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# *Angiostrongylus vasorum* in 20 dogs in the province of Chieti, Italy

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**Summary**

After a case of *Angiostrongylus vasorum* (canine lungworm) was diagnosed in the province of Chieti (Italy) in early 2008, parasitological research was conducted to investigate the presence of the parasite in dogs in the area. A total of 178 dogs, 56 carcasses and 122 stool samples were examined between January and September 2008. The carcasses were examined for the presence of adult parasites in the right ventricle and pulmonary artery, and larval forms in the internal organ and brain tissues. The faeces were inspected for larval form L1 using three diagnostic methods that are currently used to test for endoparasites and larvae of bronchopulmonary strongyles. A total of 20 cases of canine angiostrongylosis were diagnosed (8.9%), with adult parasites being identified in 5 dogs, and L1 larvae in another 15. The anatomo-pathological examination of the carcasses of the dogs infested with adult nematodes revealed pneumonia, pleurisy, reddish foam in the trachea, effusion of serohaemorrhagic fluid in the thoracic cavity and enlarged mediastinic and mesenteric lymph nodes. Histological examination of the tissues showed serious, similar syndromes with lesions caused by colonisation of the kidneys, lymph nodes and brain by the parasites. Given the large number of cases confirmed in relation to the period of study (9 months), it is essential to include angiostrongylosis among the differential diagnoses made in clinical and post-mortem examinations of dogs in the province of Chieti and in the neighbouring areas.

**Keywords**

*Angiostrongylus vasorum*, Chieti, Dog, Diagnosis, Italy, Nematode, Parasite, Pneumonia.

## Introduction

Angiostrongylosis caused by *Angiostrongylus vasorum* (Baillet, 1866), phylum Nematoda, order Strongylida, superfamily Metastrongyloidea (66), is a parasitic disease that affects dogs and other species of Canidae, including foxes.

The biological cycle of *A. vasorum*, which is only partly known, is indirect. The final host is infested by swallowing parasites belonging to genera of terrestrial and aquatic gastropods (3, 30, 35), and also the common frog or other paratenic hosts (6).

The adults of *A. vasorum* mainly live in the right ventricle and the pulmonary artery and its branches (59), with rare erratic locations due to the transition of the immature L5 forms from the capillary vessels to the pulmonary veins and subsequently to the left-hand section of the heart and, through the systemic circulation, to other organs (16, 17, 39, 41, 43,

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48, 58). The eggs produced by adult females located in the right-hand portion of the heart, reach the pulmonary capillaries where they hatch into first-stage (L1) larvae. These larvae, after reaching the lungs, perforate the capillary and alveolar wall, aided by the animal's coughing, reach the pharynx, and are swallowed and eliminated to the exterior through the faeces. Erratic locations of L1 in the brain and other organs have been described (20, 46, 48, 52, 54, 60, 62).

The free L1 larvae in the soil are consumed by intermediate hosts and reach infesting stage L3. The cycle is completed when the dog swallows the intermediate host with the L3 larvae, which mutate into L4 and L5 and migrate through the mesenteric lymph nodes to the liver and pulmonary arteries, where they develop into adult forms (30, 59).

Canine angiostrongylosis can be entirely asymptomatic or present with clinical symptoms of varying degrees of severity, up to and including the death of the animal, depending on the number of parasites and the stage of development of the disease. The disease usually has a chronic course, characterised by progressive deterioration of the respiratory and cardiac functions and altered blood coagulation. Infested dogs may present frequent coughing, dyspnoea and clouded sensorium (35). Bleeding diathesis, reduced tolerance of physical exercise, anorexia, weight loss, vomiting, diarrhoea, neurological disorders, melaena and collapse have been reported with varying frequency (12, 21, 43, 47, 52, 61, 71, 78).

Canine *A. vasorum* is endemic in well-defined areas in Denmark (5, 36, 37, 68, 70, 76, 78 ), France (22, 23, 30, 59, 64), Great Britain (13, 26, 33, 34, 42, 52, 65), Ireland (9, 21, 40, 57, 79) and Uganda (10, 11), while sporadic cases have been reported in Argentina (74), Brazil (27, 28, 38, 39), Germany (4, 50), Great Britain (52, 72), the former Soviet Union (19, 23), Switzerland (23), Turkey (69) and Spain (60).

The disease has recently been reported in previously unaffected areas of Canada (8, 14, 75), England (12, 24, 32), Germany (20, 67, 68), Greece (51), Italy (18, 61, 62, 71), the Netherlands (73), Sweden (1) and Switzerland (67).

The extension of the distributional area of canine angiostrongylosis is attributable to climatic conditions that are conducive to the development of the intermediate and paratenic hosts, the presence and high density of foxes which can act as reservoirs, and the presence of other intermediate and final hosts which are not yet known (7, 35, 44, 45, 67).

In Italy, after the first case that was described in Tuscany in 2002 (18), four cases were recently observed in Tuscany and Lazio (62), two in Abruzzo (71) and one in Puglia (61).

After a case of angiostrongylosis that was reported in January 2008 in a dog from the province of Chieti, which was examined at the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'* (*Istituto G. Caporale*), it was considered advisable to estimate the percentage of positive dogs among those referred to the laboratory from the same area.

## Materials and methods

Between January and September 2008, tests for the presence of *A. vasorum* were conducted on 178 dogs from municipalities in the province of Chieti (Table I), by means of post-mortem examination of 56 carcasses and analysis of 122 stool samples provided by veterinary surgeons who operated in the area, accompanied by a form that provided the data and clinical symptoms of the animal.

The right-hand area of the heart and the pulmonary artery were tested for adult parasites, in accordance with conventional methods (25, 29, 53).

Stool samples were taken from the intestine of positive dogs for coprological tests. Histological tests were also performed on portions of brain, heart, liver, mediastinic lymph nodes, spleen, lungs and kidneys.

The stool samples were tested for L1 larval forms using three different diagnostic methods, as follows:

- direct microscopic examination
- flotation with a heavy zinc sulphate solution (specific gravity 1 300 g/cm$^3$)

- Baermann technique

all of which are currently used in the laboratory to test for endoparasites and bronchopulmonary lungworm larvae.

In view of the intermittent elimination of the larvae (49, 52), after the first test the owner of the animal was asked for permission to take a second sample to eliminate false negative results. Using this procedure, one, two or three diagnostic tests could be performed on stool samples from the same animal. One stool sample was taken from 66 dogs, two from 9 dogs and three from 47 dogs; the samples were taken directly from the rectum of the animals at intervals of not less than 24 h.

The adult parasites and L1 larvae were identified on a morphological basis using the identification keys described by Costa *et al.* (15), Euzéby (25) and Rosen *et al.* (59).

The confidence interval (CI) of the percentage was determined by means of a Bayesian approach using the Beta distribution (s+1,*n*-s+1) where s is the number of positives and *n* the total number of animals tested.

## Results

The anatomo-histopathological and parasitological examinations indicated the presence of *A. vasorum* in 20 dogs out of the 178 examined (8.9%, 95% CI: 7.4%-16.7%).

In particular, adult parasites were observed in the right ventricle and pulmonary artery of five dogs (Figs 1, 2 and 3) and L1 larvae in the faeces of another 15 dogs (Figs 4 and 5).

The age, gender, breed and municipality of origin of the positive dogs are presented in Table II.

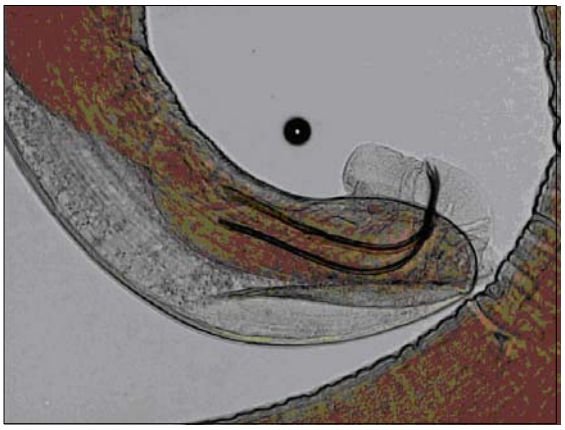
Figure 6 shows a map of the province of Chieti with the municipalities of origin of the positive dogs: 9 of a total of 20 dogs from the municipality of Torino di Sangro were infested (6 of which belonged to the same owner) (95% CI: 25.7%-66%).

Clinical examination revealed 20 positive dogs, severe dyspnoea (30%), spontaneous bleeding (25%), anaemia (20%), melaena (10%), coughing (5%), epileptic fits (5%) or no symptoms (35%) (Table III).

Table I
Municipality of origin, number of carcasses and stool samples of dogs examined

| Municipality | Carcasses | Stool samples |
|---|---|---|
| Altino | | 2 |
| Archi | 1 | 5 |
| Ari | | 1 |
| Arielli | | 1 |
| Atessa | 3 | 34 |
| Bomba | | 3 |
| Canosa Sannita | 1 | |
| Casacanditella | | 1 |
| Casalbordino | 2 | 10 |
| Casoli | 1 | |
| Castelfrentano | | 1 |
| Fallo | 1 | |
| Fara San Martino | | 1 |
| Filetto | | 1 |
| Fossacesia | 2 | 1 |
| Francavilla | 1 | |
| Frisa | 1 | |
| Gessopalena | 1 | |
| Guardiagrele | 1 | |
| Lanciano | 19 | 12 |
| Montazzoli | 1 | 2 |
| Mozzagrogna | 2 | 4 |
| Orsogna | | 1 |
| Ortona | 2 | 1 |
| Paglieta | 2 | 10 |
| Perano | | 2 |
| Pietraferrazzana | | 1 |
| Quadri | | 2 |
| Rocca San Giovanni | 1 | 4 |
| Roccamontepiano | | 1 |
| Roccaspinalveti | | 2 |
| San Salvo | 1 | |
| San Vito Chietino | | 1 |
| Sant'Eusanio del Sangro | | 3 |
| Santa Maria Imbaro | 4 | 2 |
| Scerni | | 1 |
| Torino di Sangro | 3 | 8 |
| Tornareccio | 1 | |
| Torricella Peligna | | 1 |
| Vasto | 5 | |
| Villa Alfonsina | | 1 |
| Villa Santa Maria | | 2 |
| Total | 56 | 122 |

a) Side view
Clarified in lactophenol (10×)



b) Front view
Clarified in lactophenol (10×)

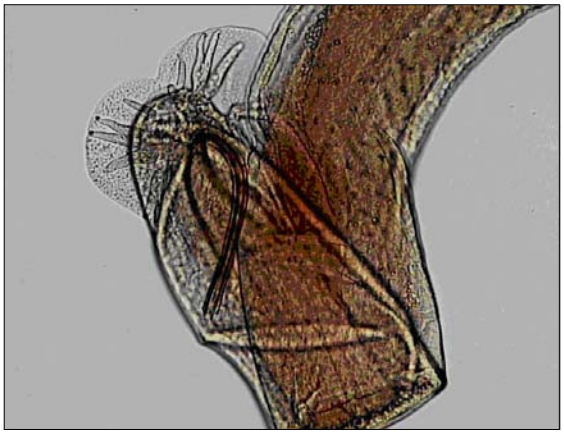


Figure 1
Rear extremity (caudal sac) of a male *Angiostrongylus vasorum* found in the right-hand portion of a canine heart

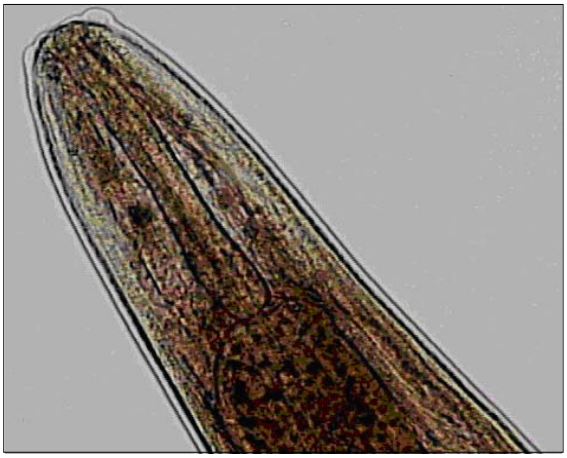


Figure 2
Front extremity (caudal sac) of a female *Angiostrongylus vasorum* found in the right-hand portion of a canine heart
Clarified in lactophenol (10×)

Figure 3
Rear extremity (caudal sac) of a female *Angiostrongylus vasorum* found in the right-hand portion of a canine heart
Clarified in lactophenol (10×)

Figure 4
L1 larva of *Angiostrongylus vasorum* sampled from canine faeces
Baermann technique (10×)

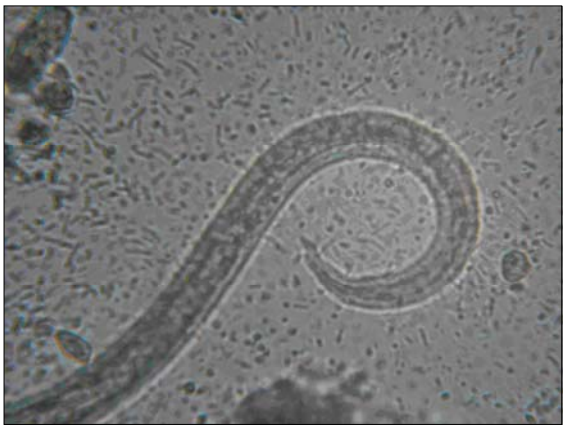


Figure 5
Caudal extremity of L1 larva of *Angiostrongylus vasorum* sampled from canine faeces
Direct microscopic examination (40×)

Table II
Age, gender, breed and origin of dogs infested with *Angiostrongylus vasorum*

| Identification number of case | Age in months | Gender | Breed | Municipality of origin |
|---|---|---|---|---|
| 1 | 8 | M | Beagle | Torino di Sangro |
| 2 | 9 | M | Beagle | Torino di Sangro |
| 3 | 6 | M | Mongrel | Torino di Sangro |
| 4 | 36 | F | Beagle | Rocca San Giovanni |
| 5 | 120 | M | Golden retriever | Perano |
| 6 | 36 | F | Alsatian | Fossacesia |
| 7 | 24 | F | Boxer | Santa Maria Imbaro |
| 8 | 84 | M | Mongrel | Lanciano |
| 9 | 18 | F | Mongrel | Lanciano |
| 10 | 144 | F | Bloodhound | Quadri |
| 11 | 12 | M | Ariegeois | Torino di Sangro |
| 12 | 60 | M | Alsatian | Torino di Sangro |
| 13 | 36 | M | Mongrel | Torino di Sangro |
| 14 | 24 | F | Mongrel | Torino di Sangro |
| 15 | 8 | F | Ariegeois | Torino di Sangro |
| 16 | 3 | M | Alsatian | Villa Alfonsina |
| 17 | 8 | F | Mongrel | Torino di Sangro |
| 18 | 18 | F | Mongrel | Mozzagrogna |
| 19 | 18 | F | Mongrel | Ortona |
| 20 | 12 | M | Doberman | Canosa Sannita |

F female
M male

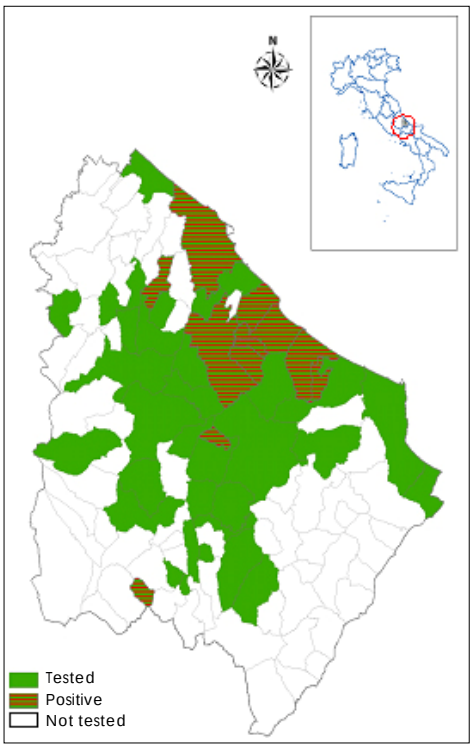



Figure 6
Province of Chieti, showing the municipalities of origin in which dogs tested positive for *Angiostrongylus vasorum*

In the five cases of angiostrongylosis diagnosed upon post-mortem examination, the following were detected:

- pneumonia characterised by areas of chronic inflammation with increased lung volume and consistency due to the presence of greyish areas of fibrosis interspersed with dark red areas of acute inflammation) (Figs 7, 8, 9 and 10)
- reddish foam in the trachea
- pleurisy, effusion of serohaemorrhagic fluid in the thoracic cavity
- enlarged mediastinic and mesenteric lymph nodes.

The parasite content ranged between 40 and 198 specimens.

Blood was found on the skin of the labial and nasal regions in two carcasses. Extensive subcutaneous haemorrhagic infiltration of the thoracic and neck region was observed in one dog and haemorrhagic petechiae in the muscles of the right ventricle in another. The

Table III
Clinical symptoms found in dogs infested with *Angiostrongylus vasorum*

| Identification number of case | Bleeding | Dyspnoea | Dry cough | Melaena | Anaemia | Epileptic fits | No symptoms |
|---|---|---|---|---|---|---|---|
| 1 | + | | | | | | |
| 2 | + | + | | | | | |
| 3 | | + | | | | | |
| 4 | + | | | | | | |
| 5 | | + | | | | | |
| 6 | | + | | + | + | | |
| 7 | + | + | | | | | |
| 8 | | | + | | | | |
| 9 | | | | | + | | |
| 10 | | + | | | + | | |
| 11 | | | | | + | | |
| 12 | | | | | | | + |
| 13 | | | | | | | + |
| 14 | | | | | | | + |
| 15 | | | | | | | + |
| 16 | | | | | | | + |
| 17 | | | | | | | + |
| 18 | | | | + | | | |
| 19 | | | | | | | + |
| 20 | + | | | | | + | |

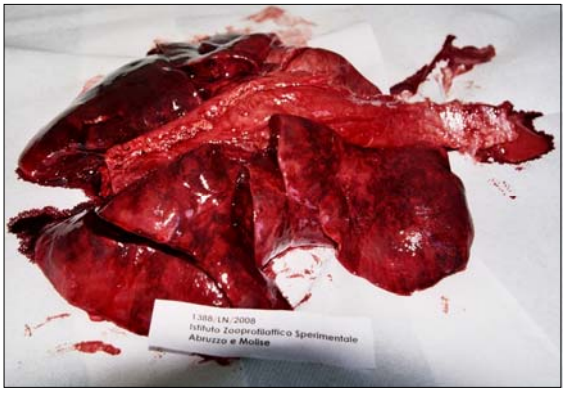



Figure 7
Lungs of dog affected by canine angiostrongylosis


Figure 8
Lungs of dog affected by canine angiostrongylosis

stool tests always showed the presence of L1 larvae.

The histological tests indicated serious, similar syndromes with organ-specific parasite colonisation of the heart, lungs, kidneys, lymph nodes and brain.

The lungs were affected by a progressive form of chronic interstitial pneumonia with prominent fibrosis surrounding the eggs and larvae, causing nodules which often converged (Fig. 11).


Figure 9
Adult *Angiostrongylus vasorum* lungworms in canine heart

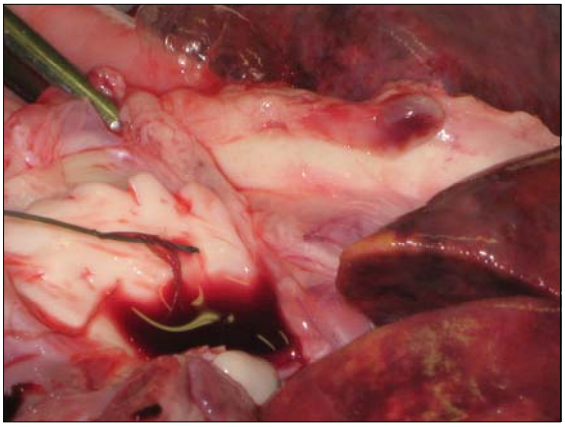


Figure 10
Adult *Angiostrongylus vasorum* lungworms in canine heart

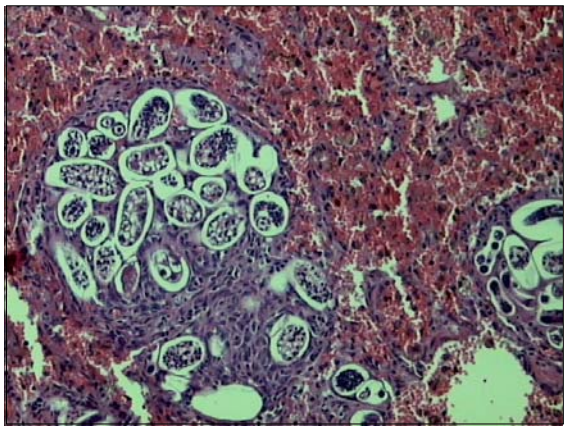


Figure 11
Microphotograph of canine lung showing haemorrhagic alveolitis and fibrous nodules with nematode eggs and larvae
Haematoxylin & eosin (40×)

The remaining parenchyma showed a haemorrhagic picture with alveolar blood engorgement and macrophagic alveolar exudation. Giant cell reactivity and the presence of adult nematodes in the arterial lumina were frequent (Fig. 12).

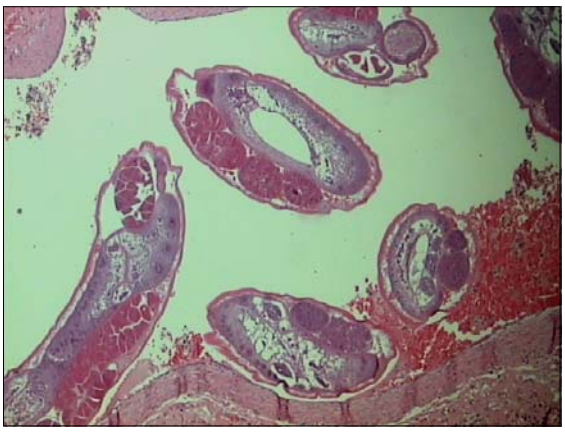


Figure 12
Microphotograph of lung section showing pulmonary endocarditis with adult nematodes in intraluminal site
Haematoxylin & eosin (5×)

The involvement of the large arterial vessels confirmed the intramural location of the eggs and larvae. The pulmonary artery was affected by inflammatory reaction and the myocardium by slight interstitial reactivity.

In all cases, ectopic locations of eggs and larvae were found in the kidneys, associated with lymphoplasmacellular inflammation (Fig. 13) and inflammation of the mediastinic lymph nodes. Adult nematodes were only observed in the pulmonary vessels and the right ventricle of the heart. In two cases, larvae were found in the brain, with inflammatory reactivity and bleeding in the perivasal site (Fig. 14) and malacia of the white matter and, in one case, chronic giant cell reactivity.

Interstitial inflammation of the hepatic parenchyma was observed in all cases, but no parasites of any kind were found.

In the dogs in which angiostrongylosis was diagnosed by stool tests, the three methods used gave different results, as shown in Table IV. The Baermann technique always revealed the presence of the L1 larvae, except

in one case, in which only the first sample of three tested positive. The tests conducted in parallel with the flotation method and direct microscopic examination did not always give positive results.

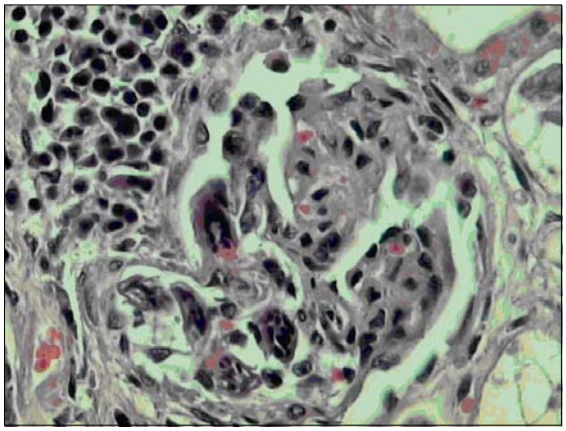


Figure 13
Microphotograph of canine kidneys showing nematode larvae in the glomerular capillaries and periglomerular lymphomonocyte inflammation
Haematoxylin & eosin (40×)

## Discussion

The results of this research indicate that a large number of dogs in the province of Chieti are infested with *A. vasorum*. Using the same methods over the same time span, equally high percentages were found in the area west of Zealand in Denmark (9.7%) (70) and on the islands of Newfoundland in Canada (23.9%) (14), while lower percentages were found in Serres, Greece (1.1%) (51), north of Copenhagen, Denmark (3.5%) (76), the Hague and Veluwue in the Netherlands (8%) (73) and in Germany (0-2% in investigations conducted over six consecutive years) (68).

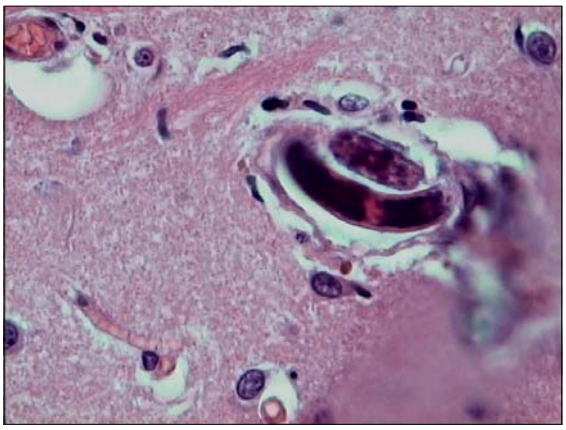


Figure 14
Microphotograph of canine central nervous system with capillary containing cross-section of nematode larvae
Slight perivasal reactivity
Haematoxylin & eosin (40×)

Table IV
Cases of canine angiostrongylosis diagnosed by stool test:
Number of positive tests performed with the three different methods used and number of samples taken

| Identification number of case | Number of stool samples taken | Direct microscopic examination | Flotation method | Baermann technique |
|---|---|---|---|---|
| 4 | 1 | 0 | 1 | 1 |
| 5 | 1 | 0 | 0 | 1 |
| 6 | 1 | 1 | 1 | 1 |
| 8 | 1 | 1 | 1 | 1 |
| 9 | 1 | 1 | 1 | 1 |
| 10 | 1 | 1 | 1 | 1 |
| 11 | 1 | 1 | 1 | 1 |
| 12 | 1 | 1 | 1 | 1 |
| 13 | 2 | 1 | 2 | 2 |
| 14 | 2 | 2 | 2 | 2 |
| 15 | 2 | 2 | 2 | 2 |
| 16 | 2 | 1 | 2 | 2 |
| 17 | 1 | 0 | 0 | 1 |
| 18 | 3 | 3 | 3 | 3 |
| 19 | 3 | 0 | 0 | 1 |

In general, cases of angiostrongylosis are likely to be underestimated due to intermittent or non-existent elimination of the larvae during the pre-patent period (49), the use of the Baermann technique with sensitivity below 99% (52, 75, 77) and the variable quantity of faeces which can produce false negative results, especially in the case of dogs for which the stool test is performed on a single sample.

The fact that six infested dogs belonged to the same breeder confirmed the hypothesis of Dodd (21) and Simpson and Neal (65) that angiostrongylosis, though an indirect cycle, is more frequent on premises where dogs live in close contact with one another.

In agreement with other authors (35, 52) no age-, gender- or breed-related predispositions were found among the positive animals. Chapman *et al*. (12) and Conboy (14) have reported the greater predisposition to parasitosis of the King Charles spaniel, Staffordshire bull terrier and beagle.

The presence of asymptomatic animals, the clinical symptoms, post-mortem examination and histology results substantially agreed with findings already reported in the literature (8, 12, 20, 35, 43, 52, 55, 56, 71).

Unlike the Koch and Willesen study (37), no granulomatous formations were found in the kidneys.

Contrary to observations of other workers (17, 41, 43, 48, 58), the ectopic location of the adult nematodes was only observed in the pulmonary arterial vessels; eggs were also found in the kidneys, and L1 larvae in the pulmonary arterial vessels, mediastinic lymph nodes, kidneys and brain.

The three methods used to detect L1 larvae of *A. vasorum* in the faeces always gave positive results for the faeces of the five dogs with nematodes in the heart, unlike the results of Patterson *et al*. (52) and Denk *et al.* (20).

According to the results of this study, the Baermann technique is the most effective method to reveal L1 larvae of *A. vasorum* in faeces, as extensively reported in the literature (7, 14).

## Conclusions

Canine angiostrongylosis was first reported in the province of Chieti on 25 January 2008, although it is suspected that the disease had been present for years, because various cases of verminous bronchopneumonia had been diagnosed by the *Istituto 'G. Caporale'* in the past when histological tests conducted on canine lungs to diagnose other disorders were examined.

It is impossible to estimate the date of appearance and the origin of the disease, i.e. whether it was spread by dogs imported from endemic areas or whether local dogs contracted the disease while travelling with their owners. A survey is being conducted to establish whether the spread of the disease is associated with its presence in foxes, as reported in Canada (8) and Denmark (5).

Factors such as mild temperature and damp air, which are conducive to the proliferation of gastropods, may explain why more positive cases are found along the Adriatic coast (13, 63).

In view of the large number of cases recorded over a nine-month period and the scarcity of reports in Italy, it is essential to include this disease in the differential diagnoses performed in clinical and post-mortem examinations of dogs in the province of Chieti and neighbouring areas.

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