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ACUTE CHAGAS' DISEASE IN WESTERN VENEZUELA: A CLINICAL, SEROPARASITOLOGIC, AND EPIDEMIOLOGIC STUDY

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Abstract. A clinical, parasitologic, and serologic study carried out between 1988 and 1996 on 59 acute-phase patients in areas of western Venezuela where Chagas' disease is endemic showed 19 symptomatic patterns or groups of symptoms appearing in combination with different frequencies. The symptomatic pattern with the highest frequency was that showing simultaneously fever, myalgia, headache, and Romaña's sign, which was detected in 20% of the acute-phase patients. Asymptomatic individuals and patients with fever as the only sign of the disease made up 15% and 11.9% of the total acute cases, respectively. Statistical correlation analysis revealed that xenodiagnosis and hemoculture were the most reliable and concordant of the five parasitologic methods used; these two methods also showed the highest proportions in detecting any clinical symptomatic pattern in acute-phase patients. A similar high reliability and concordance was obtained with a direct agglutination test, an indirect immunofluorescent antibody test, and an ELISA as serologic tests, which also showed a higher proportion of positive detection of clinical patterns than parasitologic methods ($P < 0.001$). It is recommended that individuals coming from endemic areas showing mild and/or severe clinical manifestations should be suspected of being in contact or having been in contact with *Trypanosoma cruzi*, be referred for parasitologic and serologic evaluations to confirm the presumptive clinical diagnosis of acute Chagas' disease, and start specific treatment. The epidemiologic implications of the present findings are discussed and the use of similar methodology to evaluate other areas where Chagas' disease is endemic is suggested.

Shortly after describing *Trypanosoma cruzi*, the etiologic agent of American trypanosomiasis, Carlos Chagas created the basis for future study of the disease that was named after him. He was the first to recognize the role that triatomine bugs play in the transmission of the infection and provided information about its pathology, symptomatology, epidemiology, and geographic distribution.[1] Although Chagas described the disease based on the clinical presentation of acute infection, he later showed the existence of a chronic phase.[1] However, it is now recognized that there are often no clinical manifestations with Chagas' disease, and the clinical diagnosis in an epidemiologic context is confirmed by either the presence of anti-*T. cruzi* antibodies or the parasite itself.[2] Some investigators define acute Chagas' disease as the stage in which the parasite is easily detected by direct examination of peripheral blood, and/or by recognizing a suggestive clinical picture along with the detection of anti-*T. cruzi* IgM.[3] Others, however, note that acute Chagas' disease is characterized by diverse clinical manifestations.[4] There are a small number of publications on the acute phase of Chagas' disease; this is estimated to be 5% of the total dealing with this disease.[5] Nevertheless, there appears to be an increase of the number of acute cases recorded in some Latin American countries, which has led to predictions that the situation at the beginning of the 21st century will be similar to that in the early years of this century.[6,7] Venezuela falls into this category. It is therefore important to be able to detect acute infections that would make early specific therapy possible.

In this paper, we present the results of a comprehensive study carried out on 59 acute-phase patients from western Venezuela during the period January 1988–December 1996. This includes a description of the principal clinical manifestations, a complete parasitologic study using five diagnostic methods, a serologic diagnosis following three different tests along with biochemical characterization of isolated stocks of *T. cruzi*, and epidemiologic considerations of the variables found in the study area. Special attention was paid to the reliability and concordance of the methods used and a comparison made between them to determine the possibility of accurately diagnosing the acute phase of Chagas' disease.

MATERIALS AND METHODS

Study area. The patients studied were from localities where Chagas' disease is endemic in the states of Barinas (77.6%), Merida (15.1%), Trujillo (3.3%), Zulia (1.9%), Tachira (1.3%), and Portuguesa (0.8%), which are located at different altitudes and between 69°30′ and 72°00′W and 7°30′ and 9°00′N in western Venezuela. Most of the acute-phase patients came from rural villages in Barinas located between elevations of 90 and 280 meters, with a mean annual temperature of 26.7°C, an average relative humidity of 74%, and a mean rainfall of 1,600 mm/year. In this area, the dry season is from September to March and the wet season from April to August, with an average rainfall of 87 mm/year and 209 mm/year, respectively. Most houses in which acute cases were detected were typical rural dwellings built with mud walls and a thatched roof. The houses were surrounded by palm trees in which triatomine bugs and/or other wild animals (*Didelphis, Rattus*) were present. Domestic dogs were usually present in all houses. Most patients reported triatomine bugs inside their houses and were able to identify the different nymphal stages. Vector control by spraying the dwellings with insecticide was a regular practice several years ago during the campaign against malaria; however, this practice has been reduced considerably.

Detection of acute cases. A total of 152 patients were examined from January 1988 to December 1996 following referral for treatment of symptoms of acute or chronic Chagas' disease. The procedures used in each case included clinical evaluation at the outpatient clinic of the Luis Razetti Hospital (Barinas, Venezuela) and/or the cardiovascular cen-

ter at the Universidad de Los Andes (Merida, Venezuela). After written consent was obtained from the patients, they participated in a study protocol that included a clinical examination, routine laboratory tests, resting and 24-hr continuous electrocardiograms, chest radiographs, bidimensional echocardiogram, and right heart catherization with septal endomyocardial biopsy.[8,9] Patients with a presumptive diagnosis of the acute phase of Chagas' disease were referred to the Center for Parasitological Research at the Faculty of Science, University of Los Andes (Merida, Venezuela) where they were examined using parasitologic and serologic methods. The study was approved by the Biomedical Committee of the National Research Council in Venezuela.

The parasitologic methods included fresh peripheral blood samples and blood smears stained with 10% Giemsa in phosphate buffer, pH 7.2, for microscopic examination, subcutaneous inoculation of mice using 0.5 ml of blood, hemoculture of 0.5 ml of blood in NNN culture medium with insect saline solution as an overlay, and xenodiagnosis using 10 III instar nymphs of *Rhodnius prolixus* from a closed colony kept at the laboratory. When patients reported allergies due to insect bites or unknown causes, artificial xenodiagnosis was carried out by placing 3 ml of blood into a feeding system for 20–30 bugs made from a rubber condom. Fresh and stained blood films were examined immediately. Inoculated mice (kept in an animal house at room temperature [22–25°C] and fed *ad libidum*) were examined every five days, being declared negative if no parasites were detected after 12 observations. Hemocultures (maintained in an incubator at 25°C) were examined every week for eight weeks or until they were positive for parasites. The bugs used for xenodiagnosis (kept in a constant temperature room at 25°C, a relative humidity of 75%, and a 12:12 light : dark period and fed on clean mice every 15 days) were examined every 30 days for six months. When flagellates of *T. cruzi* were detected in the inoculated mice, hemocultures, or bugs used for xenodiagnosis, they were isolated, maintained in NNN culture medium, and homogenized to be used for enzyme characterization.

Serologic methods to detect circulating anti-*T. cruzi* antibodies were carried out with sera obtained on the same day that parasitologic examination was performed. The sera were tested by the direct agglutination test (DAT) after pretreatment with 2-mercaptoethanol, the indirect immunofluoreseence antibody test (IFAT), and an ELISA following standard procedures.[10–12] Titers $\geq$ 1:64 for the DAT and IFAT and an optical absorbance $\geq$ 0.2 for the ELISA were considered positive for infection with *T. cruzi*. Patients were considered seropositive when they showed reactivity in at least two of the three serologic tests. Dogs from houses of 15 of the 59 acute-phase patients were sampled by xenodiagnosis and serology using the same procedures described for human cases.

Enzyme electrophoresis. *Trypanosoma cruzi* stocks isolated from seven acute phase patients (2-92, 1-93, 8-93, 4-94, 8-95, 36-95, and 37-95) from five different rural endemic areas (El Real, Santa Ines, Ciudad Bolivia, Quebrada Seca, and Calcetas) in the state of Barinas were grown and prepared for identification on the basis of isoenzyme profiles. Analysis was carried out using agarose gel electrophoresis according to procedures described by Momen and others.[13] Seven enzymes were used: malate dehydrogenase (MDH; E.C. 1.1.1.37), phosphoglucomutase (PGM; E.C. 1.4.1.9), glucose-6-phosphate dehydrogenase (G6PDH; E.C. 1.1.1.49), isocitrate dehydrogenase with NADP (IDHNADP; E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (6PGDH; E.C. 1.1.1.44), malic enzyme (ME; E.C. 1.1.1.40), and mannose phosphate isomerase (MPI; E.C. 5.3.1.8). Conditions used for visualizing enzymes were those recommended by Cupolillo and others.[14] Lysates of the Brazilian strain MHOM/Br/50/Y of *T. cruzi* were included in each electrophoretic run as a standard reference.

Statistical analysis. Data was analyzed using nonparametric statistical methods (statistical package SAS).[15,16] A correlation analysis was carried out to estimate the reliability of parasitologic and serologic methods using the Cronbach Coefficient Alpha (ρ^2). Concordance between methods was calculated by applying the Phi Coefficient (φ).

RESULTS

Acute cases. Of 152 individuals examined for chagasic infection, 91 (59.9%) showed positive results, with 59 of these patients (64.8%) manifesting acute Chagas' disease. Fifty-eight of the 59 acute cases (98.3%) acquired their infection in 25 endemic localities in 14 counties (municipios) in the state of Barinas. The remaining case came from the state of Trujillo and was accidentally infected in a laboratory while manipulating *T. cruzi* culture medium. Figure 1 shows the geographic location of the study area and the distribution of the acute cases detected. The 59 acute-phase patients showed a male:female ratio of 1.3 (33 males and 26 females), an mean $\pm$ SD age of 18.3 $\pm$ 13 years (18.6 years for females and 18.0 years for males) with a range from one to 51 years. Most of the acute cases were detected in individuals less than 20 years old (57.6%), with 49.1% of the cases occurring in children less than 15 years of age. No statistically significant differences were observed in the proportion of male and female acute cases. Table 1 gives details on the age and sex distribution of the acute-phase patients diagnosed during the study.

Clinical findings. The average time between the onset of symptoms and clinical evaluations was 14 and 26 days for children and adults, respectively. Most patients (84.7%) showed persistent fever lasting 21 days (range = 2–60) in adults and 15 days (range = 4–20) in children. A portal of entry was observed in 28 of the 59 acute-phase patients, Romaña's sign in 27 (45.8%), and chagoma in one (1.7%). In the remaining cases no clear portal of entry was established; however, their residence in endemic areas and the presence of other clinical symptoms suggested the diagnosis. These included weakness in 30 patients (50.8%), headache in 28 (47.5%), hepatomegaly in five (8.5%), and generalized edema in two (3.4%). Heart failure was clinically detected in only 10 patients (17%), although myocarditis was constantly found in endomyocardial biopsies obtained for histopathologic studies. Megaoesophagus or megacolon was not detected in any of the acute-phase patients studied. Details of the principal clinical symptoms detected in the 59 patients are shown in Figure 2. It is interesting to note that nine of the 59 acute-phase patients (15.3%) were completely asymptomatic and were detected when parasitologic or serologic methods were applied to people who lived with the detected

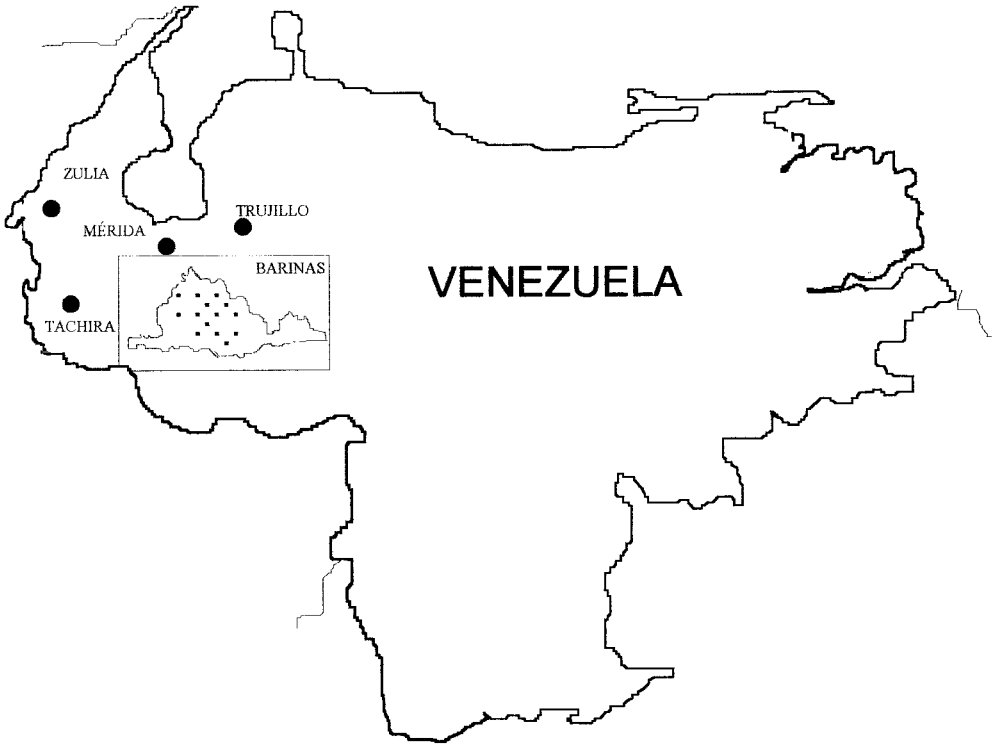

FIGURE 1. Geographic location of the study area in western Venezuela. The **inset** shows the state of Barinas and localities where 58 of the patients in the acute-phase of Chagas' disease were detected.

symptomatic cases. Two of these nine patients (four males and five females 2–29 years old) showed evidence of myocarditis when a endomyocardial biopsy specimen obtained for histopathologic study was examined. A complete account of the frequencies of the clinical symptomatic patterns or groups of clinical signs observed in the 59 cases with acute Chagas' disease is shown in Table 2.

Parasitologic findings. Of the 59 acute cases examined, 48 (81.3%) showed positive results for parasites by at least one of the five parasitologic methods used in the study. *Trypanosoma cruzi* infections were detected in 61%, 53%, 34%, 29%, and 15% of the acute-phase patients when xenodiagnosis, hemoculture, Giemsa-stained blood films, inoculation into mice, and microscopic examination of freshly obtained blood, respectively, were used as routine methods to corroborate a presumptive clinical diagnosis. A correlation analysis carried out to estimate the reliability among parasitologic methods revealed higher confidence with xenodiagnosis ($\rho 2 = 0.75$), inoculation into mice ($\rho 2 = 0.70$), and hemoculture ($\rho 2 = 0.69$) than with stained blood films and/or microscopic examination of fresh blood samples ($\rho 2 = 0.42$). The high correlation among the first three methods indicates that they have the same level of reliability, with difference compared with microscopic examination of fresh blood samples and stained blood films being statistically significant ($P < 0.001$). In addition, the correlation analysis carried out to estimate the concordance between any pair of the five parasitologic methods showed a better concordance between hemoculture and xenodiagnosis ($\varphi = 0.68$), xenodiagnosis and inoculation into mice ($\varphi = 0.66$), and hemoculture and inoculation into mice ($\varphi = 0.61$) than between any pair composed of the combination of microscopic examination of a fresh blood sample and/or Giemsa-stained blood film, which showed a maximum concordance coefficient of ($\varphi = 0.38$). Figure 2 gives details of the proportion of acute cases detected with the parasitologic methods used in the study. It was also observed that 11 (18.6%) of the 59 acute-phase patients infected with *T. cruzi* had an associated infection with *T. rangeli,* which was demonstrated by hemoculture and/or xenodiagnosis in most cases from which isolates of the parasite were obtained.

TABLE 1

Age and sex distribution of acute cases of Chagas' disease detected in western Venezuela

Age group (years)	No. (%) males	No. (%) females	Total (%)
1–5	7 (63.6)	4 (36.4)	11 (18.6)
6–10	4 (57.1)	3 (42.9)	7 (11.9)
11–15	8 (72.7)	3 (27.3)	11 (18.6)
16–20	1 (20.0)	4 (80.0)	5 (8.5)
21–25	3 (27.3)	8 (72.7)	11 (18.6)
26–30	4 (66.7)	2 (33.3)	6 (10.2)
>30	6 (75.0)	2 (25.0)	8 (13.6)
Total	33 (55.9)	26 (44.1)	59 (100.0)

Serologic findings. A high proportion of seropositivity in acute-phase patients was detected when the DAT (88%), IFAT (98%), and ELISA (97%) were used as methods of testing sera for *T. cruzi.* The coincidence of the results of at least two of the three serologic tests, a condition established to determine if a sample was seropositive, was as high as 89% in for the DAT and IFAT, 96% for the DAT and ELISA,

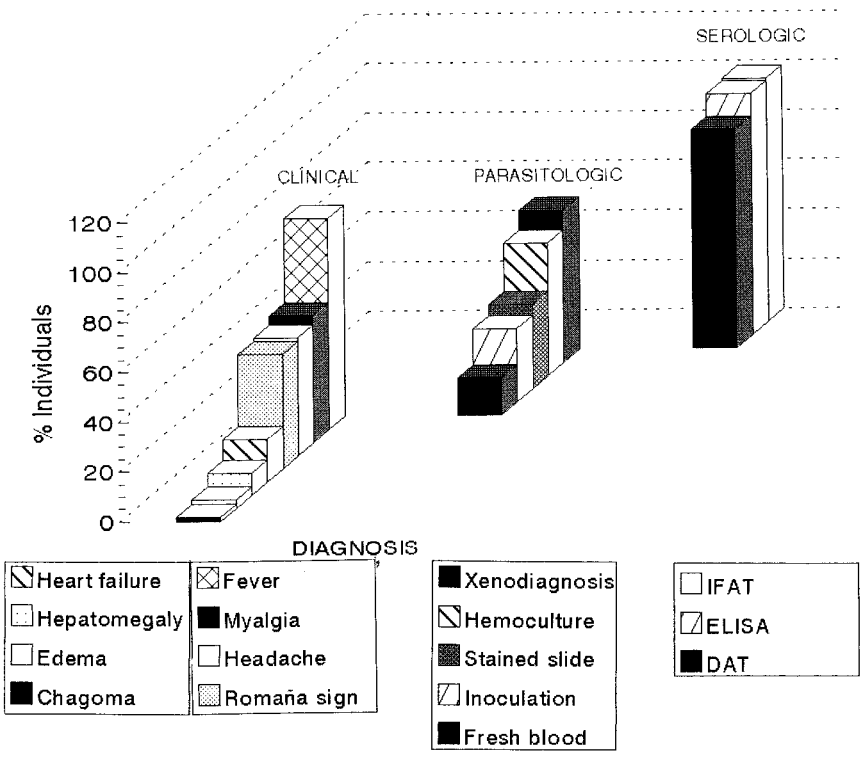

FIGURE 2. Clinical signs and seroparasitologic findings in the acute cases of Chagas' disease in western Venezuela. IFAT = indirect immunofluorescence antibody test; DAT = direct agglutination test.

and 96% for the IFAT and ELISA. Coincidences in the results of the three tests were observed in 93% of the samples. When titer frequency was compared among the three methods, the DAT and ELISA showed 59% and 68% of the patients with titers ranging from 1:64 to 1:128, while the IFAT showed 73% of the patients with titers ranging from 1:512 to 1:2,048. With respect to seronegative acute-phase patients, only two cases (3.4%) were observed. One case was asymptomatic but with parasitemia demonstrated by xenodiagnosis, hemoculture, and animal inoculation, and the other showed a typical clinical picture and positive xenodiagnosis. Four cases showed negative results only for the DAT and one each

TABLE 2
Patterns of frequency of the principal clinical signs observed on 59 acute cases of Chagas' disease detected in western Venezuela

No. of acute-phase patients	Symptoms									Relative frequency (%)
	None	Fever	Myalgia	Headache	Romaña	Heart failure	Hepatomegaly	Edema	Chagoma	
9	☐									15.2
7		☐								11.9
2		☐	☐							3.4
3		☐		☐						5.1
2		☐			☐					3.4
1		☐				☐				1.7
7		☐	☐	☐						11.9
1		☐	☐		☐					1.7
1		☐	☐			☐				1.7
2		☐			☐	☐				3.4
2		☐			☐		☐			3.4
1		☐			☐			☐		1.7
1		☐				☐	☐			1.7
12		☐	☐	☐	☐					20.2
1		☐	☐		☐	☐				1.7
1		☐	☐		☐				☐	1.7
1		☐		☐	☐		☐			1.7
3		☐	☐	☐	☐	☐				5.1
1		☐	☐	☐	☐			☐		1.7
1		☐	☐	☐		☐	☐			1.7
Total = 59	9/59	50/59	30/59	28/59	27/59	10/59	5/59	2/59	1/59	100.00
%	15.2	84.7	50.8	47.5	45.8	16.9	8.5	3.4	1.7	

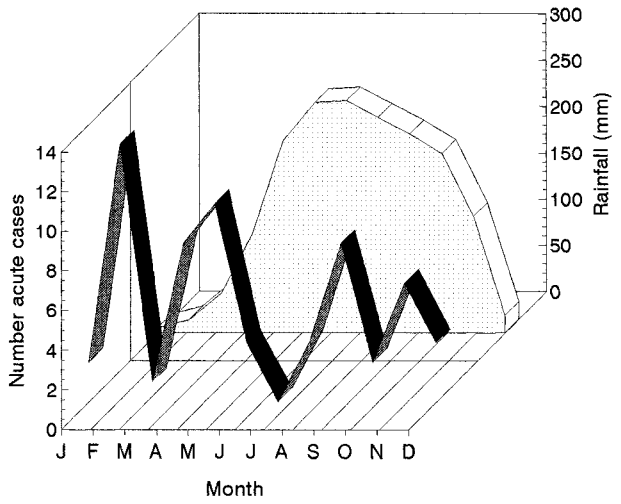

FIGURE 3. Seasonal distribution of acute cases of Chagas' disease in relation to rainfall in western Venezuela.

for the IFAT and ELISA, although they presented typical signs of the acute phase of Chagas' disease. Statistical analysis revealed a high reliability for all the serologic methods used, as indicated by the level of the estimated coefficient ($\varphi 2 = 0.78$, 0.76, and 0.70) for the IFAT, DAT, and ELISA, respectively. Similarly, a good level of concordance was also detected between the DAT and IFAT (0.77), the DAT and ELISA (0.75), and the IFAT and ELISA (0.76). In all cases the sensitivity of the tests was approximately the maximum value (0.93–1.0).

Epidemiologic findings. Acute cases were detected in every month of the year except July, with 35 (59%) of the patients becoming infected during the dry season between the end of September and March, and the remaining 24 (41%) from April to August, the period corresponding to the wet season. Figure 3 shows the seasonal distribution of the acute cases indicating months when more cases occurred in relation to rainfall in the study area. With regard to the contact with triatomine bugs, 34 of the patients (57.6%) reported the presence of vectors in their houses, and in 85% (29 of 34) infection with *T. cruzi* was detected when bugs were examined. Similarly, bugs *(R. prolixus* and *R.robustus)* infected with *T. cruzi* and/or *T. rangeli* were found when palm trees *(Acrocomyia* sp.) located near the houses of five of the acute-phase patients were sampled. Thirty dogs (20 males and 10 females) with an mean ± SD age of 1.5 ± 1 years from houses of 13 of the acute-phase patients were examined for infection with *T. cruzi.* Xenodiagnosis revealed parasites in eight (26.6%), whereas serologic tests (DAT and IFAT) carried out in 15 of them showed seropositivity in 12 (80%). *Trypanosoma cruzi*–infected dogs was also observed in seven of the 13 houses sampled. Xenodiagnosis showed that two specimens of *Didelphis marsupialis* and five of *Rattus rattus* collected from houses with acute cases were infected with *T. cruzi.*

Circulating zymodeme. Electrophoretic studies were carried out on *T. cruzi* stocks obtained from acute-phase patients living in endemic areas in which 47.5% of the total cases were detected. The results indicated one enzyme profile in the seven parasite isolates based on the high percentage of similarities among them. The similarity level ranged from 83% between stocks 2-92 and 8-93 to 100% among the other stocks (1-93, 4-94, 8-95, 36-95, and 37-95). When compared with the Brazilian standard control, the observed similarity was 94% with stock 2-92 and 100% with the rest of the local isolates. Details of the zymogram of the isozymic isolates for the seven enzymes used are given in Figure 4.

DISCUSSION

The ability to accurately diagnose the acute phase of Chagas' disease in rural areas where it is endemic has always been a difficult task due to the heterogeneity of clinical symptoms, which ranges from an absence of symptoms to severe clinical manifestations that are also indicative of other diseases. In addition, the difficulty in finding laboratories that perform parasitologic and/or serologic methods as well as skilled practicioners, leads us to believe that most of the recent infections caused by *T. cruzi* have not been diagnosed. It is generally agreed that the absence or scarcity of clinical symptoms in acute cases implies that patients may have active infections but not an active disease.[7] In this case, the acute-phase patient is only detected by chance or by extend-

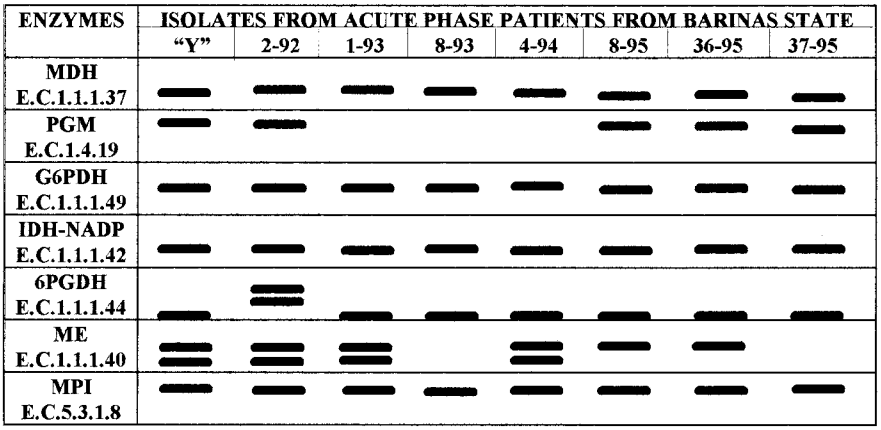

FIGURE 4. Zymogram of isolates from acute cases of Chagas'disease detected in localities of western Venezuela. The Brazilian Y strain was included as a reference. For definitions of enzymes, see Materials and Methods.

ing seroparasitologic diagnosis to people living in the vicinity of an identified acute case.

In the present study, we were able to clinically, parasitologically, and serologically diagnose 59 acute cases. Fifty-eight were natural infections from endemic localities in the state of Barinas in western Venezuela, where an outbreak of acute Chagas' disease was detected during the last eight years, and one was an accidental case caused by inoculation of culture medium containing *T. cruzi*.

In relation to the clinical pictures observed, we observed eight principal signs or symptoms in the 59 acute-phase patients that could be divided into four groups according to their frequencies. Fever was most frequent, detected in 84.7% of the acute-phase patients; a second group with a frequency of approximately 50% was made up of those with myalgia (50.8%), headache (47.5%), and Romaña's sign (45.8%); a third group was composed of those with signs of heart failure (17%) and hepatomegaly (8.5%); and a fourth group was composed of those with edema (3.4%) and chagoma (1.7%). Apart from the asymptomatic patients who represented 15.2% (9 of 59) of the total cases, statistical analysis revealed 19 patterns or groups of symptoms that appeared in combination at different frequencies in the acute-phase patients (Table 2). The symptomatic patterns with the highest frequency include those cases showing simultaneously fever, myalgia, headache, and Romaña's sign, which were detected in 20.2% of the acute-phase patients, followed by those with fever, myalgia, and headache (11.9%), and those with fever as the only sign of the disease (11.9%). The other 16 symptomatic patterns showed frequencies of 5.1% (2), 3.4% (4), and 1.7% (10) (Table 2). Thus, one may conclude that any patient from an endemic area with any of the above symptomatic patterns should be suspected of being in contact or having been in contact with chagasic infection, and should be referred for parasitologic and serologic evaluation to confirm the presumptive clinical diagnosis of acute Chagas' disease and start the specific treatment to avoid fatal consequences.

Some investigators have stated that recent infections by *T. cruzi* are not diagnosed because clinical symptoms are either nonclassic, i.e., without Romaña's sign, or too mild to be recognized.[7] In our case, classic Romaña's sign was detected in 45.8% (27 of 59) of the acute-phase patients and was always associated with other signs or symptoms. This included fever (3.4%), fever and myalgia (1.7%), fever and heart failure (3.4%), fever and hepatomegaly (3.4%), fever and general edema (1.7%), fever, myalgia, and headache (20.3%), fever, myalgia, and heart failure (1.7%), fever, myalgia, and chagoma (1.7%), fever, headache, and hepatomegaly (1.7%), fever, myalgia, headache, and heart failure (5.1%), and fever, myalgia, headache, and edema (1.7%). However, two of these cases (1-93 and 10-94) were undiagnosed by general practicioners and initially treated as ophthalmologic infections. If this occurs when patients show typical signs of chagasic infection, one can deduce a higher number of undiagnosed acute cases when patients show only fever, a common sign for almost all diseases and/or infections. This appears to be relevant to our observation that fever was the only sign of infection in 11.9% of the acute cases studied, and was associated with symptoms other than classic Romaña's sign in 27.2% of the acute-phase patients. Thus, it is important that people in charge of diagnosis at health centers in endemic areas are aware of this and recommend seroparasitologic evaluations of patients with severe clinical pictures as well as those with mild symptoms.

Another interesting aspect of this study is the fact that only 10 (17%) of the 59 acute cases showed some clinical manifestation of heart failure, six of them (10.2%) being associated with Romaña's sign. However, histopathologic observations carried out in 26 endomyocardial biopsy specimens showed acute myocarditis in all of them. Although this observation has been described,[6] we mention it to associate it with the recommendations given above.

In relation to the methods used for the diagnosis of Chagas' disease, it is recommended that the degrees of reliability and concordance are measured to avoid further difficulties in interpretation.[17] In our case, a correlation analysis was carried out to estimate the reliability among the five parasitologic methods used during the study. This analysis revealed higher confidence for xenodiagnosis, inoculation into mice, and hemoculture. These methods also showed better concordance than the fresh blood sample and examination of stained blood films. Similarly, statistical analysis revealed high reliability for the three serologic tests (DAT, IFAT, and ELISA) and a good level of concordance between any pair of these tests. The methodology carried out during the study was well controlled and homogeneous and gave accurate results. Once the reliability and concordance of the seroparasitologic methods used for the diagnosis of the acute-phase patients was estimated, we were able to determine which test showed a higher proportion of positive detection in relation to the detected clinical symptomatic patterns. To do this, each clinical symptomatic pattern was compared with the results obtained after using the eight diagnostic methods. This comparison indicated that xenodiagnosis and hemoculture are the two parasitologic methods with the highest proportion of positive detection of any clinical symptomatic pattern in acute-phase chagasic patients. On the other hand, serologic methods showed a higher proportion of positive detection of clinical symptomatic patterns than the parasitologic methods, with the differences being statistically significant ($P < 0.001$). The analysis also revealed that serologic tests are able to detect positivity in any of the clinical symptomatic patterns, showing no significant differences among them. It is concluded that xenodiagnosis and hemoculture and any of the serologic tests used in this study can be recommended for use in epidemiologic studies to confirm presumptive clinical diagnosis of acute Chagas' disease.

With regard to the age of the patients in the acute phase of Chagas' disease, Chagas stated that most cases are detected in children approximately 10 years old and many cases in older individuals are not diagnosed.[18] The detection of acute infections in children is important even if they are asymptomatic because it permits the use of timely and specific therapy.[7] In our study, we found that 30.5% of the confirmed acute cases were children less than 10 years old, which corroborate the findings of other investigators.[7,18] However, this difference was not found in asymptomatic patients. Similarly, when patients showing mild signs were analyzed, 71% of the cases with fever as the only sign of the disease were found in individuals less than 10 years old.

Contrary to data on the prevalence of Chagas' disease in

Venezuela,[19] our study has demonstrated that acute cases are still a problem in endemic areas that were considered controlled during the 1960s campaign against indoor triatomine bugs. The fact that we were able to detect 58 natural acute infections during the last eight years in just one western state demonstrate that acute Chagas' disease is not unusual in Venezuela. This also allows us to suggest an extension of this kind of study, using the methodology performed in the present work, to other endemic areas where the risk of infection is similar to the area considered in this paper. This recommendation was made after reading reports of a three-year study in the northcentral part of the country, an area considered highly endemic at the beginning of this century, and in which no acute cases were found after examining more than 700 seropositive individuals.[20] The continuous detection of acute-phase cases in the study area is due to the presence of environmental conditions that permit the circulation of *T. cruzi* in their natural reservoirs and/or vectors that are protected in the wide extensions of palm trees that characterize the area. The fact that palm trees are found all around the localities and/or dwellings in enormous numbers facilitates the presence of vectors such as *R. prolixus* and *R. robustus* that inhabit these plant species.[21] This is particularly true of *R. prolixus*, the most important vector of Chagas' disease in Venezuela, which can quickly build up substantial domestic colonies favoring indoor transmission when encountering domestic and peridomestic animals with *T. cruzi* infections. Another factor that enables us to interpret the dynamic transmission of chagasic infection in the study area is that acute cases were found in triatomine-free houses. This indicates that infected triatomine bugs are attracted to houses from their natural shelters located at different distances in the peridomiciliary or sylvatic areas and feed on and infect individuals without colonizing indoors. This behavior should be evaluated because of its epidemiologic implications. The relatively high frequency of *T. rangeli* infections detected in acute-phase patients (18.6%) constitutes a remarkable finding from an epidemiologic point of view due to its potential interference in the diagnosis of *T. cruzi* infections.

Some investigators have suggested that it is important to determine if there is any link between the *T. cruzi* zymodeme carried by patients and the severity of Chagas' disease.[22] However, others have concluded that there is no relationship between a parasite strain defined by stable biochemical markers and a particular feature of the disease.[23] This lack of a relationship between virulence and zymodeme has been reinforced by other investigators, who concluded that acute cases attributable to different zymodemes can give similar initial clinical pictures.[24] In our case, the information gathered in relation to enzyme profiles in seven stocks obtained from a similar number of acute-phase patients from Barinas at different periods and localities showed no significant differences between the clinical pictures recorded during the evaluation. This was probably due to the similarity of the biochemical phenotype of the parasites studied. Although this information will be considered in a larger study on isolates from acute-phase patients and wild and domestic animals, this sample appears to be very homogeneous, suggesting that one zymodeme circulates in the study area.

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