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Electrocardiographic evaluation of dogs experimentally infected with *Trypanosoma cruzi* during the acute and indeterminate chronic phases of infection

Avaliações eletrocardiográficas nas fases aguda e crônica indeterminada em cães experimentalmente infectados com *Trypanosoma cruzi*

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SUMMARY

Groups of dogs experimentally infected with *Trypanosoma cruzi* (1,000 trypomastigotes/kg body weight, Colombian strain) and infected and treated with a beta blocker (metoprolol) were submitted to electrocardiographic evaluation during the acute phase (weekly for 5 weeks) and during the chronic indeterminate phase (monthly for 6 months) with the objective of studying the degree of cardiac lesion. The alterations observed in the acute phase were more frequent in the infected group, with increased heart rate, mv suppression and increased PR interval, and increased heart rate persisted during the chronic indeterminate phase. In the infected group treated with metoprolol there was mv suppression in the acute phase and maintenance of heart rate within normal limits in the chronic indeterminate phase. Other alterations such as increased Q wave, 1st and 2nd degree atrioventricular block, disorders of ventricular conduction, ventricular tachycardia, ventricular extrasystole, asystole, and T wave suppression or increase, mainly occurred in the infected group during the acute phase.

UNITERMS: Electrocardiography; Dogs; Chagas' disease.

INTRODUCTION

Chagas' disease or South American trypanosomiasis, caused by the hemoflagellate protozoan *Trypanosoma cruzi* and described by Chagas¹⁰ (1909), affects approximately 16 million people on the American continent²⁹. About 5 million people are contaminated in Brazil¹¹.

The disease is clinically characterized by acute, indeterminate and chronic phases. The acute phase is easily reproduced in young dogs^{1,15,22}; the lesions are disseminated and may affect various regions of the organism, but severity of each case is related to the involvement of heart and central nervous system²⁴. The presence of countless trypanosomes in the bloodstream during this phase represents the parasitemic period when the disease can be diagnosed by blood examination under light microscope. The test of reference for serologic diagnosis of Chagas' disease is the indirect immunofluorescence reaction¹⁴. The acute phase, which usually lasts about 40 days, is followed by the chronic asymptomatic or indeterminate phase characterized by the disappearance of all clinical signs⁵. During this phase, a laboratory diagnosis can be made by xenodiagnosis and indirect immunofluorescence. The period between the asymptomatic phase and the acute cardiac phase, in which the animals may develop signs of cardiomegaly, arrhythmia and congestive heart failure, has been reported to range from 3 to 55 months^{16,19}. However, these signs have not been detected in experimental observations from 24 to 76 months^{5,16,25}.

The changes detected by the electrocardiogram (ECG) during the acute phase may reflect the progression and distribution of the inflammatory process in the atria, in the right portion of the interventricular septum and/or in the free wall of the right ventricle⁴.

The first ECG alterations detected during the acute phase by about the 7th day or from the 15th to the 45th day were sinus arrhythmia, sinus tachycardia, wandering sinus pacemaker, atrial rhythm, sinus arrest, atrial fibrillation, junctional or nodal rhythm, wide low-voltage P waves, decreased voltage of the QRS complex, inverted and deep T waves, marked elevation, axis deviation, 1st degree atrioventricular block (AVB), and full right branch block (RBB) with anterior left hemiblock (ALHB)^{1,4,6,9,16,19}.

Approximately 3 months after experimental infection, the ECG of dogs showed a tendency to normalization, a fact characterizing the asymptomatic period^{3,5,16}. However, 8 to 36 months after inoculation, most of the dogs presented prolonged QRS, decreased voltage, and 1st and 2nd degree AVB and RBB, with only one dog developing ventricular extrasystole^{5,20}, whereas another study carried out during the same phase did not show these changes². Premature ventricular beats, changes in T wave and in the ST segment (lesion current, ischemia) and frequent alterations of the electric axis were identified after one year of observation^{4,6}. Blocks, which were sometimes of low frequency, appeared during the terminal phases of infection^{1,4,16,26}.

Administration of metoprolol (a beta blocker) to human patients with dilated cardiomyopathy reduces heart rate both during

exercise and under resting conditions, improving symptoms and tolerance to exercise¹². Propranolol and metoprolol, respectively administered to human chronic chagasic patients with ventricular extrasystole^{21,23} and rats with experimental chagasic heart disease for 10 months, had a beneficial action, as shown by improved patient condition and by a reduction of the prevalence of electrocardiographic changes compatible with Chagas' heart disease⁷.

The objectives of the present study were to determine the electrocardiographic changes of dogs experimentally infected with *Trypanosoma cruzi* during the acute and chronic indeterminate phases of the disease, and to evaluate the effects of administration of the beta blocker metoprolol.

MATERIAL AND METHOD

The study was conducted in the Cardiology Laboratory, Veterinary Hospital, Faculty of Agrarian and Veterinary Sciences, State University of São Paulo, UNESP, Jaboticabal Campus, SP.

Thirty, 4 month-old mongrel dogs of both sexes were maintained in individual kennels, receiving Commercial food* twice a day and water *ad libitum*. The animals had been vaccinated against distemper, hepatitis, leptospirosis, parvovirus and coronavirus** according to a standard schedule and treated with a vermifuge***. The following experimental groups were formed:

- Infected (I) - 15 dogs studied for 5 weeks after inoculation;
- Infected and treated (IT) - 5 dogs studied for 5 weeks after inoculation and treated with the beta blocker;
- Chronically infected (CI) - 5 dogs studied for 6 months after inoculation;
- Chronically infected and treated (CIT) - 3 dogs studied for 6 months after inoculation and treated with the beta blocker;
- Control (C) - 10 noninfected animals studied for 5 weeks, 5 of which were studied for a total of 6 months.

The Colombian strain of *Trypanosoma cruzi* was used for inoculation¹³. The inocula were obtained from citrated mouse blood and utilized at the concentration of 1,000 trypomastigotes/kg body weight inoculated by the intraperitoneal route.

Parasitemia was observed by the technique of Brenner⁸ (1962), at 3-day intervals starting on the 4th day after inoculation. The indirect immunofluorescence reaction at 1:40 dilution was performed before inoculation, during the 2nd and 5th week after inoculation, and 6 months after inoculation. Xenodiagnosis using 15 3rd - to 5th-instar *Triatoma* nymphs was performed during the 5th month of infection.

Starting on the day of inoculation, animals of the IT group were medicated with metoprolol tartrate**** at the dose of 15 mg/kg body weight by the oral route, twice a day for 6 months (CIT group).

The electrocardiographic studies were carried out before inoculation, weekly for 5 weeks in the control and infected groups, and then for 6 months. The tracings were obtained in the absence

of anesthesia from the I, II and III bipolar leads and from the unipolar aVR, aVL and aVF leads at the rate of 50 mm/sec, with the recorder calibrated for 1 millivolt (mv) = 1 cm²⁸. The apparatus used was a one-channel electrocardiograph*****.

The quantitative electrocardiographic parameters were analyzed statistically by analysis of variance and the means were compared by the Tukey test²⁷.

RESULTS

Serology and parasitemic curves

Before inoculation, the indirect immunofluorescence (1:40) test was negative in all animals, whereas during the 2nd and 5th weeks and 6 months after inoculation the infected animals reacted positively to the same dilution.

The pre-patent period was of 13 days in both groups and the parasitemic curves maintained the same relative behavior, characterized by a gradual increase with a peak on the 25th day. A statistically significant difference occurred on the 28th day (Fig. 1).

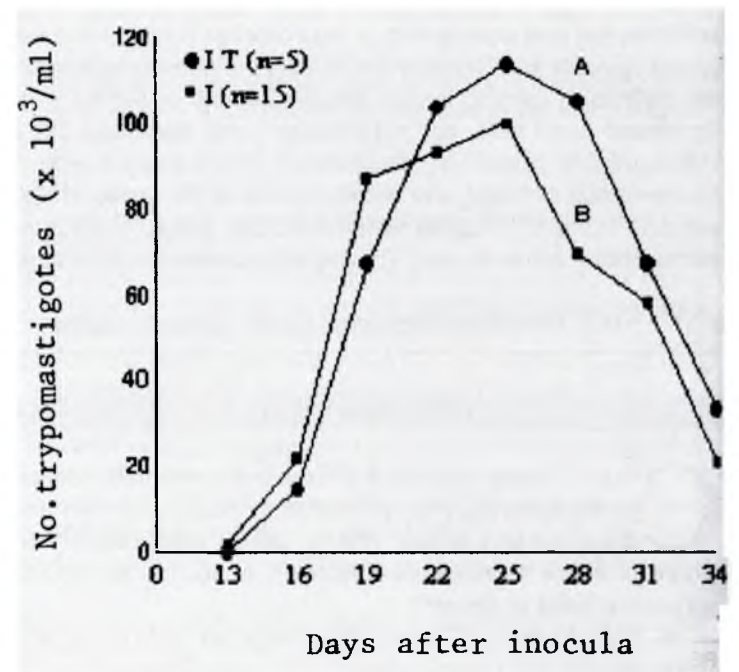


Figure 1

Parasitemia curves for a 5-week period in groups of dogs infected (I and IT) with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight. The IT group received orally 15 mg metoprolol/kg body weight (Lopressor, Ciba Geigy) twice a day. The results are reported as means. Different letters indicate significant differences ($p < 0.05$, Tukey test), Jaboticabal, 1995.

Electrocardiographic findings obtained from Lead II

A. Acute phase:

Heart rate and rhythms

During this phase, the heart rate (bpm) of group I was significantly increased from the 4th week compared to group C, and group IT remained within values close to control. The rhythms

* Pedigree Champ Filhotes - Effem Produtos Alimentícios.

** Cinovac P; Coronaguard - Solvay Saúde Animal.

*** Drontal - Bayer S.A. - Produtos Veterinários.

**** Lopressor - Biogalênica Química e Farmacêutica Ltda.

***** ECG-6 - ECAFIX.

in both infected groups ranged from respiratory sinus arrhythmia (RSA), sinus rhythm (S), and sinus tachycardia (Tab. 1);

P Waves - seconds (sec) and millivolt (mv)

No significant differences in these parameters were observed between groups;

QRS complexes (sec and mv)

Only the mv of the QRS complex (Tab. 2) presented a significant decrease during the 4th and 5th week in the infected groups compared to group C;

PR and QT intervals (sec)

The PR interval was significantly increased in the infected groups compared to group C during the 3rd week. A significant increase in QT interval occurred in both infected groups during the 3rd week (Tab. 3);

ST segments and T waves

No significant differences in either parameter were detected between the infected groups and the control;

Table 1

Weekly heart rate values ($x \pm SEM$) and individual characterization of cardiac rhythms recorded by the ECG (D II) during the acute phase in dogs of the control group (C, n = 10), the group infected with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight (I, n = 15), and the group similarly infected and treated orally with 15 mg/kg metoprolol (Lopressor - Ciba Geigy) twice a day (IT, n = 5). (Jaboticabal, São Paulo, 1995).

GROUP	TIME (weeks)					
	0	1	2	3	4	5
	HEART RATE (bpm)					
C	127 A a ± 7	127 A a ± 7	16 A a ± 6	23 A a ± 7	108 B a ± 6	101 BC a ± 7
I	127 A a ± 5	131 A a ± 6	120 A a ± 4	134 A a ± 5	146 A a ± 7	145 A a ± 8
IT	146 A a ± 4	112 A a ± 7	112 A a ± 11	120 A a ± 6	138 AB a ± 11	110 C a ± 10
	CARDIAC RHYTHMS					
C	RSA 6 S 3 ST 1	RSA 5 S 5	RSA 6 S 4	RSA 6 S 3 ST 1	RSA 8 S 2	RSA 8 S 2
I	RSA 9 S 6	RSA 7 S 7 ST 1	RSA 9 S 5 ST 1	RSA 2 S 9 ST 3	RSA 4 S 5 ST 3	RSA 6 S 1 ST 5
IT	RSA 4 ST 1	RSA 2 S 3	S 5	S 5	S 5	RSA 4 ST 1

RSA = Respiratory sinus arrhythmia; S = sinus; ST = sinus tachycardia.

Different capital letters in the columns and different lower case letters on the lines represent statistically significant differences ($p < 0.05$, Tukey test) between groups and weeks, respectively.

Cardiac axis

No significant changes were observed in this parameter.

B. Indeterminate chronic phase:

Cardiac rate (bpm) and rhythms

Cardiac rate was elevated in group IC and cardiac rhythms were normal (Tab. 4);

PR (sec) and QT (sec) intervals

PR intervals did not differ significantly between groups. QT intervals were within normal limits in group CI and were increased close to C values in group CIT, but the changes were nonsignificant (Tab. 5).

P waves (sec and mv), QRS complexes (sec and mv), ST segments, T waves and heart axis were

Unchanged when compared to the control group;

C. Other alterations:

Tab. 6 presents the prevalences of other electrocardiographic changes observed during the acute and chronic asymptomatic phases.

The electrocardiographic tracings of the alterations detected during the acute and chronic asymptomatic phases are presented in Fig. 2.

DISCUSSION

This experiment showed the susceptibility of young dogs to experimental *Trypanosoma cruzi* infection^{1,4,15-17,18}

The parasitemic curves for groups I and IT were relatively similar and characterized by a gradual increase during the course of

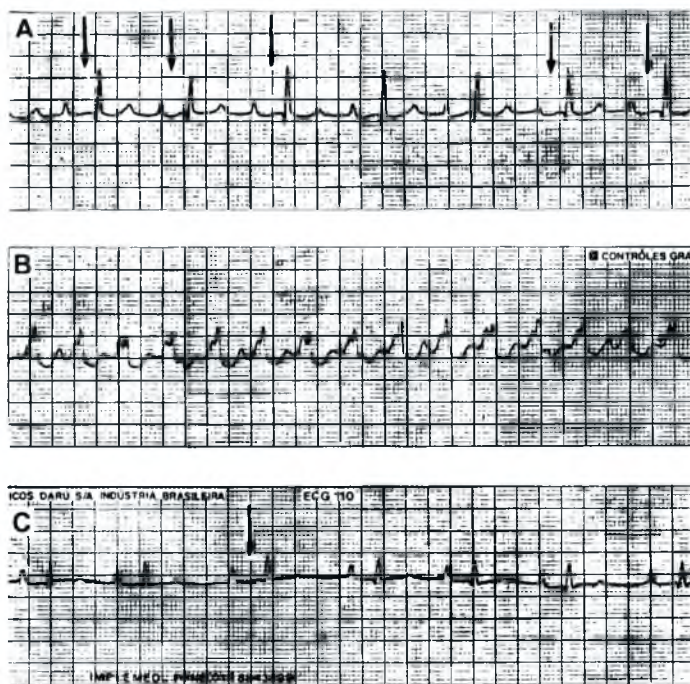


Figure 2

Electrocardiographic changes in D II detected during the 3rd week - 1st degree AVB (A); the 4th week - ventricular tachycardia (B), 1st degree AVB and mv suppression of the R wave (C), Jaboticabal, 1995.

infection, since lysis and release of trypomastigote forms occurred after intense multiplication of amastigote forms in the cytoplasm of host cells. The prepatent period of 13 days after inoculation for most animals in both groups was different from the values reported by other investigators for the same strain, which ranged from 15 to 22 days³ and from 5 to 7 days⁵ after inoculation, demonstrating the wide variation of this period.

The electrocardiographic alterations of the acute phase ranged from none to changes that might reflect the progression and diffusion of the inflammatory process on the right side of the heart, especially along the conduction system, such as complete or incomplete RBB with or without ALHB or PLHB^{3,4,5,16,19,20,26}. These changes have been reported to be usually due to the administration of inocula with high concentrations, since there is a relationship between the number of forms inoculated and the degree of pathogenicity^{13,22}.

The significant 35% increase in heart rate observed in group I was recorded starting from the 4th week, whereas the heart rate of group IT was below the values recorded for group I and close to control values, a fact possibly due to the reducing action of metoprolol¹². During this phase, the rhythms recorded in both infected groups, such as respiratory sinus arrhythmia, sinus rhythm and sinus tachycardia, are those normally and frequently occurring in dogs²⁸. Sinus tachycardia is a common arrhythmia which may be due to the animal restraint process, or which may be

Table 2

Weekly values ($\bar{x} \pm \text{SEM}$) of the QRS complexes (sec and mv) recorded by the ECG (D II) during the acute phase in dogs of the control group (C, n = 10), the group infected with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight (I, n = 15), and the group similarly infected and treated orally with 15 mg/kg metoprolol (Lopressor, Ciba Geigy) twice a day (IT, n = 5). (Jaboticabal, São Paulo, 1995).

GROUP	TIME (weeks)					
	0	1	2	3	4	5
QRS (seg)						
C	0.04 A a ± 0.001	0.04 A a ± 0.001	0.04 A a ± 0.001	0.04 A a ± 0.001	0.003 A a ± 0.001	0.04 A a ± 0
I	0.04 A a ± 0.00099	0.04 A a ± 0.001	0.04 A a ± 0.001	0.04 A a ± 0.002	0.04 A a ± 0.002	0.04 A a ± 0.002
IT	0.04 A a ± 0.002	0.04 A a ± 0	0.04 A a ± 0	0.04 A a ± 0	0.04 A a ± 0	0.04 A a ± 0
QRS (mv)						
C	1.2 A a ± 0.1	1.3 A a ± 0.1	1.4 A a ± 0.1	1.3 A a ± 0.1	1.3 B a ± 0.1	1.6 B a ± 0.01
I	1.5 A a ± 0.09	1.4 A a ± 0.09	1.5 A a ± 0.1	1.3 A a ± 0.1	0.9 A b ± 0.1	0.7 A b ± 0.09
IT	1.3 A a ± 0.2	1.3 A a ± 0.2	1.3 A a ± 0.2	0.9 A a ± 0.1	0.6 A a ± 0.1	0.7 A a ± 0.2

Different capital letters in the columns and different lower case letters on the lines represent statistically significant differences ($p < 0.05$, Tukey test) between groups and weeks, respectively.

Table 3

Weekly values ($x \pm SEM$) of PR (sec) and QT (sec) intervals recorded by the ECG (D II) during the acute phase in dogs of the control group (C, n = 10), the group infected with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight (I, n = 15), and the group similarly infected and treated orally with 15 mg/kg metoprolol (Lopressor, Ciba Geigy) twice a day (IT, n = 5). (Jaboticabal, São Paulo, 1990).

GROUP	TIME (weeks)					
	0	1	2	3	4	5
	PR intervals (sec)					
C	0.08 A a ± 0.004	0.08 A a ± 0.005	0.09 A a ± 0.004	0.08 B a ± 0.003	0.08 A a ± 0.003	0.08 BC a ± 0.005
I	0.08 A a ± 0.003	0.08 A a ± 0.003	0.08 A a ± 0.003	0.1 A b ± 0.007	0.1 A b ± 0.006	0.09 AC a ± 0.005
IT	0.08 A b ± 0.006	0.09 A b ± 0.005	0.1 A b ± 0.006	0.12 A a ± 0.01	0.1 A b ± 0.007	0.11 A a ± 0.005
	QT intervals (sec)					
C	0.17 A a ± 0.004	0.17 A a ± 0.003	0.18 A a ± 0.004	0.17 B a ± 0.004	0.18 A a ± 0.003	0.18 A a ± 0.004
I	0.17 A a ± 0.004	0.17 A a ± 0.003	0.17 A a ± 0.002	0.19 A a ± 0.002	0.17 A a ± 0.05	0.17 A a ± 0.006
IT	0.16 A b ± 0.004	0.19 B a ± 0.003	0.18 A b ± 0.007	0.2 A a ± 0.004	0.19 A a ± 0.004	0.18 A a ± 0.004

Different capital letters in the columns and different lower case letters on the lines represent statistically significant differences ($p < 0.05$, Tukey test) between groups and weeks, respectively.

pathologically associated with congestive heart failure, anemia and febrile states²⁸ with some animals in group I sporadically presenting periods of higher body temperature during the acute phase. The P waves of the two infected groups did not differ significantly from the control group, although these differences have been reported^{6,19}. Frequently described mv suppression occurred during the final weeks of the acute phase in both infected groups^{1,4,6,16,19,20,26}. First and second degree atrioventricular blocks, which are frequent during this phase^{1,4,6,17,18,26}, occurred in animals of both infected groups, although they were more frequent in group I. No significant differences in ST segment, changes of T wave polarity or cardiac axis were recorded between infected and control animals, although changes in these parameters have been reported^{3,4,6,19,24}.

The prevalence of other electrocardiographic changes, mainly recorded in group IT and compatible with the acute phase^{1,2,4,6,16,19,26}, was detected at lower proportions in group IT, indicating the possibility that the beta blocker prevented the appearance of electrocardiographic changes, in agreement with the findings reported by Bestetti⁷ (1988). These facts lead us to the conclusion that further studies are necessary to clarify this question, before a definitive conclusion can be reached.

During the chronic asymptomatic phase, the mean monthly

values of heart rate continued to be elevated in group CI compared to groups CIT and C, as observed during the previous phase, probably due to the administration of metoprolol, which acts by reducing heart rate¹². The cardiac rhythms of both infected groups were within normal limits for dogs²⁸. The increased duration of the QT interval in group CIT continued to present values close to those for group C, whereas it was lower in group CI. Since the QT interval is inversely proportional to heart rate²⁸ and since group CIT maintained values close to normal, this fact may have been related to the action of the beta blocker (metoprolol), which, when used since the day of inoculation, maintained a heart rate compatible with normal values for the species¹².

The significant differences between the infected groups and the control in terms of ST segments, changes in T wave polarity and in cardiac axis were not detected during this phase of the experiment.

CONCLUSIONS

The present results indicate that the changes that occurred during the acute phase such as elevation of heart rate, mv suppression of the QRS complex and increased PR intervals, and other parameters were mainly detected in the infected group, thus

demonstrating the intense lesion promoted by the disease. During the chronic indeterminate phase, a clear tendency to normalization was observed in the infected group treated with metoprolol, especially in terms of heart rate and maintenance of the increase in QT interval, probably due to the action of the beta blocker.

Table 4

Monthly heart rate values ($x \pm SEM$) and individual characterization of cardiac rhythms recorded by the ECG (D II) during the indeterminate chronic phase in dogs of the control group (C, n = 5), the chronic group infected with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight (CI, n = 5), and the group similarly infected and treated orally with 15 mg/kg metoprolol (Lopressor, Ciba Geigy) twice a day (CIT, n = 3). (Jaboticabal, São Paulo, 1995).

GROUP	TIME (months)				
	2	3	4	5	6
	HEART RATE (bpm)				
C	88 B a ± 6	80 B a ± 4	106 B a ± 2	92 AC a ± 9	88 B a ± 11
CI	136 A a ± 4	146 A a ± 10	122 A a ± 2	120 A a ± 13	126 A a ± 9
CIT	80 B a ± 6	76 B a ± 9	90 C a ± 0	73 BC a ± 3	73 B a ± 9
	CARDIAC RHYTHMS				
C	RSA 4 S 1	RSA 5	RSA 2 S 3	RSA 4 S 1	RSA 5
CI	RSA 1 S 4	RSA 2 S 3	RSA 4 S 1	RSA 3 S 2	RSA 3 S 2
CIT	RSA 3	RSA 2 S 1	RSA 2 S 1	RSA 3	RSA 2 S 1

Different capital letters in the columns and different lower case letters on the lines represent statistically significant differences ($p < 0.05$, Tukey test) between groups and months, respectively.
RSA = respiratory sinus arrhythmia; S = sinus; ST = sinus tachycardia.

Table 5

Monthly values ($x \pm SEM$) of PR (sec) and QT (sec) intervals recorded by the ECG (D II) during the indeterminate chronic phase in dogs of the control group (C, n = 5), the chronic group infected with 1,000 *Trypanosoma cruzi* trypomastigotes (Colombian strain) per kg body weight (CI, n = 5), and the group similarly infected and treated orally with 15 mg/kg metoprolol (Lopressor, Ciba Geigy) twice a day (CIT, n = 3). (Jaboticabal, São Paulo, 1995).

GROUP	TIME (months)				
	2	3	4	5	6
	PR interval (sec)				
C	0.10 A a ± 0.003	0.10 A a ± 0.006	0.10 A a ± 0.008	0.10 A a ± 0.008	0.10 A a ± 0.01
CI	0.10 A a ± 0.006	0.10 A a ± 0.004	0.08 A a ± 0.008	0.09 A a ± 0.008	0.09 A a ± 0.006
CIT	0.11 A a ± 0.006	0.10 A a ± 0.01	0.11 A a ± 0.006	0.11 A a ± 0.006	0.11 A a ± 0.01
	QT interval (sec)				
C	0.19 A a ± 0.004	0.21 A a ± 0.004	0.18 A a ± 0.004	0.20 A a ± 0.006	0.20 A a ± 0.007
CI	0.17 A a ± 0.004	0.14 A a ± 0.03	0.18 A a ± 0.006	0.17 A a ± 0.008	0.18 A a ± 0.007
CIT	0.20 A a ± 0.006	0.20 A a ± 0	0.19 A a ± 0.006	0.20 A a ± 0.003	0.21 A a ± 0.005

Different capital letters in the columns and different lower case letters on the lines represent statistically significant differences ($p < 0.05$, Tukey test) between groups and months, respectively.

Table 6

Prevalence of other electrocardiographic alterations observed in dogs infected with *Trypanosoma cruzi* (1,000 trypomastigotes/kg body weight, Colombian strain) (groups I and IT) and in dogs infected and treated (groups CI and CIT) during the acute and chronic indeterminate phases. (Jaboticabal, São Paulo, 1995).

ALTERATIONS	Group I	Group IT	Group CI	Group CIT
	(n = 15)	(n = 5)	(n = 5)	(n = 3)
	Acute phase		Chronic asymptomatic phase	
Increased Q wave	1	10	7	2
mv suppression	8	5	-	-
1 st degree AVB	5	1	-	-
2 nd degree AVB	1	-	-	-
Ventricular conduction disorder (intramural microinfarction)	2	-	4	-
T wave suppression	2	-	-	-
Asystole	1	-	-	-
Ventricular tachycardia	1	-	-	-
Ventricular extrasystole	1	-	-	-
Increased T wave	1	4	-	-

RESUMO

Grupos de cães experimentalmente infectados com *Trypanosoma cruzi* (1,000 tripomastigotas/kg de peso, cepa Colombiana) e infectados e tratados com beta bloqueador (metoprolol) foram avaliados pela eletrocardiografia na fase aguda (semanalmente por 5 semanas) e na fase crônica indeterminada (mensalmente até 6 meses), com o objetivo de estudar a extensão das lesões cardíacas. As alterações na fase aguda foram mais freqüentes no grupo infectado, destacando-se o aumento da freqüência cardíaca, a supressão da mv e o aumento do intervalo PR, e na fase crônica indeterminada houve persistência do aumento da freqüência cardíaca. No grupo infectado e tratado com metoprolol observou-se, durante a fase aguda, a supressão da mv, e na crônica indeterminada, a manutenção da freqüência cardíaca dentro dos limites normais. As outras alterações registradas, como aumento de onda Q, bloqueios átrio-ventriculares de 1º e 2º graus, distúrbios de condução átrio-ventricular, taquicardia ventricular, extrassístole ventricular, assistolia, supressão ou aumento de onda T, ocorreram principalmente no grupo infectado na fase aguda.

UNITERMOS: Eletrocardiografia; Cães; Doença de Chagas.

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