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A CASE STUDY OF XENODIAGNOSIS

C. O. TORNO, V. SOARES, A. VEXENAT, C. C. CUBA, A. C. BARRETO, N. J. ALVARENGA and P. D. MARSDEN

SUMMARY

The susceptibility of **Dipetalogaster maximus** to the Barreiras strain of **Trypanosoma cruzi** was studied by giving 100 third instar bugs a chance to feed on a patient with acute Chagas'disease. The subsequent infection rate in the bugs was too high to define a refractory group for breeding purposes.

INTRODUCTION

Early observations established that not all bugs ingesting blood trypanomastigotes of **T. cruzi** became infected. In patients with acute Chagas' disease and demonstrable parasites in direct blood filme a proportion of engorged bugs fail to become infected [5,7,13]. The first experimental proof of this finding was reported by PHILLIPS & BERTRAM [12]. These workers also showed that refractoriness to infection persisted in individual bugs after several infective meals and that the progeny of such bugs exibited similar behavior. MAUDLIN [9] studied the genetics of this aspect and found that the intensity of infection with **T. cruzi** in **Rhodnius prolixus** is a metric character of low heritability. Genetic mechanisms controlling susceptibility of insect vectors to infection have been demonstrated in mosquitoes in relation to their Malaria and Filaria parasites [6].

In previous studies using the bug species **D. maximus** have shown that this is a useful xenodiagnostic agent in our laboratory [8]. The question arises as to whether we could further improve our technique by selectively breeding a more susceptible bug population. For this we need information on the intraspecific susceptibility of this species and this report describes our first experiment in this direction.

MATERIAL AND METHODS

A single xenodiagnosis was done using 100 third instar **D. maximus** on a volunteer patient with acute Chagas' disease. A 29 year old man from Barreiras, Bahia, his fresh blood smears contained circulating trypanosomes. There was a relative lymphocytosis with 10% of atypical mononuclear cells. Although Chagas serology was negative (CFT, IHA) the unabsorbed Paul Bunnell was 1:1792. He complained of 10 days mild fever and is an example of acute Chagas' disease mimicing clinically infectious mononucleosis.

The 100 III instar **D. maximus** were distributed in plastic ice cream cartons closed with gauze (5 to a pot). All bugs were applied at the same time for 30 minutes. Venous and capillary blood samples were obtained and using the method of BRENER [2] the number of trypanosomes per milligram of blood was calculated. The bugs were weighted individually to the nearest milligram within 6 hours of feeding on a Sartorius balance. The mean weight of an unfed IIIrd instar (58mg) subtracted from the result. As the weight loss in the first six hours of a fed bug was a mean of 120mg due to urine excretion a calculation was made to adjust for this factor. Knowing the amount to blood ingested by each bug the number of trypanosomes in that blood could then be calculated.

Núcleo de Medicina Tropical e Nutrição, University of Brasília, Brasil

After weighing each bug was given a number and kept in an individual container. Since the main object of the experiment was to define susceptible and unsusceptible bugs with a view to breeding care was taken not to damage the genitalia. Further blood meals were offered to the individual bugs using uninfected anaesthetised mice at 30, 40, 50, 60 and 70 days after the xenodiagnosis. When bug faeces were spontaneously eliminated these were examined an each occasion for the presence of flagellates. Bugs which did during the 70 day period were examined by rectal dissection. At 70 days all surviving bugs negative for **T. cruzi** were dissected and the intestinal contents examined. 21 Known positive bugs were reexamined 232 days after the infective feed to assess if gut infections persist for this length of time in this bug species.

RESULTS

The patient had approximately 10 trypanosomes per milligram of venous blood in his circulation at the time of xenodiagnosis. He suffered no ill effects from such a large xenodiagnostic procedure and there was no evidence of an immediate or delayed skin reaction.

Table I shows the data on the feeding pattern, number of trypanosomes ingested and the subsequent infection rate in the 100 bugs. All degrees of engorgement occurred. In the table we have separated the results of bugs which died during the experiment from bugs which survived 70 days for the following reason. The protocol did not include examination of the upper intestine of the dead bugs or inoculation into mice of microscopically negative intestinal pools. There is therefore an element of doubt whether infection could have been persistent in the upper intestine of these bugs [1]. Why 26 bugs died during the experiment is not clear but table one shows it was not related to failure to feed.

However considering the 69 bugs that survived the experiment and ingested blood only three bugs were apparently refractory and this characteristic could not be related to the quantity of trypanosomes ingested. Refeeding behavior of survivor bugs is shown in Graph I. By 70 days nearly all had taken another blood meal. The 21 positive bugs (19 fifth instar and 2 fourth instar) examined at 232 days were all still infected.

TABLE I

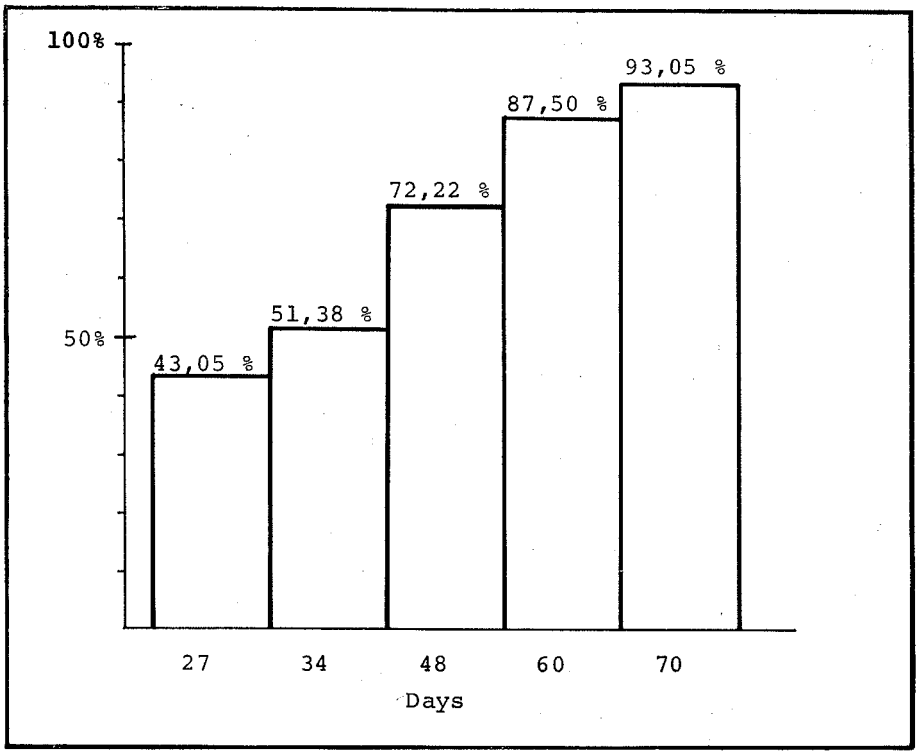
To show the results of xenodiagnosis in the 100 bugs (*)

Bugs alive after the 70 days	Number of trypanosomes ingested					
	1,000 — 2,000	2,000 — 3,000	3,000 — 4,000	4,000 — 5,000	> 5,000	Total
Positive for **T. cruzi**	1	10	46	8	1	66
Negative for **T. cruzi**	1	0	1	0	1	3
Bugs dead during the 70 days						
Positive for **T. cruzi**	0	3	3	3	0	9
Negative for **T. cruzi**	7	1	6	1	0	15

(*) Of the 100 bugs 7 did not take in any blood

DISCUSSION

We have documented that 60% of patients with no previous exposure to **D. maximus** have an immediate skin reaction and 52% a delayed at 48 hours [3]. Our volunteer in this study showed no such side effects of xenodiagnosis. The calculation of trypanosomes ingested can only be regarded as approximate since it was only possible to weigh the bugs six hours after feeding, and the correction applied for urine loss does not take into account differentials related to the degree of engorgement. However the number of bugs surviving the experiment in which trypanosomes were not found was very small (3 or 4.5%). These three bugs ingested varying



Graph I — Accumulated percentages on refeeding of **D. maxima III**

quantities of trypanosomes and one refractory bug was calculated to have ingested more than 5,000 trypanosomes. Previous experiments of this kind have suggested that refractoriness cannot necessarily be related to the number of trypanosomes ingested [10]. Another explanation of these three negative bugs is that they have spontaneously lost their infection during the observation period.

D'ALESSANDRO & MANDEL [4] found that 32% of naturally infected **R. prolixus** lost their infections in a three month observation period. However the period here is shorter and we have evidence that **D. maximus** will support infection with this strain of **T. cruzi** for 232 days. The refeeding behavior of the group of bugs was in part related to the amount of blood ingested at the test feed. Bugs ingesting little blood tended to refeed earlier but within the time of the experiment almost all bugs accepted a second feed. The high mortality (27%) of fed third instar bugs has been noted before in this laboratory. The average daily mortality rate of **D. maximus** third instar unfed was 0.32% but rose to 0.79% after feeding (Weber & Gilks, unpublished observations). The explanation for this finding is obscure.

This investigation was done in the hope of defining susceptible and refractory populations of **D. maximus** to **T. cruzi** for breeding purposes. However within the conditions of the experiment it was not possible to define a refractory group. Further experiments are indicated adjusting trypanosome ingestion to less than 2,000 organisms to define if at this end of the scale refractoriness is present. NEAL & MILES [11] showed under their experimental conditions as few as 177 trypanosomes could infect **R. prolixus.** This is likely to vary with both the bug species and the strain of **T. cruzi** but we intend to continue our investigations of the susceptibility of **D. maximus** to the Barreiras strain of **T. cruzi.**

RESUMO

Estudo de um caso de xenodiagnóstico em paciente com doença de Chagas agudo

A suscetibilidade do **D. maximus** para a cepa de Barreiras do **T. cruzi** foi estudada com 100 ninfas do terceiro estágio alimentadas em uma paciente com Doença de Chagas agudo. A taxa de infecção nestes triatomíneos foi tão alta que não foi possível identificar um grupo refratário para a criação.

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