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SUBCLINICAL *ANGIOSTRONGYLUS VASORUM* INFECTION IN A TERRIER DOG KENNEL

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Abstract

Angiostrongylus vasorum is a parasitic nematode causing severe clinical signs in infected dogs. In the past few years *A. vasorum* has been repeatedly described in both traditional endemic foci and previously free regions. Nonetheless, the infection is often neglected or unnoticed by vet practitioners, due to gaps of information on *A. vasorum* epidemiology, and to drawbacks inherent to the clinical and parasitological diagnosis. Indeed, subclinical infections may occur and, when present, clinical signs are difficult to differentiate from those of other canine cardio-pulmonary diseases. Additionally, the *gold standard* test for the aetiological diagnosis of the infection, i.e. the Baermann's method, is not commonly performed by veterinarians.

The present study describes cases of subclinical *A. vasorum* infection in a Jack Russell Terrier dog kennel in Italy and the ability of a newly marketed rapid kit (IDEXX *Angio Detect*™ *Test*) for the field diagnosis of angiostrongylosis, pre- and post-treatment with a formulation licensed for the treatment of *A. vasorum*.

SUBKLINICZNE ZARAŻENIE *ANGIOSTRONGYLUS VASORUM* W HODOWLI PSÓW TERIERÓW

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Słowa kluczowe: *Angiostrongylus vasorum*, psy, diagnostyka, Imidacloprid, Moxidectin, Włochy.

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Abstrakt

Angiostrongylus vasorum to pasożytniczy nicień powodujący ciężkie objawy kliniczne u zarażonych psów. Na przestrzeni ostatnich kilku lat wielokrotnie opisywano przypadki inwazji *A. vasorum* zarówno w endemicznych ogniskach, jak również w regionach wcześniej wolnych od tego pasożyta. Często jednak zdarza się, że możliwość inwazji zostaje pominięta lub przypadek zarażenia pozostaje niedostrzeżony przez lekarza weterynarii z powodu niedostatecznej wiedzy na temat epidemiologii *A. vasorum* oraz niedociągnięć czy błędów podczas badania klinicznego i parazytologicznego. Podkliniczna postać inwazji może jednak występować – w takiej sytuacji objawy kliniczne są trudne do odróżnienia od tych towarzyszących innym chorobom układu sercowo-naczyniowego i chorobom płuc u psów. Co więcej, standard diagnostyki etiologicznej inwazji, czyli metoda Baermanna, nie jest powszechnie wykorzystywana przez lekarzy weterynarii.

W pracy opisano przypadki podklinicznej inwazji *A. vasorum* w hodowli Jack Russel terierów we Włoszech oraz podjęto zagadnienie możliwości zastosowania nowo wprowadzonego na rynek testu (IDEXX *Angio Detect*^{TM} *Test*) w diagnostyce terenowej angiostrongylozy przed leczeniem preparatem dopuszczonym do stosowania w terapii inwazji *A. Vasorum* i po takiej kuracji.

Introduction

Canine angiostrongylosis is an emerging parasitic disease of dogs and wild animals (e.g. foxes and wolves) caused by *Angiostrongylus vasorum* (Nematoda, Metastrongyloidea). The interest on this nematode is growing due to the severe clinical outcome of the infection in dogs, the increase of reports in both traditional endemic foci and previously free areas, and for the recent insights achieved on the biology, treatment and the diagnosis of the infection (FERDUSHY and HASAN 2010, TRAVERSA et al. 2010, 2013, SCHNYDER et al. 2014). Adult stages reside in the heart and pulmonary arteries, where adult females produce eggs that embryonate and hatch within alveolar ducts and alveoli. First stage larvae (L1) penetrate into the alveoli and migrate to the pharynx, are swallowed and released into the environment *via* the feces. The life cycle of *A. vasorum* is indirect, involving slugs and snails as intermediate hosts, in which L1 develop to the third infectious stage (L3). Dogs become infected by ingesting mollusks harboring L3 (ANDERSON 2000, FERDUSHY and HASAN 2010, MORGAN and SHAW 2010). *Angiostrongylus vasorum* has been considered for a long time to be present only in well-isolated endemic foci (i.e. areas of France, UK and Denmark). However, in the last decade, the nematode has been recorded in dogs in previously free areas of northern and central Europe, e.g. Sweden, Switzerland, Germany, of the Mediterranean Basin, e.g. Greece and Italy, and of Eastern Countries, e.g. Slovakia, Poland, Hungary (MAJOROS et al. 2010, TRAVERSA et al. 2010, 2013, DI CESARE et al. 2011, HURNÍKOVÁ et al. 2013, GUARDONE et al. 2013, SCHNYDER et al. 2013).

Canine angiostrongylosis may be asymptomatic/sub-clinical or characterized by iper-acute, acute or chronic signs, that may be life-threatening when a specific therapy is not administered (TRAVERSA and GUGLIELMINI 2008,

FERDUSHY and HASAN 2010, MORGAN et al. 2010, TRAVERSA et al. 2010). Cardiac, neurologic, gastrointestinal and hematological signs may be present in infected animals, being coughing, dyspnea, and some non-specific signs, such as anorexia and weight-loss, lethargy, depression the most common. Cardiovascular signs (e.g. heart murmur, ascite, syncope) of congestive heart failure (*cor pulmonale*) may be observed. Bleeding disorders and coagulopathies may cause petechial or ecchymotic haemorrhages in the conjunctiva, episclera, gingiva and subcutis, as well as epistaxis, haemoptysis, post-surgical haematomas, gastrointestinal bleeding, haematuria and anaemia. Others common signs of angiostrongylosis include neurological (e.g vestibular signs, convulsion and paralysis), ocular signs (e.g. uveitis) due to larvae or egg embolism or haemorrhages. Infected dogs may also show vomiting, diarrhoea and anorexia (GOULD et al. 1999, CHAPMAN et al. 2004, OLIVEIRA-JÚNIOR et al. 2004, TRAVERSA and GUGLIELMINI 2008, TRAVERSA et al. 2008, 2010, 2013, KOCH et al. 2009). A recent multi-centric survey performed in dogs with clinical pictures compatible with angiostrongylosis confirmed that non-specific gastrointestinal, respiratory, hematological and neurological signs may occur and that coughing is the most prevalent (TRAVERSA et al. 2013). Given the lack of specificity of signs, the clinical diagnosis of the infection is impossible. The detection of L1 in faeces of infected animals with the Baermann's method is the most reliable approach to achieve the aetiological diagnosis of angiostrongylosis (TRAVERSA and GUGLIELMINI 2008). This technique is relatively easy to perform and cheap, although it is time-consuming (i.e. 24–36 h) and requires well-trained microscopists. In fact, L1 should be recognized based on their length (i.e. 310–400 μm) and tail, having a typical sinus wave curve with a dorsal spine. These larvae need to be discriminated from those of other free-living or parasitic nematodes (i.e. *Crenosoma vulpis*, *Oslerus osleri* or *Filaroides* spp.) which can be present in canine faeces (TRAVERSA et al. 2010).

In additional, the Baermann's method has major disadvantages like the inability to diagnose infections during the pre-patent period and when larvae are not being shed, even in presence of severe clinical signs (CONBOY 2009, TRAVERSA et al. 2010). Innovative studies have been recently performed to overcome the constraints of copromicroscopic approaches (AL-SABI et al. 2010, SCHNYDER et al. 2011, 2013, 2014, SCHUCAN et al. 2012). After some of these studies, a newly marketed rapid kit (IDEXX *Angio Detect*^{TM} *Test*) has been recently developed for the serological diagnosis of angiostrongylosis. The present study described cases of subclinical infections by *A. vasorum* in a Jack Russell Terrier dog kennel in Italy and the efficiency of this new kit in the field diagnosis of angiostrongylosis. The diagnostic performance of both the Baermann's test and the rapid kit has been evaluated before and after treatment with a parasiticide spot-on formulation licensed for the treatment of *A. vasorum*.

Materials and Methods

Study design and animals

The study was carried out in a private Jack Russell Terrier kennel located in Gallicano Municipality (Tuscany region, central Italy), selected for a previous history of angiostrongylosis. At Day -15 all the fifteen dogs living in the kennel were clinically examined and then faeces and blood were collected to be subjected, respectively, to the Baermann's method and to the detection of the circulating antigen of *A. vasorum* with the *Angio Detect™ Test*. Dogs were considered infected when positive at the Baermann's and/or the *Angio Detect™ Test*. At Day 0 positive dogs were clinically examined and treated with a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate®, Bayer). Two and four weeks after treatment (Days 14 and 28) these dogs were examined for clinical signs and samples were collected and examined as above with the Baermann's method and the *Angio Detect™ Test*.

Baermann methods and *Angio Detect™ Test*

The Baermann's test was performed as on the follow: 3–5 grams of each stool sample was put in the center of double layers of cheesecloth sheet. A pouch containing the fecal material was formed by holding the four corners of the cheesecloth sheet together and molding the cloth around the fecal material using a closing string. The pouch was placed in a funnel filled with water and kept at room temperature. After 24 hours, 15 ml of fecal fluid was drawn off the bottom funnel into a tube and centrifuged at 2000 rpm for 5 minutes. The sediment was transferred onto a slide and microscopically examined using a light microscopy at 10X, 40X and 100X.

Angiostrongylus vasorum L1s retrieved at the copromicroscopic examination were identified according to morphological and morphometrical keys features (TRAVERSA et al. 2010). The *Angio Detect™ Test* was performed and interpreted following manufacturer's instructions using the plasma collected from EDTA-blood samples.

Results and Discussion

At the clinical examinations performed before (Day-15) and after (Day 14 and 28) treatment no dogs showed clinical signs suggestive of angiostrongylosis.

The Baermann's test performed at Day-15 revealed the presence of *A. vasorum* L1 (Figure 1) in the faeces of 3 dogs. Two of them resulted positive also at the *Angio Detect*™ *Test* (Table 1). All examinations performed after treatment (Day 14 and Day 28) with the Baermann's methods and the rapid kit were negative (Table 1). The negative result of the kit in one infected dog at the pre-treatment evaluation is not indeed surprising. The possible explanations of this result could be the low level of circulating antigen due

Fig. 1. First stage larvae of *Angiostrongylus vasorum* (200x magnification)

Table 1

Results of investigations of fifteen dogs living in a dog kennel

Dog ID	D – 15		D – 14		D – 28	
	B	ADT	B	ADT	B	ADT
1	–	–	np	np	np	np
2	–	–	np	np	np	np
3	–	–	np	np	np	np
4	+	–	–	–	–	–
5	–	–	np	np	np	np
6	–	–	np	np	np	np
7	–	–	np	np	np	np
8	–	–	np	np	np	np
9	–	–	np	np	np	np
10	–	–	np	np	np	np
11	–	–	np	np	np	np
12	–	–	np	np	np	np
13	+	+	–	–	–	–
14	+	+	–	–	–	–
15	–	–	np	np	np	np

Explanation: Results of Baermann's method (B) and *Angio Detect Test IDEXX* (ADT) tests at days (D) – 15 pre-treatment with moxidectin and +14 and + 28 post-treatment with moxidectin. + positivity; – negativity; np: not performed.

an early stage of the infection (as supported by the lack of clinical signs) and/or the formation of antigen-antibody complexes, which may inhibit the detection of *A. vasorum* antigens, as described also for *Dirofilaria immitis* (SCHNYDER et al. 2014). Overall, it has been recently shown that this kit has a specificity of ~100% and a sensitivity of ~85%, which may lead to negative results in dogs positive at the BAERMANN'S test (SCHNYDER et al. 2014). Importantly, further studies on a large scale are necessary to evaluate in field conditions the concordance between the results of the Baermann's test and the rapid kit, in both clinically and subclinically infected dogs.

The results of the present study confirm the possible occurrence of subclinically infected dogs with no apparent signs compatible with the infection. A diagnosis of angiostrongylosis should be always considered in the presence of compatible clinical signs (TRAVERSA et al. 2013). However, dogs living in endemic areas, especially young animals and those that usually eat mollusks, should be routinely screened for *A. vasorum* even in absence of clinical signs. In fact, young dogs are more susceptible to the infection for their age-related level of immunity (TRAVERSA and GUGLIELMINI 2008, FERDUSHY and HASAN 2010). The *Angio Detec*^{TM} *Test* is particularly suitable for this purpose, as it can be directly used in veterinary practices and it represents a valid tool for a quick diagnosis for its high values of sensitivity and specificity (SCHNYDER et al. 2014).

Furthermore, the *Angio Detect*^{TM} *Test* is a powerful tool in dogs with clinical signs and needing a prompt anthelmintic treatment. In fact, this test may provide results in fifteen minutes, while the Baermann's test requires at least 24 hours. On the other hand, dogs presenting clinical signs compatible with angiostrongylosis but negative at the *Angio Detect*^{TM} *Test*, should be examined with the Baermann's method before excluding the infection.

A reliable diagnosis of dog angiostrongylosis is of great importance under a practical standpoint. In fact, despite the severe pathogenic impact of *A. vasorum*, the available parasiticide options are straightforward and effective (TRAVERSA et al. 2010). Hence, it is noteworthy that the present study confirmed the high efficacy of moxidectin contained in Advocate® for the therapy of canine angiostrongylosis (WILLESEN et al. 2007).

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