Xenodiagnosis

Hugo Schenone

Programa de Parasitología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 9183, Santiago, Chile

Key words: Chagas disease - xenodiagnosis - *Trypanosoma cruzi*

Xenodiagnosis (XD) is a sophisticate procedure – developed by Brumpt in 1914 – which utilize the vector, acting as a biological culture medium, for the detection of *Trypanosoma cruzi* infection in man and other mammals. In the XD non-infected laboratory reared nymphs of triatomines are used. These nymphs are fed on birds (mostly chicken) which are normally refractory to the parasite. The XD, constituted by a cylindric pot, made of wood or other similar suitable material, covered with a piece of gauze, containing 7-10 nymphs III of triatomines unfed in the previous 3-4 weeks, supported by a bracelet, is applied, during 20-30 min, to the skin surface (upper limb) of the individual to be examined. After this time the nymphs become engorged and it is advisable to keep them in entomological laboratory conditions. After about 30 days, excreta (feces and/or urine) of the insects are microscopically examined for moving *T. cruzi* trypanomastigotes.

Since its introduction numerous studies on the use of XD have been carried out in several countries of the American Continent, particularly in those where Chagas disease is a major medical and public health problem. These studies – many of them ingenuous and creative – have dealed with diverse parameters of XD and can be grouped as follows:

METHODOLOGY, STANDARIZATION OF TECHNICAL AND OPERATIONAL ASPECTS

Since the 30's an uninterrupted series of valuable contributions has been performed. These contributions have stressed on different aspects of it: (a) *generalities* (Dias 1934a, 1940, Mazza 1938, Talice 1944, Schenone et al. 1968a, Salgado 1969, Cerisola et al. 1974, Neal & Miles 1977, Pereira VL et al. 1989); (b) *quality and number of XD kits and species, instar and number of insects to be used:* according to the best of available information there have been neither uniform criteria for quality and number of kits nor for the instars and number of bugs to be used and duration of its application. Thus, a XD unit, considered as routine or natural XD, in summary, has consisted in one kit (one unit), commonly a cylindric pot of varied material, holding 5-10 nymphs of laboratory reared local triatomine species, applied on the skin surface of the individual for about 30 min. Time for the reading has not being well determined. In most of occasions the results have not been satisfactory in suspected cases of Chagas disease, particularly in chronic infections. Facing this situation, arose the idea of performing seriate XD [Dias (1940) practiced seriate XD in dogs infected with Venezuelan *T. cruzi* samples] similar to the techniques employed in other parasitic infections. In this way, we started using pairs of kits containing 7-10 nymphs III of *Triatoma infestants* starved for a period of 3-4 weeks; each pair of kits was applied during 25-30 min to each of both arms for three consecutive days, making a total of six kits with a total of 42 bugs. Routinely the triatomines are examined 30, 60 and 90 days after XD application. When this method was checked in a group of patients with chronic infection, demonstrated by positive serology, sensitivity obtained was 46.1% with one kit (one unit), 54.7% with one pair (two units), 61.8% with two pairs (four units) and 69.1% with three pairs (six units) (Schenone et al. 1974, 1991). To each of 109 children 0-10 year-old, with positive serology for *T. cruzi* infection who were going to receive specific treatment, a pair of XD kits was applied resulting 65 (59.6%) positive, figures that increased to 82 (75.2%) when an additional pair of kits was applied to those who had resulted negative (Schenone 1998). Several authors consider that one XD consists in the simultaneous application of 40 triatomine nymphs distributed into four containers with batches of 10 insects each, method with a good yield, but for us, consisting in the use of four XD kits simultaneously (Pereira JB et al. 1989, 1996, Pereira VL et al. 1989, Chiari et al. 1989, Coura et al. 1991, Menezes et al. 1992, Medeiros et al. 1994). In some

Fax: +56-2-777.4216.
E-mail: rmercado@machi.med.uchile.cl
Received 9 June 1999
Accepted 9 August 1999

instance, authors have referred to one XD with 90 insects and one XD with 120 insects (Bronfen & Alvarenga 1991); (c) *blood ingestion and mortality of insects utilized in XD*: it is necessary to consider that not all the nymphs used in the XD are going to produce suitable material for the microscopical detection of *T. cruzi* because a proportion of them does not suck blood when are applied on the skin of patients, and another proportion dies during the laboratory maintenance periods before examinations, proportions that, in average, reach to 20.5 and 7.3% respectively (Bronfen & Alvarenga 1991). In an accurate experiment comprising 592 nymphs III of *T. infestans* utilized in 30 pairs of XD kits applied during 15 consecutive days (noon and midnight) to a chronic infected patient with a permanent high parasitemia, cumulative mortality of bugs observed was 0.0, 19.4, 51.9 and 87% in examinations effected 30, 60, 90 and 120 days after applications (Schenone et al. 1977). In a total of 1890 XD kits which contained a total of 13,252 third instar of *T. infestans* nymphs, examined during the last 15 years at the laboratories of the Parasitology Program of the Biomedical Sciences Institute of the University of Chile Faculty of Medicicine, the observed average mortality rate of insects was 32.8%; (d) *artificial XD*: due to the fact that some patients present bug bite skin reaction (Mott et al. 1980). This was the reason for the introduction of this technique which has been successfully developed (Romaña & Gil 1947, Nussenzweig & Sonntag 1953, Cedillos et al. 1982b, Silva 1991). According to Santos et al. (1995), it has a higher positivity than routine or natural method. Particularly interesting is the *post mortem* artificial XD performed in blood collected from corpses during autopsy, with a preliminary 30% positivity in individuals whose serological tests for Chagas disease – performed previously to XD – were positive (Lopes et al. 1986); (e) *technique for obtaining the substratum to be examined*: abdominal compression (Schenone et al. 1968a, 1969, 1974, Pereira JB et al. 1989, Pereira VL et al. 1989, Coura et al. 1991, Silva et al. 1995); intestine dissection, grinding and homogenization (Perlowagora-Szumlewicz et al. 1982, Bronfen et al. 1989, Bronfen & Alvarenga 1991, Menezes, 1992); liquefaction of the whole insect, filtration of resultant material through cotton, and centrifugalization (Maekelt, 1964, Rohwedder et al. 1970, Cedillos et al. 1982a); examination of contents:individual (Cedillos et al. 1982a, Pereira JB et al. 1989, Bronfen & Alvarenga 1991, Coura et al. 1991, Menezes et al. 1992) and pooled (Schenone et al. 1968a, Bronfen et al. 1989); (f) *apparently sensitivity of XD* can be improved by the so-called xenoculture which consists in sowing the intestinal contents of the triatomine nymphs used in it in a modified LIT medium (Bronfen et al. 1989); (g) *reading:* must be done by well trained and skilled personnel. It is highly advisable to have in mind the eventual finding of *T. rangeli* and/or *Blastocrithidia triatomae* in the intestinal contents of triatomines (Cerisola et al. 1971a, Cedillos et al. 1982a); (h) *interpretation of result*s: a positive XD in a suspected individual (clinical picture, positive serology and/or epidemiological antecedents) means *T. cruz*i infection and may confirm a chagasic etiology, but a negative XD no necessarily indicates abscence of the parasite and the need of repeating the exam (Freitas 1952, Castro et al. 1983).

SELECTION OF PATIENTS FOR SPECIFIC TREATMENT AND ITS EVALUATION

Since the 70s XD has systematically been used as a relaible tool for the selection and evaluation of patients who have received specific chemotherapy for Chagas disease (Schenone et al. 1970, Cançado et al. 1973, Cerisola et al. 1977, Pereira VL et al. 1989, Schenone 1998).

SEARCHING FOR THE MOST EFFECTIVE TRIATOMINE SPECIES

With the aim of finding the ideal triatomine for XD, different groups of investigators have undertaken trials with this purpose. Cerisola et al. (1971b) in Argentina, working with three chronic cases of Chagas disease, with reiterated positive previous XD, tested on them six species of triatomines from different countries from Central and South America and found a high positivity in *T. infestans* and *T. pallidipennis*, low positivity in *Rhodnius prolixus* and no positivity in *T. dimidiata*, *Panstrongylus herreri* and *R. palescens*. In 1996, Pereira JB et al. applied 563 XD to 563 chronic chagasic patients, between 6 and 89 years of age, from different areas of Brazil. Each XD was constituted by four boxes containing a total of 20 nymphs IV of *P. megistus* and 20 nymphs IV of *T. infestans*. The results showed that 205 (36.4%) were positive, composed by 85 (15.1%) due only to *P. megistus* nymphs, 44 (7.8%) to *T. infestans* nymphs and 76 (13.5%) to nymphs of both species, giving in consequence a total higher positivity of 28.6% in *P. megistus* against 21.7% in *T. infestans*. Perlowagora-Szumlewicz et al. (1982) working with 13 guinea-pigs with laboratory acute infection by Y strain of *T. cruzi*, submitted them to XD containing nymphs IV of nine species of triatomines, obtained the following rates of positivity in the corresponding bugs: *P. megistus* 97.8%, *T. rubrovaria* 95%, *T. pseudomaculata* 94.3%, *R. neglectus* 93.8%, *T. sordida* 84.3%, *T. brasiliensis* 76.9%, *R. prolixus* 53.1%, *T. infestans* 51.6% and *T. dimidiata* 38.2%.

Dipetalogaster maximus, the higher known triatomine, native from Mexico with a restricted geographical distribution in a section of Baja California – a non traditional bug for XD – became a very promissory biological resourse for the detection of *T. cruzi* in infected individuals. Studies carried out in Brazil by Cuba et al. (1978), Barretto et al. (1978) and Marsden et al. (1979) in significant groups of patients with chronic infection have demonstrated that nymphs IV and V of *D. maximus* are more susceptible to *T. cruzi* than the same instars of *T. infestans*, and that nymph I of *D. maximus* is as sensitive as nymph III of *T. infestans* in detecting subpatent parasitemia. On the other side, Bronfen et al. (1989) observed that nymphs III of *T. infestans* are rather more sensitive than nymphs I of *D. maximus* and that nymphs of *D. maximus* have the practical advantage of reducing the number of insects required for XD.

EVALUATION OF PARASITEMIA, ITS RELATIONSHIP WITH CLINICAL ASPECTS AND EVOLUTION OF CHAGAS DISEASE AND ISOLATION OF STRAINS OF *T. CRUZI*

Determination of parasitemia in pre-treatment trials of chronic chagasic patients permits quantification and estimates of its levels in different clinical situations and the changes through the years (Castro et al. 1983, Contreras et al. 1988, Bronfen et al. 1989, Bronfen & Alvarenga 1991, Menezes et al. 1992, Medeiros et al. 1994, Schenone et al. 1995). XD also makes possible the isolation of *T.cruzi* strains from different infected patients (Bronfen et al. 1989, Miranda et al. 1994). Pereira JB et al. (1989) proposed a classification of parasitemia based on the rate of positiveness of nymphs: I. Not detected (0%). II. Low (< 2%). III. Medium (2.1-7%). IV. High (> 7%). High parasitemia was more frequent in patients with chagasic cardiopathy. Persistent parasitemia was seen in 100% of patients of group IV, in 22.2% of group III and in none of group II. In one chronic chagasic infected patient with previous demonstration of high presence of *T. cruzi* – 67 (93.1%) out of 72 XD practiced – two series of 30 kits of XD (with 10 *T. infestans* nymphs III each) were applied at noon and midnight during 15 consecutive days, there was no circadian variation of parasitemia evaluated by the number of positive XD, 93.3% in each series (Schenone et al. 1977).

COMPARISON OF ITS SENSITIVITY WITH OTHER PROCEDURES FOR THE DETECTION OF *T. CRUZI* IN THE BLOOD STREAM

As parasitological diagnosis has a higher complexity in chronic infection than in acute infections, description will be stressed on the first.

XD performed in Chile demonstrated a positivity of 80.3, 86.4 and 49.3% in congenital, acute and chronic infections respectively (Schenone et al. 1969, 1974).

Fifty nine chronic infected patients with positive serology were simultaneously submitted to XD – with 40 nymphs of *P. megistus*, *T. infestans* and *D. maximus* – and hemoculture in LIT medium, resulting positive XD in 23.7, 32.2 and 42.4% with one, two and three species respectively, and hemoculture in 24.1%; these figures increased to 30.5, 33.9 and 49.4% when, according to Chiari and Brener (1966) recommendations, positivity of XD and hemoculture were combined (Bronfen et al. 1989).

In other study which included 39 chronic infected chagasic patients were examined a the same time by using XD containing 40 nymphs of *T. infestans* and hemoculture in LIT medium, results showed a positivity of 36.1 and 19.4% for each of the techniques used (Pereira VL et al. 1989).

Chiari et al. (1989) performed a study with chronic infected patients with positive serology for Chagas disease, in which XD with 40 nymphs III of *T. infestans* and hemoculture were practiced being the corresponding positivity found of 27.6 and 55.2%.

In a field survey carried out in five counties of the state of São Paulo (Brazil) by using four techniques for the detection of blood flagellates – fresh smear, stained smear, hemoculture and XD nymphs III and IV of *T. infestans*, *R. prolixus* and *R. neglectus* varying in number from 3-5 to 25-30 according to the size of the animal – 22 mammals out of 440 animals tested were found infected with *T. cruzi*. In these mammals (21 marsupials and one carnivore) the positivity of each technique was two fresh smears, two stained smears, four hemocultures and 22 XD (Tolezano et al. 1989).

In an experimental study with laboratory acute and chronic *T. cruzi* infected mice, hemoculture in Warren's medium and XD with *R. prolixus* nymphs III and IV, were carried out resulting a positivity of 93 and 36% in animals with acute infection and of 31 and 14% in chronically infected animals. The authors concluded that hemoculture in Warren's medium has shown that overall blood culture is superior to XD for the detection of *T. cruzi*, being the method of choice in the acute stage of the infection, but when the infection is in the chronic phase, the use of both methods is required to give the maximum sensitivity for detection of *T. cruzi* (Neal & Miles 1977).

RELATIONSHIP BETWEEN CYCLE OF THE PARASITE AND THE VECTOR

Different approaches have been studied in considering cycle of *T. cruzi* and vectors (Dias 1934, Wood 1960, Zeledon 1974, Coura 1975, Schenone et al. 1977, Bronfen et al. 1984, Alvarenga &

Bronfen 1984, Coura et al. 1989, Perlowagora-Szumlewicz et al. 1990).

EPIDEMIOLOGICAL SURVEYS

By the use of XD as a tool, interesting studies have been carried out (Dias 1935, Neghme & Schenone 1962, Schenone et al. 1968b, Coura et al. 1991).

REMARKS

XD is a diagnostic procedure which uses the vector which acts as a biological culture medium for the detection of *T. cruzi* in the blood of infected man and other mammals.

Routine or natural XD consists in the use of one cylindric pot containing 7-10 nymphs of unfed triatomine bugs which suck blood from the skin of the individual to be examined.

After an incubation period, the abdominal contents of the utilized nymphs are examined by means of different techniques (compression of the abdomen, dissection, grinding and homogenization of the intestine, and liquefaction of the whole insect).

The yield of XD increase in direct proportion with the number of kits (units) used, for this reason seriate XD is recommended.

XD has been considered a good tool for etiological Chagas disease diagnosis, both for selection of patients for specific therapy and for its evaluation.

It has been shown useful for evaluation of parasitemia and its relationship with clinical conditions of Chagas disease.

Even though there are other efficient procedures for the detection of *T. cruzi*, XD considered an efficient diagnosis method for *T. cruzi* in the blood stream, particularly useful in chronic chagasic infection.

REFERENCES

Alvarenga NJ, Bronfen E 1984. Interação do *Trypanosoma cruzi* com diferentes vectores: uso para xenodiagnóstico. *Rev Soc Bras Med Trop 17*:145-149.

Barretto AC, Marsden PD, Cuba CC, Alvarenga NJ 1978. Estudo preliminar sobre o emprego de *Dipetalogaster maximus* (Uhler, 1894) (*Triatominae*) na técnica do xenodiagnóstico em forma crônica de doença de Chagas. *Rev Inst Med Trop São Paulo 20*: 183-189.

Bronfen E, Alvarenga NJ 1991. O xenodiagnóstico e os critérios para avaliar o nível de parasitemia do paciente chagásico crônico. *Rev Soc Bras Med Trop 24*: 37-42.

Bronfen E, Dias JCP, Gouveia SC 1984. Infecção experimental do *Triatoma infestans* e *Panstrongylus megistus* pela cepa Y do *Trypanosoma cruzi* (Silva e Nussenzweig, 1953). *Rev Pat Trop 13*: 1-7.

Bronfen E, Rocha FSA, Machado GBN, Perillo MM, Romanha AJ, Chiari E 1989. Isolamento de amostras do *Trypanosoma cruzi* por xenodiagnóstico e hemocultura de pacientes na fase crônica da doença de Chagas. *Mem Inst Oswaldo Cruz 84*: 237-240.

Brumpt E 1914. Le xenodiagnostic. Application au diagnostic de quelques infections parasitaires et en particulier à la trypanosomose de Chagas. *Bull Soc Pat Exot 7*: 706-710.

Cançado JR, Marra UD, Mourão OG, Alvares JM, Oliveira JMP, Machado JR, Salgado A 1973. Bases para avaliação do tratamento específico da doença de Chagas humana segundo a parasitemia. *Rev Soc Bras Med Trop 7*: 156-166.

Castro CN, Alves MT, Macedo VO. 1983. Importância da repetição do xenodiagnóstico para avaliação da parasitemia na fase crônica da doença de Chagas. *Rev Soc Bras Med Trop 16*: 98-103.

Cedillos RA, Hubsch R, Tonn RJ, Escalante P, Carrasquero B, Liendo H 1982a. Comparación de dos métodos de laboratorio para examinar xenodiagnósticos. *Bol Of Sanit Panam 92*: 49-56.

Cedillos RA, Torrealba, JW, Tonn RJ, Mosca W, Ortegón A 1982b. El xenodiagnóstico artificial en la enfermedad de Chagas. *Bol Of Sanit Panam 93*: 240-249.

Cerisola JA, Del Prado CE, Rohwedder R, Bozzini JP 1971a. *Blastocrithidia triatomae* n. sp. found in *Triatoma infestans* from Argentina. *J Protozool 18*: 503-506.

Cerisola JA, Rohwedder RW, Del Prado CE 1971b. Rendimiento del xenodiagnóstico en la infección chagásica crónica humana utilizadando ninfas de diferentes especies de triatominos. *Bol Chil Parasitol 26*: 57-58.

Cerisola JA, Rohwedder R, Segura EL, Del Prado CE, Alvarez, M, Wynne GJ 1974. *El Xenodiagnóstico. Normalización. Utilidad*, Imprenta Instituto Nacional de Investigaciones Cardiológicas, Buenos Aires, 127 pp.

Cerisola JA, Silva NN, Prata A, Schenone H, Rohwedder R 1977. Evaluación mediante xenodiagnóstico de la efectividad del nifurtimox en la infección chagásica crónica. *Bol Chil Parasitol 32*: 51-62.

Chiari E, Brener Z 1966. Contribução ao diagnóstico parasitológico da doença de Chagas na sua fase crônica. *Rev Inst Med Trop São Paulo 8*: 134-138.

Chiari E, Dias JC, Lana M, Chiari CA 1989. Hemocultures for the parasitological diagnosis of human chronic Chagas disease. *Rev Soc Bras Med Trop 22*: 19-23.

Contreras MC, Schenone H, Rojas A 1988. Positividad del xenodiagnóstico en personas con reacción de hemaglutinación indirecta para enfermedad de Chagas positiva, de acuerdo a los títulos de dicha reacción. *Bol Chil Parasitol 43*: 22-24.

Coura JR 1975. Evolutive pattern in Chagas disease and the life span of *Trypanosoma cruzi* in human infection. *PAHO Sci Publ 318*: 378-383.

Coura JR, Abreu LL, Willcox HPF, Petana W 1989. Xenodiagnosis in chronic Chagas disease: Why some patients are persistently positive and other are negative? *Mem Inst Oswaldo Cruz 84* (Suppl.II): 114.

Coura JR, Abreu LL, Willcox HPF, PetanaW 1991. Evaluation of the xenodiagnosis of chronic Chagas patients infected ten years or over in an area where transmission has been interrupted – Iguatama and Pains, West Minas Gerais State, Brazil. *Mem Inst Oswaldo Cruz 86*: 395-398.

Cuba CC, Alvarenga NJ, Barreto AC, Marsden PD 1978. Nuevos estudios comparativos entre *Dipetalogaster maximus* y *Triatoma infestans* en el xenodiagnóstico de la infección chagásica crónica humana. *Rev Inst Med Trop São Paulo 20*: 145-151.

Dias E 1934a. Xenodiagnostique appliqué à la trypanosomiasis américaine. *CR Soc Biol (Paris) 118*: 187-189.

Dias E 1934b. Estudos sobre o *Schizotrypanum cruzi. Mem Inst Oswaldo Cruz 28*: 1-110.

Dias E 1935. Xenodiagnóstico e algumas verificações epidemiológicas na moléstia de Chagas. *IX Reunión Soc Argent Pat Reg 1*: 89-119.

Dias E 1940. Técnica do xenodiagnóstico na moléstia de Chagas. *Mem Inst Oswaldo Cruz 35*: 335-342.

Dias E 1940. Xenodiagnósticos seriados em cães infectados com amostras venezuelanas de *Schizotrypanum cruzi. Bras Med 54*: 859-861.

Freitas JLP 1952. O diagnóstico de laboratorio da moléstia de Chagas. *Rev Clín S Paulo 28*: 1-10.

Lopes ER, Chapadeiro E, Brener Z, Franciscon JH, Cardoso JE, Adad SJ Tostes Junior S 1986. Xenodiagnóstico artificial "post-mortem" em chagásicos crónicos. *Rev Soc Bras Med Trop 19*: 252-262.

Maekelt G 1964. A modified procedure of xenodiagnosis for Chagas disease. *Amer J Trop Med Hyg 13*: 11-15.

Marsden PD, Barretto AC, Cuba CC, Gama MB, Ackers J 1979 Improvements in routine xenodiagnosis with first instar *Dipetalogaster maximus* (Uhler 1894) (Triatominae). *Am J Trop Med Hyg 28*: 649-652.

Mazza S 1938. Instrucciones para el diagnóstico de laboratorio de enfermedad de Chagas. *MEPRA 1*: 1-11.

Medeiros LB, Naves HA, Santos JF, Gomes DVA, Carvalho MESD, Oliveira RA, Nascimento MD, Luquetti A, Rassi A 1994. Tentativa de correlação do xenodiagnóstico com o nivel de parasitemia na fase crônica da doença de Chagas. *Rev Pat Trop 23* (Supl.): 8.

Menezes CAS, Bittencourt AL, Mota E, Sherlock I Ferreira J 1992. Avaliação da parasitemia em mulheres portadoras de infecção pelo *Trypanosoma cruzi* durante e após a gestação. *Rev Soc Bras Med Trop 25*: 109-113.

Miranda JAR, Oliveira EC, Campos DE, Rassi A, Rezende JM, Luquetti AO 1994. Differenças entre isolamento de estoques de *Trypanosoma cruzi* provenientes de xenodiagnóstico de pacientes na fase aguda e na fase crônica da doença de Chagas. *Rev Pat Trop 23* (Supl.): 9.

Mott KE, França JT, Barrett TV, Hoff R, Oliveira TS, Sherlock IA 1980. Cutaneous alergic reactions to *Triatoma infestans* after xenodiagnosis. *Mem Inst Oswaldo Cruz 75*: 3-10.

Neghme A, Schenone H 1962. Enfermedad de Chagas en Chile: Veinte años de investigación. *Anais do Congreso Internacional sobre Doença de Chagas. Rio de Janeiro (1959) 3*: 1069-1105.

Neal RA, Miles RA 1977. The sensitivity of culture methods to detect experimental infections of *Trypanosoma cruzi* and comparison with xenodiagnosis. *Rev Inst Med Trop São Paulo 19*: 170-176.

Nussenzweig V, Sonntag R 1953. Xenodiagnóstico artificial. Novo processo. Primeiros resultados positivos. *Rev Paulista Med 40*: 41-43.

Pereira VL, Marcos de AA, Boainain E 1989. Xenodiagnóstico, hemocultura e teste de lise mediada pelo complemento, como critérios de seleção de pacientes chagásicos crônicos para quimioterapia. *Rev Inst Med Trop São Paulo 31*: 301-307.

Pereira JB, Willcox HPF, Marcondes CB, Coura JR 1989. Parasitemia em pacientes chagásicos crônicos avaliada pelo índice de triatomíneos infectados no xenodiagnóstico. *Rev Soc Bras Med Trop 22*: 39-44.

Pereira JB, Junqueira ACV, Santos LC, Castro JAF, Araujo IB, Coura JR 1996. Xenodiagnóstico na doença de Chagas crônica. I. Sensibilidade de *Panstrongylus megistus* e *Triatoma infestans. Rev Soc Bras Med Trop 29*: 241-247.

Perlowagora-Szumlewicz A, Müller CA, Moreira CJC 1982. Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas disease. 1- Comparative xenodiagnosis with nine triatomine species of animals with acute infections by *Trypamosoma cruzi*. *Mem Inst Oswaldo Cruz 77*: 37-53.

Perlowagora-Szumlewicz A, Müller CA, Moreira CJC 1990. Studies on search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas disease. 4 – The reflection of parasite stock in the responsiveness of different vector species to chronic infection with different *Trypanosoma cruzi* stocks. *Rev Saúde Públ 24*: 165-177.

Rohwedder RW, Del Prado CE, Cerisola JA, Rebosolan JB 1970. Aportes al método del examen del xenodiagnóstico previo licuado de los triatominos. *Bol Chil Parasitol 25*: 106-110.

Romaña C, Gil L 1947. Xenodiagnóstico artificial. *An Inst Med Regional (Tucumán) 2*: 57-60.

Salgado AA 1969. Consideraciones sobre metodología y sensibilidad del xenodiagnóstico. *Bol Chil Parasitol 24*: 9-13.

Santos AE, Silva IG, Rassi A 1995. Estudo comparativo entre o xenodiagnóstico natural e o artificial em chagásicos crônicos. *Rev Soc Bras Med Trop 28*: 367-373.

Schenone H 1998. Tratamiento etiológico en la fase crónica de la enfermedad de Chagas en niños de Chile. *Rev Pat Trop 27* (Supl.): 33-34.

Schenone H, Alfaro E, Reyes H 1969. Rendimiento del xenodiagnóstico en las formas aguda y congénita de la enfermedad de Chagas. *Bol Chil Parasitol 24*: 105-106.

Schenone H, Alfaro E, Reyes H, Taucher E 1968a. Valor

del xenodiagnóstico en la infección chagásica crónica. *Bol Chil Parasitol 23*: 149-154.

Schenone H, Alfaro E, Rojas A 1974. Bases y rendimiento del xenodiagnóstico en la infección chagásica humana. *Bol Chil Parasitol 29*: 24-26.

Schenone H, Contreras MC, Rojas A 1991. Rendimiento del xenodiagnóstico, según el número de cajas utilizadas en 1.181 personas con infección chagásica crónica diagnósticada mediante la reacción de hemaglutinación indirecta. *Bol Chil Parasitol 46*: 58-61.

Schenone H, Contreras MC, Rojas A, Villarroel F 1995. Positividad del xenodiagnóstico, según edad, en personas con serología positiva para enfermedad de Chagas. *Bol Chil Parasitol 50*: 40-43.

Schenone H, Concha L, Aranda R, Rojas A, Alfaro A, Knierim F, Rojo M 1970. Valor do xenodiagnóstico na avaliação do tratamento da infecção crônica pelo *Trypanosoma cruzi*. *Rev Goiana Med 16*: 179-184.

Schenone H, Rojo M, Concha L 1977. Positividad diurna y nocturna del xenodiagnóstico en un paciente con infección chagásica crónica de parasitemia permanente. *Bol Chil Parasitol 32*: 63-66.

Schenone H, Rubinstein P, Knierim F, Sandoval J, Rojas A 1968b. Infección por *Trypanosoma cruzi* en dadores de sangre en un hospital de Santiago, Chile. *Bol Chil Parasitol 23*: 83-84.

Silva IG 1991. Dispositivo para realização do xenodiagnóstico artificial. *Rev Pat Trop 20*: 35-38.

Silva IG, Silva HHG, Luquetti AO, Rezende JM 1995. Positividade do xenodiagnóstico de acordo com a faixa etária, o sexo e a forma clínica da doença de Chagas. *Rev Pat Trop 24*: 193-197.

Talice RV 1944. *Enfermedades Parasitarias*, Ed. Científ Sindicato Médico del Uruguay.

Tolezano JE, Nunes EV, D´Andrade OM, Araujo MFL, Balanco JMF, Chieffi PP, Westphalen SR, Valentim AM, Pereira LE 1989. Técnicas parasitológicas para investigação de animais naturalmente infectados por flagelados do gênero *Trypanosoma* Gruby, 1943. *Rev Inst Adolfo Lutz 49*: 85-92.

Wood SP 1960. A potential infectivity index for *Triatoma* harboring *Trypanosoma cruzi* Chagas. *Exp Parasitol 10*: 356-365.

Zeledon R 1974. Epidemiology, modes of transmission and reservoir hosts of Chagas disease, p. 51-85. In *Trypanosomiasis and Leishmaniasis with Special Reference to Chagas Disease*. Ciba Foundation Symposium 20 (new series).