Rev. Inst. Med. trop. São Paulo
31 (2): 63-70, março-abril, 1989

NATURAL TRYPANOSOMA CRUZI INFECTION IN DOGS OF ENDEMIC AREAS OF THE ARGENTINE REPUBLIC (1)

Marta A. LAURICELLA (2), Angel J. SINAGRA (2), Irene PAULONE (2), Adelina R. RIARTE (2) & Elsa L. SEGURA (3)

SUMMARY

The population dynamics and the prevalence of chagasic infection of 352 dogs living in 108 rural houses infested by triatomines were studied. The region was divided into three sections according to increasing distances to an urban area. Each animal was identified by means of its particular characteristics and built, and its owners gave information about its habits. By means of xenodiagnosis, serology and ECG studies, prevalences of infection, parasitological-serological correlation, percentage of altered electrocardiographic outlines and percentage of houses with parasitemic dogs, were determined.

The rural area showed a characteristic **T. cruzi** infection pattern and differences in the canine population parameters with respect to the other areas were observed: a higher proportion of puppies than adult dogs, a more sedentary population, higher prevalences of infection, as measured by xenodiagnosis, in dogs, and the highest proportion of bedroom insects infected with **T. cruzi**.

It is assumed that the sedentary characteristics of the human population in that rural area impinge in the blood offer to the triatomine population, and the high percentage of parasitemic dogs of the area, contribute to the rise of "kissing bugs" infected with **T. cruzi** found in bedrooms.

KEY WORDS: Chagas' disease; Epidemiology; Dogs; **Trypanosoma cruzi**.

INTRODUCTION

The knowledge of the dynamics of transmission of **Trypanosoma cruzi** in the domestic rural environment is essential for the application of measures tending to diminish or abolish incidences of infection in humans and animals.

The elevated frequency of parasitemic dogs[6, 27] and the detection of high proportions of proteins derived from canine blood in the contents of **Triatoma infestans** promesenteron[26], is evidence of the fundamental role the dog plays in the transmission of this flagellate.

In 1916 Salvador MAZZA identified **T. cruzi** in circulating blood of young dogs in the Province of Jujuy[11], and characterized the transplacentary transmission in this host by following infected females and their youngsters[12].

(1) This work was supported by a grant from TDR WHO UNDP Bank.

(2) Professional in Research of the Instituto Nacional de Diagnostico e Investigacion de la Enfermedad de Chagas "Doctor Mario Fatala Chaben" (I.N.D.I.E.C.H.)

(3) Director of I. N. D. I. E. C. H.

Address for correspondence: Dra. Marta Alicia Lauricella. Instituto Nacional de Diagnostico e Investigacion de la Enfermedad de Chagas "Dr. Mario Fatalla Chabén". Paseo Colón, 568. Buenos Aires 1063, Argentina.

In Brazil, the prevalence of infection as measured by xenodiagnosis for naturally infected dogs, was of 18.3%[8], and in Chile of 9.3%[20]. In endemic areas of our country, the prevalence of infection detected by serology, for dogs from urban areas, was of 17.5%[5]. However, the highest indices (64.2% and 65.2% for xenodiagnosis and serology respectively) corresponded to dogs from rural areas in the Province of Santiago del Estero[27].

The area chosen for this study covers a surface of 440 km^2 and it belongs to the Rio Hondo County in the mid-west of the Province of Santiago del Estero. It's a valley one km in width, crossed by the Dulce River and it is located between 4 to 50 km from the city of Termas de Rio Hondo. The zone shows the typical vegetation of the Fitogeographic Chaco Province forested with axebreakers (Aspidosperma spp and Schinopsis spp). Partial deforestation has generated a secondary formation of xerofilus shrubs (Acacia spp and Larrea spp). This area involves a rural community of approximately 3,000 inhabitants, where the serological prevalence for Chagas' Disease in children less than five years old is of 8%[16]. The province of Santiago del Estero belongs to a vast central region of Argentina where the absolute maximum temperatures surpass 45 C and the relative annual humidity is less than 70%; this region is also coincident with the isoline of 75% triatomine domicile infestation[3].

As this rural community belongs to an area of high endemicity for Chagas' Disease, an epidemiological surveillance project consisting in the use of appropriate tools and technology to diminish the vectorial transmission through sanitary agents belonging to the same community is being implemented in this area[16, 17]. The objective of this work is to characterize the rural canine population and to obtain information on the dynamics of infection by **T. cruzi**. The realization of these objectives will permit the planning of control strategies on the domestic reservoirs, contributing to abolish their parasite offer to the triatomine and, hence, to man.

MATERIALS AND METHODS

A — Selection of the sample:

The study was performed with 352 dogs (D) living in 108 houses. Only those not found during two consecutive visits to the house, and those presumably dangerous to the operators were excluded (12/352).

The area (Fig. 1) was divided into three zones according to the distances to the city of Termas de Rio Hondo and to the structural characteristics of the houses (huts: built with indigenous materials; brick: for which industrial technology was used; and mixed: a combination of both).

The rural zone (R) contained improved huts and huts in similar proportions and was the most distant to the city; the transitional (T) zone had the three types of houses in similar proportions and the periurban (Pe) zone had a majority of brick houses.

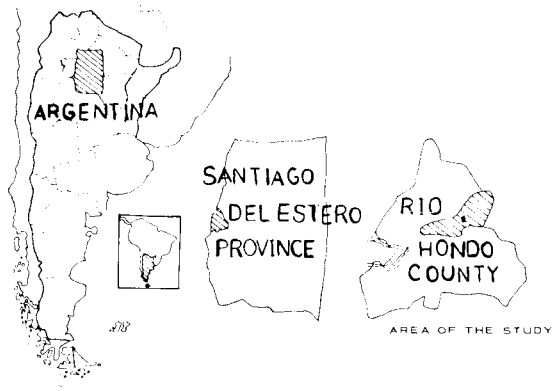

Fig. 1 — Geographical location of the Rio Hondo Department, Santiago del Estero, Argentina.

A sample of the canine population that belonged to houses that had at least four triatomines (kissing bugs) during the entomological survey performed in the zone the previous year, was selected. The search methodology used was that indicated by the Chagas National Service of one man hour of searching effort using chemical irritants[15]. Immediately after this survey the area was sprayed with Deltamethrin and Benzene-Hexachloride.

B — Characteristics of the population survey:

It consisted of an inspection of the animal's aspect (sex, color, coat, particular signs, approximate weight and physical appearance) and of a questionnaire answered by its owner concerning the animal's habits (Fig. 2).

Coding	House number Dog number	House location Owner's full name
Description	Sex Age Size Weight estimate	Built Color Kind of hair Particular signs
Questionnaire	In this area, questions made to owners about origin, hunting, nocturnal, and feeding habits and transient migrations are recorded.	
Physical appearance		

Fig. 2 — Epidemiological survey model: identification card, identikit and questionnaire.

C — Studies performed

On land — To detect parasitemia, xenodiagnosis was done in 340 D using twenty **T. infestans** according to the methodology described previously[2]. Eight ml of non-heparinized braquiocephalic-, yugular-, or saphena-vein blood were extracted for the procurement of serum for anti **T. cruzi** specific antibodies on 331 A, and looking for parasites using the STROUT enrichment method[24], on 42. With the purpose of detecting chronic injuries, electrocardiographic records of 45% (85 ECG) of the D older than two years of age, were obtained using a Fukuda Century electrocardiograph (SCC-1) with a paper velocity of 2.5 cm/sec without previous anesthesia of the animals.

The houses included the home and the peridomicile, the first constituted by the bedrooms, the gallery, and the yard or outside area next to the house, where the owners sleep in the summer. The peridomicile was represented by the kitchen, the deposit and the animal pen. The number of "kissing bugs" found in 102 houses, those found in their peridomicile, and those in the bedrooms with a previous application of piretroid stimulants, was determined.

Evaluation of infection with **T. cruzi** was performed on "kissing bugs" found in bedrooms. The Average Triatomine Infestation (A.T.I.) was determined as the average of total "kissing bugs" of the house; those corresponding to bedrooms ($A.T.I._b$) and peridomicile ($A.T.I._p$) were also calculated.

In the laboratory — The blood obtained by conserving the sera in glycerin buffer (90% glycerin — 10% stabilized saline solution in equal amounts) was processed daily. The blood of three D chosen at random was observed daily looking for the presence of **T. cruzi.** For the specific antibody search in the serum of 326 D, the indirect immunofluorescence reaction was employed, using canine antigamma-globulin fluorescein tagged (Byosis S.A.). Titles accepted as positive were 16. Commercial indirect hemmagglutination (Polychaco S.A.) was also used. Discordant samples were submitted to an ELISA reaction using an antigen prepared from cultured epimastigotes[25].

All reactions were adapted to canine sera and their sensibility was evaluated using sera of 60 parasitemic D from the Moreno County (Province of Santiago del Estero)[9].

D — Statistical studies

Statistical analysis of the data was done using the x^2 test for analysis of frequency[23]. Significance was set at P = 95%

RESULTS

A — General characteristics of the canine population

The canine population density was of 3.4 D/house and only two houses lodged more than six (11 and 13 D). The age distribution showed a 32% diminution of the D number of the 1-2 year interval with respect to D of the 0-1 year interval. Seventy five percent and 90% were between 0-4 years old and 0-6 years old respectively. The number of males (72%) was significantly higher than the number of females (28%). Seventy seven percent was over a year old and was classified according to activity, into guardians (68.8%), hunters (25.7%) and goat tenders locally called "goaters" (5.5%). See Table 1.

Seventy two percent of the dogs were born in the proximities of the houses, and the rest mostly in the cities of Termas de Rio Hondo, Santiago del Estero and some localities of the province of Tucuman; only 3% slept temporarily in the woods. Twenty nine percent of the houses

TABLE 1
Percentages and prevalences of infection (PI) by serology of dogs classified according to their role and environment.

	Role of dogs in the house		
	guardians	hunters	goaters
%	68.8	25.7	5.5
PI (%)	36.4	46.2	13.0
	Environment		
	Domicile	Storage area	Pen
%	89.4	8.4	2.2
PI (%)	59.7	36.4	33.3

TABLE 2
Age specific prevalences rates of seropositivity and **T. cruzi** parasitemia in dogs.

	Dogs examined by age groups				
Ages	0-4	5 9	10-14	15 19	Total
# dogs examined	279	45	12	4	340
# dogs xenopos.	45	13	6	2	66
# dogs seropos.	78	27	8	2	115
Px (%)*	16.1	28.9	50.0	50.0	19.4
Pi (%)**	28.0	60.0	66.7	50.0	33.8
Px Pi***	0.58	0.48	0.75	1.0	0.57

(*) Prevalence rates of infection by xenodiagnosis
(# dogs xenopos.) / (# dogs examined)
(**) Prevalence rates of infection by serology
(# dogs seropos.) / (# dogs examined)
(***) Parasitological serological correlation
(# dogs xenopos.) / (# dogs seropos.)

lodged temporary workers, most of whom went to the sugar harvest in Tucuman where they stayed up to five months of the year; this way, 25% of the D abandoned the habitat transiently.

The D received unbalanced diets and the food was of low protein content.

Differences between the three zones were observed: R showed two distinctive characteristics: a) the percentage of houses with temporary workers was significantly lower (16%) than in T (28.2%) and in Pe (40.7%) $P < 0.05$; b) the decrease in the number of D of the interval of 1-2 years with respect to the 0-1 year interval was of 68.2%, much greater ($P < 0.05$) than the one registered in T (34.5%) and in Pe (12.5%). However, there were no differences between the parameters of T and Pe.

B — Characteristics of the infection by T. cruzi

The prevalence of parasitemic infection as seen by xenodiagnosis (Px), was of 19.4% with no differences between males (20.1%) and females (17.8%). Forty six percent of the houses had at least one D with positive xenodiagnosis and in only one of 42 blood samples, **T. cruzi** was detected by the STROUT method. This sample belonged to a nine month old D that lived in the Pe area. The percentages of parasitemic dogs according to age intervals increased with each age group, and in those older than ten years old an important increment with respect to the younger ones was observed (Table 2).

Almost thirty four percent (33.8%) of the D were reactive for specific anti **T. cruzi** antibodies and no differences were observed in the prevalences of infection by serology (Pi) between males (35.5%) and females (32.2%). No differences were seen in $Pi_{(Pe)}$ = 30.0%, $Pi_{(T)}$ = 33.0%, and $Pi_{(R)}$ = 40.2%. The Pi for the different D subgroups classified according to their environment and activity in the house were determined. The Pi of the hunters and guardians resulted significantly greater than those of the goaters ($P < 0.05$), and the Pi of the dogs that slept in the houses was significantly greater than of those which slept in the peridomicile ($P < 0.05$) (Table 1).

The study of the dynamics of infection revealed that 50% of the serologically reactive D contract infection before they reach five years of age. The parasitologic-serological correlation for all the D was of 57.7%, and of 70% for the first year of life, showing a similar tendency to that observed for the age-specific parasite recovery.

Five electrocardiograms, either had evidences of conduction disorders, incomplete blockade of the right branch, left anterior hemiblockade, or both. Four of the five animals were parasitemic, all of them with positive serology.

The A.T.I. of 102 houses was of 43 + 42.9 "kissing bugs" per house. Ninety nine percent of the houses had triatomines in bedrooms (A.T. $I._{b}$ = 25 + 25.4) and 58% of the houses had "kis-

sing bugs" in the peridomicile ($A.T.I._p$ = 28 + 32.3). On the other hand, out of 66% of the bedroom-insects examined, 30% of them were infected with **T. cruzi** (Table 3).

Area R showed distinctive characteristics with respect to infection patterns. Significant differences were observed ($P < 0.05$) in Px of area R (30.5%) with respect to that of T (17.1%) and of Pe (16.2%) for similar Pi in all areas. Furthermore, the difference in the percentage of insects infected with **T. cruzi** found in the bedrooms of the R area (39.7%) with respect to those of T (25.2%) and of Pe (27.8%), was significantly different ($P < 0.05$) (See Table 3). There were no differences between the parameters of T and Pe.

DISCUSSION

The subdivision of this area according to increasing distances to an urban conglomerate had the purpose to evaluate the influence of the environment on **T. cruzi** transmission. As is frequent in rural areas, a transition of huts to brick houses as the distance to the city diminished, was observed. Taking into account that the highest prevalences of seroreactivity are usually seen in residents of non-plastered houses[13], it was not surprising to observe a characteristic epidemiological profile in this rural area. The parameters of the canine population and the characteristics of infection by **T. cruzi** in the rural area showed differences with those of the remaining areas. There was a greater proportion of cubs than adults, a more sedentary population, higher prevalences of infection as seen by xenodiagnosis in the dogs and the highest proportion of **T. cruzi**-infected insects in the bedrooms. The high number of dogs less than one year compared to the rest, is an evidence of the difficulties that this population has to overcome in the rural area, such as malnutrition, dehydration, parasitism and its consequences, which impinge upon the health of the animals. However, the possibility that in this area, infection by **T. cruzi** could well be another cause of the death of the puppies must be considered.

In the rural area, significant increases in the number of houses with sedentary residents during the whole year were detected, the migration to the sugar-cane harvest in Tucuman is an sporadic fact, in contrast to what is observed in the periurban area where over 40% of its population has migratory habits. In a house where temporary migration of its human and animal inhabitants occurs, the triatomine population could be modified because of lack of ingesta; however, this hypothesis needs to be confirmed. The detection of chagasic infection observed in Chilean sedentary paleoindian tribes, which were formerly nomads and had Bolivia as their dispersion center, would support this hypothesis[21].

The highest prevalences of infection as measured by xenodiagnosis in dogs, and the highest proportion of bedroom insects infected with **T. cruzi** were observed in the rural area. The correlation between levels of triatomine infestation and parasitemia detected, agrees with that observed in dogs of other endemic areas of Argentina[27, 28] and Brazil[14].

It is not possible to infer from this work if the infectivity by **T. cruzi** in domicile triatomines influences the prevalences of infection in dogs or vice versa. However, the influence of this reservoir in the domestic cycle is undeniable, evidenced by the higher indices of seroreactivity in children who live in houses with infected animals[14] and the high frequency of domiciliary triatomines which feed in dogs[26, 28].

TABLE 3
Detection of infection by **T. cruzi** in domiciliary triatomines found in bedrooms.

Area	rural (R)	transitional (T)	periurban (Pe)	Total
# houses	22	48	32	102
% of infected bedroom "kissing bugs"	39.7 (184/462)	25.2 (196/778)	27.8 (103/370)	30.0 (483/1610)

In this work the parasitological-serological correlation goes from 58% in young animals to 100% in senile dogs, as it was also observed in other areas of Santiago del Estero[6]. This is unlike humans where this correlation descends from 100% in children less than four years old to 33% in adults[8], and is an indication of differences between the parasitological profiles of humans and dogs. Higher chances of reinfection in dogs, due to their closer contact with the triatomines[28], and the peculiar characteristics of their immune system, could be the cause for these differences.

The numeric differences observed between males and females suggest an artificial selection made by their owners, frequent in rural areas. The similar prevalences of infection by xenodiagnosis and serology in both sexes indicate a similar susceptibility to infection, as seen in humans, in contrast to that observed in experimental infections of mice with the Brazil strain of **T. cruzi**[18] or in certain strains of rats[19].

The evidence that one out of two houses of the study area had at least one parasitemic dog, justifies the investigation of the infection mechanisms of this reservoir, evaluating the incidence of perinatal[12] and "contaminant" transmission[4]. The high proportion of perinatally infected puppies studied by MAZZA[12] and the contaminant mechanisms of infection observed in animals[4] bespeak for the evaluation of its influence specially in desinsectized houses.

The unmistakable confirmation that this reservoir contributes blood and flagellates to the triatomine population, supports the implementation of measures tending to block its influence by means of the development of vaccines[22] or the use of chemotherapy[1, 7].

RESUMO

Infecção natural pelo Trypanosoma cruzi **em cães de áreas endêmicas da República Argentina.**

Estudou-se a dinâmica populacional e a prevalência de infecção chagásica de 352 cães vivendo em 108 moradias rurais infestadas por triatomíneos. A área foi dividida em três setores de acordo com suas crescentes distâncias em relação a um povoado urbano. Cada animal foi identificado mediante suas características particulares, e seus donos deram informações a respeito de seus hábitos. As prevalências de infecção foram determinadas por sorologia, correlação parasitológica-sorológica, percentual de traçados eletrocardiográficos alterados e o percentual de moradias com cães parasitados.

A área rural mostrou um padrão característico de infecção por **T. cruzi** e observaram-se diferenças nos parâmetros de população canina em relação às restantes áreas: maior proporção de filhotes, maior população humana e canina sedentárias, maiores prevalências de infecção por xenodiagnóstico em cães e maior proporção de barbeiros infectados com **T. cruzi** nos cômodos da casa.

Supõe-se que as características sedentárias da população humana desta área rural é fundamental para a oferta de sangue para as populações triatomíneas, e a elevada porcentagem de cães parasitêmicos dessa área contribui para o aumento de barbeiros infectados com **T. cruzi** encontrados nos quartos de dormir.

ACKNOWLEDGMENTS

We thank Dr. Roberto Chuit for his professional help in the area, and Mr. Domingo Luna and Mr. Griseldo Roldan for technical collaboration. We also thank Dr. Mirta Carlomagno for editorial assistance. Our deepest thanks go to Dr. Carlos Del Prado for the photographs.

REFERENCES

1. CABEZA MECKERT, P. M.; CHAMBO, J. G. & LA GUENS, R. P. — Modification of the pattern of infection and evolution of cardiopathy in experimental Chagas disease after treatment with immunosuppressive and trypanocidal drugs. **Medicina (B. Aires), 48**: 7-11, 1988.

2. CERISOLA, J. A.; RHOWEDDER, R.; SEGURA, E. L.; DEL PRADO, C. E.; ALVAREZ, M. & MARTINI, G. W. — El xenodiagnóstico: normalización utilidad. Premio Geigy 1974. Buenos Aires, Ministerio de Bienestar Social, Secretaria de Salud Pública, 1974.

3. CURTO DE CASAS, S. I. — Problemática geográfica de la endemia chagásica y técnica para su estudio. Factores biológicos y ecológicos en la enfermedad de Chagas. In: CARCAVALLO, R. U.; RABINOVICH, J. E. & TONN, R. J., ed. — **Epidemiología-vectores**. Buenos Aires, Servicio

Nacional de Chagas; Ministerio de Salud y Acción Social, 1985. Cap. 4, p. 31-47.

4. DIAZ UNGRIA, C. — Experimental study of **T. cruzi** in dogs and other vertebrates. The problem of transmission. **Kasmera, 3:** 73-88, 1968.

5. GORODNER, O. L. Z. de; MENDIVIL, G. T.; RISSO, A.; RISSO, J. J.; PETRAGLIA, G.; DE FRANCESCO, C. & BUSTAMANTE, A. — Enfermedad de Chagas natural en perros. Estudios serológicos, anatomopatológicos y electrocardiográficos de la fase crónica indeterminada de la infección. **Medicina (B. Aires), 45:** 535-538, 1985.

6. GURTLER, R. E.; SOLARZ, N. D.; LAURICELLA, M. A.; HAEDO, A. S.; PIETROKOVSKI, S.; ALBERTI, A. A. & WISNIVESKY-COLLI, C. — Dynamics of transmission of **Trypanosoma cruzi** in a rural area of Argentina. III. Persistence of **T. cruzi** parasitemia among canine reservoirs in a two-years follow-up. **Rev. Inst. Med. trop. S. Paulo, 28:** 213-219, 1986.

7. HABERNKORN, A. & GONNERT, R. — Animal experimental investigation into the activity of Nifurtimox against **T. cruzi. Arzneim. Forsch, 22:** 1570-1582, 1972.

8. HOFF, R.; MOTT, K. E.; SILVA, J. F.; MENEZES, V.; HOFF, J. N.; BARRETT, T. V. & SHERLOCK, I. — Prevalence of parasitemia and seroreactivity to **T. cruzi** in a rural population of northeast Brazil. **Amer. J. trop. Med. Hyg., 28:** 461-466, 1979.

9. LAURICELLA, M. A.; WISNIVESKY COLLI, C.; RUIZ, A. M.; SOLARZ, N.; BUJAS, M. A.; & SEGURA, E. L. — Estudio serológico de poblaciones. In: CONGRESO ARGENTINO DE PROTOZOOLOGÍA y REUNIÓN SOBRE ENFERMEDAD DE CHAGAS, 1., Huerta Grande, Córdoba, 1984.

10. LAURICELLA, M. A.; RIARTE, A. R.; LAZZARI, J.; BAROUSSE, A. & SEGURA, E. L. — Enfermedad de Chagas en perros experimentalmente infectados con **T. cruzi. Medicina (B. Aires), 46:** 195-200, 1986.

11. MAZZA, S. — Observación de infección espontanea del perro por el **Schizotrypanum cruzi. Rev. Soc. argent. Biol., 2:** 33-41, 1926.

12. MAZZA, S. — Frecuencia e importancia de la infección natural de perros y gatos por **S. cruzi** y cuestión de la herencia del mismo en los primeros animales. In: **REUNIÓN DE LA SOCIEDAD ARGENTINA DE PATOLOGÍA REGIONAL, 9.,** Mendoza, 1935. v. 1, p. 412 417.

13. MOTT, K. E.; MUNIZ, T. M.; LEHMAN, J. S.; HOFF, R.; MORROW, R. H.; SILVA DE OLIVEIRA, T.; SHERLOCK, I. & DRAPER, C. C. — House construction, triatomine distribution and household distribution of seroreactivity to **T. cruzi** in a rural community in Northeast Brazil. **Amer. J. trop. Med. Hyg., 27:** 1116-1122, 1978.

14. MOTT, K. E.; MOTA, E. A.; SHERLOCK, I.; HOFF, R.; MUNIZ, T.; OLIVEIRA, T. & DRAPER, C. C. — **T. cruzi** infection in dogs and cats and household reactivity to **T. cruzi** in a rural community in the Northeast Brazil. **Amer. J. trop. Med. Hyg., 27:** 1123-1127, 1978.

15. NORMAS TECNICAS Y DE PROCEDIMIENTOS PARA LA LUCHA QUIMICA CONTRA EL VECTOR DE LA ENFERMEDAD DE CHAGAS. Buenos Aires, Publicación del Servicio Nacional de Chagas de la República Argentina, Ministerio de Salud Publica y Medio Ambiente, **1** (1): 29-34, Oct., 1983.

16. PAULONE, I.; CHUIT, R.; PEREZ, A.; WISNIVESKY-COLLI, C. & SEGURA, E. L. — Field research on an epidemiological surveillance alternative of Chagas disease transmission: the Primary Health Care (PHC) strategy in rural areas. **Rev. argent. Microbiol., 20** (suppl): 103-105, 1988.

17. PEREZ, A.; LANSETTI, J. C.; DE RISSIO, A. M.; PAULONE, I.; CHUIT, R. & SEGURA, E. L. — Seguimiento longitudinal de la población de niños de 0-5 años bajo vigilancia epidemiológica para la Enfermedad de Chagas en Termas de Rio Hondo, Sgo. del Estero. In: CONGRESO ARGENTINO DE PROTOZOOLOGÍA Y REUNIÓN SOBRE ENFERMEDAD DE CHAGAS, 2., La Falda, Córdoba, 1987. p. 46.

18. POSTAN, M.; MC DANIEL, J. P. & DVORAK, J. A. — Studies of **T. cruzi** clones in inbred mice. II Course of infection of C57BL/6 mice with single-cell-isolated stocks. **Amer. J. trop. Med. Hyg., 33:** 236-238, 1984.

19. REVELLI, S. S.; AMERIO, N.; MORENO, H. S.; VALENTI, J. L.; BALBARREY, H. & MORINI, J. C. — Enfermedad de Chagas crónica en la rata. Características serológicas, electrocardiográficas e histopatológicas. **Medicina (B. Aires), 40** (suppl. 1): 69-76, 1980.

20. ROJAS, A.; SOTELO, J. M.; VILLARROEL, F. & CONTRERAS, C. M. — La importancia del perro y el gato en la epidemiología de la Enfermedad de Chagas. **Bol. chil. Parasit., 28:** 42-43, 1973.

21. ROTHHAMMER, F. — Chagas' disease in Chilean mummies. **Parasit. Today, 1** (1): 3, 1985.

22. RUIZ, A. M.; ESTEVA, M.; RIARTE, A.; SUBIAS, E. & SEGURA, E. L. — Immunoprotection of mice against **T. cruzi** with a lyophilized flagellar fraction of the parasite plus adjuvant. **Immunol. Lett., 12:** 1-4, 1986.

23. SOKAL, R. R. & ROHLF, F. J. — **Introduction to biostatistics.** San Francisco, W. H. Freeman & Co., 1969.

24. STROUT, R. G. — A method for concentrating hemoflagellates. **J. Parasit., 48:** 100, 1962.

25. VOLLER, A.; DRAPER, C. C.; BIDXELL, D. E. & BARTLETT, A. — Microplate enzyme-linked immunosorbent assay for Chagas' disease. **Lancet, 1:** 426-428, 1975.

26. WISNIVESKY-COLLI, C.; GURTLER, R. E.; SOLARZ, N. D.; SALOMON, D. & RUIZ, A. M. — Feeding patterns of **T. infestans** (Hemiptera: Reduviidae) in relation to transmission of American Trypanosomiasis in Argentina. **J. med. Entomol., 19:** 645-654, 1982.

27. WISNIVESKY-COLLI, C.; GURTLER, R. E.; SOLARZ, N. D.; LAURICELLA, M. A. & SEGURA, E. L. — Epidemiological role of humans, dogs and cats in the transmission of **T. cruzi** in a central area of Argentina. **Rev. Inst. Med. trop. S. Paulo, 27:** 346-352, 1985.

LAURICELLA, M. A.; SINAGRA, A. J.; PAULONE, I.; RIARTE, A. R. & SEGURA, E. L. — Natural **Trypanosoma cruzi** infection in dogs of endemic areas of the Argentine Republic. **Rev. Inst. Med. trop. São Paulo, 31 (2):** 63 70, 1989.

28. WISNIVESKY-COLLI, C.; RUIZ, A. M.; LEDESMA, O.; GURTLER, R. E.; LAURICELLA, M. A.; SALOMON, D. O.; SOLARZ, N. & SEGURA, E. L. — Ecologia doméstica de la Trypanosomiasis americana: perfil alimentario del **T. infestans** en un area rural de la provincia de Santiago del Estero. Argentina. **Rev. Soc. bras. Med. trop., 20:** 31-39, 1987.

Recebido para publicação em 23/8/1988.