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EPIDEMIOLOGIC AND LABORATORY STUDIES OF CHAGAS' DISEASE IN NORTHEASTERN BRAZIL

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1. INTRODUCTION

The measurement of the impact of Chagas' disease on populations within endemic areas remains a significant challenge for biomedical research. Chagas' disease is characterized by the triatome vector's complicated ecology, the multiple domestic and sylvatic reservoirs of the etiologic agent, Trypanosoma cruzi, and the wide spectrum of clinical manifestations of the disease in different geographic areas. In order to determine some priorities for the control of this disease we have begun a population based longitudinal study in a rural endemic area. In this population we have measured exposure to the parasite by serology and cardiac morbidity by electrocardiograms. Baseline findings from this study have already led to specific hypotheses about this complex disease which are also being tested in hospital based clinical and laboratory studies.

Our project has the financial aid of the Wellcome Trust and the Pan American Health Organization. Investigations on Chagas' disease were initiated in 1973 in Bahia, Brazil and were made possible by collaboration of the Universidade Federal da Bahia (UFBa), its Faculdade de Medicina and the Instituto Nacional de Endemias Rurais - Fundação Oswaldo Cruz (FOC). The contributors of data presented are: from the Universidade Federal da Bahia, Faculdade de Medicina, Department of Cardiovascular Disease, Prof. Armenio C. Guimaraes, and Dr. José Pericles Esteves; Department of Preventive Medicine, Dr. Celso A. Pugliese and Dr. Eduardo Mota, ICOMI-UFBa Fellow; Institute of Mathematics and Data Processing Center, Dra. Celina Bittencourt Marques; Instituto Nacional de Endemias Rurais - FOC, Nucleo de Pesquisas da Bahia, Dr. Italo R. Sherlock, Director and Dr. Tacito Mendes Muniz; the Harvard School of Public Health, Dr. Thomas H. Weller, Dr. Richard H. Morrow, Dr. J. Stauffer Lehman, Jr., Dr. Kenneth E. Mott, and Dr. Rodney Hoff.

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2. THE PROJECT LABORATORY

A laboratory was initially established by the Harvard/Wellcome/PAHO/UFBa/INERu Project to support the epidemiological studies in Castro Alves and for our research projects in Hospital Edgard Santos (HPES-UFBa) and other clinics in the Salvador area. Laboratory space has been provided by UFBa and equipment has been purchased with aid of a PAHO research grant. In addition to serving our research needs, this equipment has been available for use by graduate students and faculty of the University and we have been asked and have performed Chagas' disease diagnostic tests for SUCAM and for physicians at HPES with this equipment.

In the area of Chagas' disease, the laboratory has capabilities for serological diagnosis and parasitological diagnosis by blood culture and xenodiagnosis. Brasilian technicians have been trained to perform these procedures. Presently immunologic methods for investigating humoral and cellular immunity in Chagas' disease are being developed.

3. CASTRO ALVES STUDY AREA

The Municipio of Castro Alves, is located in a tropical agricultural region of Baia de Todos os Santos. The area with rugged steep hills and deep valleys is 260 meters above sea level and receives 130-180 cm of rainfall annually. Tobacco and mandioc root are the principal crops but cattle raising is becoming increasingly important.

In the 1970 census, Castro Alves recorded 46,727 inhabitants of which 75% were rural. Based on our own census, we estimate the birth rate to be 50-55 per thousand, the crude mortality rate about 15 per thousand and the infant mortality about 200 per thousand.

Selection of this area was prompted by previous studies by the Nucleo de Pesquisas da Bahia, INERu-FOC who had demonstrated infestations of Panstrongylus megistus in approximately 20% of the houses. They initiated a triatomine control program using insecticides in January 1973.

The study area consisting of 10 contiguous "fazendas" was mapped and a census completed in September 1973. The population at that time was 1,087 and this report is based on 1,051 persons who were present when serum specimens and electrocardiograms were taken in December 1973 and February 1974.

4. SEROLOGICAL TESTING FOR CHAGAS' DISEASE

Several serological tests and antigens were evaluated for the epidemiological studies. We adopted the LBCF microtiter adaptation of the complement fixation test (CFT) using a protein antigen, prepared by the method of Maekelt, which was obtained commercially (Behringwerke, Marburg, Lahn, West Germany) or from Dr. I. Kagan at the CDC in Atlanta. The specificity of the CFT for Chagas' disease was ascertained by negative findings with sera from healthy individuals from the U.S. and individuals with other diseases. Sensitivity of the CFT is indicated by positive tests in all patients from whom T. cruzi was demonstrated by xenodiagnosis or positive blood cultures. Reproducibility and reliability of the CFT have been demonstrated by concordant results (97% within one dilution) on a battery of sera when tested on different days, when tested in our Boston and Salvador laboratories and when tested by Dr. I. Kagan at CDC.

In addition to the CFT we have been using the indirect immunofluorescence test (IFT). The IFT tests were initially performed by Dr. C. C. Draper at the London School of Hygiene and Tropical Medicine; however, since then we have been performing this test in Salvador. Concordance between the IFT and the CFT results was 96% (388/403) on individuals from the Castro Alves study. The main advantage of the IFT is the capability for testing blood samples dried on filter paper which we have found useful under field conditions, particularly in infants when venipuncture is difficult.

5. PREVALENCE OF SEROREACTIVITY TO T. CRUZI IN CASTRO ALVES

The presence of serological detectable T. cruzi antibody is used clinically as an indication of present or past infection with T. cruzi and can be used epidemiologically to determine the prevalence of infection

exposure in a population. The high prevalence rate of seropositivity to T. cruzi, as detected by the CF and IFA tests, in this rural population is shown in TABLE I. The gradual increase in prevalence rates with age up to 20 years in Castro Alves has also been observed in other population based studies in Brazil, Venezuela and Costa Rica, although, the rates in the younger age groups (1-9 years) in Castro Alves were higher than reported in other studies (10-15%). Most reported cases of acute Chagas' disease in endemic areas are less than age 10 which would not be expected with the gradual increasing seropositivity rates prior to age 10 in our population. The implication is that infection with T. cruzi prior to age 20 occurs at a relatively constant rate and may not necessarily be associated with a clinically apparent illness. The lower rates observed after age 55 may reflect a higher death rate among seropositive individuals, a loss of seroreactivity and/or a cohort effect.

The CF Geometric Mean Titer (CMT) and IFA GMT among age groups over age 25 varied little; thus it appears that antibody titers remain stable throughout infection. Females in age groups over age 10, however, had a generally higher GMT than males.

In this area with a long history of stable T. cruzi transmission, seropositivity was clustered in households. Ten percent of households had 5 or more seropositive individuals which accounted for 30.2% of all seropositive individuals or twice the expected rate had clustering not occurred. The presence of a seropositive child in a household was a good indication that the household would have a high prevalence of seropositivity. Specifically, the probability of other members of the household being seropositive was about 50% greater than when a child of the same age was seronegative. Thus, screening for young seropositive children provides a useful tool for identifying households with high infection rates and for application of selective control measures. In endemic areas of lower prevalence, the presence of a young seropositive child may be an even more important index of household infection than in highly endemic areas.

6. ELECTROCARDIOGRAPHIC EVIDENCE OF CHRONIC CHAGAS' CARDIOMYOPATHY

In Castro Alves abbreviated electrocardiograms (ECG) based on leads I, II, aVL, V_1 and V_5 with a 30 second V_1 rhythm strip, were taken on persons 10 years of age and older. The Minnesota Code was modified to amplify the classification of the conduction defects and the arrhythmias characteristic of chronic Chagas' cardiomyopathy. In preliminary evaluation of the abbreviated 5 lead ECG, sensitivity for detecting these abnormalities was equivalent to the routine 12 lead ECG.

Below age 55 complete right bundle branch block and bifascicular blocks were the abnormalities most highly associated with seropositivity to T. cruzi (TABLE II). Multifocal ventricular ectopic activity was found only in seropositive individuals below age 55; while above age 55 this abnormality was found only in seronegative persons. Seropositivity to T. cruzi was also associated with a P-R interval of 0.20 or greater irregardless of the heart rate, and in the presence of sinus tachycardia with a P-R interval of 0.17 or greater.

A-V conduction defects, ventricular conduction defects and arrhythmias (sections 6, 7 and 8 of the Minnesota Code) were 6 times more frequent among seropositive persons when compared with seronegative persons. This association with seropositivity was greater among males than females and greater among young persons than older persons. Seropositive males between ages 25-44, an economically important age group, had 8 times the arrhythmias and conduction defects than the seronegative group. Interestingly, the peak rate of cardiac abnormalities among seropositive individuals was found in the 25-29 year age group whereas peak rates among seronegative persons occurred in the older age group and are presumably due to heart disease of other etiologies.

The median age of individuals with conduction defects was 15 years younger than those with ventricular ectopic activity alone or in combination with ventricular conduction defects. This finding suggests that the initial detectable cardiac lesion is related to the intrinsic conduction system. Ventricular ectopic activity, on the other hand, may be a later manifestation of chronic Chagas' cardiomyopathy.

7. VECTOR DISTRIBUTION, HOUSE CONSTRUCTION AND SEROPOSITIVITY TO T. CRUZI

The type of house construction and presence of P. megistus appears to affect the rate of seropositivity in that household. P. megistus was found 6.8 times more frequently in the unplastered mud-stick houses than in mud brick houses. Seropositivity rates among inhabitants of mud-stick houses was 2.4 times that among inhabitants of mud brick houses. Almost all seropositive inhabitants of mud brick houses had lived previously in unplastered mud-stick houses.

As indicated previously, seropositivity in children below age 10 is the good indication of recent transmission of T. cruzi. No child under 10 years of age living in a mud brick house was seropositive. Children who lived in unplastered mud-stick houses had a seropositivity rate 2.6 times greater than that of children who lived in plastered or partially plastered mud-stick houses.

The presence of P. megistus infected by T. cruzi was associated with a high rate of seropositivity in the household, especially in children. With an increasing density of P. megistus house infestation (when at least one bug was infected with T. cruzi) there was a proportional increase in seropositivity in the household (TABLE III). When more than 7 P. megistus were found in a house, over 80% of the inhabitants were seropositive.

In stable endemic areas such as Castro Alves where P. megistus is the principal domestic vector without a sylvatic cycle, surveys of house type, and the density of P. megistus and its infection rate with T. cruzi may be a good indication of the prevalence of seropositivity to T. cruzi in the household.

8. ROLE OF DOMESTIC ANIMALS IN T. CRUZI TRANSMISSION

Domestic animals are susceptible to chronic T. cruzi infection and may be important reservoirs for infecting domestic bugs and therefore pose a risk to human infection. In two "fazendas", dogs and cats were examined by xenodiagnosis. In every house with infected domestic animals, the captured

P. megistus were infected with T. cruzi. Ninety-five percent of the inhabitants of these houses were seropositive. The seropositivity rate among inhabitants of houses with infected domestic animals, independent of the presence of P. megistus, was significantly higher than among inhabitants of houses with uninfected domestic animals. This suggests that infected domestic animals are important risk factors for T. cruzi transmission in Castro Alves.

9. LABORATORY RESEARCH

Dr. Hoff's research interests center on the elucidation of parasitological factors and host-immunological factors that influence the progression from primary T. cruzi infection to chronic Chagas' disease. Within these broad objectives we have begun projects that are suited to the availability of hospital patients, the interests of our Brazilian colleagues and the capabilities of the laboratory.

10. PARASITEMIA IN CHRONIC CHAGAS' DISEASE

In the chronic stage of Chagas' disease, the difficulty of demonstrating T. cruzi by direct examination requires the use of blood cultures and xenodiagnosis. For culture we have been using NNN medium with an overlay of Pan's F29 medium. Prior to culture, the parasites in 3 to 5 ml of venous blood are concentrated after hemolysis with isotonic NH_4Cl solution, centrifuging, repeating hemolysis and then inoculating the pellet into NNN culture. Xenodiagnosis is routinely performed by feeding 5 or 10 5th instar Triatoma infestans on patients and then examining the bugs' gut contents 30 days later for the presence of T. cruzi.

This study is part of a collaborative project whose overall goal is to improve the classification of patients with Chagas' disease according to clinical, electrocardiographic, parasitologic and immunologic findings. We have been studying patients hospitalized at HPES with a presumptive clinical diagnosis of chronic Chagas' cardiomyopathy using serology, blood culture and xenodiagnosis. Of the first 25 patients studied 22 were seropositive and 3 were seronegative. Utilizing a single xenodiagnosis (10 bugs) and one

venous blood culture, T. cruzi was demonstrated in 11 of 22 (50%) seropositive patients; 5 cases were positive by culture only and 2 cases were positive by xenodiagnosis only. Of the 8 cases with positive culture, all were positive by preculture concentration with NH_4Cl hemolysis-centrifugation technique while only 4 cases were positive when whole blood was cultured. Blood cultures and xenodiagnosis from the 3 seronegative patients were negative and they have since been considered to have heart disease of other etiology.

With only a single blood culture and xenodiagnosis, we have been able to demonstrate T. cruzi parasitemia in 50% of seropositive heart disease patients. Repeated attempts with larger volumes of blood will undoubtedly increase the probability of isolation of T. cruzi from seropositive patients. Thus, the accumulated tissue damage by persistant parasites needs to be considered as part of the pathophysiology of chronic Chagas' disease in addition to the immunopathologic mechanisms that have been recently proposed. This is supported by Venezuelan workers who found that ECG abnormalities were 6.3 times more frequent in seropositive patients who were xenodiagnosis positive when compared to seropositive patients with a negative xenodiagnosis (77.3% vs 12.3%). This suggests a poor prognosis for patients with demonstrable parasitemia and chronic Chagas' cardiomyopathy.

This study demonstrates the value of using both xenodiagnosis and blood culture for detecting parasitemia since one method can be positive while the other negative. Clearly, preculture concentration by the NH_4Cl hemolysis-concentration technique greatly improved sensitivity compared to inoculation of whole blood. In addition to concentrating parasites for culture this technique may increase the efficiency of isolation by diluting out growth inhibiting substances such as antibodies that are present in plasma.

11. INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM IN ACUTE CHAGAS' DISEASE

Meningoencephalitis is a severe complication of acute Chagas' disease especially in young children. In addition, chronic neurological syndromes with spastic paralysis, mental deficiency and cerebellar symptoms have been ascribed to chronic Chagas' disease. While T. cruzi has been isolated from

the cerebrospinal fluid (CSF) of patients with meningoencephalitis, it is not known whether the parasites are commonly present in the CSF in acute disease. In collaboration with Dr. Rodolfo Teixeira, Professor and Chief of the Tropical Medicine Service at HPES, we have looked for T. cruzi in the CSF of 9 patients with acute Chagas' disease who were admitted to the hospital. These patients ranged in age from 3 months to 15 years and had CNS symptoms ranging from severe convulsions to only complaints of headache. All cases were begun on treatment with an experimental antitrypanosomal drug immediately after collection of CSF by standard lumbar puncture. CSF was inoculated into NNN cultures, a total leucocyte count was performed and the remaining CSF was centrifuged. The sediment was examined for identification of leucocytes and presence of T. cruzi. The supernatant CSF was stored frozen for later testing for anti-T. cruzi antibody by the CFT and for determination of the immunoglobulin G (IgG) and albumin concentrations by radial immunodiffusion tests (Hyland, Costa Mesa, California).

Cultures were positive for T. cruzi in 7 of the 9 cases with acute Chagas' disease. In 2 of the 7 positive cases there was more than one red blood cell per mm^3 of CSF suggesting that the parasites might have originated from blood contaminating the CSF during the tap. Mononuclear cells were found in normal numbers. Anti-T. cruzi antibodies were detected in the serum of all patients but not in the CSF. Albumin levels were in the normal range (12 to 34 mg/dl) in all patients, while IgG levels were slightly elevated in 3 patients (3.8 to 4.0 mg/dl) whose cultures were also positive for T. cruzi.

It is of interest that T. cruzi in the CSF during the acute stage is associated with normal cytology and absence of detectable antibodies. The clinical and immunological significance of these findings deserves further investigation.

12. CONGENITAL TRANSMISSION OF T. CRUZI

This prospective study is designed to determine the risk of morbidity and mortality to the fetus and infants of mothers who are seropositive for

Chagas' disease. In addition whenever possible we are examining the placenta for direct evidence of infection and pathology. The study group consists of 940 pregnant women in the Northeast Amaralina suburb of Salvador whose nutritional status is being investigated by Dra. Lucila Milanese of the Department of Preventive Medicine. CFT tests on sera from 856 of the women revealed 120 seropositive (14.0%). The women in the study group have been followed through the pregnancy and the infants will be followed for one year to determine if there is any association of chronic T. cruzi infection of the mother (seropositivity) and increased risk of fetal loss, infant mortality, low birth weight or failure to thrive. This study will be completed in February 1977.

T. cruzi was detected in one of 17 placenta from the group of seropositive women. The offspring of this case were premature twins (31 weeks) with weights of 1.17 kg and 1.20 kg and T. cruzi was detected in peripheral blood. They died 5 and 7 days after birth. The tissues of the placenta, which was macroscopically normal, were heavily infected with amastigotes of T. cruzi.

At autopsy, the pathology observed in the twins was similar to that described by Bittencourt except that the urinary bladder in both cases was heavily infected. The IFT of the cord blood serum was positive for IgG antibodies but not for IgM antibodies to T. cruzi. In a follow-up study of the family, T. cruzi was detected by blood culture in the mother and in two of her three children who were examined. The mother's electrocardiogram showed right bundle branch block. She had lived in Salvador for the past 20 years and all of her children were born in the city.

Congenital transmission of T. cruzi has been demonstrated in several Latin American countries. In this case we were able to show the extent of the infection and pathologic lesions in both the placenta and the offspring. The presence of other infected children in this family suggests that congenital transmission may have occurred previously.

TABLE I

CASTRO ALVES - BAHIA

AGE AND SEX SPECIFIC SEROPOSITIVITY RATES FOR T. CRUZI *

	Males			Females			Total		
Age	Number Tested	Number Positive	% Positive	Number Tested	Number Positive	% Positive	Number Tested	Number Positive	% Positive
0-11 Months	7	0	0	9	0	0	16	0	0
1-4 Years	36	5	13.9	40	1	2.5	76	6	7.9
5-9	65	20	30.8	71	19	26.8	136	39	28.7
10-14	57	20	35.1	70	25	35.7	127	45	35.4
15-19	30	15	50.0	54	34	63.0	84	49	58.3
20-24	31	20	64.5	43	25	58.1	74	45	60.8
25-34	44	30	68.2	63	36	57.1	107	66	61.7
35-44	46	26	56.5	45	31	68.9	91	57	62.6
45-54	41	27	65.9	31	17	54.8	72	44	61.1
55-64	18	8	44.4	26	6	23.1	44	14	31.8
65+	26	10	38.5	26	14	53.8	52	24	46.2
Totals	401	181	45.1%	478	208	43.5%	879	389	44.3%

* Results were as determined by the CF test (titers of 1:8 and greater = positive). If CF was AC or not done, the IFA result as used (titers of 1:64 and greater = positive).

TABLE II

CASTRO ALVES - BAHIA

HOUSEHOLD SEROPOSITIVITY RATES FOR T. CRUZI * AND DENSITY OF PANSTRONGYLUS MEGISTUS INFESTATION

	Total Persons			Children 10 years old		
House status	Number Examined	Number Positive	% Positive	Number Examined	Number Positive	% Positive
Without P. megistus	342	122	35.7	70	6	8.6
With uninfected P. megistus	82	35	42.7	22	6	27.3
1-3 P. megistus **	49	27	55.1	16	6	37.5
4-6 P. megistus **	46	34	73.9	11	7	63.6
7-9 P. megistus **	23	19	82.6	5	4	80.0
≥ 10 P. megistus **	29	27	93.1	6	6	100.0
Totals	571	264		130	35	

* Excluding houses which were not searched (12) or where only remnants of P. megistus were found (21)

** At least one P. megistus infected with T. cruzi.

TABLE III

CASTRO ALVES - BAHIA

ECG ABNORMALITIES (VENTRICULAR CONDUCTION DEFECTS AND / OR ARRHYTHMIAS); ** COMPARISON WITH SEROREACTIVITY TO T. CRUZI BY AGE AND SEX

	Males				Females			
	CF Positive *		CF Negative		CF Positive *		CF Negative	
	Abnormal ECG /		Abnormal ECG/		Abnormal ECG/		Abnormal ECG/	
AGE	%	Total	%	Total	%	Total	%	Total
10-14	15.0	3/20	5.6	2/36	0	0/25	9.1	4/44
15-24	34.3	12/35	7.7	2/26	20.3	12/59	7.9	3/38
25-44	43.6	24/55	5.9	2/34	27.3	18/66	4.9	2/41
45-64	42.9	15/35	21.7	5/23	17.4	4/23	24.2	8/33
65+	44.4	4/9	62.5	10/16	7.1	1/14	16.7	2/12
Total		58/154		21/135		35/187		19/168

* Complement fixation positive = titer 1:8 or greater

** Abnormal ECG - Ventricular conduction defect and /or arrhythmias coded by Modified Minnesota Code as follows: 7-1, 2, 3, 4, 5, 6, 7, 8, 9; 7-0. 1, 0.2, 0.3; 8-1.1, 1.2, 1.3, 1.4, 1.7; 8.-2.1, 2.2, 2.3, 2.4, 2.5; 8-3.1, 3.2; 8-4.1, 4.2; 8-5.1; 8-6.1; 8-7.1.