7 *C. geoffroyi* Humboldt, 1812. The origin of the marmosets were as follows:

Marmoset n.º	Species	State	Municipality
1872*	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
1950	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
1952	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
1955	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
1961	<i>C. geoffroyi</i>	Minas Gerais	Araçuaí
2010	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
2026	<i>C. penicillata jordani</i>	Minas Gerais	Curvelo
2027	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
2046	<i>C. penicillata jordani</i>	Minas Gerais	Lagôa Santa
2114	<i>C. geoffroyi</i>	Minas Gerais	Pedra Azul
2129	<i>C. geoffroyi</i>	Espírito Santo	Colatina
2131	<i>C. geoffroyi</i>	Espírito Santo	Colatina
2132	<i>C. geoffroyi</i>	Espírito Santo	Colatina
2167	<i>C. geoffroyi</i>	Espírito Santo	Colatina
2168	<i>C. geoffroyi</i>	Espírito Santo	Colatina

Two series of experiments were performed: in the first 23 experiments, trypanosomes were counted in the blood collected every six hours — at 6:00, 12:00, 18:00 and 24:00, i. e., at about sunrise, midday, sunset and midnight. In the remaining experiments, nos. 24 to 30, the blood was taken every two hours — at 6:00, 8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00, 24:00, 2:00 and 4:00.

To obtain the blood samples a small cut was made at the margin of the marmoset's ear, and 5 cubic millimeters of blood was aspirated in a graduated pipette and transferred to a glass slide where a thick rectangular smear was prepared, measuring 1 by 1.5 centimeter. The blood was stained by Giem-

sa's and examined with oil-immersion, all trypanosomes found in the whole smear being counted.

As seen in Table I, 8 marmosets were used only once (nos. 1950, 1952, 1955, 1961, 2026, 2027, 2028 and 2132); 4 were used in two experiments (nos. 2010, 2029, 2031 and 2046); 1 in four (no. 2067) and 2 in five (nos. 1872 and 2114).

In experiments nos. 1 to 25 the marmosets were kept under the usual laboratory conditions as to temperature, light and noise. In experiments nos. 26 and 27 the temperature in the laboratory was maintained at 21-22°C for the whole nycthemeral period. In experiment no. 28 the laboratory was kept lighted during the night. Finally, in experiments nos. 29 and 30 we tried to simulate an inver-

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TABLE I

Parasitaemia in marmosets (*) naturally infected with *Trypanosoma minasense*, measured at six-hour intervals, starting at 6:00 ó clock

No. of experiment	No. of marmoset	Trypanosomes in 5 mm ³ of blood, according to hour									
		In numbers					In percentages				
		6:00	12:00	18:00	24:00	Total	6:00	12:00	18:00	24:00	
1	1872	26	219	206	20	471	5.5	46.5	43.7	4.3	
2	1872	37	214	215	34	500	7.4	42.8	43	6.8	
3	1872	81	242	186	26	535	15.1	45.3	34.8	4.8	
4	1872	51	13	123	126	313	16.3	4.2	39.3	40.2	
5	1872	55	177	203	55	490	11.2	36.1	41.5	11.2	
6	1950	2	8	14	7	31	6.5	25.8	45.1	22.6	
7	1952	22	10	93	39	164	13.4	6.1	56.7	23.8	
8	1955	6	8	15	2	31	19.4	25.8	48.4	6.4	
9	1961	51	212	210	46	519	9.8	40.8	40.5	8.9	
10	2010	44	156	172	37	409	10.8	38.1	42.1	9	
11	2010	117	221	229	86	653	17.9	33.8	35.1	13.2	
12	2027	19	94	117	14	244	7.8	38.5	48	5.7	
13	2026	29	113	66	16	224	12.9	50.4	29.5	7.2	
14	2046	26	106	175	43	350	7.4	30.3	50	12.3	
15	2046	45	51	192	29	317	14.2	16.1	60.6	9.1	
16	2129	7	54	68	7	136	5	40	50	5	
17	2114	41	79	211	78	409	10	19.3	51.6	19.1	
18	2132	3	79	107	2	191	1.6	41.4	56	1	
19	2114	31	79	98	77	285	10.9	27.7	34.4	27	
20	2131	6	88	68	4	166	3.6	53	41	2.4	
21	2114	33	54	173	83	343	9.6	15.8	50.4	24.2	
22	2129	17	144	153	23	337	5	42.7	45.4	6.9	
23	2131	11	88	74	2	175	6.3	50.3	42.3	1.1	
24	2167	3	112	302	19	436	0.7	25.7	69.2	4.4	
25	2168	10	121	184	5	320	3.1	37.8	57.5	1.6	
26	2167	6	224	323	12	565	1.1	39.6	57.2	2.1	
27	2167	12	71	323	7	413	2.9	17.2	78.2	1.7	
28	2114	30	14	76	38	158	19	8.9	48.1	24	
29	2167	8	261	159	6	434	1.8	60.2	36.6	1.4	
30	2114	16	41	84	50	191	8.4	21.5	43.9	26.2	
Total		845	3353	4619	993	9810	8.6	34.2	47.1	10.1	

(*) *Callithrix penicillata jordani* in experiments nos. 1 to 8 and 10 to 15, *C. geoffroyi* in experiments nos. 9 and 16 to 30.

In experiments nos. 24 to 30 the counts were made every two hours, but the figures corresponding to the 6:00, 12:00, 18:00 and 24:00 hours counts were also included in the present Table.

TABLE II
Parasitaemia in marmosets (*) naturally infected with *Trypanosoma minasense*, measured at two-hour intervals, in experiments nos. 24 to 30

Hour	Trypanosomes in 5 mm ³ of blood, according to hour															
	In numbers						In percentages									
	Exp. 24	Exp. 25	Exp. 26	Exp. 27	Exp. 28	Exp. 29	Exp. 30	Total	Exp. 24	Exp. 25	Exp. 26	Exp. 27	Exp. 28	Exp. 29	Exp. 30	Total
6:00	3	10	6	12	30	8	16	85	0.2	1.3	0.4	0.9	4.9	0.6	2.5	1.1
8:00	4	8	7	6	20	49	21	115	0.4	1	0.4	0.4	3.3	3.4	3.3	1.5
10:00	20	13	26	6	16	86	23	195	1.5	1.6	1.6	0.4	2.6	6.1	4.4	2.5
12:00	112	121	224	71	14	261	41	844	8.2	15.2	14.4	5.2	2.3	18.4	6.5	10.8
14:00	210	186	369	275	23	277	81	1421	15.3	23.2	23	20.1	3.8	19.5	12.7	18.2
16:00	508	214	530	541	225	474	156	2648	37	26.7	33.1	39.5	36.9	33.3	24.7	33.8
18:00	302	184	323	323	76	159	84	1451	22	23.1	20.1	23.6	12.5	11.2	13.2	18.6
20:00	162	30	75	108	48	70	61	554	11.7	3.9	4.7	7.9	7.9	4.9	9.6	7.1
22:00	23	11	11	14	52	9	48	168	1.6	1.4	0.7	1.0	8.5	0.6	7.6	2.2
24:00	19	5	12	7	38	6	50	137	1.3	0.6	0.7	0.5	6.2	0.4	7.9	1.8
2:00	5	6	4	2	41	4	29	91	0.4	0.8	0.3	0.1	6.7	0.3	4.6	1.2
4:00	5	10	10	5	27	18	19	94	0.4	1.2	0.6	0.4	4.4	1.3	3	1.2
Total	1373	798	1597	1370	610	1421	634	7803	100	100	100	100	100	100	100	100

(*) The marmosets used in each experiment were: no. 2167 in experiments nos. 24, 26, 27 and 29; no. 2168 in experiment no. 25; and no. 2114 in experiments nos. 28 and 30.

sion in the conditions of day and night by keeping the marmosets, for six days prior to the experiments and during the experiments, in a dark and silent room and without food during the day and in a lighted room with a radio broadcasting during the whole night, when also food was given to the animals.

RESULTS

Table I shows the parasitaemia observed at six-hours intervals. It includes not only the twenty-three experiments in which the blood was collected at 6:00, 12:00, 18:00 and 24:00 hours, but also the results obtained at these hours in the seven experiments in which the blood was taken every two hours. The percentual figures for each count were obtained in relation to the total number of trypanosomes counted in the four smears of each experiment. In the total counts for all experiments, more than 4/5 of the trypanosomes were found in the samples taken at sunset or midday and less than 1/10 at

either sunrise or midnight. The same trend was observed in the great majority of the individual experiments, the peak of parasitaemia being usually at 18:00 or 12:00 hours.

As seen in Table II and Figure 1, the results of the seven experiments in which the blood was collected every two hours were more elucidative and showed more clearly the pattern of variation in the parasitaemia. By the figures corresponding to the total experiments, a very definite curve was obtained, the peak of parasitaemia being at 16:00 hours, decreasing thereafter and remaining low during the night and early morning. In all seven experiments the highest number of parasites was also at 16:00 hours, and although the individual curves showed some variation, they kept the same trend. No difference was noted by comparing experiments nos. 24 and 25, performed under normal laboratory conditions, with experiments nos. 26, 27 and 28, in which either the temperature was kept constant or the room was lighted during the whole nycthemeral period. However, in experiments nos. 29 and 30, in which for six days before and during the

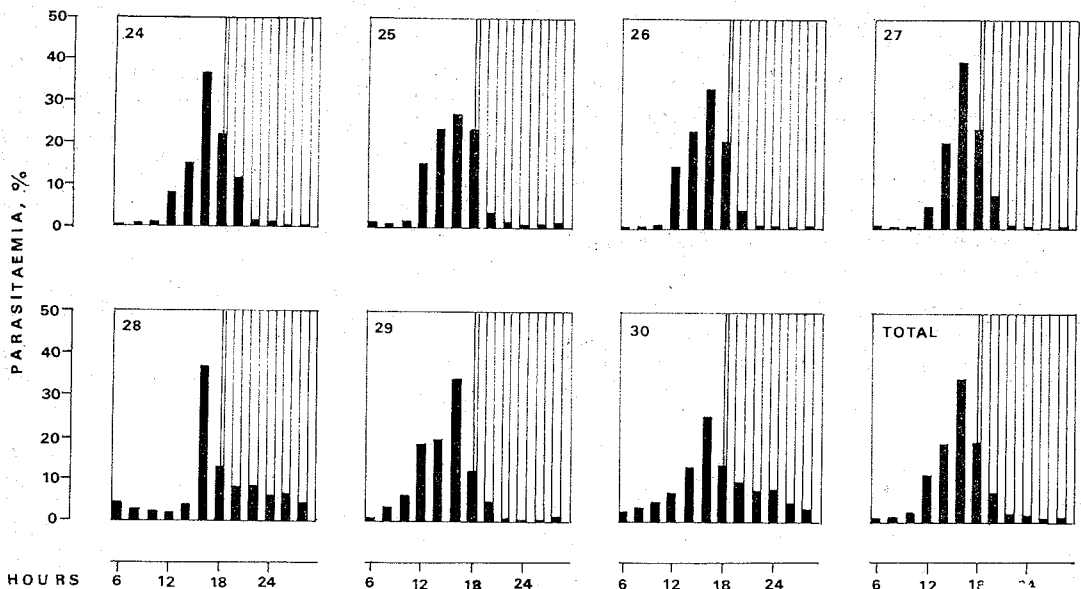


Fig. 1 — Percentual density of *Trypanosoma minasense* in the peripheral blood of *Callithrix geoffroyi* marmosets, in counts made at two-hour intervals, starting at 6:00 o'clock. Experiments nos. 24 to 30 and Totals. (Based on Table II).

experiment the marmosets were kept in a lighted and noisy room during the night and in silence and darkness during the day, although the general trend of the parasitaemia was the same as in the other experiments, a tendency for the flatness of the curve was observed.

COMMENTS

These tests show that parasitaemia of *T. minasense* in naturally infected marmosets of two species — *Callithrix penicillata jordani* and *C. geoffroyi* — is subject to a circadian rhythm. In the counts made at six-hour intervals the highest parasitaemia was found either at sunset or at noon, but the tests performed at shorter intervals revealed that the density of the parasites in the peripheral blood reaches its highest levels in the late afternoon, quite before sunset.

The discovery of a periodicity in the parasitaemia of *T. minasense* has various implications, as we have pointed out in a previous note⁴: it is interesting as a biological phenomenon by itself; it indicates that when parasitaemia is included in any study of this trypanosome, the hour when the blood is taken has to be considered; the same can be said in relation to surveys aiming at determining the rate of parasitism by *T. minasense*, because in cases of very low infections the blood taken in the morning or at night might not reveal the parasites. Finally, on the basis of the present findings, we may speculate that the natural vector of *T. minasense* is likely to be a haematophagous insect with a preference for feeding in the afternoon.

RESUMO

Variação nictemeral na parasitemia do Trypanosoma minasense em saguís do gênero Callithrix (Primates, Callithricidae) naturalmente infectados

Medimos a parasitemia do *T. minasense* em duas espécies de saguís, *Callithrix penicillata jordani* e *C. geoffroyi*, em duas séries de experiências. Na primeira, em que o sangue periférico era colhido a intervalos de seis horas — às 6, 12, 18 e 24 horas — verificamos que a densidade de tripanosomas era geralmente muito maior ao crepúsculo vespertino ou ao meio-dia do que à meia-noite ou ao amanhecer (Tabela I). Na segunda série, em que a colheita de sangue era feita cada duas horas a partir das 6, a parasitemia atingiu seu pico às 16 horas, mantendo-se baixa durante a noite e pelo menos parte da manhã (Tabela II e Fig. 1). Isto denota um ritmo circadiano, cujas implicações são discutidas no texto.

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