A SEROEPIDEMIOLOGICAL SURVEY OF CHAGAS' DISEASE AND A SEARCH FOR RESERVOIR HOSTS IN TWO ENDEMIC AREAS OF ECUADOR

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Abstract: In two Ecuadorian endemic areas of Chagas' disease, Pedro Carbo in coastal low-land and Zaruma in high-land of the Andes, a seroepidemiological survey of the human infection and a search for reservoir hosts were performed during the period between June 1983 and March 1984.

Indirect hemagglutination (IHA) test-positive rate of inhabitants was 4.3 per cent (10/233) in Pedro Carbo, and 15.5 per cent (67/433) in Zaruma. The positive rate of high school students was 2.2 per cent (10/446) and 3.9 per cent (12/305) in each area, respectively.

Trypanosoma cruzi was found in two species of peridomestic mammals, *Didelphis marsupialis* (opossum) and *Dasybus novemcinctus* (armadillo). The infection rate of *D. marsupialis* was 25.0 per cent (17/68) in Pedro Carbo and 18.5 per cent (5/27) in Zaruma. Only one of the 70 babies from pouch of *D. marsupialis* examined was found to be positive for *T. cruzi*, though the mother and the other litter mates were negative for the flagellata. One of four *D. novemcinctus* was positive for the parasite in Zaruma. No trypanosome was recognized in all the rodents and bats examined. The result suggests that *D. marsupialis* is the principal reservoir host of Chagas' disease in various endemic areas of Ecuador.

INTRODUCTION

American trypanosomiasis, Chagas' disease, caused by *Trypanosoma cruzi* is widely found between latitudes 40°N and 40°S in South and Central Americas and some of the southern states of the United States of America (Molyneux and Ashford, 1983). In Ecuador, several workers reported previously that Chagas' disease spread to various departments of the country from coastal regions to high-lands of the Andes, where it displayed a great problem of public health (Espinoza, 1955; Rodriguez, 1959).

In South and Central Americas, many mammalian orders have already been demonstrated

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to be the reservoir of *T. cruzi*, and these animals play an important role in the transmission of Chagas' disease (Zeledón, 1974). However, few investigations have been carried out on the transmission of the disease in Ecuador. Therefore, more precise information on the transmission mode of Chagas' disease has been required in various areas with different endemicity.

We performed a preliminary seroepidemiological survey of the human disease and a search for the reservoir hosts, in two areas, Pedro Carbo and Zaruma, Ecuador. This paper deals with the results of examination on the infection of inhabitants and peridomestic mammals in these areas.

MATERIALS AND METHODS

Description of study areas

Two endemic areas, Pedro Carbo and Zaruma in Ecuador, were chosen as our study areas. The first town, Pedro Carbo, in the Department of Guayas, is situated on the coastal plain of damp ground zone, about 60 km north-east of Guayaquil, the biggest commercial city in Ecuador. In this area, most of the people live in the houses made of bamboo. The second town, Zaruma, in the Department of El Oro, is located on the mountainous region of the Andes, 1,100 m above sea level, about 300 km south-west of Guayaquil. The inhabitants of this town mainly live in brick houses.

Examination of inhabitants

Serum samples were collected from 679 inhabitants, among which 446 were students of two high schools, *Colegio Pedro Carbo* and *Colegio Granja Garces*, in Pedro Carbo, and also collected from 738 inhabitants, among which 305 were students of two high schools, *Colegio 26 de Noviembre* and *Colegio Nocturno San Miguele*, in Zaruma. All the serum samples collected were transferred to the laboratory in Guayaquil and then examined by a commercially available kit of indirect hemagglutination (IHA) test (POLYCHACO, S.A.I.C., Argentine) for Chagas' disease. The serum samples thus tested were considered as positive when the reciprocal titer was 16 or more; non of control sera from 32 healthy individuals in non-endemic area for Chagas' disease was reactive at this dilution with this IHA-kit.

Examination of reservoir hosts

Blood samples of peridomestic mammals, which live surrounding or inner house, including litters of opossum from the pouch of their mothers were examined by culture method. The culture materials were taken from the heart of each mammal by using a disposable syringe with citrate solution. About 0.5 to 3 ml of each blood sample was overlaid on one to three culture tubes.

Preparation of culture medium

The culture medium used was modified from Senekjie's medium (Senekjie, 1943), and had been routinely used in the laboratory. The medium was composed of 100 g beef extract, 1 g peptone (Difco), 0.5 g sodium chloride, 2 g glucose and 2 g agar (Difco) in 100 ml distilled water. The pH was adjusted to 7.2–7.4 and this stock medium was autoclaved at 120°C for 20 minutes. Thereafter, defibrinated sheep blood was added to the melted medium in the rate of 20 per cent,

and the medium was placed in slant tube. Two ml sterile physiological saline containing 500 I.U. penicillin and 500 µg streptomycin per ml was overlaid. The inoculated culture tubes were incubated at 25°C. If no organism was found in the culture until 35 days after incubation, the sample was regarded as negative. The flagellata developed in a test tube were identified as *Trypanosoma cruzi* by the amastigote proliferation both in mice and in fibroblast cell culture infected with them.

RESULTS

Serological survey in inhabitants

The result of a total of 1,417 examinees in the two endemic areas was shown in Table 1. IHA positive inhabitants were observed in 10 (4.3%) of 233 in Pedro Carbo, and 67 (15.5%) of 433 in Zaruma. The positive rate of IHA in Zaruma was 3.6 times as much as that in Pedro Carbo. No difference was observed in the positive rate among age groups in Pedro Carbo. In Zaruma, however, positive rate apparently increased with age. There was no difference in the positive rate between males and females in both areas. Positive rate in high school students was 2.2 per cent (10 out of 446) in Pedro Carbo, while it was 3.9 per cent (12 out of 305) in Zaruma.

Examination of reservoir hosts

As shown in Table 2, *T. cruzi* was found in two species of peridomestic mammals, *Didelphis marsupialis* (opossum) and *Dasyfus novemcinctus* (armadillo). The positive rate in *D. marsupialis* was 25.0 per cent (17 out of 68) in Pedro Carbo, and 18.5 per cent (5 out of 27) in Zaruma. No difference was recognized in the positive rate between male and female *D. marsupialis* (data not

Table 1 Prevalence rate of seropositivity to *Trypanosoma cruzi* by indirect hemagglutination test in the inhabitants and high school students in Pedro Carbo and Zaruma, Ecuador

Examinees	No. positive*/No. examined							
1) Inhabitants	Pedro Carbo				Zaruma			
(Age in year)	Male	Female	Total (%)		Male	Female	Total (%)	
0-9	0/17	2/18	2/35	(5.7)	0/1	0/3	0/4	(0)
10-19	1/15	0/22	1/37	(2.7)	1/11	0/25	1/36	(2.8)
20-29	2/20	2/28	4/48	(8.3)	0/51	7/82	7/133	(5.3)
30-39	0/12	1/31	1/43	(2.3)	3/38	4/61	7/99	(7.1)
40-49	2/14	0/19	2/33	(6.1)	3/18	8/34	11/52	(21.2)
50-	0/19	0/18	0/37	(0)	23/54	18/55	41/109	(37.7)
Total	5/97	5/136	10/233	(4.3)	30/173	37/260	67/433	(15.5)
2) Students**	5/198	5/248	10/446	(2.2)	8/113	4/192	12/305	(3.9)

* The test was considered to be positive, when the reciprocal titer was 16 or more.

** 10-19 years old.

Table 2 Detection of *Trypanosoma cruzi* by hemoculture method in the blood samples from peridomestic mammals in Pedro Carbo and Zaruma, Ecuador

Mammals examined		Pedro Carbo	Zaruma
Order	Species	No. positive/ No. examined (%)	No. positive/ No. examined (%)
Marsupialia (Opossum)	<i>Didelphis marsupialis</i> (adult)	17/68 (25.0)	5/27 (18.5)
	<i>Didelphis marsupialis</i> (baby*)	1/42 (2.4)	0/28 (0)
	<i>Didelphis paraguayensis</i>	—	0/2 (0)
Edentata (Armadillo)	<i>Dasybus novemcinctus</i>	—	1/4 (25.0)
Rodentia (Rodent)	<i>Rattus spp.</i>	0/3 (0)	0/28 (0)
	<i>Mus musculus</i>	0/62 (0)	0/47 (0)
Chiroptera (Bat)	<i>Molossus molossus</i>	0/72 (0)	0/15 (0)
	<i>Artibeus fraterculus</i>	—	0/22 (0)

* A total of 70 babies derived from 3 positive and 13 negative mothers.

shown). Sixteen females of *D. marsupialis* from both areas had harboured one to eight litter mates, 70 in total, in their pouches. Only one of these litters was positive for *T. cruzi* (Table 2). However, the organism was not found in the mother and four brothers of the same litter of the positive *D. marsupialis*. One out of four *D. novemcinctus* was positive for *T. cruzi* in Zaruma. All of 140 rodents and 109 bats were negative for the trypanosome.

DISCUSSION

We performed a seroepidemiological survey of Chagas' disease in human in Pedro Carbo and Zaruma, Ecuador. Rodorigez (1959) previously reported the prevalence rate of this disease, using complement fixation test (CFT). According to him, the positive rate was 8.8 per cent (28/320) in the inhabitants from Department of Guayas, and 22.7 per cent (15/66) in those from Department of El Oro. Moreover, Espinoza (1955) reported that CFT-positive rate in school children was 4.2 per cent (7/167) in Guayas and 0.9 per cent (1/109) in El Oro. As we detected positive rate of examinees by using IHA, it will be difficult to compare the result directly with previous data. The result, however, suggests that Chagas' disease still remains as a big threat to human health in both endemic areas, Pedro Carbo in Guayas and Zaruma in El Oro, though the prevalence rate was relatively low in the present study.

In Pedro Carbo, there was no difference in the positive rate among age groups of the examinees (Table 1). As this town was located near a big city, Guayaquil, there was a frequent immigration and emigration of people. For this reason, there may be no accumulation of IHA positive persons with age in this region.

In the present study, *T. cruzi* was found in 22 adult and one baby opossums (*D. marsupialis*) in both areas examined. *T. cruzi* was found in opossum captured at Guayas, Ecuador

(Espinoza, 1953). Moreover, the population size of this species at both collecting sites was considered to be very large at the time of animal collections, suggesting its important role as a reservoir host of Chagas' disease in coastal and mountainous regions of Ecuador.

One baby opossum was positive for *T. cruzi* in the present examination. This infection might have been caused through post-partum contact with infected vectors, because its mother and brothers were all negative for the trypanosome. Zeledón *et al.* (1975), during the course of investigation on Chagas' disease, reported negative results in fresh blood examination of 145 young opossums (*D. marsupialis*) originated from 36 positive mothers in Costa Rica. It was pointed out that young opossums were more susceptible than mature ones to *T. cruzi* infection which occasionally caused a fatal case (Zeledón and Ponce, 1972). Therefore, judging from the above findings, it seems that congenital infection with *T. cruzi* is rare in opossum.

In Panama, *Rattus rattus* was found to be a major reservoir host for Chagas' disease (Edgcomb and Johnson, 1970). In Ecuador, Arzube (1966) demonstrated the presence of rat blood in the gut of *Triatoma dimidiata*, investigating the blood meal preference of the triatomine bug against various domestic and peridomestic animals. In Colombia, on the other hand, Marinkelle (1982) found *T. cruzi* infection in bats. In our study, however, the search for *T. cruzi* in rodents and bats from Pedro Carbo and Zaruma revealed a negative result.

From the present survey of peridomestic mammals, it is likely that opossum plays an important role as reservoir host of Chagas' disease, whereas the other mammals negative for the flagellata such as rats, mice and bats, play rather a minor role in the transmission of human disease in Ecuador.

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エクアドル共和国のシャーガス病流行地における住民及び保虫動物の調査

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エクアドル共和国のシャーガス病流行地の2地域, Pedro Carbo (平野部) と Zaruma (標高 1,100 m のアンデス山脈中腹部) において, 住民の血清学的検査, 及び保虫動物の調査を行った。

間接赤血球凝集反応によるシャーガス病陽性率は, 学生を除く住民では, Pedro Carbo で4.3% (10/233), Zaruma で15.5% (67/433) であった。学生の陽性率は, Pedro Carbo で2.2% (10/446), Zaruma で3.9% (12/305) であった。

保虫動物の調査において, *Trypanosoma cruzi* が *Didelphis marsupialis* (opossum) と *Dasyprocta novemcinctus* (armadillo) の2種の野性動物から分離された。*D. marsupialis* の感染率は, Pedro Carbo で25.0% (17/68), Zaruma で18.5% (5/27) であった。*D. marsupialis* の幼獣70匹のうち1匹が陽性であったが, その母獣, 及び同腹子からは原虫が確認できなかった。検査した全てのゲッ歯類, 及びコウモリは陰性であった。

これらの結果から, エクアドル共和国においては, *D. marsupialis* がシャーガス病の主保虫動物と考えられた。