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Absence of both circadian rhythm and *Trypanosoma cruzi* periodicity with xenodiagnosis in chronic chagasic individuals

Ausência de ritmo circadiano e de periodicidade do *Trypanosoma cruzi* ao xenodiagnóstico em chagásicos crônicos

Cleudson Castro¹ and Aluizio Prata²

Abstract Xenodiagnoses were performed every 3 hours using 10 *Triatoma infestans* 3rd instar for 24 to 72 hours, in 18 chronic chagasics with positive serology and/or xenodiagnosis. There was no statistically significant difference in the positivity of assays performed during the day (9:00 to 18:00h) compared to those performed at night (21:00 to 6:00h), ($\chi^2 = 0.1526$ $p = 0.696$). Xenodiagnosis was performed in ten of the patients for 13 successive days but there was no periodicity detected in the positive assays.

Key-words: Circadian rhythm. *Trypanosoma cruzi* parasitemia. Serial xenodiagnoses.

Resumo Realizamos xenodiagnósticos de 3 em 3 horas com 10 ninfas do 3^o estágio de *Triatoma infestans* por um período de 24 a 72 horas, em 18 chagásicos crônicos que tinham sorologia e/ou xenodiagnóstico positivos. Não houve diferença estatisticamente significativa na positividade entre os testes realizados durante o dia (9:00 às 18:00h) e à noite (21:00 às 6:00h), ($\chi^2 = 0,1526$ $p = 0,696$). Dez dos pacientes fizeram também xenodiagnósticos diários, durante 13 dias sucessivos, não havendo periodicidade dos testes positivos.

Palavras-chaves: Ritmo circadiano. Parasitemia pelo *Trypanosoma cruzi*. Xenodiagnósticos seriados.

Some authors have expressed interest regarding the study of the circadian rhythm of trypanosomes in primates³ and other animals^{4,8,13}. Nevertheless, despite the extensive literature at hand on *Trypanosoma cruzi* biology, until 1975, no report existed about the circadian rhythm of this parasite⁶. In 1977, the first publication reporting the nycthemeral behavior of *T. cruzi*

in the peripheral blood of a chronically infected patient appeared⁹. Only recently experimental works on this matter have been presented^{5,10,11,12}. With a view to contributing to a better knowledge of the theme, we present the result of xenodiagnoses carried out several times a day and on successive days, as previously mentioned^{1,2}.

MATERIAL AND METHODS

The work was performed in chronically *T. cruzi* infected adult male and female individuals, hospitalized in the Sanatório Espírita of Anápolis, Goiânia State, Brazil, or pertaining to the Military Police of Sobradinho city, DF. All patients were both serology and/or xenodiagnosis (xeno) positive for chagasic infection and they were

hospitalized for the study of parasitemia. A xenodiagnosis was scheduled for each patient, every three hours with 10 *Triatoma infestans* 3rd instar, totaling eight tests every 24 hours. In some patients, besides the above-mentioned assays, xenodiagnoses were scheduled once a day during 13 successive days. The results of the tests

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were expressed as either positive or negative after microscopic assay of a pool of feces between lamina and laminula, resulting from abdominal compression

to a minimum of seven and to the maximum of 10 triatomines. The tests were performed prior to specific treatment with benznidazol.

RESULTS

For the study of parasitemia 18 patients were tested, 13 men and 5 women. Ten performed tests during 24 hours, 4 during 48 hours and 4 during 72 hours, 2 during 45 hours and 1 during 21 hours consecutively (Table 1). A total of 237 xenodiagnoses were performed. Out of these, 87 (36.7%) were positive (Table 2). For 3 patients all xenodiagnoses were negative. The patient that presented greater

positivity had 22 (91.7%) positive xenodiagnoses in 24 tests. At a significance of 1%, no statistical difference was observed in the positivity of the tests in the different schedules. If we consider the daily positivity from 9 a.m. to 6 p.m., and the nocturnal period from 21 p.m. up to 6 a.m., no significant statistical difference was verified between these two periods ($p = 0.696$).

Table 1 - Results of xenodiagnoses performed in 18 patients with the number of tests and the percentage of positivity

Patients	Xenodiagnoses		
	performed	positive	%
ASS	8	3	37.5
HPC	8	7	87.5
LS	8	2	25.0
AMFL	8	1	12.5
ARS	8	2	25.0
LMS	8	4	50.0
AMA	8	6	75.0
MMC	8	4	50.0
BPS	8	5	62.5
FJO	7	6	85.7
NA	24	5	20.8
JMD	24	4	16.7
JFS	24	0	-
IAM	24	22	91.7
JCP	15	0	-
FCA	15	7	46.7
VCS	16	0	-
JFR	16	9	56.2
Total	237	87	36.7

Table 2 - Xenodiagnoses performed in 18 individuals, every 3 hours.

Hours	Xenodiagnoses		
	performed	positive	%
3	29	12	41.4
6	30	12	40.0
9	28	8	28.6
12	28	9	32.1
15	31	13	41.9
18	30	11	36.7
21	30	8	26.7
24	31	14	45.2
Total	237	87	36.7

diurnal schedule from 9 a.m. to 6 p.m. versus nocturnal from 9 p.m. to 6 a.m. $\chi^2 = 0.1526$, $p = 0.696$.

In the evaluation of the parasitemia over 13 successive days, 10 of the patients were studied as follows: 7 during each day, 2 during 12 days, and one performed 9 xenodiagnoses in the period of 13 days (Table 3). Out of the 124 xenodiagnoses in successive days, 47 (37.9%) were positive.

For 2 of the individuals with 13 successive xenodiagnoses all the tests were negative. One presented 12 positive results and another, 10 (Table 3). Observing the sequence of the tests performed (Table 3), no evidence of periodicity was detected.

Table 3 - Results of xenodiagnoses of 10 patients who performed tests several times a day and once a day, in 13 successive days.

Time	Patients									
	ASS	HPC	LS	AMFL	ARS	LMS	AMA	MMC	BPS	FJO
hour	xenodiagnosis every 3 hours									
3	+	+	-	-	+	+	+	+	+	-
6	+	+	-	-	-	+	-	+	+	+
9	+	+	-	-	-	-	-	-	+	-
12	-	+	-	+	-	-	+	-	-	+
15	-	+	+	-	-	-	+	-	-	+
18	-	+	+	-	-	+	+	+	+	+
21	-	+	-	-	-	-	+	-	-	+
24	-	-	-	-	+	+	+	+	+	+
day	daily xenodiagnosis									
1 ^o	-	-	-	+	+	-	-	+	-	+
2 ^o	-	-	-	-	-	-	-	+	-	+
3 ^o	-	-	-	+	+	-	+	-	-	+
4 ^o	-	-	-	-	+	-	+	+	+	-
5 ^o	-	+	-	-	-	-	+	+	-	+
6 ^o	-	-	-	-	+	-	-	+	-	+
7 ^o	-	-	-	-	+	-	-	+	-	-
8 ^o	+	-	-	-	-	-	-	+	-	+
9 ^o	-	+	-	-	+	-	-	+	-	+
10 ^o	-	+	-	-	+	-	+	+	-	+
11 ^o	+	+	-	-	-	-	+	+	-	+
12 ^o	-	+	-	+	+	-	+	+	-	+
13 ^o	-	-	-	-	-	-	-	+	-	-

+ = positive; - = negative

DISCUSSION

The most common and important biological rhythm is that of 24 hours known as circadian rhythm which includes both the diurnal and nocturnal periods. Its purpose is to benefit the parasite, thus facilitating the spreading of the species. Sporadically, researchers have aroused interest regarding the study of the trypanosomes' circadian rhythm. A study of this kind has already been performed with the *Trypanosoma minasense* in Brazilian primates with the observation that this parasite is more numerous in peripheral blood at midday and at sunset than in the morning and midnight³. Also the circadian rhythm of

Trypanosoma congolense was studied in the peripheral blood of mice, and the greatest number of parasites was observed at 10 p.m. *T. congolense* showed a well-established cycle in mice whereas *T. vivax*, *T. brucei* and *T. lewisi* presented less-defined cycles⁷. The fact that the circadian rhythm of *T. congolense* varies according to the host must be emphasized. Among cattle, this parasite reaches a higher parasitemia when blood is collected from the ear, at 7 a.m., with an environmental temperature of 17°C, then at noon at 36°C. The detection of a greater number of *T. rotatorium* in the blood of frogs starts at 4a.m., reaching a

maximum at 4 p.m. The periodicity of this tripanosoma seems to be related to the intensity of light to which the frog is submitted. At night the parasites are scarce¹³. In this same host, the *T. parvum* is more numerous in the blood at night than during the day⁶. The African trypanosomes do not show a defined rhythm and up to 1975 no study on *T. cruzi* circadian rhythm existed⁶.

Studies into the *T. cruzi* circadian rhythm in man and animals are equally scarce. The only study on humans as far as we know was performed by Schenone et al in 1977, in a chronic chagasic patient with high parasitemia. For this patient xenodiagnosis was performed at

midday and midnight during 15 consecutive days, and it was observed that there was an equal infection in both periods⁹. Using experimental animals, it was seen that in mice, during the acute phase, and in both guinea-pigs and mice, in the chronic phase, there is no circadian rhythm for *T. cruzi*¹¹. The study performed by hemoscope and xenodiagnosis showed that the positive tests were frequent but not cyclic during the 24-hour period.

The tests resulted positive in all the scheduled periods for each patient, thus showing that xenodiagnosis can be performed effectively at any time, both during the day or at night.

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