

Research Note

## Preliminary Investigation of Chagas' Disease and Intestinal Parasitosis in a Classical Endemic Area of Paraguay

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### Abstract

The prevalence of *Trypanosoma cruzi* infection was studied in December 1992 and January 1993 in residents (1–84 years of age) in Caroveni, a satellite community of Villarrica, Guairá Department, an endemic area of Chagas' disease in Paraguay. It was found that 11 out of 224 (4.9%) persons were positive for anti-*T. cruzi* antibodies as shown by a Chagas' test. The prevalence of intestinal parasitosis was also investigated for the sero-positive and the sero-negative residents. The formalin-ether sedimentation (M.G.L.) method showed that 14 out of 21 (66.7%) residents were infected with intestinal parasitosis with a high prevalence of hookworm (9 persons) and *Entamoeba coli* (9 persons). The correlation between the positive rates for Chagas' disease and those for intestinal parasitosis was examined statistically but was found to be insignificant by the  $\chi^2$  test.

**Key words:** Chagas' disease, classical endemic area, intestinal parasitosis, correlation, Paraguay

In Paraguay, it has been reported that a number of people have suffered from *Trypanosoma cruzi* infection and intestinal parasitosis. For instance, 9.7% of 6,000 persons (Canese, 1978) were found to be positive for anti-*T. cruzi* antibodies in a nationwide investigation. With respect to the prevalence of intestinal parasitosis, a high rate of hookworm infection (58.8% of 9,000 persons including adults and children; Canese and Canese, 1976) was reported in a nationwide inquiry conducted from 1962 to 1974 and summarized in 1976. It is important to

investigate whether the prevalence of Chagas' disease and intestinal parasitosis correlate statistically from an epidemiological standpoint. In this investigation, we attempted to confirm the prevalence of *T. cruzi* infection and intestinal parasitosis based on a survey of a rural village of an endemic area for Chagas' disease.

The surveyed area (Caroveni community) is a rural village located near Villarrica (an ancient city of ca. 44,000 population), which is about 130 km southeast of Asunción (Fig. 1). The main industry of the village is agriculture and pomiculture. This investigation was performed in cooperation with the staff of I.I.C.S. (Instituto de Investigaciones en Ciencias de la Salud, Department of Tropical Medicine, Chief, Lic. Antonieta R. de Arias) of the National Asunción University in December 1992 and January 1993.

With regard to the investigation of Chagas' disease, we first conducted an inquiry about anamnestic Chagas' disease; next, to conduct a screening test of anti-*T. cruzi* antibodies, blood samples were collected from the tips of fingers of 224 persons ranging in age from 1 to 84. Serum samples and standard sera (negative, borderline and positive) were diluted 1:20 in PBS-Tween solution and their

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Fig. 1 Scheme of Paraguay showing the studied area. (Caroveni Community in Guairá Department, marked as ●).

\*I: Central Department, II: Cordillera Department, III: Paraguari Department, IV: Guairá Department.

antibody titers were measured using a Chagas'-Test kit developed by I.I.C.S. for the detection of *T. cruzi* antibodies by ELISA. Goat anti-human IgG antiserum conjugated with horseradish peroxidase was used for detection of the antibodies. The serum samples were further diluted for titration, and each optical density was determined using an ELISA reader. The titer cut-off point for positive was 1/20. Moreover, thin smear blood specimens of the seropositive persons were examined with Giemsa staining.

For the investigation of intestinal parasitosis, fecal samples were collected in sampling bottles by each resident. Each sample (ca. 15g) was fixed with

45ml of 10% formalin and processed by the formalin-ether (M.G.L.) method with iodine staining to examine all intestinal helminths and protozoa (1.5ml suspension including sediments and physiological saline) under a microscope. Then, the number of eggs, cysts or larvae per one gram of feces was calculated for each positive sample.

From the result of the inquiry for Chagas' disease, we could not find any suspected patient except for one woman (61 years of age) who had undergone megacolon surgery and was in good health at the time of examination. There were no records of anyone having Romanã's sign, which is proper to Chagas' disease. In addition, the residents reported

that there had been no appearance of vector bugs (*Triatoma infestans* etc.) of *T. cruzi* for the last ten years. Vector bugs were not found in any wooden walls of the houses examined; most residents had mainly concrete or brick houses with electrical facilities and wells with enough height (more than one meter) for protection. In the screening test for anti-*T. cruzi* antibodies, 11 persons (4.9%) including 6 women and 5 men were found to be positive of 224 residents examined in the village of Caroveni (population 500, Table 1); no children (40 residents under 19 years of age) were found to be positive. The titers of the sero-positive samples ranged from 1/20 to 1/80 except for one sample (>1/80). In the medical inquiry for the sero-positive persons, no abnormalities were found with respect to health, and *T. cruzi* was also not found in any thin blood specimen examined.

In the investigation for intestinal parasitosis, 14 persons (66.7%) including 7 women and 7 men were found to be infected by intestinal parasites, among 21 residents in the village (Table 2). The species of the parasites identified were hookworm (42.9%),

*Entamoeba coli* (42.9%), Rhabditoid larvae (19.0%), *Ascaris lumbricoides* (9.5%) and *Giardia lamblia* (9.5%). Moreover, 9 (42.9%) out of 21 persons harbored parasites of more than two species; double infection with hookworm and *E. coli* (55.6%) was most frequently observed among the 9 persons.

The results of this study showed that the positive rate of *T. cruzi* infection in the residents (4.9%) was lower than that (9.4%) studied in this area by Canese (1978). Furthermore, no sero-positive children for *T. cruzi* were found. These data suggest a declining tendency for the prevalence of Chagas' disease in this area. However, it is possible that the area of this study was not exactly the same as that studied by Canese (1978); domestic animals such as cattle, pigs, dogs and cats could serve as animal reservoirs for Chagas' disease instead of the vector bugs, as reported in other endemic areas by Fujita et al. (1994). In recent reports, 7% of 300 soldiers (15–20 years of age, originating mainly from the Departments of Paraguari, Cordillera and Guairá, Fig. 1) from 3 military units near Asunción were found to be positive for *T. cruzi* infection (Rovira et al., 1991); and 7.6% of 158 children (4 months–13 years of age) in a rural town of Paraguari Department were found to be positive for *T. cruzi* infection (Candido et al., 1991a), indicating the needs for a nationwide investigation to determine the current prevalence of Chagas' disease.

With respect to intestinal parasitosis (Table 2), high rates of hookworm infection (49.2%) and *E. coli* infection (49.2%) were observed with a declining tendency for other parasites on the whole when compared with those of investigation in the outskirts village of Asunción (hookworm, 64.1%; *Entamoeba coli*, 14.6%; *Ascaris lumbricoides*, 23.4%; *Giardia lamblia*, 16.3%; Canese and Canese, 1976). Since Caroveni, located on the outskirts of Villarrica (Fig. 1), is primarily a farming and pomiculture village, poor sanitary conditions were observed; these included the use of human manure in farming, unhygienic lavatories and habits of walking barefoot in spite of residential improvements (wells and electrical facilities), indicating the duration of factors permitting the prevalence of intestinal parasites with special reference to the soil-transmitted parasites. Further study should be performed to confirm a more accurate number of infected persons includ-

Table 1 Prevalence of *Trypanosoma cruzi* infection in residents of Caroveni Community (Guairá Department, 224 individuals ranging from 1 to 84 years of age) in Paraguay investigated on December 2, 1992

Person No. of positive sample	Age	Sex	Titer* of anti- <i>T. cruzi</i> antibody by Chagas' test (ELISA) kit
1	24	Female	1/80
2	40	Male	1/80
3	41	Male	1/80
4	47	Female	1/20
5	47	Female	1/20
6	58	Female	1/80
7†	61	Female	1/80
8	63	Male	1/80
9	75	Female	1/80
10	76	Male	>1/80
11	80	Male	1/80

i.e. Total No. of samples (%) = 224 (100)

Positive samples (%) = 11 (4.9)

Negative samples (%) = 213 (95.1)

\* The titer cut-off point for positive was 1/20.

† She had undergone megacolon surgery and was in good health at the time of examination.

Table 2 Prevalence of intestinal parasitosis in residents of Caroveni Community (Guairá Department) in Paraguay investigated on January 6, 1993

Person No.	Age	Sex	Intestinal parasite species diagnosed, ( ); No. of eggs, cysts larvae per one gram of feces				
			*Hw.	*A. l.	*R. l.	*E. c.	*G. l.
1 <sup>§</sup>	24	Female	0	0	0	(40)	0
2	25	Female	(13)	0	0	(0.4)	(0.7)
3	38	Male	0	0	0	0	0
4 <sup>§</sup>	40	Male	(1.9)	0	0	0	0
5 <sup>§</sup>	41	Male	(3.3)	0	(0.1)	(40)	0
6	41	Male	0	0	0	0	0
7 <sup>§</sup>	47	Female	0	0	0	(13)	0
8	56	Male	0	0	0	0	0
9 <sup>§</sup>	58	Female	0	0	0	(20)	0
10	60	Female	0	(8.7) <sup>†</sup>	0	0	0
11 <sup>§</sup>	61	Female	0	0	0	0	0
12 <sup>§</sup>	63	Male	0	0	0	0	0
13	63	Male	(1.1)	0	0	(33)	0
14	64	Male	(1.5)	0	0	(27)	0
15	73	Male	(5.7)	0	0	(20)	0
16 <sup>§</sup>	75	Female	0	0	0	(33)	(0.1)
17	75	Male	(3.9)	(0.1) <sup>‡</sup>	(0.1)	0	0
18	77	Female	0	0	0	0	0
19 <sup>§</sup>	80	Male	0	0	0	0	0
20	80	Male	(0.7)	0	(1.3)	0	0
21	84	Male	(0.8)	0	(0.1)	0	0

\* Hw. = Hookworm eggs, A.l. = *Ascaris lumbricoides* eggs, R.l. = Rhabditoid larva, E.c. = *Entamoeba coli* cysts, G.l. = *Giardia lamblia* cysts. <sup>†</sup> fertilized eggs, <sup>‡</sup> unfertilized eggs.

<sup>§</sup> These persons were the sero-positive residents for Chagas' disease each with a titer of more than 1/20; they were chosen from the same population as shown in Table 1.

ing children and parasite species of soil-transmitted parasites. In recent reports for intestinal parasitosis of children in Paraguay by Cándido *et al.* (1991b) or Fujita *et al.* (1993), further species in addition to those reported by the authors, including *Hymenolepis nana*, *Trichuris trichiura*, *Blastocystis hominis*, *Chilomastix mesnili*, *Iodamoeba buetschlii* and *Endolimax nana* were recorded. These additional species are most likely a result of the differences in the numbers of samples, as well as the areas and ages examined.

For examination of the statistical differences, these residents positive for Chagas' disease (9 persons) and intestinal parasitosis (14 persons), as

shown in Table 2, were compared using the  $\chi^2$  test; however, no statistical significance was found, indicating a low correlation based on differences of focuses or infection routes of these parasites including *T. cruzi*.

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