

## CHAGAS' DISEASE IN THE AMAZON BASIN: SPECULATIONS ON TRANSMISSION PER OS

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### S U M M A R Y

Although 8 autochthonous cases of Chagas' disease have been recorded in Belém, Pará, human infection with *Trypanosoma cruzi* remains rare in the Amazon Basin due to the absence of domiciliated bugs. Sporadic cases follow the occasional invasion of houses by silvatic vectors from nearby forest, where there is a high infection rate in wild animals. It is suggested that transmission to man may be *per os*, at least on some occasions: this would explain the simultaneous development of acute Chagas' disease by four members of a family, in a house which appeared to be devoid of bugs. Epimastigotes of *T. cruzi* survived for at least 3 hours, at 26-28°C, in milk and cold, cooked foodstuffs including rice, beans, fish and minced beef. All of 30 mice fed with these contaminated foods became infected. The present low incidence of Chagas' disease in the Amazon Region appears to be a stable situation, but devastation of the forest might result in a higher incidence if silvatic vectors adapted to human dwelling-places. The main threat, however, lies in the introduction of **already infected**, domiciliated triatomines from endemic areas elsewhere in Brazil, with the establishment of new foci of "domestic" zymodemes of *T. cruzi* and transmission from man to man.

### I N T R O D U C T I O N

Chagas' disease of man was long thought to be absent from the Amazon Region of Brazil: during the past 10 years, however, 8 autochthonous cases have been described from Pará State, mostly from the suburbs of Belém<sup>2,5,6</sup>. A serological survey of 5,119 suburban inhabitants of that city revealed 14 positive reactions with immunofluorescent antibody titres greater than 1:64, and 15 equivocal reactions at 1:16. The same Authors<sup>2</sup> examined 1,171 wild mammals from Pará State and isolated *T. cruzi* from 100 (8.5%): 13 different species were indicated as hosts of this parasite. In the suburbs and nearby forests of Belém, 33 out of 189 wild animals harboured *T. cruzi* (17.4%), and some of them were trapped in the back gardens of houses. Isolates from man and animals in Pará have been shown to be similar

("types I and III") by comparative isoenzyme studies, but different from *T. cruzi* commonly associated with endemic Chagas' disease in Bahia State<sup>3</sup> ("type II").

Outstanding features in the epidemiology of the Amazonian cases of Chagas' disease were as follows: a) all patients lived in the suburbs, close to the forest, b) no triatomine bugs were found in their houses, and no-one could remember being bitten by one, c) there was a general lack of contact between infected persons and wild animals, d) all cases were in the acute phase of the infection, and 4 occurred simultaneously in one house.

Infected silvatic bugs (*Rhodnius pictipes* and *Panstrongylus geniculatus*) were on rare occasions found inside houses in the outskirts of Belém<sup>2</sup>, and it was assumed that the human

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infections were derived from such bugs. Our suggestion that transmission might have been acquired per os, on at least some occasions, was firmly rejected by some<sup>4</sup>; although BARRETTO et al.<sup>1</sup> discussed their own and other Authors' experiments on the infection of animals by the oral route, and concluded that they demonstrated "the importance of the oral route as a portal of entry of *T. cruzi*".

We wish to report, here, the results of some experiments on the survival of epimastigotes of *T. cruzi* in a variety of common local foods, and subsequent attempts to infect laboratory mice with these contaminated substances.

#### MATERIALS AND METHODS

**The parasite** — Our stock of *T. cruzi* (M 5103) was isolated from an armadillo (*Dasypus novemcinctus*) from Pará: it is highly infective to laboratory mice.

Ideally we wished to contaminate the foods with flagellates from local silvatic bugs, but no colonies of these were available at the time. The next best alternative would have been those from experimental infections in non-indigenous "domestic" species: interestingly, however, two attempts to infect batches of *Triatoma infestans*, *T. brasiliensis*, *T. sordida*, *Rhodnius prolixus* and *Dipetalogaster maximus* produced very poor results. We were, therefore, finally obliged to use epimastigotes from NNN cultures (Oxoid "CM3", with 0.85% saline overlay).

**Contaminated foods** — These included pasteurized milk (plastic-packed); boiled beans ("feijão"), flaked fish, minced beef, and rice; bottled fruit-juice (mango); a mixture of cheese and tinned guava-fruit preserve ("goiabada"); and dry mandioca flour ("farinha"). All the cooked items were allowed to cool to room temperature (26-28°C).

Approximately 2.0 g of each solid food and 2.5 ml of the liquids were placed separately in small glass pots, and to each was added 0.2 ml of the culture fluid containing the flagellates: no attempt was made to calculate the number of organisms involved. Each contaminated substance was well mixed, and the containers covered to prevent undue evaporation. They were left at room temperature for 1-3 hours, during which period a small quantity of each mixture was examined microscopically under a

cover-slip. The dryer substances were mounted in a drop of saline for this purpose.

Batches of 6 mice (adult, Swiss strain) were fed with each contaminated food. The animals had been deprived of food and water for the previous 24 hours and, in most cases, the food was simply left in their cages. We felt this preferable to force-feeding with pipette or forceps, which introduced the added complication of possible transmission by way the mouth abrasions. Exceptions were the milk and mango juice, which were fed to the mice by a plastic pipette.

Fresh preparations of tail-blood from each mouse were periodically checked for trypanosomes. Animals remaining negative were killed, one month later, and NNN cultures made from heart-blood.

#### RESULTS

Active flagellates were seen in the milk, beans, fish, minced beef and rice examined 1 hour, 1½ hrs., 2 hrs., 2½ hrs., and 3 hrs., respectively, after the addition of the parasites. No living flagellates were seen in the bottled fruit-juice or the cheese — "goiabada" mixture, in which substances the organisms appeared to be rapidly immobilized: possibly this was due to preservatives, which are commonly present in tinned and bottled products. Neither could we see parasites in the "farinha", and they were doubtless rapidly desiccated in this very dry substance.

All those mice fed with the contaminated milk, "feijão", fish, minced beef, and rice became infected (100%): the trypanosomes first became visible in the blood 12 days after the animals had ingested these foods.

No parasites were seen in the blood of the mice fed with the contaminated bottled fruit-juice, the cheese — "goiabada" mixture, or the "farinha": subsequent NNN culture of heart-blood from these animals remained negative.

#### DISCUSSION

We did not prolong our observations on the survival of epimastigotes of *T. cruzi* in household foods beyond 3 hours, but at that time the parasites remained very active: it is quite probable that they are capable of remaining viable for much longer periods, perhaps even undergoing multiplication, providing the foods re-

main moist. This, and the ease with which we infected mice by feeding them with contaminated foods, leads us to continue in our conviction that man, too, may sometimes acquire infection per os. Kitchen foods could become contaminated with flagellates from bugs faeces or, even more likely, if an entire insect falls and disintegrates in a container of food. The latter situation could well explain the simultaneous acquisition of Chagas' disease by 4 members of a family, in a house which appeared to be devoid of bugs.

In the tropical heat of the Amazon Region, much food is eaten cold, even if well cooked beforehand: furthermore, in the poorer houses, any of yesterday's "left-overs" will certainly be saved for future use. Such dishes or pans of cold food will usually stay on the stove, table or the shelves of cupboards, where contamination by the occasional intruding bug would be by no means unlikely.

The rare contact man has with the silvatic vectors accounts for the low incidence of Chagas' disease in the Amazon Region, where the only known domiciliated reduviid bug is *Triatoma rubrofasciata*. Although one extensive colony of this bug was found in the city of Belém<sup>2</sup>, it was located in a rat-infested factory on the waterfront, far from contact with any silvatic host of *T. cruzi*.

The fact that local, silvatic vectors have shown no indication of adaption to man's dwelling-places in north Brazil is reassuring, and probably a reflection of the general absence of major ecological upheavals caused by so sparse a population. Elsewhere in Latin-America man has profoundly disrupted the balance of nature by his avid destruction of the primary forest: subsequent domiciliation of some of the more adaptable triatomine vectors of *T. cruzi* has transformed an innocuous parasite of wild animals into a common pathogen of man. It is to be hoped that future plans for the development of the Amazon Region might consider the danger of repeating this process.

In the meantime, the major hazard which could lead to a dramatic increase of Chagas' disease in the north of Brazil is clearly the introduction of domiciliated bug species from elsewhere. Until recently, the geographic isolation of the Amazon Basin has proved an efficient barrier: but the situation is rapidly chang-

ing, and there now exist major highways directly linking the north with the highly endemic areas of the northeast and the south. It has been optimistically suggested that domiciliated vectors from these areas (e.g. *T. infestans*, *T. brasiliensis*, or *Panstrongylus megistus*) might be unable to survive the climatic conditions of the Amazon Region: colonies of these species, however, thrive ominously well in our laboratory in Belém, even at normal room temperature and humidity.

More comparative studies are needed on the development of Amazonian isolates of *T. cruzi* in a wide range of bug species. Thus, it is of great interest that the armadillo parasite from Pará developed very poorly in a number of well-known vectors from elsewhere, whereas our isolates from other animals grow well in the same triatomine species. Interestingly, this difference is also seen in NNN culture: we have had considerable difficulty in maintaining isolates from armadillos in this medium which, however, supports luxuriant growth of isolates from other animals. Hopefully, then, even if non-indigenous triatomine species did establish themselves in the Amazon Region, they might not prove to be effective vectors of some of the local zymodemes of *T. cruzi*.

The real threat to north Brazil, of course, lies in the introduction of already infected bugs from the northeast and south, with the consequent establishment of new foci of domestic zymodemes and transmission from man to man.

## RESUMO

### **Doença de Chagas na Bacia Amazônica: especulações sobre a transmissão oral.**

Embora 8 casos autóctones de doença de Chagas tenham sido registrados em Belém, Estado do Pará, a infecção humana por *Trypanosoma cruzi* continua a ser rara na área amazônica, devido à ausência de vetores triatomíneos domiciliares. Casos esporádicos da moléstia surgiram em consequência da invasão ocasional de domicílios por vetores silváticos, procedentes da mata circunvizinha, onde existe uma grande fonte de infecção entre os mamíferos. Sugere-se que a transmissão talvez se tenha dado por via oral, pelo menos em alguns dos casos. Isto poderia explicar o desenvolvimento simultâneo da forma aguda da doença

de Chagas em 4 membros da mesma família, numa casa sem qualquer evidência de barbeiros. Epimastigotas de *T. cruzi* sobreviveram, por um tempo mínimo de 3 horas a 26-28°C, em vários alimentos, incluindo-se leite, arroz, feijão, peixe e carne picada cozidos. Cem por cento dos 30 camundongos que foram alimentados com a comida contaminada, acima referida, tornaram-se infectados. A incidência atualmente baixa de doença de Chagas na área amazônica parece ser uma situação estável, mas a devastação da floresta pode vir a resultar num aumento da incidência da doença humana, caso alguma espécie silvestre do vector triatomíneo venha a se adaptar às moradias locais. A maior ameaça, entretanto, reside na possibilidade de introdução de barbeiros domiciliares, já infectados, procedentes de áreas endêmicas de outras partes do Brasil, com o estabelecimento de focos de zimodemas "domésticos" de *T. cruzi*, e a transmissão de homem para homem.

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