

EVALUATION OF THE XENODIAGNOSIS OF CHRONIC CHAGAS PATIENTS INFECTED TEN YEARS OR OVER IN AN AREA WHERE TRANSMISSION HAS BEEN INTERRUPTED – IGUATAMA AND PAINS, WEST MINAS GERAIS STATE, BRAZIL

JOSÉ RODRIGUES COURA; LAÉRCIO LUIZ DE ABREU^{*}; HENRY P. F. WILLCOX & WALTER PETANA

Faculdade de Medicina, Universidade Federal do Rio de Janeiro and Instituto Oswaldo Cruz Departamento de Medicina Tropical, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

To evaluate the results of xenodiagnosis in chronic Chagas patients infected for ten years or over in an area where transmission has been stemmed as well as the performance of these tests applied one or more times to determine the presence of the parasite in serum-positive patients for Trypanosoma cruzi infection, 570 xenodiagnosis were performed in 246 patients by exposing each patient to 40 Triatoma infestans nymphs of 3rd/4th stage once, twice or three times, at 30 days intervals.

The 570 xenodiagnosis showed overall positive results in 50.7% with a peak 78% in patients under 20 years of age, and 60.5% in those over 60. Of the 158 patients who underwent three xenodiagnosis, 51 (32.3%) had three positive tests, 48 (30.3%) had all negative results, and the remainder had alternating positive and negative findings. There was no difference in number of positive results between the 1st, 2nd and 3rd tests; however, the 1st and 2nd trials added up to 53.2% and the sum total of all three trials was 57.7%.

Key words: xenodiagnosis – *Triatoma infestans* – chronic Chagas infection – *Trypanosoma cruzi*

The xenodiagnosis of Chagas infection, introduced by Brumpt in 1914, is still the most effective method available to isolate *Trypanosoma cruzi* during the chronic phase of the infection, either for a parasitologic diagnosis or in assessing the efficacy of specific therapy (Chiari & Brener, 1966; Schenone et al., 1968; Cançado et al., 1973; Salgado, 1975; Cerisola et al., 1977). Some authors, like Chiari et al. (1979) subsequently found that hemocultures in 30 ml of heparin-blood samples, grown in LIT medium in six test tubes carefully checked at 15, 30, 45 and 60 day intervals, would provide better results than xenodiagnosis. However, Junqueira et al. (1991) did a comparative study and found that by using the same procedure as Chiari et al. (1979) with an identical amount of blood ingested by the

triatomids, xenodiagnosis techniques were markedly more accurate.

Several studies on xenodiagnosis have been performed in Brazil and in other countries of this hemisphere to: enhance the technique (Dias, 1940; Schenone et al., 1974; Bronfen, 1989); evaluate its effectiveness versus other methods (Pifano, 1955; Salgado, 1969; Neal & Miles, 1977; Junqueira et al., 1991); evaluate specific therapies (Cançado et al., 1973; Cerisola et al., 1977); find a more effective insect (Cuba et al., 1979; Perlowagora-Szumlewicz & Müller, 1979, 1982, 1987; Perlowagora-Szumlewicz et al., 1988, 1990); study the cycle of *T. cruzi* and vector interaction (Dias, 1934; Zeledon, 1975; Bronfen et al., 1984; Alvarenga & Bronfen, 1984); and lately to evaluate its parasitemia and impact on the clinical aspects and evolution of Chagas disease (Castro, 1980; Castro et al., 1983; Coura, 1975; Coura et al., 1984; Pereira et al., 1989).

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The longer and shorter persistence of *T. cruzi* in the bloodstream of chronic Chagas

patients has been under speculation. Escape mechanisms of the parasite, shape and strain differences and parasite-host interactions have been studied. However, little is known as to why some chronic patients remain with patent parasitemia (persistently positive xenodiagnosis) for many years, while others are persistently negative (Coura et al., 1989).

The objective of this study is to look into the performance of xenodiagnosis in an endemic area where natural transmission of the Chagas infection had been interrupted for 10 years or more by vector control with BHC spraying.

PATIENTS AND METHODS

Two hundred and forty-six patients all showing at least two positive serum reactions to Chagas infection were submitted to xenodiagnosis with 40 3rd/4th stage nymphs of *T. infestans* distributed into four containers of ten nymphs each, applied two to each inner forearm of patients and left to feed for 30 min, according to the method recommended by a group of experts from the Brazilian National Council for Scientific and Technological Development-CNPq (1974).

The patients included 106 males and 140 females, born and raised in either Iguatama or Pains counties in western Minas Gerais state, with ages ranging from 10 to 100, with the average at 42.6.

The *T. infestans* nymphs applied had been fasting for at least 20 days and were checked individually 30 days after feeding. Feces were collected by slight abdominal pressure and deposited on slides containing one drop of PBS at 7.2, homogenized, covered by a 22 x 22 mm film and examined under a microscope which magnified their diameter 400 fold. Negative triatomids were again fed with chicken blood and checked 30 days later; if their were again negative, the entire intestine content was dissected, homogenized and examined with the same technique.

Of the total 246 patients, 570 xenodiagnosis were performed with 22,800 triatomids; 158 patients underwent three xenodiagnosis each at 30 day intervals, eight patients underwent two tests, and 80 had one xenodiagnosis only. In average, 2.3 xenodiagnosis were done per

patient, and there was no significant variation ($P < 0.05$ $X^2 = 0,00035$) in the number of tests performed per age group (Table).

TABLE
Xenodiagnosis positive by age group

Age group	Patients examined	Xenodiagnosis			
		Performed No.	<i>a</i>	Positives	% of positives
10 - 19	16	41	2,5	32	78,0
20 - 29	36	84	2,3	44	52,3
30 - 39	54	120	2,2	57	47,5
40 - 49	59	138	2,3	65	47,1
50 - 59	49	116	2,3	48	41,3
60 e +	32	71	2,2	43	60,5
Total	246	570	2,3	289	50,7

a: Average of xenodiagnosis per patient
 $P < 0.05$, $X^2 = 20.33$.

RESULTS

Out of the 570 xenodiagnosis performed, 289 (50.7%) were positive, 52.6% in women and 47.4% in males. As to age group, a high incidence of positive was found in the under-20 group (78%) and among those 60 or over (60.5% positive), ranging in other age groups from 41.3 to 52.3% positive (Table). It is interesting to note that the positivity curve for these patients sloped down to 50 years of age, going up sharply for individuals 60 and older.

Of the 158 patients submitted to three xenodiagnosis, 48 (30.3%) were consistently negative, 30 (18.9%) had one xenopositive each, 29 (18.3%) had two xenopositive each, and 51 (32.3%) had all three tests positive. It is clear that one group of patients is persistently positive, other is persistently negative while an intermediate group sometimes shows positive and sometimes negative to the xenodiagnosis.

In terms of sequencing, the 1st xenodiagnosis was positive in 46.3% of the patients, the 2nd in 50.6%, and the 3rd in 47.8%; the 1st and 2nd tests together raised the percentage of positives to 53.2%, and the three tests gave a total positive response of 57.7%, indicating a diagnostic improvement of only 6.9% for the 2nd xenodiagnosis and 4.5% for the 3rd, which

can be explained by the majority of persistently positives or negatives (62.6%), as explained in the previous paragraph. On the other hand, there were no significant differences in the percentage of positives between the 1st, 2nd and 3rd xenodiagnosis performed at one month intervals (test of differences between proportions $P < 0.05$).

DISCUSSION

This study draws attention to the high percentage of positive results shown by the xenodiagnosis of chronic patients infected for over ten years in an area where transmission had been interrupted, totalling over 50% of overall positive results, 78% in the 10-19 age group, and 60.5% in the over 60s, while most other studies show positive findings as low as 20 to 30% when a single xenodiagnosis is employed. It is true in our trial we used three xenodiagnosis in 158 patients (65.8% of the total), two in eight (3.2%), and one xenodiagnosis in 80 patients (32.5%). However, Pereira et al. (1989) employed three xenodiagnosis in 206 patients at six-month intervals in an area where transmission had been interrupted more recently – Virgem da Lapa, Minas Gerais state – and found positive results in 49% of the cases for the three xenodiagnosis added up, a figure similar to our findings from a single test.

The percentage of positives in the xenodiagnosis we studied was similar to the findings by Castro (1980) in 292 patients in Mambai, Goiás state, where the 1st test yielded 41.1% positives, the 2nd 50.3%, and for the third 50.7%. However, the total percentage of positive tests in the three xenodiagnosis performed was higher than our figures.

The higher overall positive percentage found in the three xenodiagnosis by Castro (1980), in comparison to our cases and to the trials by Pereira et al. (1989), is probably due to the fact that he worked with a younger population in an area where transmission was either not stopped or had been interrupted only recently and especially because in most of his xenodiagnosis he used *Dipetalogaster maximus*, which presents a much higher yield than *T. infestans*, as evidenced in his own paper.

Finally, a word about the higher incidence of positives in patients under 20 and over 60

found by many researchers, for which a clear explanation has yet to be developed. In the under-20 group, this might be justified by their more recent of “less chronic” infections; but what about patients 60 and older? One assumption has been the lowered immunity of older patients, but there is no conclusive evidence to that effect, particularly for Chagas disease. On the other hand, we do not know why some patients show persistently positive test results while others are persistently negative. One possibility that we have raised recently (Coura et al., 1989) is failure of the lysis system allowing *T. cruzi* to circulate freely in blood, or yet other unknown “escape mechanisms” which might release the parasite into the bloodstream.

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