

## CARDIAC PLEXUS OF DOGS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*: INFLAMMATORY LESIONS AND QUANTITATIVE STUDIES

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*Qualitative and quantitative aspects of the superficial and profound cardiac plexus of dogs experimentally infected with Be-62 and Be-78 strains of Trypanosoma cruzi were studied. Animals were autopsied in the acute phase of infection. The inflammatory process, lesions and number of parasites were more intense and frequent in animals infected with the Be-78 strain than in those infected with Be-62. Despite this, no statistically significant differences could be found between the number of neuron bodies in the ganglia of infected and control dogs.*

*Key-words: Cardiac plexus. Trypanosoma cruzi. Dog. Acute chagasic cardiopathy.*

The pathogenesis of chagasic cardiopathy has been studied by several authors<sup>12-11</sup>. Many factors seem to be involved: inflammation, autoimmunity, fibrosis and denervation<sup>18-19</sup>. For example, denervation has an important role in the pathology of the disease but is not always the main factor<sup>13-14</sup>. Based on personal experience, mainly with dogs, although it has been found that chagasic cardiopathy is not counterbalanced in the acute and chronic phases, intense systematic lesions are not observed in the intracardial nervous system, mainly in its numerical reduction<sup>11</sup>.

The aims of this paper are: a) to study the inflammatory phenomena, lesions and parasitism of the superficial and profound cardiac plexus of dogs experimentally infected with Be-62 and Be-78 strains of *T. cruzi*; b) to carry out a quantitative study of ganglia and neuron bodies of these plexus in infected and control dogs.

### MATERIAL AND METHODS

Twelve outbred dogs, 65-80 days old, born and

maintained in the laboratory, were used. Before inoculation, each animal was examined to exclude the possibility of prior *T. cruzi* infection. Two groups, each of four dogs, were inoculated intraperitoneally with 2000 blood trypomastigotes/kg body weight, respectively, with Be-62<sup>17</sup> and Be-78<sup>10</sup> *T. cruzi* strains. Both strains were isolated, on different occasions, from Berenice, accepted to be the first known human patient of Chagas' disease<sup>6</sup>. Inocula were obtained from albino mice in the acute phase of infection and counted according to Brener (1962). Four normal dogs were used as controls.

Dogs were maintained on an *ad libitum* diet and observed daily. Parasitemia was assessed according to Brener<sup>4</sup>. Dogs were sacrificed about the 37<sup>th</sup> day of infection and necropsied. The atria were removed and fixed *in totum* in buffered formalin at pH 7.2, dehydrated, cleared and infiltrated with paraffin with the vessels in a basal position. Semi-serial sections (1:10), 4 $\mu$ m thick, were stained with HE and Gomori's thricromic.

Ganglia were analysed in each section to determine the following aspects: inflammatory reactions, lesions and parasitism. Inflammation was classified as discreet (+), moderate (++) or intense (+++) according to the degree of cellular infiltration. Each ganglion and its neurons (normal and degenerated) were counted systematically. The number of neuron bodies per ganglia of infected dogs were compared with control dogs by Student "t" test.

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## RESULTS

Dogs infected with Be-78 strain showed intense acute myocarditis characterized by a focal and diffuse exudation of mononuclear cells, with an endomysial distribution and parasitism of the myocardium (Figure 1). In contrast, dogs infected with Be-62 showed only discreet foci of inflammation and amastigote nests.

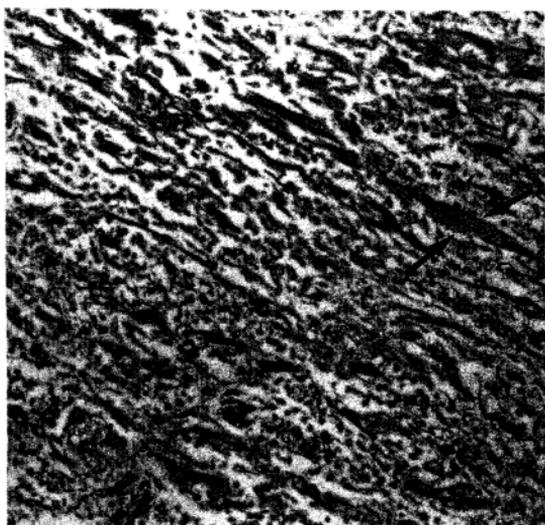


Figure 1 - Myocardium of a dog inoculated with Be-78 strain and sacrificed in the acute phase of the infection. Note the intense exudate of diffuse mononuclear cells and a nest of amastigotes (arrows). HE. 160x.

Lesions of ganglia and nerves (ganglionitis, periganglionitis, neuritis and perineuritis) with degenerative phenomena of neurons (tigrolisis, picnosis, cariólisis, tumefaction, vacuolisation and retraction) were more intense and more frequent in dogs infected with Be-78 (Figure 2; Figure 3; Table 1) than with Be-62 (Table 2). Apparently normal ganglia were often seen when inflamed and injured ganglia were recorded.

Parasites were seen only in Schwann cells and in fibroblasts of the capsule of dogs infected with Be-78 (Table 1).

The total number of ganglia and neuron bodies in each animal are shown in Tables 1, 2 and 3. The averages of neuron bodies per ganglion were

$8.92 \pm 1.02$ ,  $9.72 \pm 0.79$  and  $9.75 \pm 0.38$  respectively in dogs infected with Be-78 strain, Be-62 and controls. The Student "t" test did not show statistically significant differences between groups of dogs infected with each strain and controls (Be-78 x controls,  $t=-1.56$ ;  $p>0.05$ . Be-62 x controls,  $t=-0.069$ ;  $p>0.05$ ).



Figure 2 - Atrium of a dog inoculated with Be-78 strain and sacrificed in the acute phase of the infection. Note the sub-epicardic ganglion with pronounced periganglionitis, perineuritis and ganglionitis. HE. 160x.

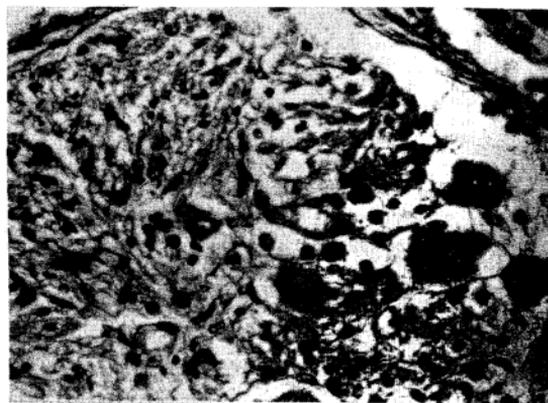


Figure 3 - Atrium of a dog inoculated with Be-78 strain and sacrificed in the acute phase of the infection. Note focal ganglionitis, with an accentuated exudate of mononuclear cells, together with pronounced regressive phenomena of several neurons. HE. 400x.

Table 1 - Results of quantitative and qualitative evaluation of cardiac ganglia in dogs experimentally infected with Be-78 strain of *Trypanosoma cruzi*.

Dog	Total number of neurons	Total number of ganglia	Inflamed ganglia			Parasited ganglia
			+	++	+++	
1	8516	951	237	25	2	78
2	6823	907	112	5	0	1
3	9966	1042	321	46	14	4
4	7276	735	129	20	1	0

+ discreet; ++ moderate; +++ intense.

Table 2 - Results of quantitative and qualitative evaluation of cardiac ganglia in dogs experimentally infected with Be-62 strain of *Trypanosoma cruzi*.

Dog	Total number of neurons	Total number of ganglia	Inflamed ganglia			Parasited ganglia
			+	++	+++	
1	6642	624	5	2	0	0
2	8437	843	6	0	0	0
3	8948	923	11	3	0	0
4	7135	815	3	5	0	0

+ discreet; ++ moderate; +++ intense.

Table 3 - Results of quantitative evaluation of cardiac ganglia in control dogs.

Dog	Total number of neurons	Total number of ganglia
1	8466	910
2	6466	670
3	7564	761
4	8927	873

## DISCUSSION

Köberle (1956) found that Autonomous Nervous System (ANS) injuries develop at an early stage of the acute phase of Chagas' disease<sup>9</sup>. His subsequent studies showed that the course of the disease and type of lesion that emerged in the chronic phase, were predetermined in the acute phase. In fact, alterations in the ANS are so constant in Chagas' disease that Köberle called it a parasympathicoprive disease, because parasympathetic denervation be could responsible for the occurrence of different

anatomoclinic forms of the disease: digestive and cardiac.

Although denervation could be one of the factors responsible, it has been demonstrated that other factors of equal, or greater importance<sup>8 21</sup>, could explain the pathogenesis and physiopathology of the disease. Lopes (1965)<sup>13</sup> and Lopes et al (1983)<sup>14</sup> agree with Köberle as much as the presence of neuron lesions, but they observed that different degrees of denervation may occur in Chagas' disease or may be absent in symptomatic patients who eventually die from the disease.

Rocha et al (1993)<sup>16</sup> showed that the ANS may suffer slight injury when patients are developing chronic chagasic cardiopathy. Also, Almeida-Ribeiro et al, in a systematic study of the atrium of a human acute case, did not encounter significant lesions of intracardial nervous system and admitted that they were due to the propagation of nearby epicarditis<sup>2</sup>. These findings refute the hypothesis that ANS lesions are caused by functional disturbances. In contrast, Davila et al (1993)<sup>7</sup> suggested that parasympathetic abnormalities of

cardiopathies are the result of progressive ventricular dilatation. Thus, even when there are no ANS lesions, functional alterations of parasympathetic ANS may occur. Our preliminary results with dogs chronically infected with Be-78 strain show acute electrocardiogram changes. Two dogs have been sacrificed and practically no lesions were seen in intracardiac nervous system, even though the entire atrium was examined.

Regional differences between *T. cruzi* populations of different zymodemes and schizodemes are also related to different anatomoclinic forms of the disease, including the intensity of neurotropism<sup>5</sup>. Many authors have studied neurotropic strains of *T. cruzi* in different experimental models and the results obtained are very questionable<sup>1 3 15 20</sup>.

So far, no systematic study of serial sections of the entire atrium was undertaken. Indeed, 2,400 sections were stained and, subjectively, a clear impression was gained that Be-78 strain is more neurotropic than Be-62 strain. The lesions of the intracardiac nervous system are mainly based on two mechanisms: directly related to the presence of parasites either in Schwann's cells or in neurons resulting sometimes in ganglionitis or then by periganglionitis with secondary ganglion infiltration. Because there might be more inflammation and more parasites in infections with Be-78 strain, obviously more serious lesions occurred with this strain. Although the denervation has not been evident, some dogs infected with Be-78, showed intense and diffuse myocarditis with the development of the symptomatic fibrosing chronic chagasic cardiopathy, similar to human disease. Some of these dogs died in consequence of the disease at different periods of the acute or chronic phase<sup>12</sup>.

Since all diseases are multifactorial, the same may occur with Chagas' disease. All of the foregoing factors discussed, and others, need to be analysed to obtain a better understanding of the pathogenesis and physiopathology of the development of the disease.

## RESUMO

Foi realizado estudo qualitativo e quantitativo dos plexos cardíacos superficiais e profundos em cães inoculados com o *Trypanosoma cruzi* das cepas Be-62 e Be-78 e sacrificados na fase aguda. O processo

inflamatório, as lesões e o parasitismo dos plexos foram mais intensos e frequentes nos animais inoculados com a cepa Be-78 do que naqueles inoculados com a cepa Be-62. Apesar deste fato, não foi verificada diferença estatisticamente significativa entre o número de corpos de neurônio por gânglio dos animais chagásicos e os controles.

*Palavras-chaves:* Plexo cardíaco. *Trypanosoma cruzi*. Cão. *Cardiopatía chagásica aguda*.

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