

***Trypanosoma cruzi*: blood parasitism kinetics and their correlation with heart parasitism intensity during long-term infection of Beagle dogs**

Vanja M Veloso⁺, Paulo MM Guedes, Isabel M Andrade, Ivo S Caldas, Helen R Martins, Cláudia M Carneiro¹, George LL Machado-Coelho², Marta de Lana¹, Lúcia MC Galvão³, Maria T Bahia, Egler Chiari³

Departamento de Ciências Biológicas, Instituto de Ciências Exatas e Biológicas ¹Departamento de Análises Clínicas ²Departamento de Ciências Médicas, Escola de Farmácia, Campus Universitário, Universidade Federal de Ouro Preto, 35400-000 Ouro Preto, MG, Brasil ³Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

The goals of the present study were to evaluate the kinetics of blood parasitism by examination of fresh blood, blood culture (BC) and PCR assays and their correlation with heart parasitism during two years of infection in Beagle dogs inoculated with the Be-78, Y and ABC Trypanosoma cruzi strains. Our results showed that the parasite or its kDNA is easily detected during the acute phase in all infected animals. On the other hand, a reduced number of positive tests were verified during the chronic phase of the infection. The frequency of positive tests was correlated with T. cruzi strain. The percentage of positive BC and blood PCR performed in samples from animals inoculated with Be-78 and ABC strains were similar and significantly larger in relation to animals infected with the Y strain. Comparison of the positivity of PCR tests performed using blood and heart tissue samples obtained two years after infection showed two different patterns associated with the inoculated T. cruzi strain: (1) high PCR positivity for both blood and tissue was observed in animals infected with Be-78 or ABC strains; (2) lower and higher PCR positivity for the blood and tissue, respectively, was detected in animals infected with Y strains. These data suggest that the sensitivity of BC and blood PCR was T. cruzi strain dependent and, in contrast, the heart tissue PCR revealed higher sensitivity regardless of the parasite stock.

Key words: *Trypanosoma cruzi* - Beagle dogs - parasitemia - blood culture - blood and tissue PCR

American trypanosomiasis is a protozoan infection caused by the flagellate *Trypanosoma (Schizotrypanum) cruzi*, and it is widespread throughout the American Continent. Approximately 13 million people living Central and South America are currently estimated to be infected and 14,000 annual deaths are associated with the infection (WHO 2005).

Different approaches have been used to diagnose Chagas disease. Serologic tests are regularly used to detect antibodies against *T. cruzi*. These tests have high sensitivity, but they lack specificity due to antigenic cross-reactivity with parasites such as *Leishmania* and *Trypanosoma rangeli* (Marcon et al. 2002). In addition, positive results from blood culture (BC) and PCR have been found in patients previously demonstrating consistently negative serological tests (Ávila et al. 1993, Gomes et al. 1999, Castro et al. 2002), reinforcing the importance and need for simultaneous use of parasitological and molecular tests in Chagas disease diagnosis.

The literature strongly suggests that the variable efficacies of parasitological methods, such as BC and xenodiagnosis, in Chagas disease diagnosis (Portela-Lindoso & Shikanai-Yasuda 2003) are highly specific, but with low sensitivity (Chiari 1999).

Thus, molecular assays such as PCR have been proposed as good alternative tools for detection of *T. cruzi* kDNA in human (Russomando et al. 1992, Wincker et al. 1994b, Gomes et al. 1999) and animal blood (Araújo et al. 2002, Guedes et al. 2004). According to Castro et al. (2002), PCR is more sensitive than BC in detecting circulating parasites in blood samples collected from chagasic patients during different time periods. Previous studies have shown PCR sensitivities of 45% to 100% for blood from chronic chagasic patients when compared to serological, xenodiagnosis or BC tests (Ávila et al. 1993, Wincker et al. 1994a, Gomes et al. 1998, Chiamonte et al. 1999). These differences could be explained by variable levels of parasitemia in infected individuals living in different endemic areas.

These observations raise the question of whether the parasitemia detected in the peripheral blood could be related to the tissue parasitism (TP) level and, consequently, to the pathogenesis of the chronic phase of the disease.

However, the kinetics of blood parasitism during long-term chronic infection and the correlation between parasitemia levels in the peripheral blood and TP are difficult to evaluate in humans. Considering that previous studies from our group using the canine model for Chagas disease have shown that these animals develop clini-

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+ Corresponding author: vanjamv@iceb.ufop.br

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cal manifestations identical to those observed in human patients (Lana et al. 1988, 1992, Bahia et al. 2002), this model would be more suitable for long-term evaluations. Therefore, this investigation was undertaken to evaluate the blood parasitism level during long-term infection of Beagle dogs inoculated with three different strains of *T. cruzi* and its correlation with heart TP.

MATERIALS AND METHODS

T. cruzi strains - *T. cruzi* Berenice-78 (Be-78) strain (*T. cruzi* II) was isolated by xenodiagnosis in 1978 (Lana & Chiari 1986) from the first human case of Chagas disease; the Y strain (*T. cruzi* II) was isolated from an acute human case (Silva & Nussenzweig 1953); and the ABC strain (*T. cruzi*) was isolated from a chronic human case (Brener 1965).

Experimental animals - 24-month-old Beagle dogs representing both sexes from the kennel of the Universidade Federal de Ouro Preto (UFOP), MG, Brazil, were used in this study. Animals were fed with commercial chow and water *ad libitum*. Before the study, they were treated with anti-helminthic drugs and vaccinated against several infectious diseases. Animals were inoculated intraperitoneally with 4.0×10^3 blood trypomastigotes/kg of body weight of the *T. cruzi* Be-78, Y and ABC strains.

This project was approved by the ethics committee for animal experimentation of UFOP. All procedures and experimental protocols were performed according to Colégio Brasileiro de Experimentação Animal instructions for the use of animals in experimental conditions.

Fresh blood test (FB) - Parasitemia was determined microscopically by fresh blood collected from the marginal ear vein daily from the 10th-30th day post-infection.

BC - Parasite detection was performed by culturing 10 mL blood samples and processing them according to Chiari et al. (1989) with some modifications. Blood samples were collected 30 days after inoculation during the acute phase. During the chronic phase, BC was carried out at regular intervals of six months (four BC/dog). Immediately after collection, the blood was centrifuged at 1,000 g, 4°C for 10 min, in order to remove the plasma.

The packed blood cells were washed by centrifugation at 4°C in 5 mL of liver infusion tryptose (LIT) medium, resuspended in 5 mL of LIT medium and uniformly distributed between two plastic tubes. Cultures were maintained at 28°C, homogenized weekly, and examined monthly for 120 days.

Considering the number of positive BC tubes during the chronic phase, the animals were arbitrarily classified into one of three levels: "low parasitemia": dogs with 0-1 positive tube; "medium parasitemia": dogs with 2-4 positive tubes; and "high parasitemia": dogs with 5-8 positive tubes.

Necropsy of the animals - All dogs were euthanized and 0.5cm³ fragments of the left and right atria, left and right ventricles, interventricular septum and apex were obtained from each dog, cut and frozen at -70°C for posterior parasite kDNA detection by PCR (T-PCR) or preserved in 10% formalin for examination of TP by immunohistochemistry (IMH).

In blood PCR (B-PCR) - For PCR of blood, 10mL of blood was collected in parallel with BC. Blood samples were mixed with an equal volume of a 6 M guanidine hydrochloride-0.2 M EDTA solution and stored for two weeks at rt (Ávila et al. 1990), followed by boiling for 15 min before DNA extraction from 200 µL of the sample (Gomes et al. 1998).

In tissue PCR (T-PCR) - Tissue samples collected 24 months after infection were thawed, minced, subjected to alkaline lysis with 50 mM NaOH, heated in boiling water for 10 min and neutralized with 130 mM Tris-HCl (pH 7.0). The DNA was extracted according to Vago et al. (1996), using positive and negative controls.

PCR conditions - PCR amplification was performed in a total volume of 20 µL containing 0.1% Triton X-100, 10 mM Tris-HCl (pH 9.0), 75 mM KCl, 5 mM MgCl₂, 0.2 mM (each) dATP, dTTP, dGTP and dCTP (Sigma), 1 µL of Taq DNA polymerase (Invitrogen), 20 pmol of S35 (5' AAATAATGTACGGG(T/G)GAGATGCATGA3') and S36 (5' GGGTTCGATTGGGGTTGGTGT3') primers and 2 µL of DNA for each sample (Ávila et al. 1990). The reaction mixture was overlaid with 30 µL of mineral oil (Sigma) and subjected to 35 cycles of amplification in an automatic thermocycler (MJ Research programmable thermal controller PTC-100). The temperature profile was as follows: denaturation at 95°C for 1 min (with a longer initial time of 5 min at 95°C), 65°C for 1 min for primer annealing and 72°C for 1 min for extension, with a final incubation at 72°C for 10 min to extend the annealed primers. The PCR products were visualized by 6% polyacrylamide gel electrophoresis followed by silver staining (Santos et al. 1993). All DNA extraction steps and reaction mixtures used for PCR were monitored and compared to positive and negative controls. The PCR was considered as negative after three DNA extractions of each sample and two amplifications of each extraction.

IMH - Tissue fragments were processed for embedding in paraffin and 5 µm-thick sections were examined. IMH staining was then performed according to the methodology of Barbosa (1985). Canine heart sections obtained during the acute phase of *T. cruzi* infection were used as positive control, and the absorbed antiserum samples or normal rabbit's serum samples were used instead of rabbit anti-*T. cruzi* sera as negative controls. Examination was carried out using an optical microscope at 40X magnification and 30 fields were assessed per sample. The presence of *T. cruzi* antigens and amastigotes was assessed.

Statistical analyses - To compare the positivity among the different methods, the chi-square test was applied. The results obtained by BC, B-PCR and T-PCR were evaluated by the McNemar chi-square test. Results were considered significant at $p < 0.05$.

RESULTS

Blood Parasitism

Acute phase (FB, BC and B-PCR) - All infected dogs exhibited a positive result for parasitemia upon examination of FB. However, the parasite was detected at dif-

ferent times depending on the *T. cruzi* strain inoculated (from the 19th-30th, 13th-21st and 13th-30th days post-inoculation in animals infected with Be-78, Y and ABC *T. cruzi* strains, respectively). In addition, the number of tests necessary for parasite detection was variable among animals inoculated with different strains. The cumulative positivity of FB was similar among animals inoculated with Be-78 (18.7%) and Y (26.2%) and significantly higher in those inoculated with ABC (73.7%) (Table I). Thus, it was necessary to perform 5.3, 3.8 or 1.4 tests to detect the parasites in animals inoculated with strain Be-78, Y or ABC strain, respectively.

Similar results were observed for BC and PCR assays performed during the acute phase. In general, the parasite was detected in the blood of infected animals by both tests, with the exception of a blood sample obtained from one dog inoculated with ABC strain that revealed negative results even after six repetitions.

Chronic phase (BC and B-PCR) - During the chronic phase, 100% of the animals inoculated with Be-78 and ABC strains presented at least one positive BC, whereas only 25% of the animals inoculated with Y strain revealed a positive BC. In addition, the positive test frequency for two years of evaluation was also similar among animals inoculated with strains Be-78 (75%) and ABC strains (62.5%); however, a significantly lower ($p < 0.05$) number of positive BC were observed in animals inoculated with Y strain (6.2%) (Table I).

Considering all positive BC tests performed during the chronic phase of infection, 61% (14/23) and 39% (9/23) were obtained in the first and second years, respectively, after inoculation. These results reveal a decrease in test positivity after the first year of evaluation. Interestingly, this decrease was only observed in animals inoculated with the ABC strain. In these dogs, 87.5% (7/8) of the positive BC were carried out during the first year of infection and 37.5% ($p < 0.05$) (3/8) were detected during the second year of infection. In contrast, the frequencies of positive BC tests performed using samples obtained from dogs inoculated with the Be-78 (75% and 75%) and Y (12.5% and 0%) strains were similar during the first and second year of examination, respectively.

Parasitemia of animals was also evaluated by analysis of the frequency of positive tubes (eight tubes/dog) in relation to the number of positive BC per animal. Fig. 1 shows the frequency of positive tubes and BC in animals infected with the different *T. cruzi* strains. No significant differences were observed between the frequencies of positive tubes and the number of BC among the animals inoculated with Be-78 and ABC strains, but these values were significantly higher ($p < 0.05$) than those observed for animals inoculated with Y strain.

Considering the number of positive tubes in BC, 33% of the animals were classified as presenting "low parasitemia", 50% "median parasitemia" and 17% "high parasitemia".

T. cruzi kDNA was detected in 40 (83.3%) out of the 48 blood samples obtained from dogs inoculated with different *T. cruzi* strains. In addition, B-PCR was positive for at least one sample obtained from each evaluated animal. No significant difference was found in blood samples obtained from dogs inoculated with Be-

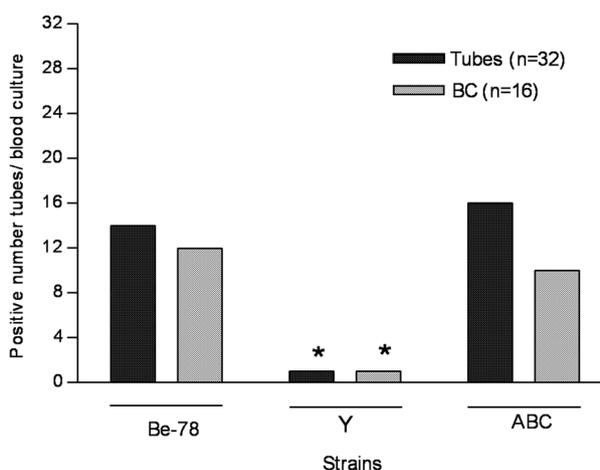


Fig. 1: frequency of positive tubes in blood culture (BC) and of positive BC performed during the chronic phase of *Trypanosoma cruzi* infection of Beagle dogs inoculated with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC strains; * $p < 0.05$.

TABLE I

Fresh blood (FB) examination, blood culture (BC) and blood-PCR (B-PCR) results performed during the acute phase and chronic phase of the infection in Beagle dogs inoculated with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC *Trypanosoma cruzi* strains

Method/Total of tests	Time of infection					
	Acute phase			Chronic phase		
	<i>T. cruzi</i> strains					
	Be-78	Y	ABC	Be-78	Y	ABC
+FB ^b /Total of FB (%)	15/80 (18.7)	21/80 (26.2)	59/80 (73.7) ^a	NP	NP	NP
+BC/Total of BC (%)	4/4 (100)	4/4 (100)	4/4 (100)	12/16 (75)	1/16 (6.2) ^a	10/16 (62.5)
+B-PCR ^c /Total of B-PCR (%)	4/4 (100)	4/4 (100)	3/4 (75)	16/16 (100)	9/16 (56.3) ^a	15/16 (93.7)

a: $p < 0.05$: significant difference; NP: not performed; +BC: positive blood culture; +B-PCR: positive B-PCR (c: performed in four samples of blood from each dog at regular intervals of six months during the chronic phase); +FB: positive fresh blood test (b: performed for 20 consecutive days on each dog in the acute phase of infection).

78 (100%) and ABC strains (93.7%). These indexes were significantly higher than those observed for animals inoculated with strain Y (56.3%) (Table I). Considering all positive B-PCR reactions performed during the CP of infection, 52.5% (21/40) were obtained during the first and 47.5% (19/40) during the second year after inoculation of dogs, with similar positivity observed for both groups during the two years of observation. Representative B-PCR samples obtained from groups of dogs infected with different *T. cruzi* strains are shown in Fig. 2.

Tissue Parasitism

Acute phase (IMH) - In all animals necropsied during the acute phase of infection, amastigotes were detected in at least one of the six heart fragments examined. In a total of 24 fragments, the rate of positivity (amastigotes isolated/nests) according to the strain was 95.8% (23/24) for animals inoculated with Be-78 strain, 70.8% (17/24) for animals inoculated with ABC strain and only 29.2% (7/24) for dogs inoculated with Y strain.

The number of amastigotes varied among animals inoculated with different *T. cruzi* strains. Thus, 101 isolated amastigotes and 183 nests were observed in 720 microscopic fields of fragments obtained from the hearts of animals inoculated with Be-78 strain. Among animals inoculated with Y strain, an intense inflammatory processes and few amastigote nests (4/720 fields) were observed. However, a greater number of isolated amastigotes (52/720 fields) or small groups of amastigotes were observed to be associated with inflammatory process. Moreover, parasitism of the cardiac tissue in animals inoculated with ABC strain showed intermediary intensity between animals inoculated with the Be-78 or Y strains, with 20 isolated amastigotes and 53 nests/720 fields observed. In these animals, TP was associated with the inflammatory process (Fig. 3).

Chronic phase (IMH and T-PCR) - No parasites were found in any heart fragments obtained from animals during the chronic phase (24 months after inoculation) by IMH. However, DNA fragments of *T. cruzi* were detected in 84.5% of the tissue samples obtained from the left and right atria, left and right ventricles, interventricular septum and apex of dogs infected with Be-78, Y or ABC strains by T-PCR. Considering the different heart areas, T-PCR was positive for at least three samples from each animal evaluated. A total of 75%, 87.5% and 91.3% of the tissue samples were positive for animals infected

strain Be-78, Y or ABC. There was no significant difference among animals infected with the different strains (Table II).

BC and B-PCR - During the acute phase of infection, 91.6% of the samples analyzed were positive for both BC and B-PCR, and 8.4% only for BC. These results indicate that BC and B-PCR showed similar sensitivity during this phase of the disease (Table III). During the chronic phase, 47.9% of the samples analyzed were positive for BC and B-PCR, 35.4% for B-PCR alone and 16.7% were negative for both tests (Table III). Positive B-PCR was significantly more frequent than positive BC. The sensitivities of BC in relation to B-PCR during the first and second years of infection for dogs inoculated with different strains were 66.7% and 47.4%, respectively (Table III).

BC, B-PCR and T-PCR - The correlation between blood and TP was evaluated for BC, B-PCR and T-PCR performed in paired form 24 months after inoculation of

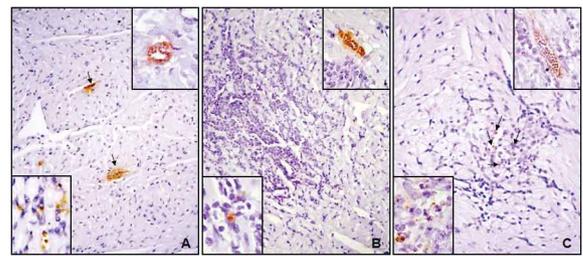


Fig 3: photomicrography of Beagle dogs myocardium infected with 4.0×10^3 blood trypomastigotes of Be-78, Y or ABC *Trypanosoma cruzi* strains and euthanized at the acute phase. A: Be-78 strain: nests or isolated amastigotes (arrows) with absence of inflammatory process; B: Y strain: intense inflammatory process and absence of parasitism; C: ABC strain: moderate inflammatory process and isolated amastigotes; inserts: nests (upper) or isolated amastigotes (lower) observed in all strains. Immunohistochemistry Peroxidase Anti-Peroxidase. A, B and C: 160X; inserts: 1000X.

TABLE II

PCRs in different heart areas performed after necropsy of 12 Beagle dogs two years after inoculation with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC strains of *Trypanosoma cruzi*

	<i>T. cruzi</i> strain		
	Be-78	Y	ABC
	+PCR/n° dogs		
RA	3/4	4/4	4/4
LA	2/4	2/4	4/4
RV	4/4	4/4	3/3
LV	2/4	4/4	3/4
IS	3/4	3/4	3/4
AP	4/4	4/4	4/4
+ frag /Total frag/ n° dogs %	18/24/4 75	21/24/4 87.5	21/23/4 91.3

AP: apex; frag.: fragment; IS: interventricular septum; LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle.

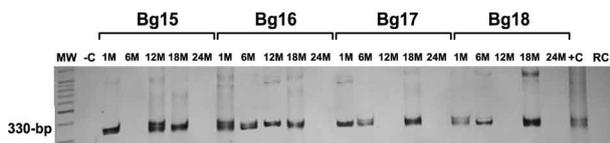


Fig. 2: representative gel showing the specific amplification of 330 bp fragments of kDNA minicircles of *Trypanosoma cruzi* in the blood of infected Beagle dogs (Bg 15, Bg 16, Bg 17 and Bg 18) with 4.0×10^3 blood trypomastigotes of the Y strain during 24 months of evaluation. MW-DNA (100 pb ladder); Bg: Beagle dog; M: month; RC: reagents control; +C: infected/positive control dog; -C: negative control dog.

TABLE III

Comparison between blood culture (BC) and blood-PCR (B-PCR) results obtained during the acute phase and chronic phase (two years) of the *Trypanosoma cruzi* infection of the 12 Beagle dogs inoculated with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC strains

	Positive tests (%)			
	Acute phase n = 12	Chronic phase		
		1st year n = 24	2nd year n = 24	total n = 48
+BC/+B-PCR	11 (91.6%)	14 (58.3%)	9 (37.5%)	23 (47.9%)
+BC/-B-PCR	1 (8.4%)	0	0	0
-BC/+B-PCR	0	7 (29.2%)	10 (41.7%)	17 (35.4%)
-BC/-B-PCR	0	3 (12.5%)	5 (20.8%)	8 (16.7%)
χ^2 McNemar	NS	$p < 0.05^a$	$p < 0.05^a$	$p < 0.05^a$
Sensitivity BC	100%	66.7%	47.4%	57.5%
Specificity BC	-	100%	100%	100%

a: $p < 0.05$, significant difference, when comparing positivity BC to B-PCR.

12 Beagle dogs with Be-78, Y or ABC *T. cruzi* strains (Fig. 4). The positivity was 33% (4/12) for BC, 66.7% (8/12) for B-PCR and 100% (12/12) for T-PCR. Considering all dogs evaluated, a higher sensitivity was observed for T-PCR compared to that observed for the other tests performed. The sensitivity of BC in relation to B-PCR, BC in relation to T-PCR and B-PCR in relation to T-PCR were 50%, 33.3% and 66.7%, respectively (Table IV). On the other hand, the parasite was only detected in heart tissue by T-PCR in animals infected with Y strain. blood parasitism was not detected in any animal evaluated by BC or B-PCR (Fig. 4).

All tests performed using blood or tissue samples obtained from animals infected with Be-78 strain exhibited a similar frequency of positivity. A high correlation was observed between parasite levels in the peripheral blood and in heart tissue. In animals inoculated with ABC strain, the positivity of B-PCR and T-PCR was also similar, with the parasite detected with significantly lower frequency by BC. In animals infected with Y strain, the parasite was only detected in heart tissue by T-PCR. Blood parasitism was not detected in any animal evaluated by BC or B-PCR (Fig. 4).

DISCUSSION

Evaluation of the parasitism level of peripheral blood in chronic Chagas disease has been evaluated by several studies. In general, the sensitivity of the parasitological tests, xenodiagnosis and BC assays is related to the blood parasitism level, which could be related to genetic characteristics of the *T. cruzi* strains. These genetic characteristics vary according to geographic regions (Coura et al. 1984). However, due to ethical reasons, most of these studies were performed in humans during the acute or chronic phases of the infection and did not evaluate the relationship between the intensity of heart tissue and blood parasitism.

During the acute phase of the infection, the presence of the parasite was easily demonstrated in all animals by the three techniques used herein (FB, BC and B-PCR).

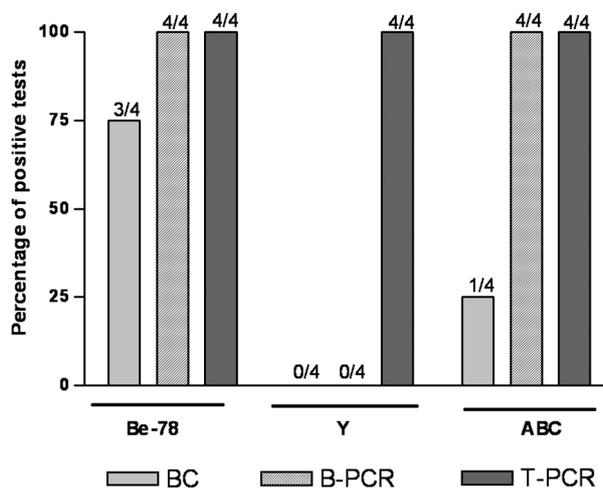


Fig. 4: comparison among the results obtained by blood culture (BC), blood-PCR (B-PCR) and tissue-PCR (T-PCR) performed after 24 months of the *Trypanosoma cruzi* infection of the 12 Beagle dogs inoculated with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC strains.

However, the variation in parasitemia levels among animals infected with different *T. cruzi* strains was demonstrated by the frequency of positive FB tests. Considering the frequency of positive tests, we verified the necessity of performing a greater number of tests to detect parasites in animals inoculated with the Be-78 strain compared to those inoculated with the Y and ABC strains. No correlation was observed between blood parasitism levels during the acute and chronic phases of infection because, during the chronic phase, animals inoculated with the Y strain showed significantly lower blood parasitism levels than those inoculated with Be-78 and ABC, as evidenced by BC and B-PCR.

In the present work, a reduction in parasitemia levels throughout the chronic phase of the infection was demonstrated by the decrease in positive BC observed during the second year compared to the first year of evaluation.

TABLE IV
Comparison between blood culture (BC), blood-PCR (B-PCR) and tissue-PCR (T-PCR) results obtained after 24 months of *Trypanosoma cruzi* infection of the 12 Beagle dogs inoculated with 4.0×10^3 blood trypomastigotes of the Be-78, Y or ABC strains

	Chronic phase (24 months after inoculation)		
	n positive/n examined (%)		
+BC/+B-PCR/+T-PCR	4/12 (33.3%)		
+BC/-B-PCR/+T-PCR	4/12 (33.3%)		
-BC/+B-PCR/+T-PCR	8/12 (66.7%)		
-BC/-B-PCR/+T-PCR	4/12 (33.3%)		
-BC/-B-PCR/-T-PCR	0		
	BC x B-PCR	BC x T-PCR	B-PCR x T-PCR
χ^2 McNemar	NS	$p < 0.05^a$	NS
Sensitivity	50%	33.3%	66.7%

a: $p < 0.05$: significant difference when comparing positivity BC to T-PCR; NS: not significant.

Interestingly, this decrease was detected in only animals infected with the ABC strain. On the other hand, the reduction in parasite load throughout *T. cruzi* infection was not verified by B-PCR. In addition, PCR was more sensitive than BC for the detection of parasitemia or parasite kDNA in distinct blood samples collected from dogs infected with each parasite strain. The present results suggest that BC and B-PCR sensitivities are related to the parasite strain. An important difference was observed for BC, as well as PCR positivity performed using blood samples from animals infected with the three different *T. cruzi* strains studied. BC and/or PCR detected the parasite or parasite kDNA in approximately 97% of the tests carried out for animals infected with Be-78 and ABC strains. On the other hand, only 56.3% of the animals infected with Y strain were positive. The difference in positivity for BC could be explained by morphological differences in the strains considered, as previously has demonstrated that *T. cruzi* strains with a predominance of slender blood trypomastigotes are more sensitive to host immune mechanisms than strains with a predominance of large or stout trypomastigotes (Brener 1969). This hypothesis was suggested by other authors who verified different courses of parasitemia in dogs inoculated with Be-62 or Be-78 strains, with a predominance of slender and large trypomastigotes (Lana et al. 1992, Araújo et al. 2002) associated with lower and higher BC, xenodiagnosis and PCR, respectively. According to Coura et al. (1984), the sensitivity of parasitological tests seems to reflect the different genetic constitution of circulating *T. cruzi* strains, which vary according to geographical regions and naturally with the polymorphism of the blood trypomastigote.

Arbitrary classification of the animals according to the parasitemic level based on BC was also associated with the *T. cruzi* strain assessed. Thus, animals with “high parasitemia”, presented a higher frequency of positive B-PCR and IMH, while the opposite was observed in animals classified as “low parasitemia”. In this scenario, the low positivity of BC and PCR performed using peripheral blood samples obtained from animals infected with strain Y reflect the low parasitemia of these

animals. Additionally, negative BC and PCR results for patients and animals with positive serology have been reported. This fact has been explained by the occurrence of patients with low parasitemia, for whom parasites could rarely or never be detected (Castro et al. 2002).

A high frequency of *T. cruzi* kDNA in representative portions of the heart was observed for all animals evaluated 24 months after infection. These results showed, as previously reported in human chronic Chagas disease (Lages-Silva et al. 2001, Elias et al. 2003), that fragments of *T. cruzi* kDNA remain in the heart tissue after long-term infection of dogs. On the other hand, Machado et al. (2001) did not detect *T. cruzi* DNA in the heart tissue of dogs inoculated with different *T. cruzi* strains using primers that amplify a 195 bp repetitive sequence of the genomic DNA, in contrast to the PCR condition used herein. However, the differences in the primers and PCR methodologies used require consideration.

With regard to the IMH results, the parasitism and inflammatory process pattern was again different, suggesting a large influence of the parasite strain on the results obtained for injuries incurred during the acute phase of infection. During the chronic phase, the IMH reaction was negative for all heart fragments. The low sensitivity of the IMH at this stage of infection has already been demonstrated for dog tissues by other authors (Caliari et al. 1994, Machado et al. 2001, Cruz et al. 2006). Lages-Silva et al. (2001) also verified that PCR exhibits greater sensitivity than IMH for detecting the presence of *T. cruzi* in the esophagus of chagasic patients during the chronic phase of the infection.

Taken together, these results corroborate the importance of *T. cruzi* strains in the sensitivity of parasitological techniques (FB, BC, B-PCR and IMH) used for diagnosis of infection. Furthermore, our data provide definitive evidence of long-term persistence of *T. cruzi* in the heart tissue of dogs infected with different *T. cruzi* strains.

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