

POSSIBLE ORAL TRANSMISSION OF ACUTE CHAGAS' DISEASE IN BRAZIL

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SUMMARY

In October, 1986, 7 to 22 days after a meeting at a farm in Paraíba state, 26 individuals presented with a febrile illness associated with bilateral eyelid and lower limb edema, mild hepatosplenomegaly, lymphadenopathy and, occasionally a skin rash. A 11-year-old boy exhibited atrial premature complexes and a 74-year-old patient developed acute heart failure. In two patients hospitalized in São Paulo city, acute Chagas' disease was diagnosed by the demonstration of circulating *Trypanosoma cruzi*. At autopsy in a fatal case, acute Chagas' cardiomyopathy was demonstrated. Xenodiagnosis were positive in 9 out of 14 tested patients. A specific IgG immune response was found in all patients and specific IgM antibodies were identified in 20 out of 22 tested patients. An epidemiological survey showed the existence of *Triatoma brasiliensis* in the outbuildings of this farm, but none in the house where most of the guests stayed. A high rate of infection with *Trypanosoma cruzi* was found in opossums. These observations together with those related to the food consumed by the patients, lead the authors to suggest that the human infections resulted from oral contamination probably originating from naturally infected marsupials in the area or crushed infected bugs.

INTRODUCTION

The present report describes the clinical features of an outbreak of acute Chagas' disease in the interior of Paraíba state and suggests, based on epidemiological data, that the infection has occurred by ingestion of contaminated food.

The Municipality of Catolé do Rocha is in a semi-arid region in the northwest of Paraíba State, with maximum and minimum temperatures of 34° C and 19° C, and an average annual rainfall of 800 mm (VAREJÃO-SILVA et al., 1984). The main activities of Fazenda Arocira, about 16 km from the town, are cattle and sheep ranching together with crops, such as sugar cane, bananas, beans and maize.

The common element of the Chagas' disease outbreak, detected in November 1986, was the

presence of all 26 seropositive patients at the Fazenda during three days of a festive reunion in the end of September 1986. Ninety four inhabitants and visitors to the Fazenda were serologically tested for IgG antibodies for *T. cruzi* by indirect immunofluorescence in blood collected in filter paper.

This report includes 26 patients with acute disease, 15 males and 11 females, whose ages ranged as follows: 5 under 15 years old, 7 from 16 to 30 years old, 7 from 31 to 45 years old, 4 from 46 to 60 years old and 3 from 61 to 75 years old. None of them had received any recent blood transfusion. All but one had attended a meeting held on a farm, in Catolé do Rocha county, in the interior of Paraíba state; one of them had stayed in the meeting for two hours and only ingested sugar-cane juice, and one patient who

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had not gone to the meeting drank only sugar-cane juice brought from there. Twelve individuals did not live in the neighbourhood and sought help in distant great cities.

MATERIAL AND METHODS

Patients were tested by the following parasitological methods: A) Direct demonstration of *T. cruzi* in buffy coat, (STROUT, 1962); B) Xenodiagnosis performed with 40 *Triatoma infestans* nymphs in 3 patients (35th day of illness) and 15 nymphs in 12 patients (40th and 55th day of illness) with insect feces analysis after 20, 30, 60 and 90 days.

Other laboratory tests included: serological reactions for IgG and IgM anti-*T. cruzi* antibodies demonstration by the indirect immunofluorescence test (IFA). In three patients examined in Hospital das Clínicas da Faculdade de Medicina da USP, blood cultures for bacteria and fungi, toxoplasmosis IFA test, typhoid and brucellosis agglutination reaction were performed and in one of them complement fixation reaction for groups B and C arborvirosis was also performed.

A survey for triatomine bugs was carried out by Superintendência de Campanhas de Saúde Pública, Ministério da Saúde (SUCAM) in the 25 dwellings and annexes of the Fazenda, and in some rocky outcrops. This study was also extended to some neighbouring localities. Bug capture was detailed and systematic, using dislodgent sprays (Neopynamim) and removing household items for study, so that the search of each house occupied one complete man-hour. Most triatomine bugs that were captured were examined for infection with *T. cruzi*, and their intestinal contents prepared for precipitin analysis of blood meals.

Domestic and sylvatic animals captured in various localities of the Fazenda were examined by xeno-diagnosis, usually with 20-40 3rd instar nymphs of laboratory-reared *Triatoma infestans* and *Panstrongylus megistus*. Blood from 50 domestic animals was also subinoculated into laboratory mice.

CASE REPORTS

Clinical and laboratory features

The incubation period, starting from the mo-

ment of food ingestion, ranged from 7 to 10 days in 4 patients, 11 to 14 days in 5; 15 to 18 days in 13 and 19 to 22 days in 4.

Clinical signs and symptoms are presented on table 1.

Table 1
Clinical symptoms and signs in 26 acute Chagas' disease patients.

Clinical signs and symptoms	Number of patients (N = 26)
Fever	26
Myalgia	26
Headache	26
Bilateral palpebral oedema	24
Lower limb oedema	22
Lymphadenopathy	13
Hepatomegaly	9
Palpable mild splenomegaly	9
Maculopapular skin rash	9
Cardiac arrhythmia*	1
Congestive heart failure	1

* - Atrial premature complexes in electrocardiogram.

Fever ranged from 37,5° C to 40° C and lasted from 5 to 50 days. Bilateral eyelid edema was noticed from the 20th to the 25th day of illness and lasted 3 to over 22 days. A maculopapular skin rash (sometimes only macular or with papular lesions smaller than 3 cm in diameter) involved primarily upper and lower limbs and trunk, and was noticed between the 3rd and the 15th day of illness, lasting 3 to 20 days.

Considering the 8 patients examined during febrile illness, lymphadenopathy was present in all of them, hepatomegaly in 5 and palpable mild splenomegaly in 3.

Nine patients received ampicillin, chloramphenicol and cotrimoxazole during 5 to 6 days, due to a supposed diagnosis of typhoid fever but exhibited no fever defervescence. Five patients received betamethasone for 5 days or prednisone for 4 days, due to a supposed diagnosis of allergic reaction. Three out of four patients with most severe edema received corticosteroids. In one of them, right bundle branch conduction disturbances were noticed.

Hematological abnormalities included relative and absolute lymphocytosis in 8 patients out of 12 tested on the 30 - 50th day of illness. Blood cultures and toxoplasmosis and brucellosis serological tests were negative in three patients tested. No serological evidence of B and C arbovirus groups was detected in one patients. Anti-O typhoid agglutinins over 80 dilution sera were seen in 4 out of 5 tested patients and similar titres of anti-H agglutinins in 2 of them.

Cardiac disturbances

An electrocardiographic study was performed in all 24 patients from the 33rd to the 55th day of illness and yielded the following results: antero-superior division block in one patient; antero-superior division block, and disturbance in ventricular repolarization in one; antero-superior division block, disturbance in the right bundle branch conduction, ventricular premature complex and first degree A-V block in one, atrial premature complexes in one; disturbance of right bundle branch conduction and tachycardia in one; disturbance of ventricular repolarization in three patients. The results were normal in 12 patients and sinus tachycardia was found in 4 of them.

Two patients with antero-superior division block had already lived in the area for a long time, but exhibited signs of acute disease without cardiac abnormalities at clinical examination and were shown to have IgM anti-T. *cruzi* antibodies. Chest X-rays were performed in 16 patients, but cardiac enlargement was found only in the patient who died.

Two out of 26 patients developed signs of cardiac involvement during clinical follow-up of the acute disease: a 11-year-old boy exhibited atrial premature complexes on the 32nd day of illness, which subsequently subsided on the 70th day. A 74-year-old man died of acute heart failure with antero-superior division block and disturbances in ventricular repolarization on electrocardiogram. Histopathological studies revealed acute Chagas' cardiomyopathy and acute Chagas' esophagitis with amastigote parasites in both tissues and inflammatory infiltration near ganglionic nerve cells in the large intestine. Cardiac abnormalities were not noticed at clinical examination of the other 24 patients.

Parasitological and serological studies

The results of parasitological and serological studies are presented on table 2.

Table 2
Results of parasitological tests in acute Chagas' disease patients.

Tests	Number of tested patients	Without specific treatment, tested between		With specific treatment**, tested between
		33 and 39 days of illness	40 and 55 days of illness	40 and 55 days of illness
Buffy coat	22	2/3*	0/14	0/5
Xenodiagnosis	14	3/3*	4/6	2/5

* - Number of positive patients/number of tested patients.

** - Patients had already received Benznidazole for three days.

Xenodiagnosis was performed only on the 14 patients presenting major clinical features.

Four patients sera revealed IgG specific antibodies in 1/40 dilution and were not available for this titration in higher dilutions and for IgM specific antibodies titrations. Table 3 shows the serological results observed in the first serum sample, obtained from the 33rd to the 55th day of illness in the other 22 patients. The two patients with nega-

tive IgM tests presented fever, lymphadenopathy, hepatomegaly, splenomegaly, eyelid edema and one of them presented cardiac arrhythmia and positive buffy coat test.

Therapy and follow-up

Fever defervescence and regression of edema and skin rashes were seen in most of the patients even before specific chemotherapy was introduced.

Table 3

Results of serological immunofluorescence reaction in 22 acute Chagas' disease patients.

Antibody class	Negative	Positive		
		Reciprocal of serum dilution		
		40-80	160-320	640
IgM	2	16	3	1
IgG	0	9	12	1

One patient exhibited acute cardiac failure and mental disorientation on the 37th day of illness. Even though specific chemotherapy with benznidazole was introduced on the 40th day, he died three days later. His chest X-ray revealed cardiac enlargement, bilateral pleural effusion and pulmonary edema.

Twenty five patients received benznidazole in a 7 mg/kg/day schedule for children and 5 mg/kg/day for adults during 60 days. One patient had her chemotherapy interrupted on the 30th day of the therapy due to agranulocytosis, which subsided after drug removal. Fever had disappeared in 17 patients, and was in defervescence in the others before the introduction of the drug. At present, 25 patients are in good clinical condition and are being followed.

Epidemiological studies [With financial help of FINEP (Proc. 4.86.0736.00) and Sudene (Proc. 28110.00.0127/87-3)].

Of 94 people living at the Fazenda who were not suspected of acute disease, only 3 had a positive IgG immunofluorescence reaction, on filter paper samples. Subsequent venipuncture samples of these individuals were negative.

All patients with acute disease, plus 2 of the 3 initially serological positive residents, had participated in two days of festivities, including meals at the Fazenda. Some of these individuals had spent the night at the Fazenda, but most had spent only one day there. The meals consisted of rice, salad, beef, lamb, pork and raw cane juice. With the exception of two adults and, perhaps, one child, all the infected people had drunk raw cane juice. The two individuals claiming not to have drunk the juice probably been in contact with it; one had

spooned juice from the cane crusher and the other had helped to prepare the cane. However, there can not be total confidence in these recollections, which were noted 60 days after the events.

No raw meat was consumed at these meals. All the meat had been cooked for several hours in a kitchen maintained in a very hygienic state.

The houses where the festive meals were taken are of plastered brick with tiled roof without ceiling. The cane crusher was partly covered by branches and leaves and partly by tiles, but with incomplete walls.

During the entomological surveys, 56 Triatominae were collected (45 *Triatoma brasiliensis*, 8 *T. pseudomaculata*, and 3 *Panstrongylus megistus*), of which 17 were captured inside 5 dwellings, 24 in two piles of rocks, and the rest in the outbuildings. This total includes insects captured before and soon after the spraying of all houses. 6 of these bugs were infected with *T. cruzi*, 3 adults inside houses and 3 nymphs from annexes. The main house of the Fazenda and its annexes, where most of patients stayed, were rigorously inspected but showed no evidence of infestation with triatomine bugs. In the group of 11 buildings, with a diameter of 160 m, including the main house, 3 infected *T. brasiliensis* were found, one in a house in the extreme north, 70 m from the cane crushing building and 120 m from the main house, and two in a henhouse near the same house.

Blood meal of 35 bugs from the farm was analysed, including 3 positive for *T. cruzi*. Twenty nine bugs (82.8%) were positive for marsupial blood, including all the positive for *T. cruzi*. Two of these were also positive for bird blood and one of these was also positive for rodent blood (MARCONDES at al., 1990).

None of 35 sheep and 15 cows examined by xenodiagnosis and subinoculation into mice, proved positive for *T. cruzi*. However, all of 11 opossums (*Didelphis albiventris*) were positive by xenodiagnosis: 9 at the first examination and 2 at the second. One of them was captured on the top of the wall, under the ceiling, of a warehouse, near the main house and the cane crusher building. Examination of the anal glands of the opossums showed epimastigotes in 4 of them. All other sylvatic mammals proved negative (4 *Galea spixi*, 2

Rattus rattus, 2 *Trichomys aperioides* and 1 *Mus musculus*).

DISCUSSION

The small number of triatomine bugs captured during our surveys suggests that bug domestic populations were very small in this region. Insecticidal spraying under anti-Chagas programme had been carried out in Catolé do Rocha during the past 5 years and a survey of the municipality in 1985 revealed only two houses infested with *T. brasiliensis* and/or *T. pseudomaculata*.

Blood meal analysis indicated that marsupials (probably *D. albiventris*) are important sources of blood and of *T. cruzi* infection for the bugs. The proportion of positivity for marsupial blood was much higher than previously observed in the north-east of Brazil (FORATTINI et al., 1981). Moreover, the infection rate of opossums was higher than that observed in other regions (BARRETO, 1979).

No evidence of direct contact of most patients with triatomine bugs, the low density of *Triatominae* in the houses of the Fazenda, the absence of *Triatominae* in the house, where most of patients stayed, together with the fact that most patients did not reside there and yet the onset of the disease appeared simultaneously in all the patients, without any putative portal of entry for parasites, leads us to conclude that direct vector transmission is most unlikely to have accounted for this outbreak. Thus, since none of the patients had any history of blood transfusion, the fact that all patients had been eating together on the same occasion strongly suggests infection by oral contamination.

Experimental evidence exists of infection by oral route. Animal infections with blood trypomastigotes inoculated by this via was observed in 1921 by NATTAN-LARRIER. The infectivity of metacyclic forms of bug feces was several times demonstrated when administered by digestive tract. KOFOID & DONAT (1933) and CARDOSO (1938) demonstrated experimental infection with metacyclic trypanosomes from bug feces via buccal mucosae, and MARSDEN (1967) infected mice via intragastric instillation. Evidence of animal infection following the ingestion of contaminated food was also showed (MAYER, 1961, LAINSON et al., 1980). Possible human contamination via breastfeeding was reported by MAZZA et al., (1936) and

by MEDINA-LOPES (1988). Besides this, oral transmission may be common in wild animals feeding on other infected animals (DIAS, 1940).

It is highly improbable in the present outbreak that meat from an infected animal would have remained infective after the lengthy cooking. Oral infection is more likely to have occurred after ingestion of raw foodstuffs (or after the cooked food had been heated). Contamination of domestic utensils may have been possible, but we consider this unlikely to have been an effective route to infect so many people.

Although *T. cruzi* can remain infective in various foods (LAINSON et al., 1980) and in milk (MAYER, 1961), in the present case very few of the foodstuffs were ingested by all the patients. Apart from the meat, only rice and sugar cane juice appear to have been universally consumed. The rice was well cooked and served on several occasions, so does not appear likely as the source of contamination. In contrast, the sugar cane juice was obtained fresh from the mill in the morning and was drunk, usually chilled, by virtually all patients. A mechanic who spent only two hours at the mill but who drank the juice, presented acute Chagas' disease, and so did a girl who drank the juice elsewhere. The precarious conditions at the sugar cane mill suggest that the juice could have been contaminated there, and we believe that this outbreak of infection has been due to ingestion of sugar cane juice contaminated with *T. cruzi* trypomastigotes possibly from crushed bug guts or from infected opossum secretions.

The installations of the sugar cane mill were carefully examined by SUCAM personnel about 50 days after the meals but no triatomine bugs were found there. This does not exclude the possibility that excretion from an infected bug may have contaminated the juice, but in this case the infective material would have been greatly diluted. *T. cruzi* can remain infective after remaining 1 and 24 hours in sugar cane juice (SOARES et al., 1987), and up to 24 hours in stored milk at room temperature (MAYER, 1961). MARSDEN et al. (1977) showed that one trypomastigote can infect mice by intraperitoneal route, but the minimal quantity of trypomastigotes required for oral infection is not known.

Considering that all the examined opossums were infected and several had flagellates in their anal glands, it seems in this case also possible that an infected opossum could have passed infective anal gland secretions over the sugar cane crusher. Mate-

rial from infected anal glands is known to be infective to mice that ingest it (JANSEN & DEANE, 1985), and according to Jansen (Dr. Ana Jansen (personnal communication) - Depto. de Protozoologia, Instituto Oswaldo Cruz, Rio de Janeiro - RJ, Brasil) a single anal gland of *D. marsupialis* can contain 0.5 ml of liquid. We did not examine the urine of opossums, but this may also contain *T. cruzi*, as observed in *D. marsupialis* in USA (McKEEVER et al., 1958; OLSEN et al., 1964).

The clinical features of the present outbreak closely resemble the previous report of an outbreak in Teutônia, Brazil (SILVA et al., 1968), in which animal reservoirs are suspected to contaminate food ingested by patients. On that occasion suspicion was raised that the use of corticosteroids in the majority of patients might have contributed to a possible reactivation of chronic disease (Di PRIMIO, 1971), some patients were shown not to have taken corticosteroids and thus the hypothesis was refuted.

In the present outbreak only one 74-year-old patient died. In contrast, the case-fatality rate seen in the Teutônia outbreak was very high, suggesting that corticosteroids may have played a role in increasing the disease severity, large number of parasites initially present in the food, or different virulence of the strains involved.

In both outbreaks, patients were initially diagnosed as typhoid fever cases, due to epidemic features of the disease. However, patients were not toxemic as they might have been in typhoid fever, so acute toxoplasmosis was suspected. Both diagnoses were discarded based on clinical and laboratory evidences.

If our conclusions are correct it would seem that the risk of an outbreak of Chagas disease with serious consequences can occur even in an area with low levels of domestic infestation with triatomine bugs. This would indicate a great need to improve our understanding of nonvectorial routes of *T. cruzi* transmission, and the ways in which man can be infected through such cycles.

RESUMO

Possível transmissão oral de doença de Chagas aguda, no Brasil.

Em outubro de 1986, 7 a 22 dias após uma reu-

nião em uma fazenda no estado da Paraíba, 26 pessoas apresentaram doença febril, associada a edema bipalpebral bilateral, e de membros inferiores, hepatoesplenomegalia leve, linfadenopatia e, mais raramente, a um exantema. Um menino de 11 anos apresentou arritmia atrial ao eletrocardiograma e um paciente de 74 anos desenvolveu insuficiência cardíaca aguda. Em 2 pacientes hospitalizados em São Paulo, foi estabelecido o diagnóstico de Doença de Chagas aguda por observação de *T. cruzi* em creme leucocitário. Em autópsia de um caso fatal foi demonstrada cardiomiopatia chagásica aguda. O xenodiagnóstico foi positivo em 9 de 14 pacientes testados. Anticorpos específicos de classe IgG foram encontrados em todos os pacientes e da classe IgM em 20 de 22 doentes examinados. Estudo epidemiológico revelou *Triatoma brasiliensis* nas vizinhanças desta fazenda, porém tal vetor não foi encontrado na casa onde a maioria dos hóspedes pernitoiu. Observou-se alta taxa de gambás infectados por *Trypanosoma cruzi*. Essas observações, associadas as informações relativas aos alimentos consumidos, sugerem que a contaminação de alimentos tenha se originado de secreções de marsupiais naturalmente infectados ou de triatomíneos infectados, que poderiam ter sido esmagados durante o preparo do caldo de cana.

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REFERENCES

1. BARRETO, M.P. - Epidemiologia. In: BRENER, Z. &

- ANDRADE, Z. - *Trypanosoma cruzi* e doença de Chagas. Rio de Janeiro, Guanabara Koogan, 1979.
2. CAMARGO, M.E.; HOSHINO-SHIMIZU, S.; MACEDO, V.; PERES, B.A. & CASTRO, C. - Diagnóstico sorológico da infecção pelo *T. cruzi*. Estudo comparativo dos testes de fixação de Complemento, imunofluorescência, hemaglutinação e floculação em 3624 soros. *Rev. Inst. Med. trop. S. Paulo*, 19: 254-260, 1977.
 3. CARDOSO, F.A. - Sur le mécanisme de la transmission de la maladie de Chagas. *Ann. Parasit. hum. Comp.*, 16: 341-349, 1933.
 4. DIAS, E. - Serviço de estudos de grandes endemias. Transmissão do "*Schizotrypanum cruzi*" entre vertebrados, por via digestiva. *Brasil - méd.*, 54: 775, 1940.
 5. DI PRIMIO, R. - Erros e deficiência no diagnóstico da Doença de Chagas no Rio Grande do Sul. O problema de provável contaminação por via oral do *Trypanosoma cruzi* em Teutônia, RS. *Hospital (Rio de J.)*, 80: 150-165, 1971.
 6. FORATTINI, O.P.; BARATA, J.M.S.; SANTOS, J.L.F. & SILVEIRA, A.C. - Hábitos alimentares, infecção natural e distribuição de triatomíneos domiciliados na região nordeste do Brasil. *Rev. Saúde públ.*, 15: 113-164, 1981.
 7. JANSEN, A.M. & DEANE, M.P. - *Trypanosoma cruzi* infection of mice by ingestion of food contaminated with material of the anal glands of the opossum *Didelphis marsupialis*. In: REUNIÃO ANUAL SOBRE PESQUISA BÁSICA EM DOENÇA DE CHAGAS, Caxambu, 1985. Programa e resumos. Rio de Janeiro, CNPq; FINEP, 1985. p. 39.
 8. KOFOID, C.A. & DONAT, F. - Experimental infection with *Trypanosoma cruzi* from the intestine of cone-nose bug; *Triatoma protracta*. *Proc. Soc. exp. Biol. Med. (N.Y.)*, 30: 489-491, 1933.
 9. LAINSON, R.; SHAW, J.J. & NAIFF, R.D. - Chagas disease in the Amazon basin: speculations on transmission "per os". *Rev. Inst. Med. trop. S. Paulo*, 22: 294-297, 1980.
 10. MARCONDES, C.B.; DIAS, J.C.P.; GUEDES, L.A.; FERRAZ FILHO, A.N.; RODRIGUES, V.L.C.C. & MENDONÇA, D.D. - Blood feeding source of triatomine bugs from some localities of Paraíba, Brazil. *Mem. do Inst. Oswaldo Cruz*, 85 (supl. 1): 110, 1990.
 11. MARSDEN, P.D. - *Trypanosoma cruzi* infection in Cifnice. II - infection induced by different routes. *Ann. trop. Med. Parasit.*, 61: 62-67, 1967.
 12. MARSDEN, P.D.; SEAH, S.K.K. & LECHERMAN, H. The infectivity of single flagellates of *Trypanosoma cruzi*. *Rev. Soc. bras. Med. trop.*, 11: 141-146, 1977.
 13. MAYER, H.F. - Infección experimental con *Trypanosoma cruzi* por via digestiva. *An. Inst. Med. region. (Corrientes)*, 5: 43-48, 1961.
 14. MAZZA, S.; MONTANA, A.; BENITEZ, C. & JANZI, E.Z. - Transmission del *Schizotrypanum cruzi* al niño por leche de la madre con enfermedad de Chagas. *Mepra, Mision de Estudios de Patologia Regional Argentina*, 28: 41-46, 1936.
 15. MCKEEVER, S.; GORMAN, G.W. & NORMAN, L. - Occurrence of a *Trypanosoma cruzi* - like organism in some mammals from southwestern Georgia and northwestern Florida. *J. Parasit.*, 44: 583-587, 1958.
 16. MEDINA LOPES, M.D. - Transmissão do *Trypanosoma cruzi* em um caso, durante aleitamento, em área não endêmica. *Rev. Soc. bras. Med. trop.*, 21: 151-153, 1988.
 17. NATTAN-LARRIER, L. - Infections à Trypanosomes et voies de penetration des virus. *Bull. Soc. Path. exot.*, 14: 537-542, 1921.
 18. NERY-GUIMARÃES, F.; SILVA, N.; CLAUSELL, D.T.; MELLO, A.L.; RAPONE, T.; SNELL, T. & RODRIGUES, N. - Um surto epidêmico de doença de Chagas de provável transmissão digestiva, ocorrido em Teutônia (Estrela, Rio Grande do Sul). *Hospital (Rio de J.)*, 73: 1767-1804, 1968.
 19. OLSEN, P.F.; SHOBMAKER, J.P.; TURNER, H.F. & HAYS, K.L. - Incidence of *Trypanosoma cruzi* (Chagas) in wild vectors and reservoirs in East-central Alabama. *J. Parasit.*, 50: 599-603, 1964.
 20. ROMAÑA, C. - Falta de la transmission hereditaria de *Trypanosoma (Schizotrypanum) cruzi* em *Didelphis paraguayenses* y comentarios sobre herencia del parasito en otros mamíferos. *Mepra, Mision de Estudios de Patologia Regional Argentina*, 4: 149-154, 1955.
 21. SHAW, J.; LAINSON, R. & FRAIHA, H. - Considerações sobre a epidemiologia dos primeiros casos autóctones da Doença de Chagas registrados em Belém, Pará, Brasil. *Rev. Saúde públ. (S. Paulo)*, 3: 153-157, 1969.
 22. SILVA, N.N.; CLAUSELL, D.T.; NÚBILOS, H.; MELLO, A.L.; OSSANAI, J.; RAPONE, T. & SNELL, T. - Surto epidêmico de Doença de Chagas com provável contaminação oral. *Rev. Inst. Med. trop. S. Paulo*, 10: 265-276, 1968.
 23. SIQUEIRA, Z.A. - Investigação imunopatológica em crias de camundongos infectados com *Trypanosoma cruzi*. Salvador, 1975. (Tese - Faculdade de Medicina da Universidade Federal da Bahia).
 24. STROUT, R.G. - A method for concentrating hemoflagellates. *J. Parasit.*, 48: 100, 1962.
 25. SOARES, V.A.; DIAS, J.C.P.; MARSDEN, P.D. & GARCIA-ZAPATA, M.T. - Sobrevivência do *T. cruzi* em caldo de cana: resultados preliminares. *Rev. Soc. bras. Med. trop.*, 20 (supl. 2): 38, 1987.
 26. VAREJÃO-SILVA, M.A.; BRAGA, C.; AGUIAR, N.J.N.; NIRTJEH, M.H.; SILVA, B.B. & VENTURA, E. - Atlas climatológico da Paraíba. Campina Grande, Universidade Federal da Paraíba, 1989.