

## Morphometry of Submucous and Myenteric Esophageic Plexus of Dogs Experimentally Reinfected with *Trypanosoma cruzi*

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*We carried out a morphometric study of the esophagus of cross-bred dogs experimentally infected or consecutively reinfected with Trypanosoma cruzi 147 and SC-1 strains, in order to verify denervation and/or neuronal hypertrophy in the intramural plexus. The animals were sacrificed in the chronic stage, 38 months after the initial infection. Neither nests of amastigotes, nor myositis or ganglionitis, were observed in all third inferior portions of esophageal rings analyzed. No nerve cell was identified in the submucous of this organ. There was no significant difference ( $p > 0.05$ ) between the number, maximum diameter, perimeter, or area and volume of the nerve cells of the myenteric plexus of infected and/or reinfected dogs and of the non-infected ones. In view of these results we may conclude that the 147 and SC-1 strains have little neurotropism and do not determine denervation and/or hypertrophy in the intramural esophageal plexuses in the animals studied, independent of the reinfections.*

Key words: Chagas disease - *Trypanosoma cruzi* - dog - reinfection - esophagus - myenteric plexus

Although it is known that dogs can develop the acute stage (Andrade 1984, Lana et al. 1992), the cardiac (Lana et al. 1988, 1992) and indeterminate (Lopes et al. 1980, Machado et al. 2001) forms of the chronic stage of the Chagas disease, showing clinical, morphological and electrocardiographic aspects similar to those observed in human, little is known about the digestive form of the infection in this animal. Few experimental anatomo-pathological studies refer to the esophageal or intestinal dilation. Koeberle (1957) found dogs with megae-sophagus and/or megacolon, however, the animals were naturally infected by *Trypanosoma cruzi* and the author did not describe how long the dogs have been infected. Two systematic studies of esophagus submucous nerve cells and myenteric plexuses in experimental Chagas disease in dogs were made (Caliari et al. 1996, Santos 1998), both in the acute phase of the disease, where no denervation was

found. In this work, we analyzed the morphometry of the esophagus, from dogs experimentally infected and/or reinfected with *T. cruzi*, to evaluate causal denervation and/or hypertrophy in the intramural plexuses of this organ as well as the possible influence of reinfection in this process.

### MATERIALS AND METHODS

Twelve 4 month-old cross-bred dogs of both sexes were used in the study. The animals were maintained in individual kennels in the animal house of the "Posto Avançado de Estudos Emmanuel Dias", Bambuí, State of Minas Gerais, according to the code of ethics of the CobeA (Colégio Brasileiro de Experimentação Animal). The strains of *T. cruzi* used were the 147 and the SC-1. The first one, isolated by haemoculture from a patient with chronic Chagas disease from Bambuí, belonging to zymodeme B, characteristic of strains circulating in the domiciliary environment (Carneiro et al. 1990) and the SC-1, isolated from a naturally infected triatomine *Panstrongylus megistus*, from the State of Santa Catarina, typed as zymodeme 1, which is characteristic of sylvatic strains (Steindel et al. 1993). Four dogs remained uninfected as controls. Eight dogs were inoculated or reinoculated intraperitoneally with  $10^3$  blood trypomastigotes/kg body weight and subdivided into groups as shown in Table I. Five reinfections were made at average intervals of six months.

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The dogs were observed clinically and electrocardiographically and sacrificed according to the code of ethics of the Cobeia at chronic phase, 38 months after the initial infection. During the necropsy, the esophagus was examined in the open and afterwards it was fixed in 4% formaldehyde for at least 48 h. A ring of 5 mm of thickness was taken from the inferior third from all dogs. The rings were processed for paraffin sectioning at 7 µm, and sections stained with haematoxylin-eosin (HE) or Giemsa. Forty serial sections were obtained from each ring and one of every four sections was used for the neuronal count (ten subseriated cuts). Since the diameter of these cells had been found previously to average 28 µm, this sampling of sections helped avoid counting the same neuron twice. In the same sections, we also sought nests of amastigotes and inflammation in the muscular and in the intramural plexuses.

The perimeter of the pericarium of 30 neurons of each esophagus whose cytoplasm presented clear limits and evident nuclei were measured by light microscopy coupled to a high resolution video camera. The image was also passed to a graphic measurement table connected to a semi-automatic image analyzer (MOP-VIDEOPLAN, Kontron Elektronik; Germany). The maximum diameter, area, and volume of the neurons were obtained automatically by this system. The ideal size of the sample of the number of neurons, to be measured in each esophagus, was obtained after calculating the accumulated means for appropriate statistic quantities (Williams 1981).

To guarantee a normal distribution, the Mann-Whitney test was used, where the level of significance considered was of 5% (p<0.05). The Stat View program was used for statistic analysis.

**RESULTS**

All infected dogs manifested evident parasitaemia during the acute phase and maintained a positive serology for all the course of the chronic infection. At the necropsy, visceromegaly were not observed. Microscopically no nest of amastigotes, nor myositis, nor gangliolitis, was found in any animal. Periganglionitis was identified in a single dog, which was not inoculated with *T. cruzi*.

No neuron was identified in the submucosa of the esophagus in the ten en-echelon sections from any dog. In the myenteric plexus, from 99 to 296 neurons were counted in infected and/or reinfected dogs and from 111 to 316 in the non-infected dogs (Table II). Table III presents the median, minimum and maximum values of all parameters studied in neurons – number, maximum diameter, perimeter, area, and volume. There was no statistically significant difference among the groups of infected/reinfected and non-infected (p>0.05).

TABLE I

Groups of non-infected, infected, reinfected dogs, and chronogram of inoculation

Groups	Inoculated strain					
	Chronogram of inoculation of <i>Trypanosoma cruzi</i> (month of inoculum)					
	0	2	8	15	21	28
Reinfected						
A (n = 3)	147	SC-1	147	SC-1	147	SC-1
B (n = 3)	SC-1	147	SC-1	147	SC-1	147
Infected						
C (n = 1)	147					
D (n = 1)	SC-1					
Non-infected						
E (n = 4)						

Inoculum: 10<sup>3</sup> tripomastigotes/kg of body weight/animal; inoculated strains: 147 e SC-1.

TABLE II

Result of the neuronal count of the myenteric plexus of the esophagus in ten subseriated cuts according to the experimental group

Experimental groups (n)	Dog	Number of neurons
Group A – Dogs reinfected (3)	1	99
	2	164
	3	124
Group B – Dogs reinfected (3)	4	111
	5	142
	6	250
Group C – Dogs prime-infected (1)	7	260
Group D – Dogs prime-infected (1)	8	296
Group E – Dogs non-infected (4)	9	166
	10	316
	11	111
	12	235

**DISCUSSION**

In this study it was not possible to induce the digestive form of the Chagas disease in the dogs, though they have been accompanied for 38 months after the first inoculation. Koeberle (1957) found mega due to chagasic infection in dogs, however, the author did not mention the duration of the infection and neither if was only happened dilation or if there was really a hypertrophy. Using dogs experimentally infected with *T. cruzi*, Okumura and Côrrea Netto (1961), clearly refered that they have only found thinning of the esophagus wall, which

TABLE III

Median, minimum and maximum values of parameters studied in neurons of the myenteric plexus of infected/reinfected and non-infected dogs

Parameters	Infected/Reinfected (n = 8)	Non-infected (n = 4)	Mann-Whitney test
Number of neurons	153 (99-296)	200.5 (138.5-275.5)	p = 0,55
Maximum diameter ( $\mu\text{m}$ )	25.8 (20.1-32.9)	26.2 (21.1-29.8)	p = 0,79
Perimeter ( $\mu\text{m}$ )	71.4 (49.4-91.3)	77.0 (55.6-84.2)	p = 0,50
Area ( $\mu\text{m}^2$ )	256.8 (118.3-462.6)	351.6 (154.9-395)	p = 0,31
Volume ( $\mu\text{m}^3$ )	3246 (1161-7786)	5269 (1489-6244)	p = 0,31

is not an indicative of mega. However, the authors named this thinning as a dilatation, which does not agree to the real concept of mega. Besides, the authors used the Y strain and a larger inoculum than that we used.

The dogs infected with *T. cruzi* presented alterations compatible with those described as the indeterminate form of Chagas disease (Machado et al. 2001). The results of the present work indicate that under systematic anatomic-pathological analysis of the esophagus, morphological alterations were not detected. The single dog with periganglionitis in the myenteric plexus of the esophagus was not infected. However, in case it is possible to find a parallel with the works with human beings, the present finding does not seem so remarkable considering that the alteration is relatively frequent in healthy, non-chagasic, humans deceased in a violent way (Adad et al. 1991).

No neuron was found in the submucosal plexus and the number of ganglionic cells in the myenteric plexus of infected and/or reinfected dogs was not different statistically from the non infected ones. These data are in accordance with Caliari et al. (1996) that, analyzing a greater number of esophageal sections of dogs sacrificed in the acute phase, also did not find denervation and only identified rare neurons in the submucosal plexus, suggesting that this plexus has a small number of neurons. In the present study, no denervation was observed maybe because the strains SC-1 and 147 have little destructive action on the myenteric plexus.

The denervation absence observed in the esophagus of the dogs, agree with results of Caliari et al. (1996), Santos (1998) and Santos et al. (1998), that have studied the acute phase of Chagas disease in dogs. However, our findings of neurons size between chagasic and non-chagasic dogs differ from Santos (1998). The average of the neuronal diameter, observed in the inferior third of the esophagus, in our animals (26.2  $\mu\text{m}$  in the non-infected dogs and 25.9  $\mu\text{m}$  in the infected and/or

reinfected) it was larger than the average obtained by Santos (1998) (19.6  $\mu\text{m}$  in the non-infected dogs and 20.7  $\mu\text{m}$  in the infected). It was possible that this difference have occurred because we have measured only the neurons with evident nuclei and nucleolus, given a more real result in maximum diameter measure. Santos (1998) measured all neurons, even that ones that the esophagic tissue was not cut in the pericarium center, resulting in lower values. We also did not find differences in neurons measurements between chagasic and non-chagasic dogs, which agree with Santos (1998) results. On the other hand, our findings agree with the observed in humans, where only in patients with megacolon was observed neuronal hypertrophy, indicating that this hypertrophy seems to be related to intense denervation and/or muscular hypertrophy (Adad et al. 2001).

In conclusion, there was no evidence, with the strains SC-1 and 147, of denervation and/or neuronal hypertrophy in the myenteric plexus of the esophagus of chagasic dogs independent of the animals being reinfected or not although they have presented the characteristic acute phase of Chagas disease.

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