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## FREQUENCY AND DISTRIBUTION OF *TRYPANOSOMA* *CRUZI* AND *TRYPANOSOMA RANGELI* IN THE REPUBLIC OF PANAMÁ\*

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**ABSTRACT:** To provide information on the frequency and distribution of human trypanosomiasis in the Republic of Panamá, we carried out a survey of the rural population in the nine provinces and the San Blas Territory. After direct examination of blood and hemoculture procedures, we examined 12,975 blood samples, corresponding to 10,570 persons, for trypanosomes from January 1965 through December 1969. *Trypanosoma cruzi* and *Trypanosoma rangeli* were more prevalent in the rural areas of Central Panamá, including the provinces of Panamá, Colón, and Coclé. Infections with *T. rangeli* were also recorded in Darién and Bocas del Toro Provinces. Parasitemia was not detected in blood samples examined from Herrera, Los Santos, Veraguas, and Chiriquí Provinces. The samples from the San Blas Territory were also negative. In Central Panamá, both *T. cruzi* and *T. rangeli* occur in the same localities. In 16 communities studied in Panamá Province, the infection rate varied from 0.5% to 8.8%. *T. rangeli* was six times more prevalent than *T. cruzi*. The annual variation in the prevalence of infections during the 5 years of this study was determined for the villages of Mendoza, Altos del Jobo, and Cerro Cama. Of 160 persons found positive for trypanosomes, 75% were less than 16 years old. Trypanosomes were more frequently detected in children 6 to 10 years old.

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The objective of this article is to provide information on the frequency and distribution of human trypanosomiasis in the Republic of Panamá. We record the results of a survey of the prevalence of trypanosome infections by direct examination of blood and hemoculture.

In Panamá, both *Trypanosoma cruzi* and *Trypanosoma rangeli* may be found in man. Although human trypanosomiasis may involve either or both of these species, only *T. cruzi* is considered pathogenic, causing Chagas' disease. *T. rangeli* is generally considered a nonpathogenic parasite of man. The frequency and distribution of both species is considered in this survey.

According to Miller,<sup>1</sup> the first case of Chagas' disease was recorded in Panamá, 1 December 1930. Clark and Dunn<sup>2</sup> demonstrated through animal inoculations that the trypanosome involved in this case was *T. cruzi*. The first infection with *T. rangeli* in man in this country was found in 1957. This infection was diagnosed by culture methods in a child from Santa Rita Village, about 35 miles from Panamá City.<sup>3</sup>

Johnson and Kelser,<sup>4</sup> using the complement-fixation reaction, carried out a serological survey in 1,251 persons from various parts of Panamá. They found Chagas' disease to be more prevalent in the central region of the Isthmus, close to the Canal Zone. Since then, little additional information has been published on the prevalence of human trypanosomiasis in the Republic. The presence of both *T. cruzi* and *T. rangeli* in endemic areas of Chagas' disease in Panamá has stressed the need for studying the frequency of infections in man and for determining the incidence by parasitological methods and the standard serological tests.

Except for the early work of Miller, Clark and Dunn, and Johnson and Kelser, little attention was given to the problem of Chagas' disease in Panamá until the early 1960's. However, the staff of Gorgas Memorial Laboratory maintained a constant surveillance for acute and chronic cases as they appeared in a few communities involved in malaria-control programs. The information gathered suggested the need for more intensive work on this disease to define the ecological and biological factors characterizing the infection in Panamá.

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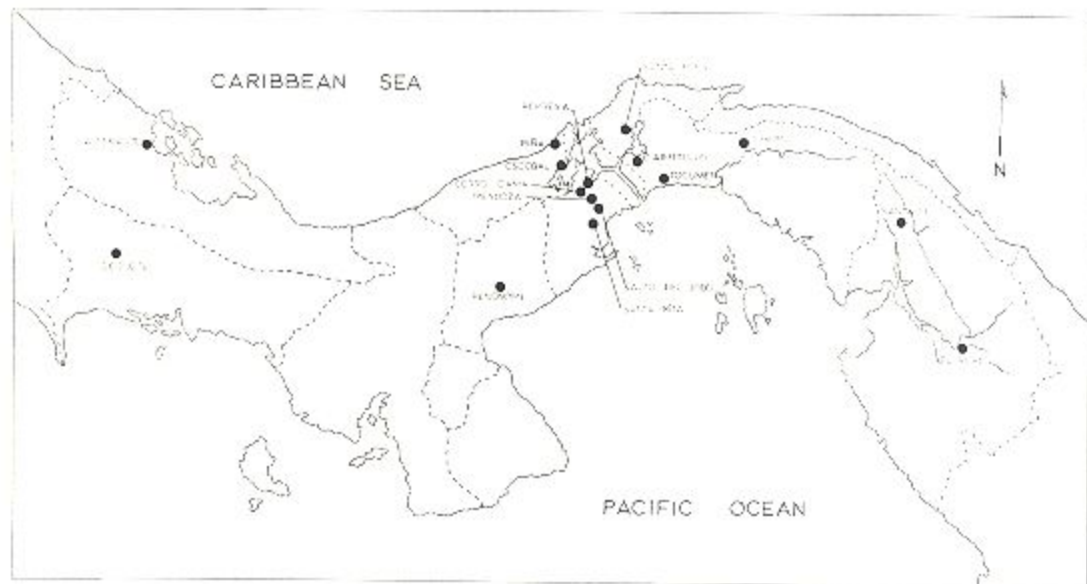


FIGURE 1. Map of the Republic of Panamá, showing the relative locations of some communities of interest in the study of human trypanosomiasis.

#### MATERIALS AND METHODS

##### *Source of Material*

The survey of the rural population of Panamá was initiated in January 1965 and extended through December 1969. Blood samples were collected from the nine provinces of the Republic and from the San Blas Territory (Fig. 1). A total of 12,975 blood samples were collected from 10,570 persons (average 1.2 samples per person) and fully processed for the presence of trypanosomes. Single samples from each person were obtained except in the provinces of Panamá (1.2 samples per person), Colón (1.5 samples per person), and Bocas del Toro (2.4 samples per person). As indicated in Table 1, most persons examined (63%) lived in Panamá and Colón Provinces in the central region of the Isthmus, close to the Interoceanic Canal. Some of this work was performed as part of the studies on nutrition of Instituto de Nutrición de Centro América y Panamá (INCAP) in Panamá.

##### *Techniques and Procedures*

In all instances, we drew blood by venipuncture, using disposable syringes and needles (Vacutainers). Direct microscopical examination of the blood involved its suspension in fresh saline solution, microhematocrit, and Giemsa-stained thick-

film preparations. Samples were observed at varying magnifications from 100 to 400 $\times$  for the fresh preparations and 400 to 1,000 $\times$  for the permanently stained slides.

We prepared blood cultures with Senekjic's diphasic medium,<sup>3</sup> (15% rabbit's blood) with 1 cc of Locke's solution as the overlay fluid. Each culture tube was inoculated with 1.0 cc of blood (1 tube per person). Cultures were incubated at 26°C for 60 days before they were discarded as negative. Each culture tube was examined 7, 15, 30, 45, and 60 days after inoculation. To distinguish between *T. cruzi* and *T. rangeli*, we carefully studied the morphology of the culture forms (epimastigotes and trypomastigotes) on the 15th and 30th days of incubation, and the infectivity to the laboratory mouse and laboratory-reared triatomid bugs was ascertained.

To study the morphology of the blood trypanosomes and the development of tissue form (amastigotes), we inoculated about 1.5 to 3.0  $\times 10^6$  culture forms per gram of body weight intraperitoneally into each of three juvenile (24- to 30-day-old) Carworth Farms white (CFW) mice. Inoculated animals were examined daily for 14 days and bled for hemoculture preparations 30 days after inoculation. Tissues were fixed in formalin and stained with the conventional hematoxylin and eosin technique.



TABLE 1

*Human trypanosomiasis in the Republic of Panamá as determined by hemoculture procedure, 1965-69*

Provinces	Samples examined	Persons examined	Persons positive		Parasite*			
					<i>T. cruzi</i>		<i>T. rangeli</i>	
			(No.)	(%)	(No.)	(%)	(No.)	(%)
Panamá	7,600	6,207	175	2.8	32	18.28	160	91.42
Colón	1,295	892	17	1.9	2	11.76	16	94.11
San Blas	98	98	0	0.0	—	—	—	—
Darién	476	476	1	0.2	—	—	1	100.00
Coeló	598	598	2	0.3	—	—	2	100.00
Herrera	267	267	0	0.0	—	—	—	—
Los Santos	284	284	0	0.0	—	—	—	—
Veraguas	291	291	0	0.0	—	—	—	—
Chiriquí	1,016	1,016	0	0.0	—	—	—	—
Bocas del Toro	1,050	441	2	0.45	—	—	2	100.00
Totals	12,975	10,570	197	1.86	34	17.25	181	91.87

\* Including some mixed infections.

The development of cruzi-like blood forms or amastigotes in the inoculated mice was required for the diagnosis of *T. cruzi* isolates. The diagnosis of *T. rangeli* isolates was based on the presence of rangeli-like blood trypanosomes in inoculated mice and the appearance of metacyclic forms in the salivary glands of *Rhodnius pallescens* after inoculation of parasites into the hemocoel of the bugs. The inoculation of culture forms into the hemocoel of the insect was performed according to the technique described by Tobie.<sup>10</sup>

## RESULTS

*Human Trypanosomiasis*

Infection with trypanosomes was more prevalent in the rural areas of Panamá (2.8%) and Colón Provinces (1.9%), reduced prevalence of human infections being detected in the rural areas of Darién (0.2%), Coeló (0.3%), and Bocas del Toro (0.45%). Negative findings were obtained (Table 1) from the samples from Herrera, Los Santos, Veraguas, and Chiriquí Provinces along the southwestern part of Panamá. The samples from the territory of San Blas were also negative, though the small number of the samples makes this less significant. Although Chagas' disease occurs in Chiriquí Province, no trypanosomes were detected in blood samples of 1,016 persons tested. Some of the corresponding serum samples from these persons gave a positive reaction to *T. cruzi* by the complement-fixation procedure.

*T. cruzi* infections were detected only in the endemic area of Chagas' disease in central Panamá (Chorrera District) and from small rural communities in Colón Province. *T. rangeli* infections in man were found in the rural population of Bocas del Toro, Coeló, Darién, Colón, and Panamá.

*Frequency of Infection in Central Panamá*

Since the first case of Chagas' disease in Panamá reported by Miller in 1931, Johnson and Kelsler<sup>1</sup> have suggested that human infections occur more frequently in the rural communities of central Panamá than in rural communities of Darién and Chiriquí Provinces. For this reason, some communities in the provinces of Panamá and Colón were sampled several times during the 5 years of the study. In Panamá Province, rural communities like Caimitillo, Buenos Aires, and those with strong urban influences, such as Tocumen, showed negative findings; no human infections were detected in 598 persons examined. However, in the study of 16 rural communities more closely associated with the humid forest, east and west of the Canal Zone, the infection rates varied from 0.5% to 8.8%. In this area, from a total of 4,844 persons examined, 167 persons (3.4%) were found positive for trypanosomes in the blood (Table 2). The villages with the highest rate of infection were Altos del Jobo (8.8%), Mendoza (4.8%), Santa Rita (3.7%), and Represa (3.1%) in La Chorrera District,

TABLE 2

Frequency of human trypanosomiasis in some endemic areas of Chagas' disease in Panamá Province. Blood culture results, 1965-69

Locality	Samples examined	Persons examined	Persons positive		Parasite	
			(No.)	(%)	<i>T. cruzi</i>	<i>T. rangeli</i>
Chepo	61	61	3	5.0	1	2
Bique	541	467	17	3.6	5	16
Cerros Silvestre	199	199	1	0.5	—	1
Arraiján	48	48	1	2.2	1	—
Represa	167	160	5	3.1	1	5
Cerro Cama	589	482	8	1.7	—	8
Altos del Jobo	794	434	38	8.8	4	38
Mendoza	1,088	630	30	4.8	5	27
Bajo Grande	375	353	10	2.8	2	9
Santa Rita	673	594	22	3.7	2	22
Zanguenga	76	76	1	1.3	—	1
Los Mortales	136	136	2	1.4	—	2
Río Congo	209	209	5	2.4	3	4
Lidice	666	474	8	1.7	2	7
Cerro Campana	220	230	4	2.8	—	4
Caimito	395	301	12	4.0	2	11
Totals	6,237	4,844	167	3.4	28	157

Caimito (4.0%) in Capira District, and Chepo (5.0%) in Chepo District east of the Canal.

Both *T. cruzi* and *T. rangeli* isolates were identified, as determined by direct and hemoculture procedures. Some persons harbored mixed infections when bled. In five cases of mixed infections, *T. cruzi* was first isolated; *T. rangeli* was isolated later. The detection of *T. rangeli* in some mixed infections was possible after the *T. cruzi* forms declined or disappeared from the peripheral circulation. In most instances, the two parasites were detected, in a mixed isolate, through laboratory procedures and inoculation into animals.

In the endemic area of Chagas' disease in Central Panamá, *T. rangeli* occurred almost six times as frequently as *T. cruzi*. In 167 infected persons, *T. cruzi* was detected in 28 and *T. rangeli* in 157; 18 persons had mixed infections.

#### Annual Incidence of Trypanosome Infections in Central Panamá

To determine the annual fluctuation in the frequency of human trypanosomiasis in known endemic areas of Chagas' disease, we selected three neighboring communities from the Mendoza area in La Chorrera District. This area, within Panamá Province, showed the highest rate of trypanosome infections during the period of this

study. The communities selected, Mendoza Village, Altos del Jobo, and Cerro Cama, are close to Gatun Lake and represent typical rural communities in the east-central section of Panamá.<sup>7</sup> The population of these communities is estimated to be 650 persons in Mendoza, 450 in Altos del Jobo, and 700 in Cerro Cama.

During 1965-69, the over-all annual rate of new infections varied from 1.2% to 6.1% (Table 3). The population of Altos del Jobo presented a higher average annual rate of infection (6.4%) than the other two communities. The frequency of new infections in this community was more than twice the average frequency for Mendoza Village (2.8%). The population of Cerro Cama showed the lowest rate of infection (1.4% to 3.3%) with an average annual rate of 2.0%. The average annual rate of patent infection for the three communities varied from 2.0% to 6.4%. We can thus predict that 20 to 64 new cases with parasitemia per 1,000 population per year occur in the Mendoza area in La Chorrera District. The figures presented in Table 3 show that during 1967 the annual rate of infection declined. During that year the infection rate dropped to 0.6% in Mendoza and to 1.4% in Altos del Jobo, the lowest rate for each location. By contrast, the infection rate in Cerro Cama was maintained at comparable levels (1.4% to 3.3%,

TABLE 3

Annual incidence of trypanosome infections in selected communities from Mendoza Area in La Chorrera District, Panamá Province, detected by direct examination and hemoculture procedures, expressed as percentage

Locality	Percentage					Average annual rate
	1965	1966	1967	1968	1969	
Mendoza	6.3	5.8	0.6	2.1	3.1	2.8 (220)*
Altos del Jobo	30.0†	9.9	1.4	5.7	7.4	6.4 (141)
Cerro Cama	3.3	1.4	1.7	2.1	2.2	2.0 (125)
Total	6.1	5.0	1.2	2.9	4.6	3.6 (491)

\* Figures in parentheses = average number of persons examined per year.  
 † Sample size for Altos del Jobo in 1965 was too small to be significant.

average, 2.0%) throughout the 5 years of the study. The annual average number of new cases in Mendoza Village was 6.0, in Altos del Jobo 7.6, and in Cerro Cama 2.6.

Frequency of Infection by Age of Persons Tested

The ages of 160 persons with positive tests, recorded at the time of the first trypanosome isolation, were grouped to determined their relation to frequency of infection. Of the 160 cases studied, 27 had *T. cruzi* infections and 149 had *T. rangeli*. The youngest infected person was a 10-month-old boy from Chepo, with *T. rangeli* in the blood. The youngest person with *T. cruzi* was a 12-month-old infant from La Chorrera. The oldest person was a 67-year-old man from Bique who carried a mixed infection. A single *T. cruzi* infection was also detected in a 66-year-old woman from Santa Rosa. Even though trypanosomes were detected in persons of almost all age groups, their presence was more frequent in young persons. As shown in Table 4, almost 80% of the positive cases studied were persons less than 20 years old, and 50% were not over 10 years old. Trypanosomes were most frequently detected in children 6 to 10 years old. In the trypanosome-positive population, 75% were less than 16 years old, while in the total population this percentile corresponded to the 26th year. The average age for the positive group was 16.9 ± 0.349 years.

The age distribution of 1,012 persons tested for trypanosomes in Central Panamá showed that 58.3% of the population was less than 15 years old. The average age for this population was 21.1 ± 0.50 years. Thus, the frequency among the younger persons was significantly lower in

the total population than in the trypanosome-positive group.

DISCUSSION

This report provides information on the frequency and distribution of trypanosomes (*T. cruzi* and *T. rangeli*) in the population of the Republic of Panamá. It is the first general survey in this country to include samples from all the provinces and the San Blas Territory. Although Chagas' disease may occur in almost any part of this country, a fact revealed by complement-fixation reactions, the presence of trypanosomes in the blood of man has been detected only in Panamá, Colón, Coclé, Darién, and Bocas del Toro Provinces. *T. cruzi* and *T. rangeli* infections were found more frequently in rural communities of Central Panamá where 1.9% to 2.0% of the population was infected. Such villages as Santa

TABLE 4

Frequency of infections, by age of patient, in 160 cases of trypanosomiasis in Panamá, based on results of blood cultures

Age group (years)	Persons infected		Cumulative (%)	<i>T. cruzi</i>	<i>T. rangeli</i>
	(No.)	(%)			
0-5	29	18.1	18.1	8	24
6-10	54	33.7	51.8	7	53
11-15	32	20.0	71.8	4	32
16-20	11	6.9	78.7	3	9
21-25	7	4.4	83.1	1	6
26-30	2	1.2	84.3	—	2
31-35	5	3.1	87.4	—	5
36-40	8	5.0	92.4	1	7
41-45	5	3.1	95.5	—	5
46-50	2	1.2	96.7	1	2
Over 50	5	3.3	100.0	2	4
Totals	160	100.0	—	27	149



Rita, Mendoza, and Altos del Jobo showed the highest rates of infection (3.7% to 8.8%).

Our unpublished data on the complement-fixation reaction in this area reveal that in Altos del Jobo 20% of the persons tested give a positive reaction; this agrees with our parasitological findings. This village had the highest rate of trypanosome-positive persons in Panamá. Complement-fixation tests in other villages from Central Panamá have demonstrated infection rates that vary from 5.6% to 11.2%.

The area of Boquete in Chiriquí Province shows a marked variation from areas in Central Panamá. In this area, no person was found with trypanosomes in the blood, but 10% of the serum from these persons gave a positive CF reaction for *T. cruzi*. We can thus state that, although trypanosomes are not often seen in the blood of man in Chiriquí, infections with *T. cruzi* occur, as shown by serological methods. *T. rangeli* appears to be absent from Chiriquí Province, because no infections were detected in 1,016 persons examined by hemoculture procedures. The common triatome species found in Boquete is *Triatoma dimidiata*. *R. pallescens*, the apparent or indicated vector in other parts of Panamá, has not been recovered in this locality. *T. dimidiata*, adults and nymphs, are found frequently within the homes of Boquete residents, and *T. cruzi* has been detected in the intestinal tract of these bugs.

In Central Panamá *T. rangeli* is more frequently found in the blood of man than is *T. cruzi*. This may be the result of a more efficient transmission mechanism for *T. rangeli* (inoculation vs. contamination) and of a longer course of patency, making the diagnosis by blood culture more effective. Transmission by bite, as in the

instance of *T. rangeli*, is generally accepted as being more effective than transmission through contamination with fecal material as required by *T. cruzi*.

Although infection with trypanosomes may be acquired at any age, the risk of infection seems to be greater at earlier ages. Johnson and Kelser<sup>4</sup> have already noted that acute cases of Chagas' disease occur primarily in children and may be fatal.

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