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Histopathological criteria for the diagnosis of abdominal angiostrongyliasis

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Abstract. An increasing number of cases of abdominal angiostrongyliasis have recently been diagnosed in southern Brazil. A comparative study of 19 confirmed cases and 15 suspected cases was undertaken to review the anatomo-pathology of the disease and to establish histopathological criteria for its diagnosis. The results were similar in both groups, except for the identification of the worm in the confirmed cases. Macroscopic features comprised two types: a predominant thickening of the intestinal wall (pseudo-neoplasic pattern) and a congestive necrotic lesion (ischemic-congestive pattern). Microscopically, three fundamental histopathological findings were detected: (1) a massive infiltration of eosinophils in all layers of the intestinal wall, (2) a granulomatous reaction and (3) eosinophilic vasculitis affecting arteries, veins, lymphatics and capillaries. We conclude that observation of this histopathological triplet excludes other causes of "eosinophilic gastroenteritis" and establishes the diagnosis of probable abdominal angiostrongyliasis.

For several years, a well-defined clinical and histopathological entity had been detected in Costa Rica, characterized by granulomatous and eosinophilic inflammatory lesions in the ileo-cecal region caused by an intra-arterial metastrongylid nematode (Cespedes et al. 1967; Morera 1967). The recovery of a few worms from surgical specimens led to the description of *Angiostrongylus costaricensis* (Morera and Cespedes 1971). The disease was named abdominal angiostrongyliasis to distinguish it from the cerebral angiostrongyliasis caused by *A. cantonensis*, another metastrongylid occurring especially in Oceania and Asia (Koo et al. 1980). Identification of the definitive (rodents) and intermediate hosts (slugs) and the establishment of the parasite's life cycle in the laboratory led to a detailed characterization of this new species. The infective larvae develop within the fibromuscular tissue in the mollusc, and ingestion by the vertebrate is required for the completion of the cycle (Morera 1973).

In Asia, a potential etiological agent of abdominal angiostrongyliasis is *A. siamensis*. Like *A. costaricensis*, it develops into an adult worm inside the mesenteric arterial system in vertebrates (Kudo et al. 1983). At present, both parasitological and epidemiological data support the identification of *A. costaricensis* as being the only known cause of human abdominal disease in the Americas (Morera 1986; Morera et al. 1983; Kaminsky et al. 1987). In southern Brazil, *A. costaricensis* was identified from slugs collected near the homes of two patients whose pathological findings were included in the present study (Graeff-Teixeira et al. 1989).

The disease is known to occur from Mexico to Argentina (Morera 1986), and a suspected case has recently been reported in Zaire (Baird et al. 1987). In Brazil, cases have been reported in the Federal District of Brasilia (Barbosa et al. 1980) and in the southern States of Sao Paulo, Parana, Santa Catarina and Rio Grande do Sul (Ziliotto et al. 1975; Agostini et al. 1984; Ayala 1987).

To date, a serological test (latex agglutination) is available only in Costa Rica, and neither eggs nor larvae have been detected in stools, since their elimination is prevented by the inflammatory reaction in the intestinal wall. Therefore, the diagnosis in many areas relies on the histopathological examination of biopsies or surgical specimens, and pathologists may not be able to identify parasite structures in hypertrophic-pseudoneoplasic lesions, which are probably the most common type associated with abdominal angiostrongyliasis. Indeed, a significant number of the cases of so-called eosinophilic ileocolitis with granulomatous reaction are known to occur in endemic areas (Cespedes et al. 1967; Zambrano 1973). To review the anatomo-pathological presentation of the disease and to define histopathological criteria for its diagnosis, a detailed comparative study was undertaken in 34 cases.

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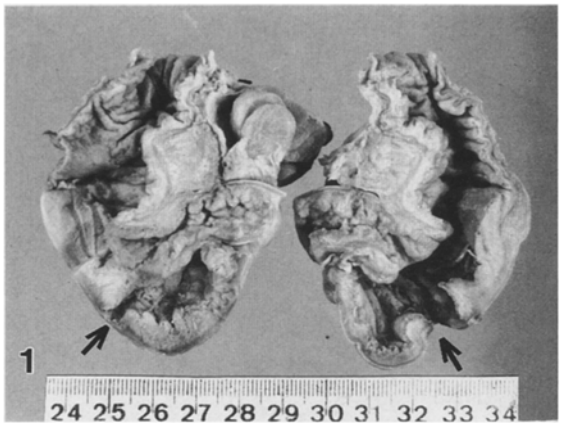
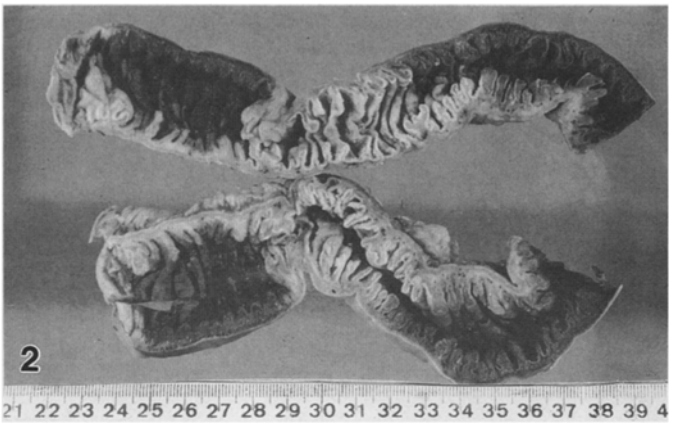
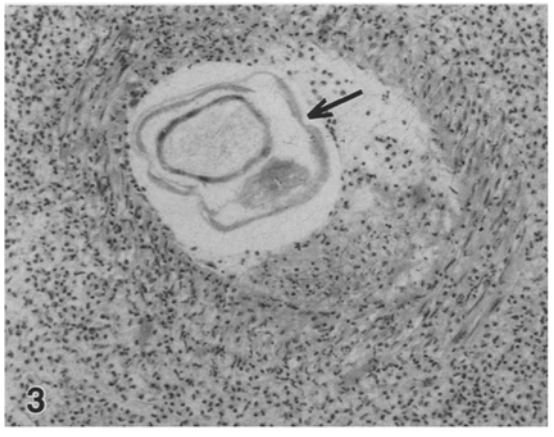
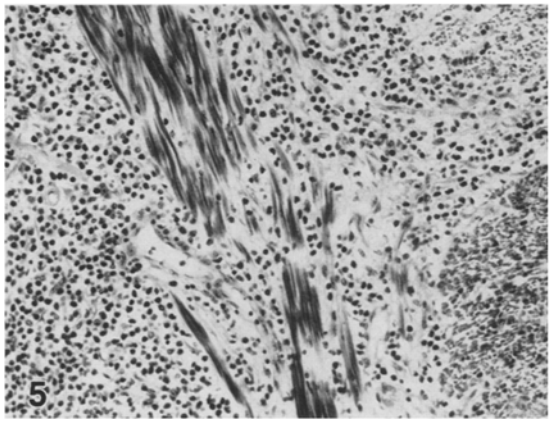
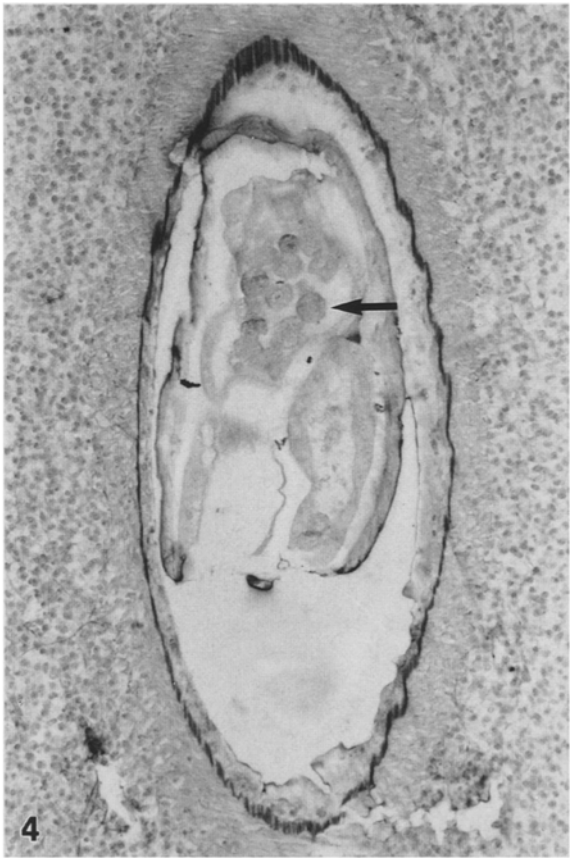




Figs. 1, 2. Macroscopic patterns of abdominal angiostrongyliasis. **Fig. 1.** Pseudo-neoplasic lesion in the ileo-cecal region, showing predominant thickening of the intestinal wall and lymph node enlargement; note the perforation in the cecum (*arrow*). **Fig. 2.** Ischemic-congestive pattern exhibiting segmented necrotic areas and widespread congestion in the small intestine





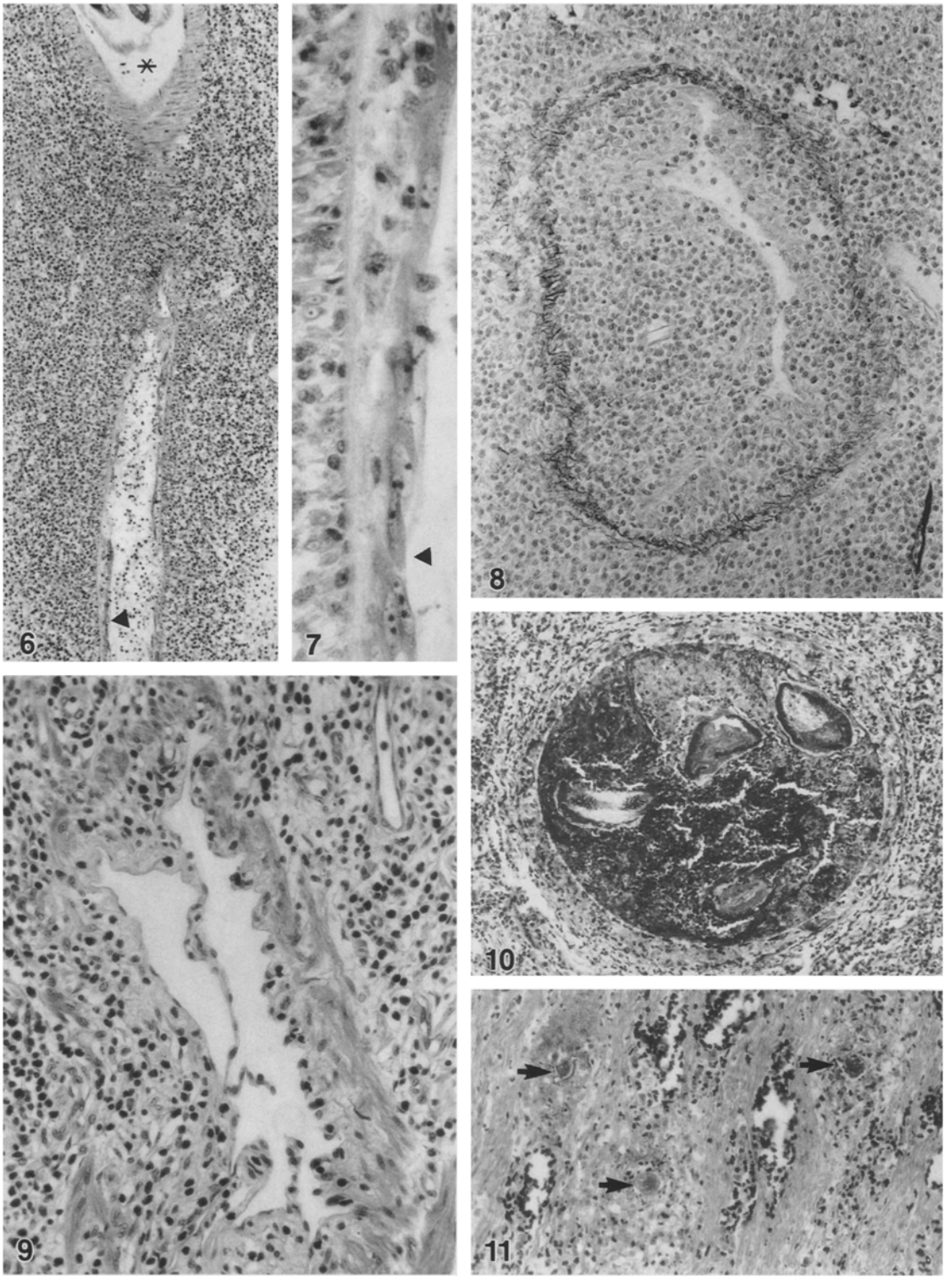


Figs. 3–5. Microscopic findings: the worm and the inflammatory reaction. **Fig. 3.** The sub-mucosa is heavily infiltrated by eosinophils; note the section of an intra-arterial nematode (*arrow*). H & E, ×125. **Fig. 4.** Demarcation of the internal elastic lamina of a parasitized artery; the reproductive tract of the worm contains many oocytes (*arrow*). Weigert's resorcin-fucsin, ×160. **Fig. 5.** Eosinophilic infiltration "bundles" in the muscular layer. Masson's trichrome, ×200

Materials and methods

In Rio Grande do Sul, Brazil's southernmost State, the records from 22 of 25 pathology laboratories were reviewed for the period of 1975–1984. The screening criterion was the citation of eosinophilia or related key words (eosinophil, eosinophilic) in the conclusions of pathological reports based on biopsies or surgical specimens from the gastro-intestinal tract. Of 654 cases, 27 exhibited intense eosinophilia and were selected after the examination of new sections that had been stained with hematoxylin and eosin. A mail survey was undertaken to cover other States of Brazil, asking for confirmed or suspected cases. Material obtained from seven patients was sent from Sao Paulo, Parana and Santa Catarina. Ten cases had previously been reported (Iabuki and Montenegro 1979; Agostini et al. 1984).

The cases were classified into two groups according to the following criteria, which were taken from data in the literature (Cespedes et al. 1967; Agostini et al. 1984; Ayala 1987): group 1 (19



Figs. 6–11. Histopathology of abdominal angiostrongyliasis: vasculitis. **Figs. 6, 7.** Ramifications (▲) of the parasitized artery (*asterisk*) shown in Fig. 4, showing intense vasculitic changes; note the endothelial proliferation and eosinophilic infiltration throughout the wall. Giemsa staining. Fig. 6, × 80; Fig. 7, × 500. **Fig. 8.** Venulitis with dense inflammatory infiltration in the intima. Weigert's resorcin-fucsin, × 125. **Fig. 9.** Sub-endothelial eosinophils in a subserosal lymphatic vessel. Masson's trichrome, × 125. **Fig. 10.** Arterial thrombosis and worm residues. Masson's trichrome, × 125. **Fig. 11.** Degenerate eggs (*arrows*) inside small vessels. Masson's trichrome, × 200

confirmed diagnoses), identification of an intra-arterial nematode; group 2 (15 suspected diagnoses) occurrence of intense eosinophilic inflammatory reaction, granuloma with eosinophilia and eosinophilic infiltration into the wall of blood or lymphatic vessels. The description of macroscopic features was based on the original pathologist's report and on six available surgical specimens that had been preserved in formalin. Serial sections were obtained and stained for light microscopy using the following methods: hematoxylin and eosin, Giemsa, Gomori's periodic acid-methenamine silver, Masson's trichrome, Weigert's resorcin-fuchsin, Sirius red, and PAS-alcian blue.

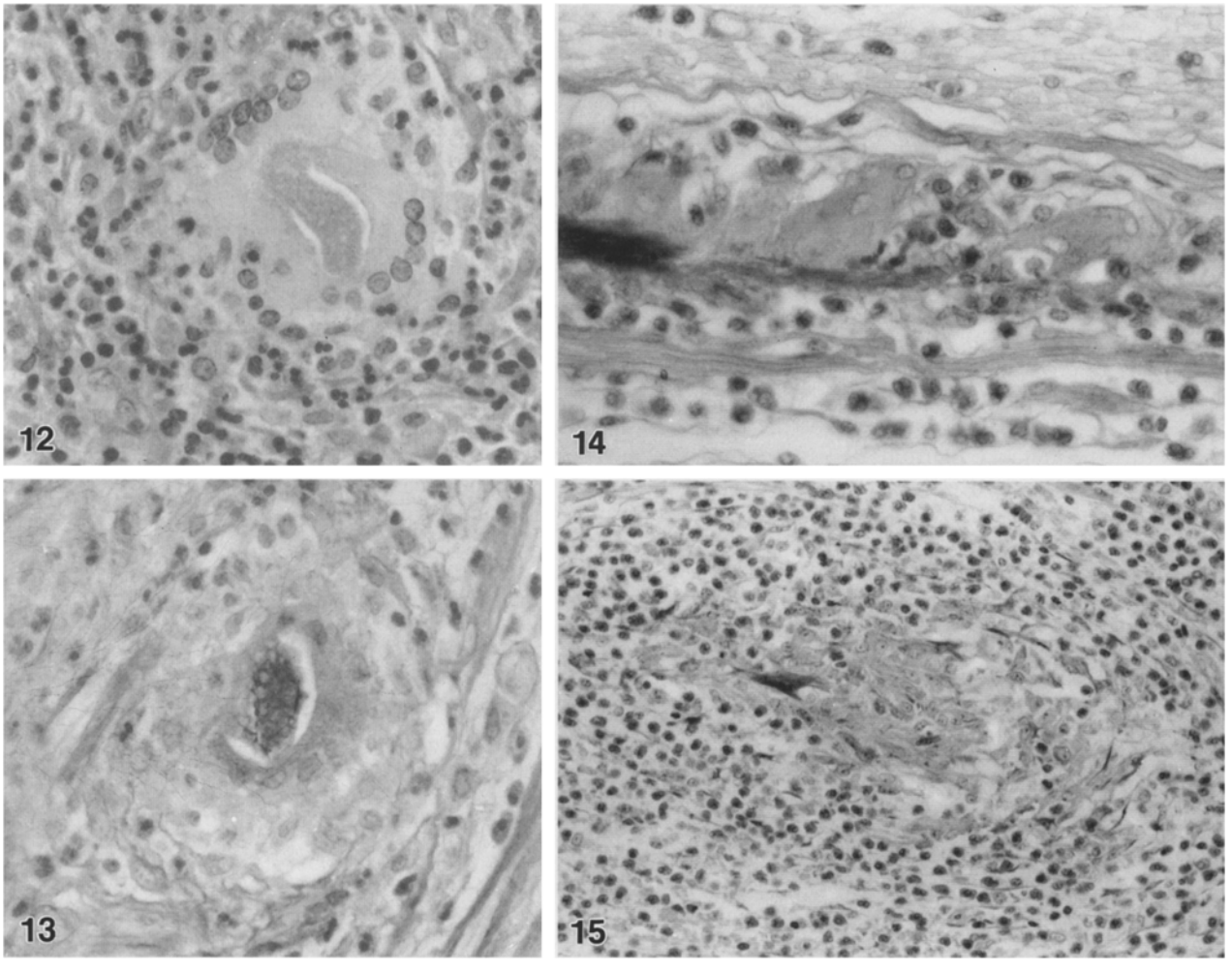
Results

The anatomo-pathological findings in both groups were similar, except for the positive identification of the worm in group 1. For this reason, the results are generally presented without mention of the group, unless necessary.

The small intestine was affected more frequently than the appendix or the cecum. In some cases the intestinal wall was enormously thickened (by up to 1.2 cm) and constricted the lumen. Together with the enlargement of the lymph nodes, this aspect had the appearance of a pseudo-neoplasic lesion (Fig. 1). Other specimens showed predominantly necrotic and congested areas involving all the layers (Fig. 2). In both patterns we detected focal flattening of the mucosa, ulcerations and perforations of the intestinal wall. The serosal layer could be thickened or covered with fibrin deposits.

Massive eosinophilic infiltration could be found throughout all layers of the intestinal wall (Fig. 3). Its intensity was higher in the muscular layer, in which fiber dissociation occurred and eosinophils could be found in close apposition to the fibers, denoting severe myositis (Fig. 5). The route of vessels through the muscular layer was enlarged by the cellular infiltrate associated with a discrete deposition of collagen fibers arranged in an inflammatory "bundle". This perivascular distribution of inflammatory cells was also noted in other layers, showing a nodular pattern in areas displaying a higher cellular density.

In every case, moderate plasmocytosis was observed in the corion of the mucosa, together with an increased number of mast cells. These cells and lymphocytes could



Figs. 12–15. Histopathology of abdominal angiostrongyliasis: the granulomatous reaction. **Figs. 12, 13.** An egg surrounded by giant cells and an egg in the center of a granuloma, respectively. H & E, ×500. **Fig. 14.** Giant cells along the trajectory of the vessel through the muscular layer. Giemsa, ×500. **Fig. 15.** Granuloma with monocytes/macrophages, eosinophils and a giant cell. Giemsa, ×256

be found in focal arrangements around some vessels in any layer. The intra-epithelial population of eosinophils and mast cells was apparently increased in segments in which the inflammatory reaction was more severe.

Worms detected inside the arteries exhibited a cuticle, a muscular layer, a general cavity without an epithelial lining, a digestive tube and the reproductive organ (Fig. 3). In some sections a uterus with eggs could be identified (Fig. 4). Eggs were occasionally seen to be trapped in small vessels (Fig. 11), engulfed or surrounded by giant cells (Fig. 12) or lying in the center of small granulomas (Fig. 13).

Vascular changes were detected in arteries (Fig. 6), veins (Fig. 8) and lymphatics (Fig. 9), and their intensity varied from isolated small, PAS-positive deposits on the endothelial surface to a severe vasculitic picture comprising an intense eosinophilic infiltration throughout the wall, focal necrotic areas and fibrinoid deposits. Sometimes the endothelium was either vacuolated or hyperplasic and hypertrophic and arranged in multilayers (Fig. 7). Thrombotic phenomena were more common in arteries, sometimes being associated with degenerate parasites (Fig. 10).

Isolated giant cells of the foreign-body type were very frequently observed and, like the granulomas, they were distributed around vessels (Fig. 14), especially in the muscular layers. Macrophages and eosinophils were the predominant cellular components of granulomas (Fig. 15). Epitheliod cell transformation and necrotic central material were unusual.

In the lymph nodes, we observed a constant folicular hyperplasia, sinusoidal histiocytosis, eosinophilic infiltration, isolated giant cells and, in one instance, a degenerate worm inside an artery showing severe vasculitis. In the intestinal wall, fibrotic areas were always present in various degrees due to the deposition of collagen type I. Eventually the fibers were anomalous, showing a homogeneous aspect, thickened and rich in sulphated proteoglycans as detected by PAS-alcian blue (pH 1). In areas containing the highest amount of inflammatory infiltrate, there was a significant increase in reticular fibers (Gomori's reticulin method) that was not associated with collagen type I deposition (Sirius red, polarized light). Ulcerations of the mucosa, reduction in the goblet-cell population, discrete fibrosis in the villi and thickened muscularis mucosae were less important findings.

Discussion

The predominance of lesions in the ileo-cecal region has been well documented in the literature (Cespedes et al. 1967; Loria-Cortes and Lobo-Sanahuja 1980). The most frequently affected site in the present study was the small intestine, recognized as the jejunum or ileum by the histological characteristics, when a surgical specimen was not available. In the most severe cases, probably those with the highest parasitic burden, very extensive intestinal damage occurred, ranging from the more proximal jejunum to the descending colon.

Two non-exclusive patterns of macroscopic lesions, both showing segmental distribution, were observed: a hypertrophic-pseudoneoplasic pattern (HP) involving predominant thickening of the intestinal wall (Fig. 1) and an ischemic-congestive pattern (IC) involving necrotic and/or congested areas (Fig. 2). The segmental aspect of the lesions has been stressed in previous studies (Agostini et al. 1984).

The impressive intensity of the eosinophilic infiltration and its extension and distribution throughout all layers are unique to abdominal angiostrongyliasis and enable a clear-cut differential diagnosis from lesions produced by other migrating parasites in the intestinal wall (Van Thiel et al. 1960; Cespedes et al. 1967; Marcial-Rojas 1977). The microscopic examination demonstrated that the enlargement in the intestinal wall was due to cellular infiltration rather than to edema or fibrosis. In acute intestinal anisakiasis, an edematous thickening of the bowel occurs and eosinophils are accompanied by neutrophils and histiocytes (Smith and Wootten 1978). In eosinophilic gastroenteritis, proximal segments are usually affected, the lesions are of a spotty nature and the eosinophilic infiltrate predominates in one intestinal layer (Klein et al. 1970).

The increased meshwork of reticular fibers detected by Gomori's reticulin method indicates an extracellular matrix rich in collagen type III, proteoglycans and fibronectin (Wolman and Kasten 1986), an environment that favors the migration of inflammatory cells.

Many reports describe vascular alterations such as proliferation of the endothelium, vacuolization of muscular cells, edema and necrosis (Zambrano 1973; Iabuki and Montenegro 1979; Ayala 1987), and Agostini et al. (1984) have proposed the observed eosinophilic arteritis and intravascular granuloma as being peculiar to the disease. In the present study we detected widespread vasculitic changes: capillaries, veins, lymphatics and arteries were affected. Serial sections of parasitized arteries demonstrated that the arterial wall close to the worm was usually not as heavily affected by the vasculitic process as were the distal segments (Fig. 6). The downstream drainage of antigens would lead to the observed distribution of vascular lesions and to eosinophilic infiltration into the intestinal wall.

The HP pattern may represent a milder degree of worm burden and/or a more adaptative behavior of *Angiostrongylus costaricensis* whereby the parasites live within arteries in the mesentery and avoid the self-destructive migration to the distal arterial segments. Indeed, the observation of intra-arterial parasites in the thickened intestinal wall of the HP pattern is unusual. Material from one patient in group 2 showed a complete spontaneous remission of a tumoral ileo-cecal mass. These chronic and possibly reversible HP lesions may predominate in the majority of uncomplicated cases of the disease that are clinically recognized in the endemic area.

It is possible that whith the occurrence of a large number of worms and/or the stimulation of their migration, acute and necrotic lesions are produced. This is exactly the case for the IC pattern, whereby congestion, thrombosis and necrosis predominate, the cellular infil-

tration is scarce and many degenerate worms can be found within the thrombi (Fig. 10).

The observation of highly activated eosinophils and endothelial cells, their close interactions and the absence of thrombi around live worms bring into consideration substances with anticoagulant activity. The complex interaction of these factors, derived either from the parasite or from the host, could eventually favor the survival of such a large intra-arterial nematode. However, the discussion of this issue is beyond the scope of the present paper.

The granulomatous reaction in abdominal angiostrongyliasis has very peculiar characteristics: the perivascular distribution (especially in the muscular layer, which appears to be a retention site for eggs), the absence of stratification or fibrosis and the intra-vascular location cited by Agostini et al. (1984). Isolated foreign-body giant cells were found more frequently than fully developed granulomas and can be interpreted as a residual reaction, similar to the involution of granulomas previously reported in paracoccidiodomycosis (Kerr et al. 1988).

The validity of the proposed criteria is supported not only by the similarity of the histopathological picture in the suspected and confirmed cases, but also by their similar epidemiological and clinical data (unpublished). Moreover, *A. costaricensis* has been recovered from the slug *Phyllocaulis variegatus* and from two wild rodents of the genus *Oryzomys* (*O. nigripes* and *O. ratticeps*) that were captured near the houses of patients whose disease had been diagnosed according to the proposed criteria (Graeff-Teixeira et al. 1989, 1990).

The definitive diagnosis relies on the identification of the parasite in histological sections and the demonstration of a specific and acute antibody response. The present study provides evidence that the observation of three fundamental histopathological findings in ileo-cecal lesions – eosinophilia, vascular alterations and granulomatous reactions – support the diagnosis of probable abdominal angiostrongyliasis.

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