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Direct Micromethod for Diagnosis of Acute and Congenital Chagas' Disease

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A microhematocrit concentration method (MH) for immediate diagnosis of Chagas' disease during the acute stage or in congenital cases was standardized. Parasitemia as low as 1,000 parasites per ml was detected, after centrifugation of six 50-μl capillary tubes, by 10-min microscopic observation of each buffy coat spread between slide and cover glass. Operator's time was reduced by at least one-third when compared with a fresh blood observation (FB). In 12 of the 15 patients studied, diagnosis was performed in 4.9 ± 3.08 min with MH, whereas 27.0 ± 12.1 min were necessary when FB was used. In the three remaining patients whose FB results were negative, MH became positive after 13, 16, and 40 min. In our experience, FB proved to be more sensitive than previously reported. Suckling mouse inoculation also proved to be sensitive but, as in xenodiagnosis and in hemoculture, the delay in getting the final result was a limiting factor.

Rapid diagnostic methods for Chagas' disease during the acute stage of infection or in congenital cases are most necessary; specific treatment with parasiticidal drugs is more efficient the sooner it is begun after infection (3). Indirect diagnosis might be helpful but the most specific one is direct detection of the parasite (13, 14, 20). Xenodiagnosis (XD), although an excellent method as regards sensitivity, fails to provide immediate results (5). Strout's method (21) is both rapid and sensitive, but the blood amounts needed disqualify it for pediatric use. Fresh blood observation (FB) and other methods have been reported to be less sensitive during the acute stage (5).

A micromethod to detect African trypomastigotes by means of direct microscopy of a microhematocrit has proved to be efficient (24). In Chagas' disease, circulating parasites are usually present in lower numbers than in African trypanosomiasis, so they often escape observation by this method. However, a modification of this microconcentration procedure results in a more sensitive test, useful for direct diagnosis of either congenital or acute *Trypanosoma cruzi* infections in children; a semiquantification of the number of circulating parasites is also feasible.

MATERIALS AND METHODS

Parasites. Rockland mice, infected with *T. cruzi*, either strain CA-I (11) or strain RA (10) (predominantly stout and slender, respectively; 4), were bled from the retroorbital sinus at the peak of parasitemia, using 10 U of heparin per ml of blood. Both strains were selected because stout and slender forms possess different movement (rotation for the former and translation for the latter) which might influence the test's sensitivity. The initial number of parasites was counted by Pizzi's technique (16), and dilutions with normal mouse blood were performed to achieve a 50 to 8,000/ml range in parasite concentration.

Standardization of the microhematocrit method (MH) for parasite concentration. Six 50-μl microhematocrit capillary tubes were filled with each parasite concentration sample, except those containing 50 and 500 parasites per ml, for which 12 capillaries each were prepared. Tubes were centrifuged in a microhematocrit rotor at 3,000 × *g* for 40 s, having been sealed at one end. After centrifugation, they were kept vertical. Each tube was cut between the buffy coat and the erythrocyte pellet. The buffy coat was poured onto a slide, a cover glass was applied, and the slide was microscopically examined for 10 min, using ×400 magnification; the number of motile parasites found per tube was registered.

FB. Six 10-μl fresh blood drops prepared between the slide and the cover glass were observed microscopically for 10 min each, under ×400 magnification. The number of motile parasites seen was also registered.

Other techniques used for parasite detection. XD (5) and suckling mouse inoculation (SMI) (15) were performed. These methods were only used with the samples containing the lowest parasite concentrations (1,000, 500, and 50/ml): (i) 40 *Triatoma infestans* nymphs of the third stage were artificially fed (9) with the infected blood samples, and insects were dissected 30 days later to search for parasites in the digestive tract; (ii) 50 μl of infected blood was injected subcutaneously into each of 8 to 10 suckling mice, and

TABLE 1. Direct detection of circulating parasites in acute and congenital patients

Patient[a]	Age	Time for positive test (min)		XD	SMI		Clinical signs
		FB	MH		Days[b]	% Positive mice	
1 A	5 mo	12	2	+	11	10[c]	Generalized edema
2 A	8 mo	37	10	ND[d]	10	90[c]	Chagoma
3 A	9 mo	40	7	ND	20	50[e]	Chagoma
4 A	2 yr	31	5	ND	11	50[e]	Chagoma
5 A	3 yr	18	4	+	18	60[c]	Submaxillar adenopathy
6 A	6 yr	25	4	ND	16	25[e]	Chagoma
7 A	6 yr	15	2	+	21	40[c]	Chagoma
8 A	8 yr	Negative	13	ND	24	50[c]	Chagoma
9 A	9 yr	38	5	ND	15	30[c]	Transfusional infection
10 C	2 mo	30	3	ND	30	10[c]	Retarded growth
11 C	2 mo	5	1	+	17	75[e]	Hepatitis
12 C	3 mo	32	11	+	21	20[c]	Retarded growth
13 C	12 mo	42	5	+	33	10[c]	Asymptomatic
14 C	15 mo	Negative	16	+	Negative	Negative[e]	Asymptomatic
15 C	19 mo	Negative	40	+	Negative	Negative[e]	Asymptomatic
$\bar{x}$		27.0	4.90		19.00	42.50	
SD		12.10	3.08		7.01	25.98	

[a] A, Acute; C, congenital.
[b] Time in which parasitemia became apparent.
[c] Percent positive among 10 animals.
[d] ND, Not done.
[e] Percent positive among eight animals.

parasitemia was evaluated in blood samples, taken from the tails, three times weekly for 40 days starting on day 15.

MH, FB, XD, and SMI were performed simultaneously, each strain being evaluated twice.

Patient evaluation. Fifteen patients, between 2 months and 9 years of age, were studied (Table 1). Those numbered 1 to 4 and 6 to 8, from endemic areas, showed common clinical signs: chagoma and generalized edema. Patient 5 was sent to our hospital because of an adenopathy; as he came also from an endemic area with symptoms of acute infectious disease, parasitological studies to detect *T. cruzi* infection were performed. Patient 9 had a congenital immunosuppressive disease with a history of periodic blood transfusions. He underwent surgery because of an endocranial hypertensive syndrome compatible with a tumor mass; as amastigote-like forms were seen in the histological studies, further parasitological investigation was carried out to confirm the suspected *T. cruzi* infection diagnosis. Children under 2 years old, born in a nonendemic area from a mother serologically positive for Chagas' disease, are usually studied to discard possible congenital *T. cruzi* infections; patients 10 to 15 were recognized during this procedure.

The following tests were conducted on each patient: (i) MH, using six heparinized capillary tubes; (ii) FB on six fresh blood drops (MH and FB observations were interrupted when the first parasite was seen); (iii) 50-μl blood inoculation into each of 8 to 10 suckling mice (the shortest time to prove patent parasitemia was recorded, starting on day 10 post-inoculation).

XD with 10 to 40 *Triatoma infestans* (third nymph stage), depending on the age of the patient, were performed in 8 of the 15 patients.

Simultaneously, serological tests were conducted: immunoglobulin G (IgG)- and IgM-specific antibodies were assayed, using indirect immunofluorescence and direct agglutination tests (2, 23).

RESULTS

The results obtained in the four experiments performed with strains CA-I and RA were essentially the same. The comparison between MH and FB in one experimental run is registered in Fig. 1. With the concentration procedure, the number of parasites seen during a similar observation period was 3 to 10 times higher than with FB; parasitemia as low as 1,000 to 500 parasites per ml was detected, two of the six capillary tubes for samples with 1,000 parasites per ml proving positive. Therefore, direct diagnosis of *T. cruzi* infections with such a low parasitemia level may last about 30 min. XD and SMI performed with strains RA and CA-I were positive even for samples containing as few as 50 parasites per ml.

When the study was conducted on patients, MH was positive in all 15 cases, whereas FB was negative in 3 of the cases (patients 8, 14, and 15) even after 60 min of searching (Table 1). The mean time used to detect the first parasite in FB was 27.0 ± 12.1 min; for the same samples MH was positive in 4.9 ± 3.08 min. In those three patients with negative FB, parasites were detected by MH after 13, 16, and 40 min of

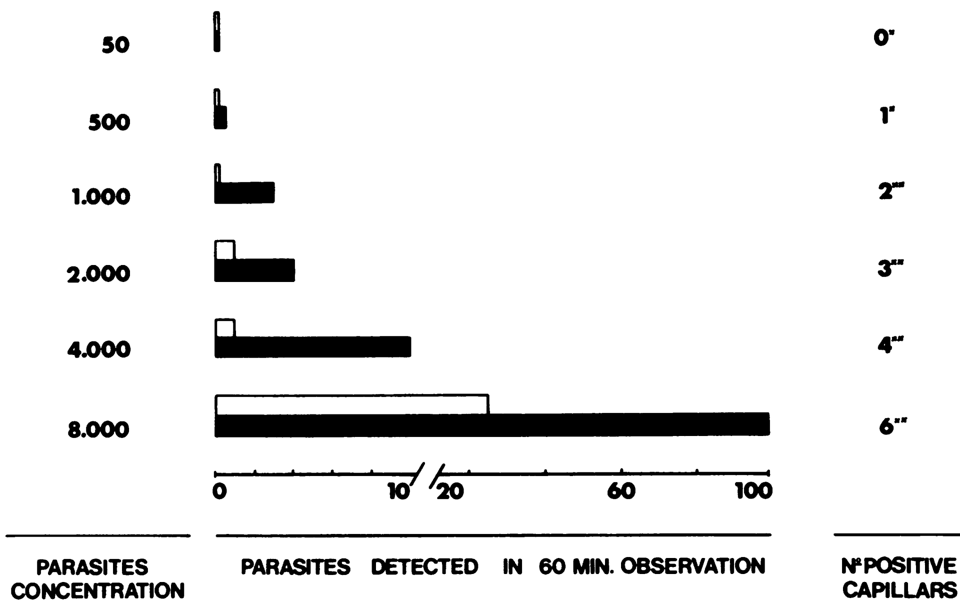

FIG. 1. Direct parasite observation by MH or FB. Open bars indicate the total number of parasites detected by FB, and closed bars are those detected by MH. Total number of capillaries: [x]12 or [xx]6.

searching, respectively. SMI proved to be sensitive for parasite detection in acute cases; for congenital ones, those studied soon after birth (2 to 3 months) were also positive, but two of the three patients over 1 year old were negative. Patent parasitemia was detected from 10 to 33 days post-inoculation in a variable number (10 to 90%) of mice (Table 1). When performed, XD was uniformly positive (Table 1).

Except for patient 9, who was serologically unreactive, the rest possessed specific IgG antibodies; in patients 1 to 8 and 10 to 12 specific IgM antibodies were detected also.

DISCUSSION

Diagnosis of *T. cruzi* infection may be performed by serology or by detection of the circulating parasites. Interpretation of antibody response proves difficult in immunosuppressive states, soon after infection, or in congenital cases. In the later two, specific IgM detection is useful, but either false-positive or false-negative reactivity is likely, as has been communicated for other protozoan infections (17, 18). Moreover, in congenital Chagas' disease IgM antibodies may appear several days after birth (22). In all of the above cases the direct observation of the etiological agent is the only reliable way to confirm diagnosis.

Direct diagnosis is feasible, especially during the acute stage of the infection. Seventeen to 100% sensitivity was reported for several techniques during this period, and the highest value was provided by XD (5, 14, 19). This latter method is not always practical due to the need of an insect house, and results are not available immediately but rather 30 or even 60 days later. Delay is also a limiting factor in hemoculture, another sensitive method (1, 14).

Since Chagas' time, the suckling mouse has been known to be a sensitive laboratory host for the isolation of *T. cruzi* (6, 15). In the present work SMI proved to be just as sensitive as XD for strains CA-I and RA. However, it should be kept in mind that both strains have become adapted to this host over many years and parasitemia can become apparent even when injecting less than 50 parasites. This might explain its higher sensitivity observed during the experimental phase of this work compared with the results achieved in children. As in XD and in hemoculture, the usefulness of SMI is limited by the delay in getting results and the operator's time required.

Instant diagnosis in recently acquired infections and in congenital cases is essential because parasiticidal treatment is more effective the sooner it is started. In Strout's method, with 95% sensitivity (5), diagnosis is immediate but the 10-ml blood volume is a limiting factor, especially considering that human *T. cruzi* infections in endemic areas are often acquired during the first years of life. FB has been reported to be less sensitive than Strout's method, detecting 54% of the acute human infections (5); in our experience its sensitivity was higher (80% in the present study). With MH, all patients studied

here were positive; compared with FB, this method proved to be capable of concentrating at least three times the number of parasites, resulting in a proportional reduction of the operator's time (see Fig. 1 and Table 1). Strains with either predominantly slender or stout bloodstream forms (11) were equally detected.

It is well known that chronic infections usually have extremely low parasitemia levels which hinder direct diagnosis (5, 7). However, circulating parasites were observed by MH in the three congenital cases studied after 1 year of age, which could be considered chronic cases, whereas only one case proved to be SMI positive and none was positive by FB.

A technique sensitive enough to follow the course of parasitemia in patients during treatment would be worthwhile. In this regard MH semiquantification should be evaluated. It might help to avoid full-term treatment with a specific drug in patients infected with a strain resistant to that particular drug (3).

As MH is highly sensitive and simple enough to be performed in any pediatric laboratory with only a centrifuge and a microscope, requiring at most 600 μl of blood from finger or heel, we propose to use it for routine diagnosis in suspected acute infections of Chagas' disease in children and in congenital infections, being cautious in those areas where *T. rangeli* coexists with *T. cruzi* (8).

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