

## THE INCIDENCE OF CHAGAS' DISEASE IN PANAMA AS DETERMINED BY THE COMPLEMENT- FIXATION TEST<sup>1</sup>

CARL M. JOHNSON AND RAYMOND A. KELSER

*From the Gorgas Memorial Laboratory and the Army Medical Research Board*

Received for publication November 18, 1936

Many studies on the distribution of Chagas' disease have been made since it was first described by Carlos Chagas in Brazil in 1909, and although the disease has been reported from Central America and naturally infected insect vectors have been found in North America, in California and Arizona, very little attention has been given to it in these places. Thus, the extent of the infection among the population is not definitely known. In Panama infection of man by *Trypanosoma cruzi* was first noted by Miller (1), and from time to time since then a few other cases have been added to the originals by Clark and Dunn (2), DeCoursey (3) and Johnson and DeRivas (4).

The difficulties encountered in the diagnosis of this malady, as observed in Panama, have been in a large part responsible for our lack of knowledge concerning it. In addition to the direct microscopical examination of the blood, which is the commonest method of diagnosis, various types of supplementary tests have been used as aids from time to time. Those which have been most commonly employed are the inoculation of susceptible animals, attempts to cultivate the organism from the blood of suspected cases, and the xenodiagnosis of Brumpt. These have all proven only of occasional value because of the scarcity of the parasites in the circulating blood and the irregularity of their appearance. With a view to obtaining a more accurate method

<sup>1</sup> Read at the annual meeting of the American Society of Tropical Medicine at Baltimore, Maryland, November 18, 19 and 20, 1936.

of diagnosis, Machado and Guerreiro (5) introduced the complement-fixation test, using antigen prepared from tissues of heavily infected animals. While their test appeared to constitute a distinct advantage over the older methods, considerable difficulty has been experienced in the preparation of uniformly satisfactory antigens. Recently one of us (Kelser (6)) has shown that a very satisfactory antigen can be easily prepared from cultures of *T. cruzi* and that it is apparently trustworthy in identifying Chagas' disease in man. It has thus made the use of the complement-fixation test a valuable and dependable one in the detection of infection of the human host with this parasite.

The purpose of the work herein reported is twofold. First, it appears important to determine the degree of infection by *T. cruzi* in a representative group of the population. Secondly, such an investigation offers the opportunity to study further the reliability and usefulness of the complement-fixation test.

Since the first cases of Chagas' disease recorded from Panama came from a group of villages situated in the Chagres River basin, this region was naturally chosen as a starting point for the survey. The villages have been under observation and experimentation for the past six years and rather extensive data have been collected on the blood parasites present in the inhabitants of this area. These villages, Santa Rosa, Guayabalito, Las Guacas, Gatuncillo, New San Juan and Chilibre, represent the central portion of the Republic. Ventorillo, a small place situated in the mountains about 30 miles northeast of Panama City, is included in this central area. From the eastern end of Panama, El Real, Yape and the San Blas Islands are representative. In the western section of Panama, specimens were taken at Puerto Armuelles. Various other groups were studied, such as the inmates of the Panama insane asylum, a few individuals from Chorrera and Aguas Buenas and several from Panama City. These last mentioned places are grouped under the miscellaneous heading in table 1.

The age, sex and length of residence at their present location, occurrence and nature of previous illnesses, and other pertinent data were noted for each individual at the time the blood was

taken for the test. Further, thick blood smears were made for the direct microscopical examination.

The technique of the complement-fixation test was the same as described by Kelsner (6). Fresh antigen was used for each batch of sera tested. All specimens giving positive and suspicious reactions were run a second time with varying amounts of serum. This was done for the purpose of checking the initial test and also to obtain information on the complement-fixation titer of the positive sera.

TABLE 1  
*Incidence of Chagas' disease by localities*

PLACE	NUMBER EXAMINED	NUMBER POSITIVE	NUMBER SUSPICIOUS	PER CENT POSITIVE OR SUSPICIOUS
Chilibre.....	130	6	1	5.38
Santa Rosa.....	122	4	0	3.27
Gatuncillo.....	87	2	0	2.30
Guayabalito.....	96	1	1	2.08
Las Guacas.....	31	2	0	6.45
New San Juan.....	208	7	5	5.77
Ventorillo.....	36	6	2	22.22
Matias Hernandez.....	90	2	0	2.22
San Blas.....	64	2	0	3.13
El Real.....	96	0	0	0.00
Yape.....	101	5	2	6.93
Puerto Armuelles.....	150	0	0	0.00
Miscellaneous.....	40	0	0	0.00
Total.....	1,251	37	11	3.83

#### RESULTS

In table 1 the results of the survey are recorded in terms of localities and total numbers. The total number of specimens examined was 1,251, of which 37 or 2.95 per cent were positive (4-plus and 3-plus reactions) and 11 or 0.88 per cent suspicious (2-plus and 1-plus reactions).

Ventorillo was highest in incidence. Here 22.22 per cent gave positive or suspicious reactions to the test. Although the number of specimens was small—only 36—this nevertheless represents practically the entire population. There were some nine

or ten babies who were too small to bleed under existent conditions, and one or two adults who refused to be bled. This was the poorest group dealt with, both economically and socially, living under the most primitive conditions to be found in Panama.

The other places ranged from 2.08 to 6.93 per cent positive and suspicious. Three of the areas surveyed were negative, *viz.* El Real, Puerto Armuelles and those comprising the miscellaneous group.

An analysis of the data with respect to the degree of infection in the various age groups is also of some interest, particularly

TABLE 2  
*Incidence of Chagas' disease—various age groups*

AGE GROUP	NUMBER EXAMINED	NUMBER POSITIVE	NUMBER SUSPICIOUS	PER CENT POSITIVE AND SUSPICIOUS
<i>years</i>				
3-5	34	0	0	0.00
6-10	208	3	1	1.93
11-15	254	0	1	0.39
16-20	154	8	1	5.84
21-30	265	12	3	5.66
31-40	164	5	2	4.27
41-50	97	5	3	8.25
50 and over	67	4	0	5.97
3-15	496	3	2	1.01
16 and over	747	34	9	5.76

Eight individuals were omitted from this table due to the fact that no age was recorded for them.

so since in other countries infections in children are reported to be common. Table 2 shows the results. In the first three groups the infection rate is very low, whereas in the groups above the age of 15 it immediately rises to a high level.

In the microscopical examination of the blood one case was found positive for the parasite. This case was a woman, 58 years of age, who was living in Las Guacas, one of the river villages. She had been examined, more or less regularly, every month for a period of four or five years and never during that period had *T. cruzi* been found in blood smears. The parasites persisted in the blood for a period of two weeks and then disap-

peared. Although frequent examinations have been made since, recurrence of the trypanosomes in her circulation has not been demonstrated. The complement-fixation test was strongly positive at the time the trypanosomes were found in the blood and is at the present time—eight months later—still a four-plus reaction.

Four of the original 19 cases reported from Panama (Johnson and DeRivas) were included in this survey. Two of these were discovered in 1931, approximately five years ago; one of the remainder was diagnosed in 1933, and the other in 1935. All of these were similar with respect to numbers of parasites found and the length of time during which they were present in the peripheral circulation. The trypanosomes in all cases were present in small numbers and persisted in the circulation for only a short period of time. These cases have been checked from time to time and the parasites have never recurred in numbers great enough to be found in microscopic examinations of thick and thin blood films, but all continue to be strongly positive to the complement-fixation test.

In two of the cases positive only to the complement-fixation test, and in one of the four mentioned above, attempts were made to demonstrate the presence of the parasites. A quantity of blood (40 cc.) was obtained by venipuncture and of this amount 10 cc. were used for animal and culture inoculation. The remainder of the blood was laked and centrifugalized and the sediment examined both in wet and stained preparations. The animals and cultures were entirely negative, but in the stained smears of one case, one trypanosome was found.

In the total group there were 161 individuals positive for malaria and 21 positive for filaria. Seven of the cases of malaria and 3 of those with filaria were also positive to the complement-fixation test for Chagas' disease.

The sera which responded to the complement-fixation test grouped as to degree of reaction as follows:

4-plus.....	27 specimens
3-plus.....	10 specimens
2-plus.....	8 specimens
1-plus.....	3 specimens

The 4-plus and the 3-plus reactions are considered positive; the 2-plus and 1-plus suspicious. A few plus-minus reactions occurred and were recorded as negative. The strongest reaction noted in the five positives of the children's group was 3-plus and occurred in 3 cases; the remaining two were 2-plus reactions.

#### DISCUSSION

There are no reports in the literature which have come to our notice with which we can compare our results. With the data covered by the present paper and the previous reports of Miller, Clark and Dunn, DeCoursey, and Johnson and DeRivas, the conclusion is drawn that the acute cases occur primarily in children and that they are commonly fatal. This would partially explain the low figures in our children group, but it does not explain the jump in incidence after the age of fifteen years is past. On the basis of our present knowledge of the transmission of the disease, the children run the same risks as the adult in acquiring the infection.

An interesting point brought out by the survey is the number of infections per family. The 48 cases which were found positive or suspicious represent forty-four families. In only two instances were more than one in any given family infected; in each of these there were two infections. Many of the families have two or more individuals in them, all living under the same conditions in the small hut or house. It is surprising that under these circumstances more were not infected. One family of eight, living in Chorrera, and in whose house a number of infected *Triatoma dimidiata* were found by Rozeboom (7), showed no infection either by blood examination or by the complement-fixation test. The above would seem to indicate that either the chances of getting the infection from infected bugs are few or the virulence of the trypanosome is so low that it has difficulty in establishing itself in the human host. Another factor to be considered is the presence of numerous animals such as dogs and cats upon which the bugs might prefer to feed. This would coincide with the opinion expressed by Reichenow (8). He states that the chief source of blood for the bugs, apart from man, is the dog.

None of the cases found by the survey presented any appreciable symptoms attributable to the infection. Contrary to the opinion of Reichenow (8), who states that he found no evidence to support the view that there is a chronic state associated with the disease, we have 4 cases in whom trypanosomes were found from one to five years ago and in whom no trypanosomes have been demonstrated since the initial period of appearance. These four were all strongly positive when recently subjected to the complement-fixation test. The same thing has been noted in experimentally infected dogs. Animals infected more than two years ago, and in which no trypanosomes have been found for over a year, are still giving 4-plus reactions to the test. If the positive complement-fixation reactions in these cases can be taken as evidence that *T. cruzi* is still harbored somewhere in the body of the individual or the lower animal, then a number of chronic cases or carriers have been encountered in this survey. We believe this to be true. It is characteristic for other trypanosome diseases to occur in a low-grade, chronic form in individuals or animals not especially susceptible to the organism and it is believed that a similar situation obtains in the case of Chagas' disease as it occurs in Panama.

Since the development of the complement-fixation test employed in this survey, advantage has been taken of the opportunities to test its specificity. Thus, sera from animals infected with several types of trypanosomes have been tested against *T. cruzi* antigens and conversely sera from cases of Chagas' disease have been subjected to tests in which antigens from other trypanosomes were used. This phase of the study included work with *T. equiperdum*, *T. hippicum* and a bat trypanosome which has not yet been definitely identified. No cross reactions of any kind were obtained with antigen or sera in which *T. cruzi* and *T. equiperdum*, or *T. hippicum* figured. A marked cross reaction was obtained, however, with *T. cruzi* antigen in tests of sera from a large number of bats infected with the unidentified parasite. It was thought at first that this parasite was probably *T. cruzi* but subsequent investigation failed to substantiate this view. While the investigation of this parasite is not yet com-

plete, it appears that it is strictly a bat trypanosome and possesses little or no pathogenicity for either the bat host or for laboratory animals.

Tests were made of a number of sera giving positive Wassermann reactions and none were positive to the Chagas' disease test.

#### CONCLUSIONS

A survey of the incidence of Chagas' disease in Panama, made through the use of the complement-fixation test, using cultural antigen, indicates, from the blood serum specimens thus far tested, that the disease and human carriers of the organisms are more common than has been generally supposed. Out of 1,251 sera thus far collected from various places in Panama, 37 were positive to the test and 11 gave suspicious reactions, a combined rate of 3.83 per cent.

The infection rate as determined by the test is low for children under 15 years of age but rises sharply above this age. This may possibly be explained on the basis of a relatively high mortality from the disease in children. This possibility is strengthened by the few clinical observations which have been made in Panama.

The complement-fixation test, as employed in this survey, is of distinct value, not only in identifying active cases of Chagas' disease, but in revealing the incidence of the infection, past and present.

#### REFERENCES

- (1) MILLER, J. W.: *Southern Med. Jour.*, 1931, **24**, No. 7.
- (2) CLARK, H. C., AND DUNN, L. H.: *Jour. Trop. Med.*, 1932, **12**, No. 1.
- (3) DECOURSEY, E.: *Amer. Jour. Trop. Med.*, 1935, **15**, No. 1.
- (4) JOHNSON, C. M., AND DERIVAS, C. T.: *Amer. Jour. Trop. Med.*, 1936, **16**, No. 1.
- (5) GUERREIRO, C., AND MACHADO, A.: *Brasil-Medico*, 1913, **27**, No. 23.
- (6) KELSER, R. A.: *Amer. Jour. Trop. Med.*, 1936, **16**, No. 4.
- (7) ROZEBOOM, L. E.: *Amer. Jour. Trop. Med.*, 1936, **16**, No. 4.
- (8) REICHENOW, E.: *Arch. f. Schiffs-u. Trop.-Hyg.*, 1934, **38**, Nos. 11 and 12.