

Diagnosis of Chagas' Disease

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ABSTRACT. A review is presented on diagnosis of american trypanosomiasis or Chagas' disease, an endemic infectious disease from the American continent. The etiologic agent, *Trypanosoma cruzi* is transmitted in nature by the reduviidae bugs. In order to select the best diagnostic procedures, relevant concepts of the immune response and physiopathology of the human host, are summarized. The complexity of Chagas' disease and the difficulties for its diagnosis and treatment evaluation, impose the need to define the best criteria for selecting diagnosis procedures which offer the highest sensitivity and specificity not only to confirm the efficacy of treatment and surveillance for disease transmission, but specially for their utilization at the primary health care level. Timely, sensitive and specific diagnosis of Chagas' disease is linked to the structure and evolution of the parasite populations, the mechanism of pathogenesis and the immune response of human host. Proper diagnosis also depends on the knowledge and evolutionary consequences of medical, entomological and public health interventions. Diagnosis based on parasite characteristics and on human host immune response will contribute to the understanding of Chagas' disease as a collective health problem and for the design and evaluation of interventions for its prevention, integral management and surveillance.

Key words: Chagas' disease, Diagnosis.

RESUMEN. En esta revisión se discuten los diferentes procedimientos de diagnóstico de la enfermedad de Chagas o tripanosomiasis americana, la cual es exclusiva del continente americano. Esta enfermedad es causada por el *Trypanosoma cruzi* el cual es transmitido al hombre principalmente a través de la picadura del vector *Triatoma* sp. En este trabajo se analizan conceptos relevantes de la respuesta inmune del huésped, las características del parásito y de la patología asociada con las diferentes etapas de la enfermedad que permitan seleccionar y utilizar de modo racional los procedimientos de diagnóstico. La complejidad de la enfermedad de Chagas y las dificultades para su diagnóstico, la evaluación del tratamiento y de las medidas de intervención, requiere la necesidad de definir los mejores criterios para seleccionar los procedimientos de diagnóstico más sensibles y específicos cuya aplicación sea útil para confirmar la eficacia del tratamiento y la vigilancia de la transmisión de la enfermedad. Un diagnóstico oportuno y adecuado contribuirá al entendimiento de la enfermedad de Chagas como un problema de salud pública y para el diseño y evaluación de las estrategias de intervención para su prevención, manejo integral y vigilancia.

Palabras clave: Enfermedad de Chagas- Diagnóstico.

INTRODUCTION

The infectious agent of Chagas' disease is the flagellate protozoon *Trypanosoma cruzi* and is naturally transmitted by a triatomine haematophagous bug.⁹ The disease is distributed from Southern South America to Northern Mexico.⁶⁵ It is believed that 12 million people are infected, mostly in rural areas, resulting in estimated from 23,000 to 43,000 deaths annually.^{55,46} *T. cruzi* is characterized by the presence of one flagellum and a single mitochondrion in which is situated the kinetoplast, a specialized DNA-containing organelle. There is not a homogeneous population of this parasite and is composed rather by a pool of strains, which circulate in both the domestic and sylvatic cycles

involving humans, vectors and animal reservoirs of the parasite.⁶² *T. cruzi* infects most mammalian cells and the parasite is highly pleomorphic, exhibiting several distinct forms in its life cycle. Humans are infected when the insect feces containing infective trypomastigotes become rubbed into the wound caused by the bite of an infected triatomine or when the conjunctiva, mucous membranes or abrasions become contaminated. The trypomastigotes forms invade a diversity of cells and are differentiated in amastigotes, which multiply in macrophages and later produce trypomastigotes, which are reproduced in the peripheral blood.⁸

Adaptation of triatomid vector to the human domestic environment facilitates transfer of infection between animals, from animals to human beings or from man to man.⁶⁷



Domestic animals such as porcine, caprin, sheep livestock, and poultry are the main *T. cruzi* reservoirs. Wild rodents, marsupials and non-human primates maintain circulation of the parasite in zoonotic endemic areas. Acute and inapparent infections occur in wild animals and chronic disease is often observed in dogs.⁵ Transmission by blood transfusions from infected persons, congenital infection, and laboratory accidents are another possible routes of parasite transmission.²⁷

So far, chemotherapy is rather unsatisfactory; the used drugs are toxic and often ineffective. However in acute phase, cure is usually possible by using diverse available drug.³⁶ Improvement of the dwellings, screen blood donors as well as environmental and vector management is of paramount importance for the control and elimination of Chagas' disease. The control, elimination or eradication of the triatomine vectors, and its cost-effectiveness are directly related to the grade of their intradomiciliary behavior. The use of pyrethroid pesticides impregnated nets could be effective to prevent bites. Chemical vector control programs have been successfully implemented in several countries of Latin America.⁵⁷

Several vaccines have been tried in experimental Chagas' disease employing live attenuated parasites, killed intact organisms, cell homogenates, subcellular fractions, and purified antigens.⁵⁸ The use of experimental vaccines only could be accepted to be used if they reduce the incidence, mortality and morbidity of the infections produced by *T. cruzi* in human beings.

CLINICAL DIAGNOSIS

To make a proper and timely diagnosis of Chagas' disease it is of paramount importance to keep in mind the modes of transmission, the different phases of the infection, as well as the physiopathology and the clinical manifestations of the disease. In medical practice, procedures utilized for Chagas' disease diagnosis should be technically and operationally feasible, simple, inexpensive, as well as highly sensible and specific.

The Acute phase. This phase usually occurs in children with a furuncle at the site of infection (chagoma). Clinical signs include fever, malaise, enlarged lymph nodes and hepatosplenomegaly. The infection in the eye occurs with unilateral edema of eyelids and conjunctivitis, with retroocular adenitis (Romaña's Sign). In addition, myocarditis and meningoencephalitis may occur.¹⁸ Chronic Chagas' heart disease, is a slowly evolving inflammatory cardiomyopathy that may lead to severe cardiac dilatation, congestive heart failure and death. The occurrence of an autoimmune process leading to chronic myocardial injury may result from a parasite-induced deregulation of the immune system, leading to loss of tolerance for self-antigens or to a *T. cruzi*-induced cross-reactive immune response to self antigens (molecular mimicry).²⁰ The mobile trypomas-

tigotes could be observed in fresh parasitized blood during the acute phase. The presence of the parasite also could be demonstrated by means of haemoculture, inoculation into newborn mice, xenodiagnosis or by molecular procedures such as Polymerase Chain Reaction (PCR).⁴

The Indeterminate phase. Parasitemia is commonly low or difficult to demonstrate in the indeterminate phase, but patients show specific antibodies using different techniques.

The Chronic phase. This phase is characterized by a slow evolution of symptoms in adults mainly resulting in pathological findings characterized by cardiomegaly, megaesophagus and megacolon. However hepatosplenomegaly, and enlargement of intestine, urethra and bladder, may occur.^{59,44,10,7,25}

The type of anatomic and physiologic damage gives clinical symptoms during the chronic phase in the heart conduction system and of the contractile fiber. Borges-Pereira *et al*⁶ evaluated the clinical and epidemiological characteristics of the aneurysm, the global systolic function, and the segmental contractility, palpitations, predominant ventricular extra-systoles followed by changes in conduction, impairment of the ventricular function, regardless of the affected segment. In view of the above, the apical aneurysm of the left ventricle could be considered as a marker of Chagas' disease and as an indicator of high morbidity of the human *T. cruzi* infection within the studied population.

Liechti *et al*³⁵ reported cases with heart problems and positive serologic tests or characteristic histological lesions for Chagas' disease, one of them having presented with acute anterior myocardial infarction with only minor coronary lesions. It has also been termed "emboligenic cardiomyopathy" since arterial embolism is a very frequent complication. Embolic obstruction of a coronary artery may therefore well be the most probable cause of myocardial infarction in young people with Chagas' disease, although other mechanisms can not be excluded. Spasm and thrombosis of the coronary microcirculation has been implicated in the pathogenesis of the cardiomyopathy of Chagas' disease. Tanowitz *et al*⁶⁰ demonstrated that increases in platelet adherence and aggregation accompany *T. cruzi* infection and may contribute to the observed microvascular pathology. These data support the hypothesis that heightened platelet reactivity and endothelial cell dysfunction are associated with acute Chagas' disease and may cause coronary microvascular spasm and/or occlusion.

Wisnivesky-Colli *et al*,⁶⁶ carried out serological tests and electrocardiographic study. The prevalence of complete right bundle branch block (RBBB) was higher in seropositive than in seronegative people.

Gloss *et al*²⁴ cumulated experience from 1977 to 1988 in regard of Chagas' disease and its sequelae, the Chronic Chagasic Cardiopathy. Myocarditis with severe heart compromise, heart failure, arrhythmia, Adams syndrome and pulmonary embolism were documented. The ECG was al-

ways abnormal and heart enlargement was noted on chest X-rays. All patients had serologic evidence of anti-trypanosoma antibodies, polyclonal hyper γ -globulinemia and some showed rheumatoid factor. Although once considered an exotic disease, Chronic Chagasic Cardiopathy is probably underdiagnosed because the lack of methodic studies looking for epidemiologic, clinical and seroimmunologic features in patients with dilated myocardopathy. Cardiologist should be aware of this heart ailment.

Dilatation of viscera is due to destruction of the neurons of the intramural plexi and particularly the Auerbach's plexus. Patients diagnosed as having chronic chagasic esophagopathy are classified on the basis of radiological findings. Megacolon, the most common complication of intestinal trypanosomosis, results in severe constipation, for which surgery is indicated. A variety of procedures have been proposed for the correction of this disabling condition including sigmoidectomy, abdominal rectosigmoidectomy, left colectomy, and subtotal colectomy.

Destruction of neurons and nervous fibers observed in different phases of Chagas' disease partially explains the deficit of the autonomous nervous system over the affected organs but the mechanism for destruction is still controversial.

Moya et al,⁴² described the parasitological and serological studies carried out during the first year of life in pediatric patients born to mothers serologically positive for Chagas' disease. The search for circulating trypomastigotes was performed by Strout, blood culture and/or xenodiagnosis. In some cases, amastigotes were also detected in placenta and umbilical cord. Complement fixation test, indirect hemagglutination and indirect immunofluorescence were used to detect *T. cruzi* antibodies. The results showed a correlation between parasite detection and the persistence of antibodies after six months of life. The methodology employed in this work is accessible to laboratories of medium complexity, and permits the diagnosis of congenital or neonatal chagasic infection with a high degree of reliability. On the other hand, it avoids unnecessary administration of trypanosomocidal drugs in a number of newborn and infants who have only received maternal antibodies at birth and were not infected by *T. cruzi*.

LABORATORY DIAGNOSIS

Laboratory diagnosis of Chagas' disease depends on the stage of the disease. Although the infection by *T. cruzi* stimulates host antibody production, its concentration is low during the acute phase, and diagnosis relies on the confirmation of parasites in blood by microscopy, hemoculture, xenodiagnosis or molecular procedures. Serology is more useful during the chronic phase, especially for screening in blood banks, for epidemiological studies and for the prevention, control and surveillance of the disease.

Complement fixation was the first test to be described by Machado & Guerreiro,²⁶ followed by many other procedures which although of wide-spread use, have different pitfalls, such as difficulties for automation, subjective interpretation of results and cross-reactivity with antibodies related to other parasites. All this may have caused inconsistent results between laboratories.²⁹

Several research groups have recently described a new technique for direct identification of *T. cruzi* stocks in the feces of triatomine vectors and in mammalian blood based on polymerase chain reaction (PCR) amplification.^{4,63,11} The PCR has the advantage of providing a more specific diagnosis than one that relies only on the detection of flagellated parasites in areas where other parasites such as *T. rangeli* could be present. In addition the PCR-based analysis is a powerful tool for determining the presence of parasites because of the method in dealing with large numbers of samples in a short period of time.

To confirm diagnosis it is recommended to perform at least two serologic tests. Nevertheless, caution is also recommended, since sensitivity and specificity could vary within a wide range of results. In areas where both species *T. cruzi* and *T. rangeli* are suspected to be present, it is recommended to use specific-purified antigens. The need to demonstrate the efficacy of chemotherapy for the treatment of Chagas' disease depends upon the availability of valid diagnosis tests. It is important to consider the presence of cases positive to xenodiagnosis and negative to serology, cases with megaviscera and negative serology and the spontaneous cure in absence of specific treatment.

A variety of serological tests are available and depending upon the predictive value it is recommended to make at least two tests for diagnosis confirmation.^{33,38} The immunofluorescence antibodies test (IFA) demonstrates the evidence that the host has been exposed to *T. cruzi* antigens. It has been observed that these antibody tests could remain positive in about 90% of the cases, even after treatment and cure of the human host.³⁰ In experimental animals treated and parasitologically negative, the clearance of marked regression of the myocardium and skeletal muscles was confirmed.³ The acute disease is confirmed with the direct examination of *T. cruzi* in the blood; the haematocrit test concentrates parasites and facilitates their observation. This method is rapid and confident. In coinfecting children with HIV the infection is severe and medication could cause mild adverse effects. Several clinical aspects of the congenital Chagas' disease diagnosis have been studied in children between 2 days and 10 years of age. In < 6 months of age children, the disease was diagnosed by observation of *T. cruzi* in the blood; the microhaematocrit test was positive in 97% of 39 cases.²²

The ideal serological technique for Chagas' disease should be easy to perform, quick in reading results, reliable and unexpensive. Sensitivity and specificity should be high. Other research groups have employed haemagglutination tests, which are currently used with success. Indirect



haemagglutination is the easiest of the available techniques, with high specificity but its sensitivity is lower than other commonly employed techniques, so it is not appropriate as a single diagnostic test.

Indirect Immunofluorescence has been used for serodiagnostic surveys of Chagas' Disease by Camargo¹² and others since 1966 and is still employed extensively, with success. Among other advantages, it is cheaper than IHA, and allows to process a great number of samples simultaneously. Nevertheless, the readings is subjective, mainly in borderline cases, and its specificity is not high, giving cross reaction in low titers with other parasitic infections, mainly visceral leishmaniasis.

Direct agglutination has been a successful technique, mainly after the treatment of sera with 2 mercaptoethanol, which increases its specificity. It is employed routinely because of its higher sensitivity. Nevertheless, it is not employed in the majority of the laboratories because it is as expensive as IHA, has a further technical step and is not so easy to read because of the white color of trypsinized epimastigotes.

Other tested techniques as latex-coated particles, yielded poor results, mainly due to low specificity and sensitivity. Radioimmunoassays have been described, but they may be not suitable for routine laboratories.

In 1977 Voller⁶⁴ introduced the ELISA technique. It has been a good improvement for *T. cruzi* infection diagnosis, but not used as routine in non-specialized laboratories, mainly due to many steps involved. It proves to have high sensitivity, but its specificity with crude antigens is far from ideal, mainly by an array of borderline results. The cut-off point should be adjusted precisely for each plate and each experiment with which the borderline results may be handled better. Also differences in optical density are detected depending on the position of the well under reading, so duplicates of each sera are mandatory, preferentially sprat away in the plate. It is necessary also to include several controls for each plate, with which the actual number of sera to be tested decreases and consequently the cost per tested sample is increased. Lemesre *et al*³³ developed a competitive antibody enzyme immunoassay using a *T. cruzi*-species-specific monoclonal antibody which allowed the development of a specific serodiagnosis of Chagas' disease with a high sensitivity. This assay can differentiate Chagas' disease from other cross-reacting parasitic diseases in areas where concomitant infections are unknown or suspected.

Methods used to diagnose *T. cruzi* infection differ in their ability to discriminate between sera from infected and uninfected individuals. Zicker *et al*⁶⁸ compared the results of an immunofluorescence (IF) test, a haemagglutination (HA) test, and an enzyme-linked immunosorbent assay (ELISA) for the diagnosis of *T. cruzi* infections in a large population-based survey in central Brazil using blood eluates from filter-paper and venous blood samples. The sensitivities of the tests on eluates, compared with results on

serum samples were low. The level of agreement between the tests on eluates was very poor, with the best positivity for IF and ELISA. Both the positive and negative predictive values of the three tests on eluates were similar (around 96%) to those for sera. Higher positive values were obtained for the three tests on sera. These results are important in relation to blood screening, routine medical practice, sero-epidemiological surveys, and the follow-up of patients admitted to therapeutic trials.

Other techniques as complement mediated lysis are time consuming, requiring the use of alive parasites which makes them dangerous for routine work, and their results have had similar problems to the conventional techniques.

The mobile trypanosomes could be observed in fresh parasited blood and a simple stained thick blood film could show trypomastigotes of *T. cruzi*, during the acute phase. During the undetermined and chronic phases of the disease, the presence of the parasite only could be demonstrated by means of haemoculture, inoculation into newborn mice or xenodiagnosis.⁴¹ Serological diagnosis of a clinically suspected case of Chagas' disease could be useful for the physician to confirm the etiology in-patients showing the clinical manifestations of the disease and the epidemiological characteristics. Tests utilized should be highly specific and sensitive. False negative result may lead to a wrong management of the case and to the missed opportunity to administer timely treatment. A false positive result could be even worst, causing iatrogenic consequences, false expectations and waist of resources.

Pinho *et al*⁴⁹ applied the ELISA to saliva to detect chronic infection by *T. cruzi* in humans. Results demonstrate no significant correlation between the antibody titre and cardiac or gastrointestinal tract disease. This assay possesses some advantages over other methods as saliva collection is non-invasive, requires no special equipment and whole saliva gave reproducible results. Although serology remains the gold standard for *T. cruzi* infection, these results suggest that *T. cruzi* specific salivary antibody detection may provide a screening diagnostic test and contribute to epidemiological studies of chronic trypanosomiasis infection in endemic areas.

The use of a complex mixture of antigens does not allow us to detect variations in antibody specificity in acute, chronic, and congenital infections, and there is an obvious need for better characterized reagents. The use of defined parasite material is clearly an essential prerequisite not only to improve serodiagnostic testing or development of a test suitable for screening transfusion blood, but also to identify target molecules for successful host-parasite interaction and to study the immunopathology of Chagas' disease. The construction of a genomic library from *T. cruzi* is making it possible to identify antigens capable of detecting specific immune responses during the acute and chronic phases of congenital and acquired Chagas' disease.³⁷ Jazin *et al*²⁸ studied secreted-excreted immunogens in human patients infected with *T. cruzi*. A pair of 45- to 55-kDa an-

tigens and a family of shed antigens characterized the acute phase, while 160- to 170-kDa immunogen appeared at the chronic phase of the disease.

Paranhos et al⁴⁷ described the characterization of a *T. cruzi* DNA sequence (clone A13) that codes for a polypeptide recognized by IgM and IgG antibodies from sera of acute and congenital chagasic patients. Antibodies to A13 antigen are also detected in the sera of chronic patients with different clinical forms of Chagas' disease, but not in sera of patients with leishmaniasis or other parasitic diseases. The antigenic determinants encoded by clone A13 are found in amastigotes and trypomastigotes of several *T. cruzi* strains, but not in the noninfective epimastigotes. The DNA sequence of the recombinant clone reveals one open reading frame encoding 251 amino acids without tandemly repeated sequences. This antigen may be useful for the development of serodiagnostic procedures. Krieger et al,³² used cytoplasmic and flagellar repetitive recombinant antigens of *T. cruzi* to detect the immune complexes by ELISA in chagasic patients. This procedure avoids false positive results when compared with techniques using conventional antigens.

For epidemiological studies, serodiagnosis techniques also require the highest sensitivity. Positive predictive values depend upon the prevalence of antibodies in the human population. Furthermore, to be efficient, a quick and feasible method of collection, labeling, protection and delivery of blood/sera samples should be used. Blood collection in filter papers, facilitates transportation from the field to the laboratory, but capillary tubes assure the possibility to better preserve the amount of antibodies. The employed techniques should be quick, easy to execute and inexpensive. It is preferable to employ at least two different techniques. The results of serological available tests for diagnosis of Chagas' disease are very useful. Nevertheless, the use of purified antigens is highly recommended when studying areas in which *T. rangeli*, *T. cruzi* and *Leishmania* infections coexist. There are few studies comparing cross-reactions with different antigens.¹⁶

Carbonetto et al,¹³ isolated a *T. cruzi* antigen which could be useful for differential diagnosis of Chagas' disease from leishmaniasis. This antigen, a 52 kDa protein, reacted by ELISA with all sera from Chagas' disease patients tested but not with sera from patients with leishmaniasis. The 52 kDa antigen is widely distributed in the *Trypanosoma* genus since monoclonal antibody reacts with *T. rangeli* and *T. cruzi* parasites.

Blood bank screening. Because of the high prevalence of Chagas' infection in Latin America, antibody screening is mandatory in this area.⁵¹ For the screening operation, a maximum of true positive results must be detected, so the highest sensitivity is required, even with low specificity. To obtain these results, it is recommended to lower the cut-off point of the techniques employed, and to use at least two, preferable three serological tests. If by chance a donor has its blood rejected, his serological diagnosis may be

confirmed thereafter. The important issue is to reject that blood for transfusion. Schmunis et al⁵⁶ reported the potential risk for infectious diseases through blood transfusion in several countries in America, and estimates that the risk of acquiring HIV through blood transfusion was much lower than for acquiring Hepatitis B Virus, Hepatitis C Virus, or *T. cruzi*. These data reinforce the need for an information system to assess the level of screening for infectious diseases in the blood supply, including *T. cruzi*. Zicker et al⁶⁸ studied the trends of *T. cruzi* infection based on data from blood bank screening in Brazil. Antibody detection against *T. cruzi* was performed by indirect hemagglutination test (IHA) and complement fixation test (CFT). The overall seroprevalence of *T. cruzi* infection among first-time donors was 3.5%. The seroprevalence rate increased with age up to 45 years and then decreased. Migrants from rural areas had higher seroprevalence rates than subjects from urban counties. The potential usefulness of blood banks as a source of epidemiological information to monitor trends of *T. cruzi* infection in an urban adult population was stressed.

The employment of screening tests is mandatory for all blood banks in Brazil throughout the country. Oelemann et al,⁴³ compared the diagnostic results of three commercially available assays used in routine testing in Brazilian blood banks: the Abbott Chagas antibody enzyme immunoassay (Abbott Laboratorios do Brasil, Sao Paulo), the BIO-ELISA CRUZI kit (Biolab-Merieux, Rio de Janeiro, Brazil), and the BIOZIMA Chagas kit (Polychaco S.A.I.C., Buenos Aires, Argentina). The evaluation was performed with sera obtained from chagasic patients and healthy residents of four different areas in Brazil where Chagas' disease is either endemic or emergent and where clinical manifestations of the disease and circulating parasite strains vary. The results obtained with each kit were compared to matched in-house ELISA and immunofluorescence assay data obtained for each sample. Depending on the area under investigation, the three commercial kits produced specificity values between 93% and 100%, sensitivity values between 97 and 100%, and accuracies ranging from 93 to 100%.

For the evaluation of chemotherapy and susceptibility of trypanosomes to drugs, there is an urgent need for the development of highly specific methods *in vivo* and *in vitro*. Doubtful results are the rule in treated patients, mainly during the chronic phase of the infection. The follow-up of treated patients is a more difficult situation because a highest specificity is needed, high sensitivity and the use of as many techniques as possible. It may require years of follow-up and very often, a non-defined situation is obtained when some of the techniques gave positive, negative and other borderline results. Multiple experimental and clinical trials with trypanozomocidal drugs, have shown effective clinical and parasitologic response, even in children with less than 3 years of age. Reappearance of parasitemia or seropositivity could be due to either relapses or reinfection.



tions. Classical symptoms such as Romaña's syndrome, inoculation chagoma, hematogenous chagoma, fever and other symptoms commonly disappear in two weeks. Hepatosplenomegalia and generalized adenomegalies could persist up to 60-90 days.^{34,19,17,15} Cure criteria include clearance of parasitemia and negative serology. To confirm negative results, at least two techniques are required for serology.^{39,14,45} Other diagnostic methods include Strout and microStrout,^{21,61} hemoculture.^{23,1,48,31} These methods show different sensitivity and specificity. During acute phase, xenodiagnosis is the most sensitive test. Xenodiagnosis and antibodies anti-living trypomastigote could also be used for the study of the susceptibility of different strains of *T. cruzi*.^{52,53,54}

Both humoral and cell mediated immunity have been involved in *T. cruzi* infection.⁵⁰ Antibody mediated protection has been associated with a particular functional type of antibody, as detected by viable immunofluorescence (VIF), which is able to bind to living trypomastigotes, and is produced during infection but not following immunisation with killed parasites. Antibodies that lyse trypomastigotes in a complement-mediated reaction are believed to be the main participants in the protection against virulent *T. cruzi*. Sera from patients chronically infected with *T. cruzi* display antibodies that bind to epitopes of living trypomastigotes, known as lytic antibodies (LA), and are detected by a complement-mediated lysis test. Conventional serology antibodies (CSA) are also present in sera from patients with chronic infections but in contrast to LA, are unable to recognize viable trypomastigotes. The presence of LA has been used as an important element in the criterion of cure in human Chagas' disease.⁴⁰ Antibodies with specificity for alpha-galactosyl-containing determinants generally called anti-Gal were studied to determine their role in the lysis of trypomastigote forms. Almeida et al² suggested that in vivo high-affinity anti-Gal antibodies may significantly contribute to the destruction of parasite.

Timely, sensitive and specific diagnosis of Chagas' disease is linked to the structure and evolution of the parasite populations, the mechanism of pathogenesis and the immune response of the human host. Proper diagnosis also depends from the knowledge and evolutionary consequences of medical, entomological and public health interventions. Clinical, parasitological, serological, entomological and epidemiological diagnosis with population-biological approaches contributes to the understanding of Chagas' disease and for the design and evaluation of interventions for its prevention, integral management and surveillance.

ACKNOWLEDGEMENTS

The authors are in debt with excellent typing assistance of Miss Claudia Gómez-Cerón.

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