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Angiostrongylus vasorum infection in dogs: Presentation and risk factors

E.R. Morgan[a,*], R. Jefferies[a,b], L. van Otterdijk[a,c], R.B. McEniry[a], F. Allen[a], M. Bakewell[a], S.E. Shaw[b]

[a] *School of Biological Sciences, University of Bristol, UK*
[b] *School of Clinical Veterinary Science, University of Bristol, UK*
[c] *School of Veterinary Science, University of Utrecht, The Netherlands*

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ABSTRACT

Infection with the nematode *Angiostrongylus vasorum* is an emerging cause of canine disease in Europe and part of North America, yet published data on its epidemiology in endemic areas are lacking. This study tested faecal samples from 897 dogs attending veterinary practices in the southern part of Great Britain, a long standing endemic focus. Among 790 dogs presenting with respiratory or other signs broadly suggestive of angiostrongylosis, 16% tested positive on a single Baermann's examination, compared with 2% of healthy dogs in the same catchment areas. Risk factors for positive tests included age (higher risk in younger dogs), season (more cases earlier in the calendar year), and worming history (lower risk if given milbemycin oxime in the past 12 weeks). Sex, neutering status and breed were not significant in terms of risk of testing positive. The most common clinical signs in infected dogs were respiratory, along with non-specific signs such as lethargy and exercise intolerance, while bleeding, neurological and gastrointestinal signs were also recorded. Around half the dogs sampled that showed signs of extra-pulmonary disease also had respiratory signs. Direct faecal smears and Baermann's tests read after one hour detected 56% and 83% of diagnosed cases respectively. The data confirm that *A. vasorum* is commonly associated with disease in endemic areas, which manifests with a broad range of signs at primary care level. Information on risk factors is useful in diagnosis and control, and forms a basis for further epidemiological investigation.

1. Introduction

The metastrongyloid nematode *Angiostrongylus vasorum* is parasitic in the heart and pulmonary arterial circulation of dogs and foxes. Clinical consequences range from mild respiratory disease to severe dyspnoea or coagulopathies, which can be fatal, as well as a wide range of other signs including neurological and gastrointestinal disease (Koch and Willesen, 2009; Helm et al., 2010). Presentation of the patient and prevention of the disease are not straightforward. Although long established in Europe and South America (Bolt et al., 1994), the parasite is spreading locally and through longer range introductions (Morgan et al., 2005; Jefferies et al., 2010). Given that the potential range of the parasite in terms of climatic suitability for transmission is probably much greater than that currently occupied (Morgan et al., 2009), it is fair to assume that an increasing population of dogs will be at risk of disease in future.

Recent studies primarily concern arrival and expansion of the parasite in new countries or regions (Bourque et al., 2008; Traversa et al., 2008; Barutzki and Schaper, 2009; Helm et al., 2009; Taubert et al., 2009; Yamakawa et al., 2009), disease in new definitive host species (Bridger et al.,

* Corresponding author. Tel.: +44 1179287485; fax: +44 1173317985.
E-mail address: eric.morgan@bristol.ac.uk (E.R. Morgan).

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2009; Patterson-Kane et al., 2009; Bertelsen et al., 2010), and new options for diagnosis (Traversa and Guglielmini, 2008; Verzberger-Epshtein et al., 2008; Jefferies et al., 2009; Schnyder et al., 2009a) and treatment (Conboy, 2004; Willesen et al., 2007; Schnyder et al., 2009b). However, there is a need to more fully understand the epidemiology, to support efforts to control infection and disease. Case reports and series have tended to focus on the more severe and interesting clinical presentations (Brennan et al., 2004; Chapman et al., 2004; Humm and Boag, 2008; Negrin et al., 2008; Sasanelli et al., 2008; Willesen et al., 2008; Schmitz and Moritz, 2009; Denk et al., 2009), and the extent to which these reflect the picture in first opinion (primary care) veterinary practice remains uncertain. This paper sets out to document the most common presenting signs in infected dogs at primary care level, and to determine risk factors for infection, in the interests of improved detection and control. The performance of quick methods for detection of larvae in faeces is also assessed, since such tests could prove useful in diagnosis or screening.

2. Materials and methods

2.1. Sampling regime

The study lasted from 2005 to 2008. Veterinary practices throughout England, Wales and Scotland were invited to participate in the study by personal contact, advertisement in the national veterinary press, and through visiting pharmaceutical representatives. Clinicians were asked to submit a single faecal sample from dogs that presented with respiratory, cardiovascular, neurological or bleeding disorders, which were not easily accounted for by a specific non-parasitological diagnosis. The case definition was therefore intentionally broad, especially since cardiovascular signs included lethargy, exercise intolerance or syncope. In addition, participating clinicians were asked, throughout the study and in all regions, to submit samples from a few healthy dogs presenting for elective procedures. This control population was supplemented by collections directly from dogs being exercised by their owners in the catchment areas of the same practices.

2.2. Questionnaire

Veterinarians were asked to complete a simple questionnaire for each dog, at the time of sampling. This included the date and information on the dog's breed, age, sex, neutering status, and worming history. Clinical signs on presentation were noted by the veterinarian using closed questions on the presence of coughing, dyspnoea, haemorrhage, collapse, exercise intolerance, gastrointestinal disorder, lethargy, neurological signs, or ocular abnormalities, and an open section for additional comments. Veterinarians were also invited to judge the likelihood of a diagnosis of *A. vasorum* infection on a six-point scale of 0 (not sick, healthy control) to 5 (very likely to be angiostrongylosis). Sample containers and pre-paid envelopes were provided, and samples dispatched on the day of collection.

2.3. Modified Baermann's test

In the laboratory, faeces were weighed, and the entire sample placed in a modified Baermann's apparatus, consisting of a muslin bag suspended in tap water in an inverse conical beaker, and left overnight at room temperature. The bag was then removed and water siphoned from the top of the beaker until the lower 15 ml remained, including the sediment. This was transferred to a test tube and centrifuged at 1500 rpm for two minutes. The sediment at the bottom of the test tube was removed, placed on a cavity slide, and a cover slip (18×18 mm) added. The slide was examined under the microscope until larvae were found, or until the entire area under the cover slip had been examined. First stage larvae of *A. vasorum* were identified on the basis of their size and characteristic tail morphology (Guilhon and Cens, 1973; McGarry and Morgan, 2009). For a subset of samples, a direct faecal smear was also prepared and examined prior to the Baermann's test, and in some cases the Baermann's test was read after one hour, with the faeces transferred to a fresh beaker and the test read again the next day as above.

2.4. Statistical analyses

The relationship between aspects of the dogs' histories and the chance of a sample testing positive for *A. vasorum* larvae was assessed using binary logistic regression. Factors that were not significant were excluded in turn until only significant factors remained. Missing values for age were replaced with the mean before analysis. The effect of preventative worming treatment was initially included as a categorical variable of wormed or not wormed, then investigated further by replacing this variable with the specific anthelmintic drugs fenbendazole, milbemycin oxime or moxidectin, all of which have been demonstrated to have some effect against *A. vasorum* (Conboy, 2004; Willesen et al., 2007), and a category for other drugs or those not specified. At the time of the study, veterinary recommendations for preventative worming generally comprised treatment every 12 weeks. Dogs given anthelmintics more than 12 weeks before sampling were considered to be not wormed, since in these cases there would be ample opportunity for re-infection. This was appropriate because the aim was to determine the extent to which routine, regular preventative worming reduced risk of testing positive, and very few dogs were claimed to be wormed regularly at intervals of more than 12 weeks. Dogs wormed in the week before sampling were also excluded from this analysis since they were likely to be under treatment for active *A. vasorum* infection, which could confound results given the tendency of larvae to disappear slowly from the faeces following effective treatment. Finally, the effect of age was further assessed by replacing the continuous variable age with categories representing the 1st, 2nd-3rd, and 4th quartiles, so that the relative risk of testing positive in different age groups could be determined. To check that healthy controls were representative of the sampled population, and not biased with respect to month, region, breed, age, sex or neutered status, logistic regression was conducted with these explanatory variables and the response variable