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Biochemical serum profiles in dogs experimentally infected with *Angiostrongylus vasorum* (Baillet, 1866)

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Abstract

The biochemical profiles of crossbred dogs experimentally infected with the parasite *Angiostrongylus vasorum* were studied. Two groups of five dogs were experimentally inoculated with 50 and 100 third stage infective larvae (L_3) of *A. vasorum* per kilogram of body weight. A third group of five uninfected animals were used as control. Serum from these animals were used for biochemical tests to measure total and fractioned proteins, urea, creatinine and to determine the activities of aspartate (AST), alanine (ALT) aminotransferase, gamma-glutamyl transferase (GGT), alkaline phosphatase (PAL) and creatine kinase isoenzyme MB (CK-MB). The α-1, α-2 and β-globulins fractions showed alterations during acute phase of the infection. No modifications were observed in the biochemical profiles of ALT, AST, GGT, PAL, urea and creatinine. CK-MB was shown to be a good early indicator of cardiac injury in dogs experimentally infected with *A. vasorum*.

Keywords: *Angiostrongylus vasorum*; Dogs; Biochemical profile; Creatine kinase (CK-MB)

1. Introduction

Angiostrongylus vasorum (Baillet, 1866; Kamensky, 1905) is a helminth from the Protostrongylidae family whose adult worms are found in the right ventricle, pulmonary artery and its branches of domestic dogs and wild carnivores. It is a cosmopolitan helminth which is endemic in France, England and Denmark (Guilhon and Cens, 1973; Jones et al., 1980; Bolt et al., 1993) with reports of natural infections in Uganda, the United States, Canada and Brazil (Cury and Lima, 1995).

Aquatic or terrestrial snails are involved as intermediate hosts in the life cycle. Dogs become infected by

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ingesting snails containing infective third stage larvae which invade the mesenteric lymph nodes where they undergo their third and fourth moults. Young adult nematodes migrate via lymphatic or hepatic portal vessels to the right side of the heart and pulmonary arteries where they develop to sexual maturity. The reported prepatent period varies from 38 to 60 days (Rosen et al., 1970; Guilhon and Cens, 1973).

Canine angiostrongylosis presents with inflammatory reactions and subsequent alterations in both pulmonary parenchyma and in the circulatory system which may lead to severe manifestations or even to the animal's death. The pathogenic determining factors may be related to the location of the parasite in the host and to the route taken during its cycle. Its possible passage in the liver and the establishment of its habitat in the heart and in the pulmonary artery may lead to alterations in both the hematological and biochemical profiles.

The tests selected for the biochemical profile were parameters which reflect clinical and pathogenic characteristics of the disease. The hepatic function was evaluated by measuring total and fractionated proteins which may reflect the synthesis capacity of the organ. Alanine (ALT) and aspartate (AST) enzymes reflect cell damage and more specifically are indicators of acute and chronic injury, respectively. The excretory liver capacity is determined by the gamma-glutamyl transferase (GGT) and alkaline phosphatase (PAL) activities. Creatinine and urea dosages are the most reliable tests of renal function, while the MB isoenzyme of CK is the standard laboratory marker to indicate cardiac injury.

Biochemical profiles of serum from dogs experimentally infected with *A. vasorum* are discussed in this paper by measuring enzyme activities and several analytes in order to determine which organs underwent alterations and what mechanisms may be involved.

2. Materials and methods

Fifteen six to eight-month old crossbreed dogs were used in this study. Before the experimental period faeces from these animals were collected and examined for helminth eggs and larvae. All the dogs were treated with an anti-helminthic drug in two doses (Nitroscanate, 50 mg/kg). Additional exams were performed at 7-day intervals to confirm that animals remained free of parasites. All the dogs were immunized against parvovirus, coronavirus and distemper virus in three doses at 1-month intervals.

The dogs were divided according to their weight in three groups. Groups A and B were inoculated orally with 50 and 100 larvae/kg of body weight, respectively. The control Group C animals were not inoculated.

The third stage infective larvae (L_3) were obtained by experimental infection. The aquatic snail *Biomphalaria glabrata* was maintained in the laboratory and each snail was experimentally infected with 400 first-stage larvae. After the 25th day of infection (d.i.) faeces were colleted daily and examined by Baermann's method to determine the prepatent period.

Samples of blood (5 mL) were obtained from each animal by atraumatic jugular venipuncture using vacutainer tubes. The first blood sampling was performed before the larval inoculation and subsequently on the 10th, 20th, 30th and 45th d.i. and then at intervals of 30 days for the 210-day experimental period. These samples were centrifuged at $700 \times g$, for 15 min and the serum obtained was immediately used for running the following biochemical tests.

2.1. Assessment of hepatic function

2.1.1. Total and fractionated proteins

The colorimetric Biuret's method (Gornal et al., 1949) (LABTEST Diagnóstica S.A.) was used for measuring total proteins. The protein fractioning was performed by electrophoresis using cellulose acetate membranes as support. The electrophoresis was performed in a electrophoresis chamber with 0.04 M sodium veronal buffer at pH 8.6. Serum samples from each dog were applied on the membranes using a semi-micro applicator. After a 40 min run, which was necessary for the migration, the membranes were stained with Ponceau's dye and discoloured in 5% acetic acid. Quantification of the individually stained zones was accomplished by direct densitometry, performed at 520 nm.

2.1.2. Aspartate aminotransferase and alanine aminotransferase activities

The colorimetric Reitman and Frankel's method (1957) (LABTEST Diagnóstica S.A.) was used to determine AST and ALT activities.

2.1.3. Gamma-glutamyl transferase activity

The kinetic Szasz's method (1969), modified by LABTEST Diagnóstica S.A. was used for this evaluation. The procedures to read and obtain of results as well as the calculations were performed according to the manufacturer's instructions.

2.1.4. Alkaline phosphatase activity

The activity of PAL was determined by the modified colorimetric Roy's method (1970) (LABTEST Diagnóstica S.A.).

2.2. Assessment of renal function

2.2.1. Urea

The colorimetric Marsh & Kirch's method (1957) which was modified by LABTEST Diagnóstica S.A. was used for this determination.

2.2.2. Creatinine

The colorimetric Heinegard and Tiderstram's method modified by Lopes et al. (1984) (LABTEST Diagnóstica S.A.) was used for this determination.

2.3. Assessment of cardiac function

2.3.1. Creatinine kinase isoenzyme MB activity

Six animals were randomly chosen, two from each Group (A, B and Control, respectively) to perform this test. The activity of CK-MB isoenzyme was determined before the infection and at intervals of 30 days throughout the 7-month experiment. The methodology used was based on a kinetic method, modified from Szasz et al. (1976) and developed by Diagnostica Merck S.A.

All the biochemical tests were carried out using serum from both control and infected animals and commercial serum (Labortest-Brasil) was also used for quality assurance.

All the parameters analyzed were submitted to analysis of variance using the STA statistical pack and the means were compared by the Student *t*-test. Unless stated otherwise, values of $p \leq 0.05$ were judged significantly different.

After the end of the experimental period all the dogs were submitted to treatment with Levamisol (10 mg/kg of live weight) on three consecutive days. After the treatment, faeces analyses of all animals were performed by the Baermann's method to confirm the absence of infection with *A. vasorum*.

3. Results

The observed values for total proteins showed a tendency to peak on the 100th d.i. for group A and on the 72nd d.i. for group B. However, when compared to the control group this was not significant ($p \geq 0.05$) (Fig. 1).

With respect to the electrophoresis of serum proteins we observed that among the five fractions analyzed the α2-globulin increased on the 100th and 130th d.i., for groups A and B compared to group C (Fig. 2) with significant statistical differences on these collecting days ($p \leq 0.05$). The β-globulin in inoculated groups reduced on the 20th d.i., followed by an emphatic increase on the 30th d.i. which persisted until the 45th d.i. (Fig. 2). There was a significant difference between groups A and C on the 20th, 45th and 100th d.i.; in groups B and C, on the 20th and 45th d.i. and in groups A and B, on the 100th d.i. ($p \leq 0.05$).

ALT activity was shown to be slightly higher in groups A and B compared to the control group on the 10th and 30th d.i. and lower on the 20th and from the 45th to the 130th d.i. We observed that the mean of AST activity was increased for groups A and B from the 30th d.i. until the 160th d.i. An increased value was observed in groups A and B on the 45th d.i. and for group B on the 160th d.i. No statistical difference was observed among the groups for the activities of these enzymes ($p \geq 0.05$).

The variation of the GGT activity in the serum of inoculated dogs (groups A and B) was not significant ($p \geq 0.05$) when compared to the control group, although the inoculated groups increased on the 30th d.i. The activity of PAL and the means of the urea and creatinine measurements in the inoculated groups varied relative to the control group but it was not significant ($p \geq 0.05$).

The averages of the CK-MB isoenzyme presented an increase for infected dogs from the 30th d.i. with peaks on the 60th d.i. for group B and on the 90th and 150th d.i., for group A. A significant difference was observed when comparing the infected groups to the control group from the 30th to the 120th d.i. ($p \leq 0.05$) (Fig. 3).

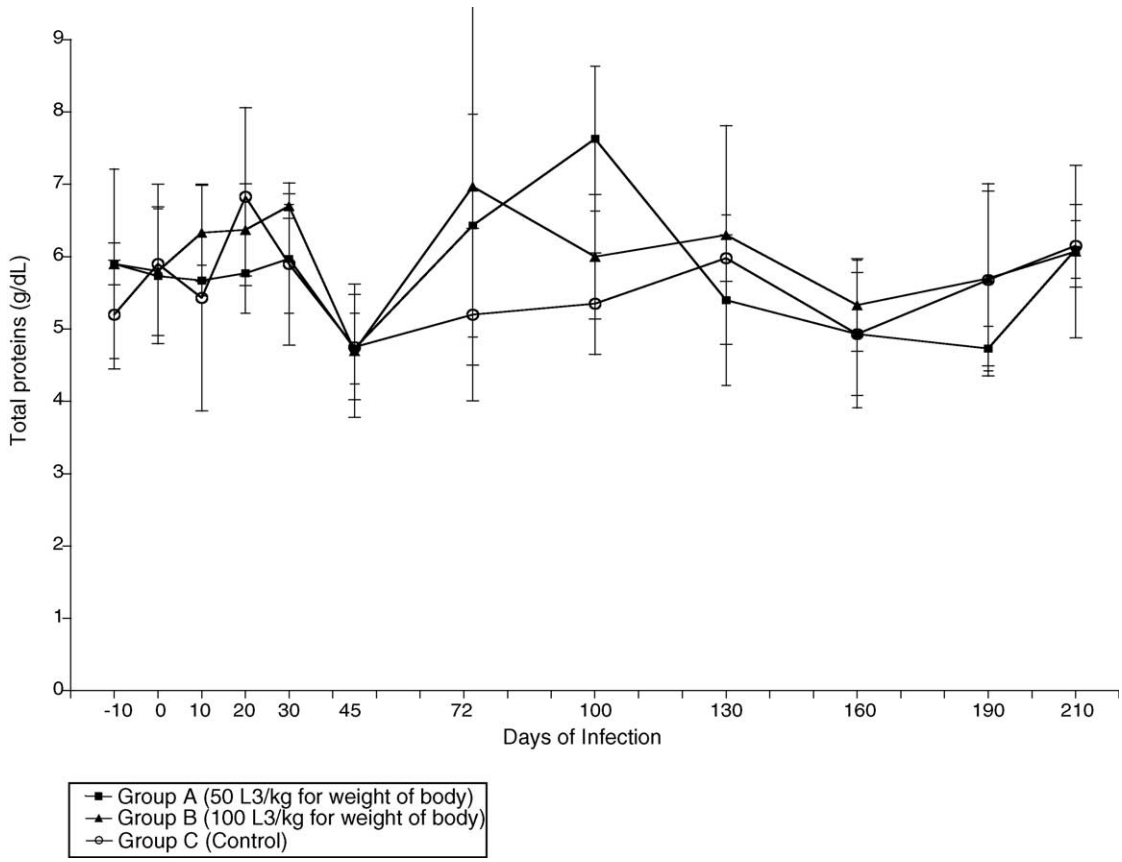

Fig. 1. Mean values of total proteins in serum of control and experimentally infected dogs with different number of larvae of *Angiotrongylus vasorum*.

4. Discussion

We observed that infection with *A. vasorum* did not alter the levels of total proteins in the infected dogs. Reports in the literature with respect to the profile of total proteins in infected dogs were not found. It is important to note that the profile of total proteins presents little diagnostic reliability in isolation without the use of other parameters. When it is analyzed with the electrophoretic running however, it is possible to know which fraction is altered. An analysis of the electrophoretic profile revealed an increase in both fractions α1 and α2-globulins, mainly on the 100th d.i. and β-globulin between the 30th and the 45th d.i. The alterations found in the proteic fractions occurred in the clinical acute phase of the disease. According to Mebus and Coles (1965), the increase of these globulins occurs in inflammatory processes, mainly related to α2-globuline, which is more sensitive in bacterial and viral infections. Dogs with angiostrongylosis may present pulmonary alterations due to the presence of the parasite in this organ which may provide secondary bacterial infection. The mechanism which promotes the increase of the concentration of this fraction is not yet clear. The alterations related to the β-globulin were previously mentioned by Groulade (1963), Mishra and Cens (1971) and Patteson et al. (1993). These probably occurred due to the severity of the pulmonary infection. Changes in the concentration of the proteic fraction are observed in enteropathies and in inflammatory processes. The number of larvae did not interfere in the response in terms of immunoglobulin concentration, as the animals of the inoculated groups showed similar responses to the infection.

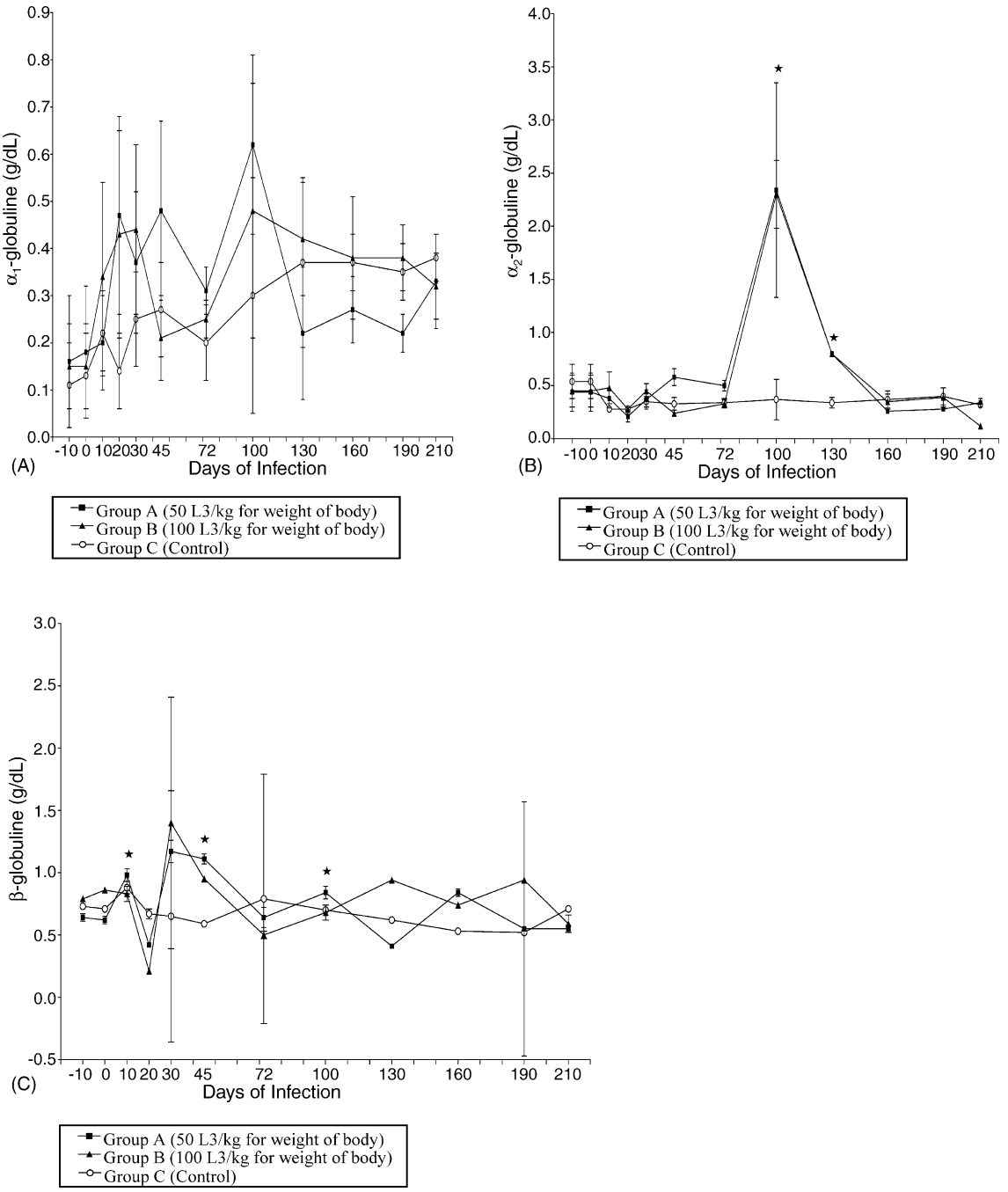

Fig. 2. Mean values of α_1-globuline (A), α_2-globuline (B) and β-globuline (C) in serum of control and experimentally infected dogs with different number of larvae of *Angiotrongylus vasorum*. Significant difference between groups ($^{*}p \leq 0.05$).

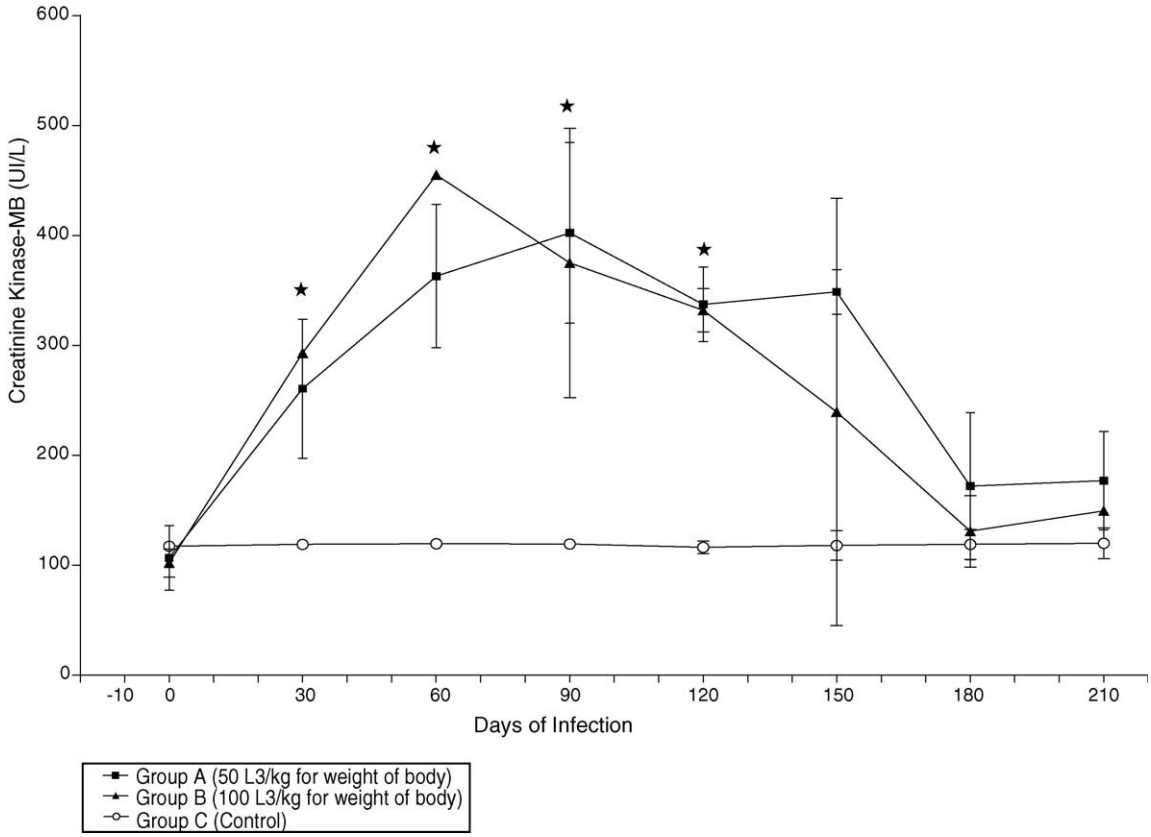

Fig. 3. Means values of activity of creatinine kinase-MB isoenzyme in serum of control and experimentally infected dogs with different number of larvae of *Angiotrongylus vasorum*. Significant difference between groups ($^{*}p \leq 0.05$).

No significant ALT differences were observed between the average activities of the groups. Some days later however, groups A and B presented higher activity compared to the control group which may be related to minor hepatic injury induced by parasitic activity. Koch and Jensen (1992) have also found normal levels of ALT in naturally infected dogs. This enzyme is important in the evaluation of hepatic injury and it is a good marker of hepatocellular injury in the acute phase. No significant alterations in AST activity were observed. However, on the 45th d.i. AST activity was higher compared to the control group in both inoculated groups, although no statistical differences were observed. This enzyme, besides being present in the hepatocytes, is also present in muscular cardiac cells. The increase of its activity coincides with the presence of the parasite in the heart. This increase is related to the cardiac injury produced by the parasite and is therefore also associated with the increase of CK-MB activity.

GGT and PAL activities, as well as urea and creatinine concentrations did not alter although Mishra and Cens (1971) have reported a significant increase in the concentration of urea in experimentally infected dogs. Since we used two markers for the renal injury and we measured creatinine which is the most reliable biochemical test of glomerular function we can conclude that no alteration in renal function occurred in our study.

The analysis of the CK-MB activity showed that this enzyme is a highly sensitive indicator of the cardiac muscle injury produced by *A. vasorum*. Increased levels were also observed between the 30th and the 45th d.i. which coincides with the arrival

of the parasite in the heart and lung when it becomes an adult worm and this damage to the heart may be caused by mechanical and/or antigenic activity. These injuries are detected very early by determining the CK-MB activity. When this enzyme was associated to the analysis of AST for evaluating cardiac injury (Puleo and Guadagno, 1990) we observed a parallelism between these two measurements indicating that this is an active process. We have no explanation why CK-MB activity come down after 150 d.i.. The possible explanation are the reduction of adult worms in the heart considering that no reinfection has occurred, this fact may implicate in the reduction of cardiac damage by mechanical process and due to the reduction of cardiac damage it would occur a concomitant reduction of the enzyme activity. There are no reports in the literature in related to the analysis of this enzyme for evaluating cardiac alterations produced by *A. vasorum.*

The analysis of these results allows us to conclude that infections of dogs by *A. vasorum* are not able to produce alterations in the hepatic and renal functions, detected by biochemical tests. These may be performed for clinical interest in case of alterations observed in the liver and/or kidneys. However, it is important to emphasize that fractionated serum proteins present alterations in their concentrations, which are compatible to the clinical status, produced by the parasite in dogs. Thus, the effectiveness of CK-MB is confirmed in detecting cardiac injuries produced by the presence and or action of the adult parasite in the organ. Our main intention was to describe that laboratory analysis are potentially useful for evaluation of clinical status in dogs experimentally infected by *A. vasorum*. This test may be helpful as an auxiliary method for follow-up the clinical evolution of angiostrongylosis.

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