

# EXPERIMENTAL STUDIES ON TRYPANOSOMA (SCHIZOTRYPANUM) CRUZI STRAINS ISOLATED FROM MAN, FROM ANIMALS AND FROM TRIATOMINE BUGS IN BRAZIL \*

W. B. Petana and J. R. Coura \*

*A study on nine strains of T. cruzi isolated from man, from animals and from triatomine bugs in Brazil are described. The parasites were slightly viscerotropic in white mice in six of the strains, highly viscerotropic and cardiotropic in two strains, and asymptomatic on one strain. Mechanical and cyclical passage from infected to healthy mice, and treatment of the infected mice with immunosuppressant drug, did not increase the blood parasitaemia or strain virulence. The results of biometric studies on the blood trypanosomes from each strain are also described. The various aspects on the importance of T. cruzi strain identification are emphasized and discussed.*

## INTRODUCTION

Since the pioneering work by Chagas (9, 10, 11) the various aspects of the causative organism *Trypanosoma (Schizotrypanum) cruzi* have been studied quite extensively. The investigations were directed on the pathological aspects of the parasite as well as on the biological, morphological and immunological characteristics among the various strains. Some comparative studies were also carried out on the differentiation between Brazilian and other South American strains. In Brazil the following investigators have notably contributed in this field of work: Villella and Torres (32), Campos (8), Dias (14), Nussenzweig *et al.* (22, 23), Silva and Nussenzweig (28), Brener (5, 6), Brener and Chiari (7), Deane *et al.* (13), Coura *et al.* (12), Andrade and Andrade (1, 2), Andrade *et al.* (3) and Kramer (15). From outside Brazil studies on *T. cruzi* strains

from the following countries may be mentioned. Argentina, Belize (British Honduras), Chile, Colombia, Costa Rica, México, Peru, Venezuela and the U.S.A. (3, 4, 17, 18, 19, 20, 21, 25, 26, 27, 30, 31, 33, 34).

One part of our present research project on Chagas' disease in Brazil includes the isolation of *T. cruzi* strains from man, from animals, and from triatomine bugs. The purpose for the study is to compare the behaviour of the isolates under experimental conditions in white mice, inclusive of grade of parasitaemia, virulence, morphological and biometric studies on the blood trypanosomes, organotropism, and pathological alterations in the organs of the infected animals.

## MATERIALS AND METHODS

A total of 12 strains were isolated of which nine were investigated conclusively and are described here. Five of the isolates

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\*\* Department of Preventive Medicine, Federal University of Rio de Janeiro, Caixa Postal 1859, Rio de Janeiro, Brazil.

were from human patients with Chagas' disease, two strains originated from domestic dogs and two isolates were obtained from naturally infected domestic *Triatoma infestans*. For convenience a brief history of the source from each of the strains was obtained is given below.

*Strain No. 1* — Was isolated by xenodiagnosis from 11 years old female in a locality, Piranema, in the municipality of Caxias, close to the city of Rio de Janeiro. The immunofluorescence and complement fixation test (IFT and CFT) were positive. Detailed clinical examination suggested an asymptomatic form of Chagas' disease. In the same household three more children were serologically and parasitologically positive for Chagas' disease, and from two dogs, living in the same household, *T. cruzi* was isolated by xenodiagnosis. The presence of domestic *T. infestans* infected with *T. cruzi* was also confirmed on several occasions.

*Strain No. 2* — Was obtained by xenodiagnosis from eight years old female, also from Piranema. Infection in the child was confirmed serologically and parasitologically. Clinical examination confirmed an asymptomatic form of Chagas' disease. Further four children in the same household were found serologically positive, and from three *T. cruzi* was isolated by xenodiagnosis. The xenodiagnosis test, carried out on six dogs living in the same household, was negative. Large number of *T. infestans* were captured in the house, many of them infected with *T. cruzi*.

*Strain No. 3* — Originated from 48 years old farmer by xenodiagnosis. The patient also lives in Piranema, and was found serologically positive for Chagas' disease in 1968 and 1971. The infection was diagnosed as asymptomatic, and the man is still engaged in farming. His wife also had a positive serological test for Chagas' disease, but no trypanosomes were isolated from her by xenodiagnosis. The house was searched on several occasions for triatomine bugs, but only once three adult *T. infestans* had been caught, one of which was infected with *T. cruzi*.

*Strain No. 4* — Was isolated by xenodiagnosis from a 30 years old female pa-

tient, native from Bahia. Clinical examination revealed a chronic chagasic cardiomyopathy and schistosomiasis with *S. mansoni*. The serological test (IFT and CFT) was positive on several occasions over a long period of time.

*Strain No. 5* — Originated from a 41 years old female patient by xenodiagnosis. The patient, a native from Bahia, attended our out-patient clinic and was diagnosed for schistosomiasis, and a chronic and asymptomatic form of Chagas' disease. The IFT and CFT were positive on several occasions.

*Strain No. 6 and 7* — The strains were isolated by xenodiagnosis from two dogs. The animals belong to the household in Piranema from which the human *T. cruzi* strain no. 1 was obtained.

*Strain No. 8 and 9* — Originated from domestic *T. infestans* captured in the two houses in Piranema from which the human *T. cruzi* strains were isolated (no. 1 and no. 2).

All the strains were established in adult white mice (Swiss strain), after inoculation with faeces from bugs used in the xenodiagnosis test, and from naturally infected domestic *T. infestans*. The faeces from each group of ten 5th-instar nymphs of *T. infestans*, used in xenodiagnosis, were pooled and suspended in 2.5 ml of saline solution. In the case of the naturally-infected domestic bugs, the faeces from only three adult *T. infestans* from each house were used, because of the very high gut parasitaemia with *T. cruzi*.

The number of metatrypanosomes in the faeces of bugs used in the xenodiagnosis test on humans and on the dogs was very low. It was estimated that the inoculum of each mouse contained between 30,000 and 50,000 metatrypanosomes in 0.5 ml of the pooled faecal suspension. In the case of the naturally-infected bugs the gut parasitaemia was very much higher, with between 200,000 and 400,000 infective forms per 0.5 ml of the inoculum. Prior to the inoculation Giemsa-stained smears were prepared from each batch of the faeces for confirmation of

the parasite morphology. In each case the flagellates were a mixture of epimastigote and metacyclic *T. cruzi*.

Four mice were used for each strain, and were inoculated intraperitoneally with 0.5 ml of the faecal suspension. After the inoculation the mice were divided into two groups. Two mice in group one were investigated every 10 days for blood parasitaemia, and their blood was used for further passage to healthy animals as well as for cyclical passage with *T. infestans*. Thin blood films were prepared from each mouse in group one for morphological and biometric studies of the blood trypanosomes. Because of the very scanty presence of parasites in Giemsa-stained film only 25 trypanosomes were examined and measured in each strain.

The other two mice from each strain in group two were reserved for a long-term observation on the progress of the infection, and their blood was examined only once a month in order not to disturb the animals unduly. At the end of the observation period (between eight months and one year) the mice were subject to a xenodiagnosis test with five 3rd instar *T. infestans* per mouse. Thereafter each mouse was treated for 10 successive days with an immunosuppressant drug (cyclophosphamide), in doses of 50 mg/kg body weight, inoculated intraperitoneally. This procedure was used to see if the blood parasitaemia or the strain virulence may be increased by blockage of the immunodefense system. After the treatment the mice were sacrificed and subject to a post mortem examination. A part of the heart muscle, colon and esophagus were prepared for histological scrutiny.

## RESULTS

The course of infection in the mice was generally similar in each strain. A very low blood parasitaemia developed between 10 and 30 days after the inoculation, and the number of trypanosomes remained very scanty on each subsequent examinations, never exceeding more than 20 parasites per whole of the coverslip (1cm x 1cm), using the 40x dry objective and x10 eyepiece. The morphology of the blood forms was in each case typical for

*T. cruzi*. The biometric details of the blood trypanosomes from each strain are summarized in Table 1.

The mice showed no sign of illness or distress, and eventually no trypanosomes could be detected by a direct wet blood film examination from two to four months after the inoculation. Subinoculation of blood from mice in group one to further clean mice, or cyclical passages with *T. infestans* have not increased the blood parasitaemia or strain virulence. The xenodiagnosis test on mice in group two at the end of the observation period was positive in all strains, with exception of strain number nine, where the result was negative. The post mortem and histopathological findings in mice from group two are summarized in Table 2.

The colon in one mouse from strain number seven (dog strain), and in one mouse from strain number eight (bug strain), showed a well pronounced megaly (Plate I, figs. A and B). Both colons contained a mass of hard faeces.

The hearts of mice from strain number seven and eight, both of which had a megacolon, were distinctly enlarged (Plate I, figs. C and D). On cross-section both hearts showed enlargement of the ventricular cavity, a thinning of the left ventricular apex, and hypertrophy of the intraventricular septum, mostly on the left side (Plate I, fig. G). In histological sections very scanty and small pseudocysts with amastigote form of *T. cruzi* were seen in both hearts (Plate I, figs. E and F), as well as a considerable decrease of ganglion cells.

## DISCUSSION AND CONCLUSION

The results obtained suggested that all the strains, with exception of strain number nine, were viscerotropic to a lesser degree in mice from strains number one to six, and in two mice from strain number seven and eight, with a well developed megacolon in one of each mice in strains number seven and eight. Strains number seven and eight were also cardiotropic in the two mice with a megacolon.

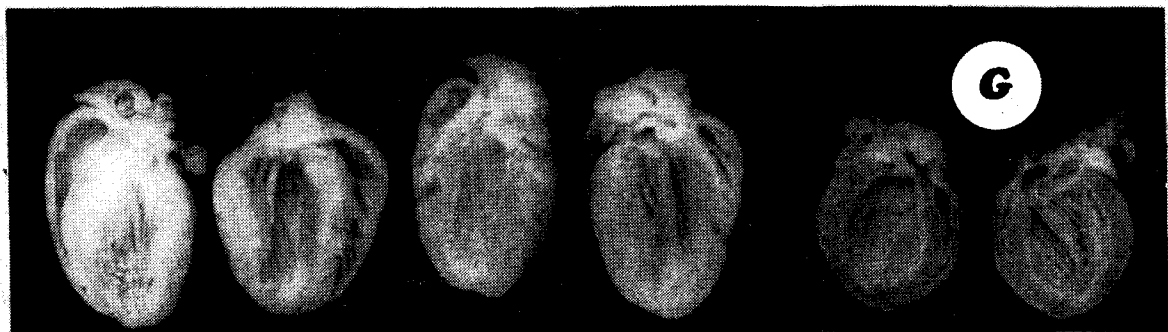
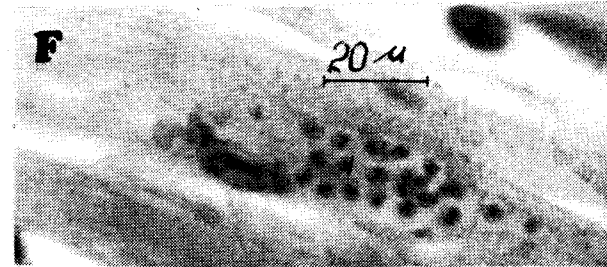
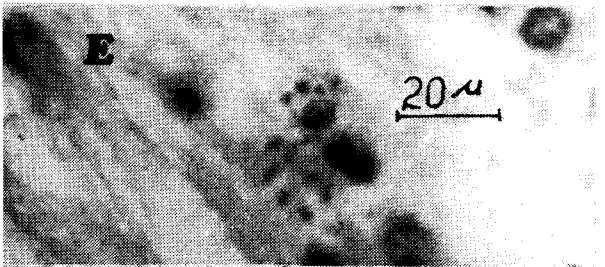
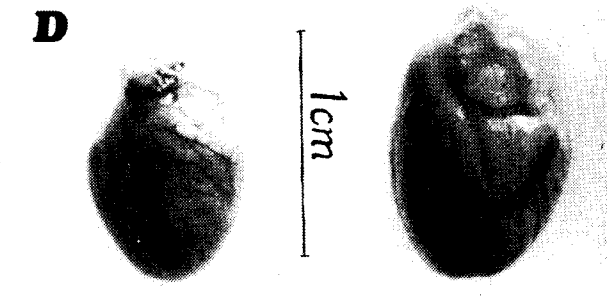
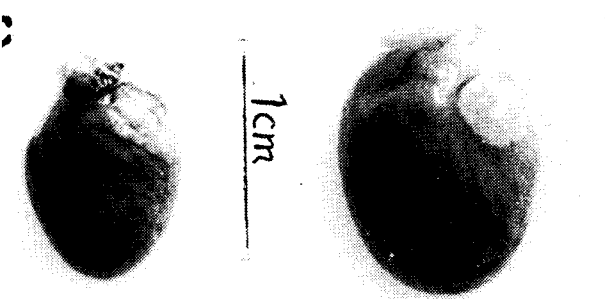
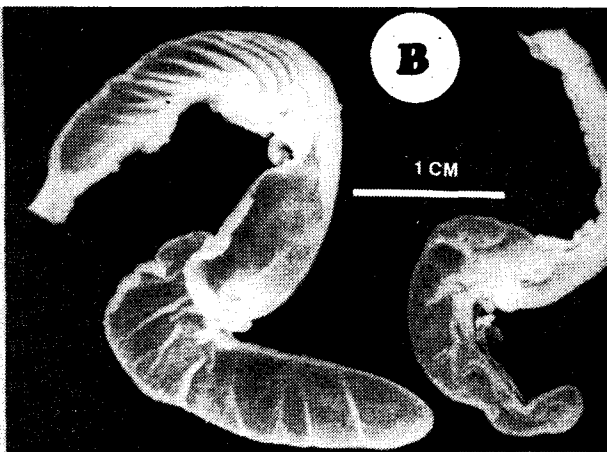
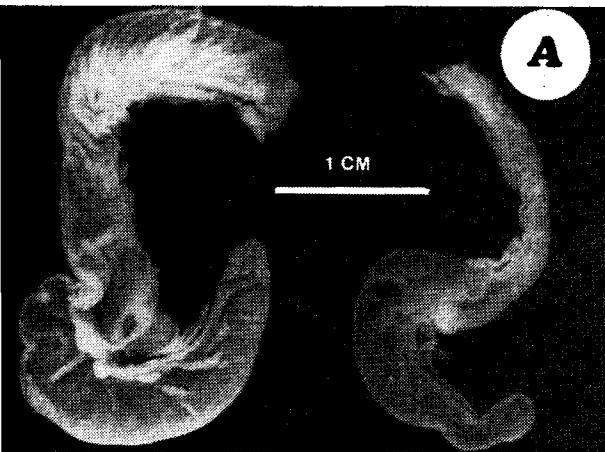
The main point of interest is that the megacolon and megaheart developed in

TABLE 1 — Showing the mean length and the nuclear index (NI) of the blood trypanosomes from each strain

Strain N <sup>o</sup>	1	2	3	4	5	6	7	8	9
Mean length (microns)	19.7	21.3	19.9	21.1	21.7	20.6	21.0	20.9	19.8
NI	1.6	1.7	1.2	1.7	1.6	1.2	1.6	1.7	1.4

TABLE 2 — Summarizing the pathology and histopathology in mice from each of the nine *T. cruzi* strains

Strain N. <sup>o</sup> :	Origin:	Post mortem findings:	Histopathology:
1	human	slight enlargement of colon; heart and esophagus normal	a slight decrease in ganglion cells in colon
2	human	" "	" "
3	human	" "	" "
4	human	" "	" "
5	human	" "	" "
6	dog	" "	" "
7	dog	megacolon and cardiomegally; esophagus normal	significant decrease of ganglion cells in colon and heart, pseud-ocysts with <i>T. cruzi</i> in heart muscle
8	<i>T. infestans</i>	" "	" "
9	<i>T. infestans</i>	all organs normal	no changes



Figs. A and B — show the enlarged colons in mice from strain number seven and number eight (a colon from a normal mouse of the same age is shown on the right).

Figs. C and D — cardiomegaly in mice from strain seven and eight (the heart from a normal mouse of the same age is shown on the left).

Figs. E and F — a small pseudocyst in the myocardium in mice from strain number seven and eight with amastigote forms of *T. cruzi*.

Fig. G — cross-section of hearts from mice in strain number seven and eight showing the cross-pathology of the myocardium as described in the text (a heart from a normal mouse is shown for comparison on the right).

mice infected with a dog and bug strains of *T. cruzi*. The dog and the insects came from the same household in which four children have been diagnosed serologically and parasitologically for Chagas' disease, but the trypanosomes isolated from one of the children (strain number one) did not produce pathological changes in the mice, comparable with those produced by the non-human strains obtained from the same circle of co-habitation. At the present the children have an asymptomatic form of Chagas' disease, and it is not possible to postulate which course the infection may take after several more years.

An added interest is, why only one mouse should develop such a severe pathological abnormality whilst the other animal, which received an identical inoculum, was free from any cross-pathological changes. A further feature of interest is that the identical pathological abnormality of the heart and of the colon occurred in mice one of which received a very small number of parasites, and the other about ten times more in the inoculum.

The negative xenodiagnosis test in the six dogs, living in the same house with infected persons and triatomine bugs, was also of interest as there is no doubt that at some time the dogs must have come into contact with infected triatomines whilst sleeping in the house. If there was a definitive mean of distinguishing *T. cruzi* and its affinity for a specific host, it may be postulated that the strain number nine was a human strain not transferrable to mice or dogs. This assumption is favoured on grounds that the *T. cruzi* isolated from the child (strain number two) did not produce any pathological condition in the mice, nor were there infected dogs in the house, though the dwelling was infested with triatomine bugs with a high rate of infection with *T. cruzi*.

The peculiarities in the behaviour of *T. cruzi* in the various mammalian hosts, and under experimental condition, are well-known. Unfortunately, however, there seems to be no satisfactory explanation for the behaviour of this flagellate from one host to another. Nussenzweig *et*

*al.* (19, 20) divide *T. cruzi* strains from Brazil into two immunologically distinct types, on which basis they offer an explanation for the clinical diversity of Chagas' disease in the various vertebrate hosts. However, it is known how immunologically unstable trypanosomes can become after a series of passages, mechanical or cyclical, or on transfer from one host to another.

A further mean of *T. cruzi* strain identification was suggested by a Panel of the WHO (24). The Panel is of the opinion that the mean nuclear index (NI) for a 'typical' *T. cruzi* is 1.5 with a range of between 1.2 and 1.7. The experts stressed, however, that both in man and in animals some strains have been found with a very low and high NI, and that the variations do not appear to be related to the pathogenicity of such strains. The point of interest here is the definition for a "typical" *T. cruzi*, which, it may be assumed, applies to strains of human origin. Such definition is not only arbitrary but also contradictory in several aspects. Since *T. cruzi* is not a specific parasite for man, its pathogenicity, histotropism and the mode of reproduction should be considered of a greater importance than the biometrical features, irrespective if the strain originated from a vertebrate or from an insect host. As shown in Table 1, the nuclear index in the nine strains described here varied between 1.2 and 1.7. This would suggest that each strain could be defined of either human or animal origin. The parasites were isolated from three different hosts, and it would be difficult to decide on biometric grounds which was a 'typical' or 'atypical' *T. cruzi*.

The movement of infected hosts and vectors, and the interchange of *T. cruzi* by several passages in different hosts and vectors may also affect the virulence of the parasite, and can result in the formation of a 'new strain' as suggested by Ryckman (28) and Lambrecht (16). There is a need for the revision of our concepts of *T. cruzi* strains, and to try to find a definitive and acceptable mean for their identification.

We are unable to offer an explanation on the differences in the strain behaviour found during our present studies. However, it is known that in some human patients

with Chagas' disease, living in the environment with infected triatomines, some of the affected members of the family may develop a severe form of Chagas' disease, whilst in others the infection may be asymptomatic for life time. The result of our investigation have once again shown the variability of *T. cruzi* under experimental conditions. This complexity among *T. cruzi* strains is perhaps one of the last intricate factors for the true understanding in the biological and therapeutic approaches for the study and control of Chagas' disease.

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#### S U M Á R I O

*Nove cepas de T. cruzi, 5 isoladas do homem, 2 isoladas do cão e 2 outras isoladas de Triatoma infestans domiciliados, foram estudadas do ponto de vista morfológico e de sua virulência e patogenicidade para o camundongo, em um período de observação de 8 a 12 meses.*

*As cinco cepas isoladas do homem mostraram baixa parasitemia quando inoculadas no camundongo e provocaram nesse animal apenas redução dos glânglios mioentéricos do colon e discreto aumento desse órgão. Das duas cepas isoladas do cão uma provocou megacolon e cardiomegalia no camundongo e a outra comportou-se de forma semelhante às cepas isoladas do homem. Das cepas isoladas do T. infestans uma foi absolutamente avirulenta para o camundongo e a outra provocou redução dos gânglios do colon e do miocárdio, cardiomegalia e megacolon.*

*Aditem os autores que a passagem "cíclica" de uma cepa de um reservatório para outro possa modificar o seu comportamento, servindo o homem como "reservatório estabilizador" da baixa virulência e patogenicidade das cepas, pelo menos daquelas isoladas de casos crônicos.*

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