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Autochthonous *Angiostrongylus vasorum* in Finland

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ABSTRACT

Angiostrongylus vasorum has spread farther north in Europe. In this study, two autochthonous findings from dogs from Finland are described: in February 2014, the infection was diagnosed in a 10-month-old labrador retriever, and in February 2017, in a three-year-old French bulldog. These diagnoses were based on direct detection of the larvae from faeces of the dogs. The dogs had no history of travel to or import from abroad; the first lived in Southern Finland and the other in Western Finland, about 150 km apart. The dogs had no clinical signs attributable to angiostrongylosis. An online questionnaire was used to survey the extent to which veterinarians in Finland have self-reportedly observed canine *A vasorum* infections. A total of 38 veterinarians authorised to work in Finland answered the questionnaire in December 2017, and 9 (24%) of them reported having seen one or more dogs with *A vasorum* infection in Finland. The results suggest that at least five individual dogs with *A vasorum* infection would have been seen in Finland, three of which had an apparently autochthonous infection. While the geographical distribution of *A vasorum* in Finland remains largely unknown, findings have started to appear from domestic dogs. It remains possible that some veterinarians could have misdiagnosed, for example, *Crenosoma vulpis* larvae as those of *A vasorum*, and the findings without confirmation using antigen test could be due to coprophagy and passage of ingested larvae; however, this does not change the main conclusion that can be made: *A vasorum* is already multifocally present in Finland. Increasing awareness about *A vasorum* is important in areas where it is emerging and spreading.

INTRODUCTION

Angiostrongylus vasorum is a metastrongyloid nematode of domestic dogs and other animals. The parasite resides in the pulmonary blood arteries and the heart of its definitive hosts and has an indirect life cycle with gastropods such as snails and slugs as intermediate hosts. *A vasorum* is of high veterinary importance as a parasite of domestic dogs, because it can cause a complex disease with at worst life-threatening manifestations, including severe respiratory distress, coagulopathy and seizures.^{1–3}

In recent years, while *A vasorum* has been increasingly recognised as a cause of clinical disease in dogs in the known endemic areas,

it has also spread to new areas.^{4–8} The Nordic-Baltic region in northeastern Europe is currently an interesting region in this regard, as it includes a country where the parasite is common and well known (Denmark) as well as countries where the parasite has been rarely reported (eg, Finland).

Literature search found no published reports of *A vasorum* infection in domestic dogs from the Baltic countries Estonia, Latvia and Lithuania. In Estonia, which is located south of Finland, *A vasorum* has been described in three of 105 investigated red foxes (*Vulpes vulpes*) and three of 240 raccoon dogs (*Nyctereutes procyonoides*) that were hunted in 2010–2012.^{9–10} No *A vasorum* infections were detected in 34 wolves (*Canis lupus*) in Latvia,¹¹ nor in 310 red foxes and 99 raccoon dogs in Lithuania.¹²

A vasorum has been found in domestic dogs and in foxes in Denmark^{13–16} and in Sweden.^{17–18} In Denmark, 2.2% of faecal samples from hunting dogs tested positive for *A vasorum*,¹⁵ and the prevalence has been estimated to be up to 80%–90% in red foxes in Northern Zealand.^{13–16} In Sweden, 0.7% of faecal samples from domestic dogs tested positive for *A vasorum*, while the infection prevalence was 0.3% in red foxes.¹⁸ No cases in domestic dogs have been reported from Norway, where *A vasorum* was detected in a red fox for the first time in 2016.¹⁹

In Finland, *A vasorum* was described in red foxes already in the 1960s.^{20–21} More recently, it was described in a single red fox from Pyhtää, which is located in Southern Finland, in the Kymenlaakso region.²² Literature search yielded a single case report of *A vasorum* infection in a domestic dog from Finland; the infection was diagnosed in a boxer that had been imported from France.²³

The aim of this study was to obtain insight into the extent to which autochthonous canine *A vasorum* infections have been observed by veterinarians in Finland.



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MATERIALS AND METHODS

Case descriptions

With consent from the dog owners, apparently autochthonous *A. vasorum* findings from two dogs (dog 1 and dog 2) from Finland are described. The clinical approach, from diagnosis to treatment, remained unaffected by this retrospective observational study; that is, the diagnostic approaches and treatment choices are reported based on the medical files of the dogs.

Questionnaire

An online questionnaire was designed using Google Forms (online supplementary file 1). The questionnaire was in Finnish and in English. Participants were recruited via three closed social media groups and one closed online discussion forum for veterinarians and by mentioning the study during a talk given at the national Annual Veterinary Congress on December 13, 2017. The period for answering was from December 1 to December 15, 2017. Participation was voluntary and anonymous, and no personal information was collected. The participants gave consent to having their responses used by submission of these after completing the questionnaire.

The first question, whether the participant was a licenced veterinarian in Finland, served as the inclusion criterion: those answering 'Yes' were included in the study. The participating veterinarians were asked whether they had seen one or more dogs that had *A. vasorum* infection in Finland. The answer options were 'Yes', 'No' and 'I am not sure'. Those answering 'Yes' received further questions about the number of dogs, and whether the infections were probably acquired in Finland (ie, no history of travel to or import from abroad). Answering these further questions was voluntary.

Statistical analyses

The CIs (Mid-P exact) for the proportions were calculated using OpenEpi software.²⁴

Map

A map was created in Google Maps. The closest town or city mentioned was used to place the cases on the map.

RESULTS

Case descriptions

Dog 1

Dog 1, a female labrador retriever, was 10 months old at the time of diagnosis in February 2014. It was born in Finland and had no history of travelling abroad. The dog was living in Southern Finland, in the Uusimaa region, in Kirkkonummi, which is located 60°07'N 24°26'E, approximately 30 km to the west from the capital Helsinki. The reason why the dog had been taken to a general health examination was coprophagy; the dog had a habit of eating faeces of its own, of other dogs and of wild animals. At the time of examination, it was winter, and the ground was covered with snow. The dog had no clear clinical signs of illness.

A faecal sample collected over three consecutive days was sent to a commercial laboratory (IDEXX laboratories). Faecal flotation was negative, but larvae of *A. vasorum* were detected with the Baermann method. The larvae present in the faeces of this dog were confirmed as *A. vasorum* based on morphological key characteristics also by two veterinary parasitologists.

On clinical examination, otitis externa was diagnosed; otherwise, the dog was clinically in good health. Complete blood count, blood biochemistry and coagulation factors prothrombin time and activated partial thromboplastin time were within reference intervals. Radiographs of the thorax, in three views, showed no abnormalities.

The dog was treated with imidacloprid and moxidectin spot-on (Advocate, Bayer Animal Health) and was retested a month later for *A. vasorum* with a negative result. Since then, the Baermann test has been repeated once a year, and results have been negative.

Dog 2

Dog 2, an intact female French bulldog, was three years old at the time of diagnosis in February 2017. It was born in Finland and had never travelled abroad. The dog was living in Western Finland, in the Pirkanmaa region, in Tampere, which is located 61°30'N 23°46'E, approximately 160 km northwest of the capital Helsinki.

The dog was fed a raw diet. According to its owner, the dog had had a short episode of diarrhoea and vomiting in November 2016, but since then it had been apparently healthy. The dog was taken to the clinic for a check-up for internal parasites.

A faecal sample was sent for flotation to a commercial laboratory (IDEXX laboratories). The result was positive; *A. vasorum* larvae were detected. One week later, a second faecal sample, collected over three consecutive days, was sent for analyses. The result of the flotation test was negative, with 'side finding: *Eimeria* species oocysts of herbivores (intestinal passage or secondary contamination)', but *A. vasorum* larvae were detected using the Baermann test.

No abnormalities were found on clinical examination. Cardiac and respiratory auscultation were normal. Radiographic and echocardiographic examinations were performed following the second faecal sample. The thorax radiographs showed mild alveolar and interstitial infiltration in the lungs. In the echocardiographic examination, the heart was found normal. Two parallel hyperechoic lines were noted in the right ventricle in right-sided long-axis and short-axis views.

A third faecal sample from the dog was available for additional testing with molecular methods at Statens Serum Institut, Copenhagen, Denmark. This sample was a new sample collected after the results from the two earlier samples were available but before treatment was started. It was submitted to DNA extraction and analysis for nematode DNA using a targeted conventional PCR (primers Angiostrong_F 5'-CCGTTCTTAGTTGGTGGAGC-3' and Angiostrong_R 5'-AATCCCTATCCCGGTCAC-3') and

a 16S/18S-based PCR approach followed by Illumina sequencing (primer sequences available in online supplementary data²⁵). No *A. vasorum* DNA nor other nematode DNA was detected by these molecular approaches.

The dog was treated with milbemycin oxime tablets (Interceptor vet, Elanco Animal Health) orally, 0.55 mg/kg four times once a week. Two weeks after completed treatment, a faecal sample collected over three consecutive days was tested using the Baermann method. The test result was negative.

Questionnaire survey

A total of 38 veterinarians completed the questionnaire. Of these, nine (24%, 95% CI 12% to 39%) reported that they had seen one or more dogs with *A. vasorum* infection in Finland, 28 had not seen a dog with the infection and one was not sure.

Assuming no overlap among the questionnaire-reported cases and including the unsure case, the veterinarians who participated had reportedly seen altogether at least 13 dogs with *A. vasorum* infection in Finland. Six of the veterinarians reported seeing a single dog with the infection, two reported seeing two dogs with the infection and one reported seeing at least two dogs with the infection. Of the dogs with the infection, two dogs were mentioned to be imported as rescue dogs and one dog had visited France. The veterinarian reporting uncertainty on seeing a dog with the infection described that a single larva, suspected to be *A. vasorum*, had been observed 10 years ago in a faecal sample of a canine patient in an intensive care unit. This dog had had lung problems and was euthanased.

Three veterinarians reported having seen a single dog with *A. vasorum* infection without a history of travel to or import from abroad, and one veterinarian reported having seen one or two such dogs. One of the dogs was explicitly reported as one of the cases described in this article. Another one was mentioned to be from a municipality in Southern Finland, the Uusimaa region, and to have had the habit of eating snails and slugs and to live in a house with a yard seen visited by red foxes. No details were provided for the other reported apparently autochthonous cases. A total of four to five dogs were thus questionnaire-reported as probably having acquired *A. vasorum* infection in Finland.

Summary of the described and the questionnaire-reported cases

A. vasorum findings in two dogs (dog 1 and dog 2) are described in this article, and altogether at least 13 dogs with the infection were questionnaire reported. One of the described cases was also a questionnaire-reported case. Assuming no other overlaps, the results suggest that there would have been at least 14 dogs with *A. vasorum* infection.

The two cases described in this article were considered autochthonous infections, and four to five dogs with autochthonous infection were questionnaire reported.



Figure 1 A map showing where autochthonous *Angiostrongylus vasorum* infections or findings have been observed in Finland. Yellow dots indicate the places where the three domestic dogs lived, and the orange dot indicates the location where the recently described infected red fox (*Vulpes vulpes*) originated from.²² The map was created using Google Maps.

As one of the described cases was also questionnaire reported, assuming no other overlaps, the results suggest that there would have been at least five dogs with apparently autochthonous *A. vasorum* infection.

Counting only the dogs that were described to an extent so that overlap between them could be confidently ruled out, the results suggest that at least five individual dogs with *A. vasorum* infection would have been seen in Finland, including three individual dogs with an apparently autochthonous *A. vasorum* infection.

The distance between the places where dog 1 and dog 2 live is about 160 km, while the municipality mentioned for one of the questionnaire-reported dogs with an apparently autochthonous *A. vasorum* infection is about 25 km from the place where dog 1 lives. These three locations and the location where the recently described infected red fox originated from²² are shown in figure 1.

DISCUSSION

Together, the findings described from dog 1 and dog 2 illustrate that autochthonous *A. vasorum* findings have been observed in domestic dogs in Finland in the recent years, and the results of the questionnaire study add to the knowledge on the current situation. The *A. vasorum* infection diagnosed in 2014 in dog 1 is considered the first autochthonous infection in a domestic dog in Finland.²¹

The parasite is present in Finland, but it needs to be emphasised that the occurrence of true autochthonous infections in dogs could not be ultimately confirmed nor their frequency estimated in this study. Increased awareness and applying specific diagnostic approaches are necessary to acquire more clinical and epidemiological data.

More than 70% of the veterinarians participating in the survey had reportedly not seen a dog with *A vasorum* infection. The true proportion may be higher, as the title of the questionnaire included the name of the parasite, and therefore, the questionnaire might have been more appealing to veterinarians who had in fact seen a dog with the infection. A higher number of veterinarians participating could have been obtained by keeping the questionnaire open for a longer period than two weeks or using additional approaches to recruit participants. We did not confirm that the participants were veterinarians, but the recruiting took place within closed discussion forums of veterinarians and at a professional event of veterinarians, the target group was explicitly mentioned in the questionnaire and the participants were asked whether they were veterinarians licenced in Finland at the very beginning of the questionnaire. Moreover, the questions used medical expressions, making it less likely for any non-veterinarians to have participated. The number of participants was limited, and the results should thus be interpreted cautiously. Veterinarians who are active on social media and attending further professional education can be over-represented.

It should be emphasised that the questionnaire survey was designed to obtain an impression of the proportion of veterinarians who self-reportedly had seen a dog with the infection, not the proportion or number of dogs that had the infection. Overlap is possible, as the same dog may have been seen by several veterinarians and the cases were not traced. Moreover, recall bias is possible, and the possibility of incorrect diagnoses is not ruled out, as details were not asked. In particular, some veterinarians could have misdiagnosed, for example, *Crenosoma vulpis* larvae as those of *A vasorum*. However, *C vulpis* is expected to be quite well known to the veterinary profession in Finland.^{21 26} Moreover, some findings could have been due to passage of ingested larvae from faeces of other infected hosts, such as red foxes.

Despite limitations of questionnaire-based studies, they can be useful for obtaining an updated overview of spread of a disease or pathogen^{8 27} as well as for formulating further research questions or planning monitoring. For example, a questionnaire survey in UK in 2009 confirmed that *A vasorum* had spread beyond previously known endemic foci.⁸ Repeated questionnaires could be used to monitor changes and trends. For emerging and spreading diseases and pathogens, questionnaire-based studies could also serve to increase awareness. This is of particular relevance for pathogens that can cause aspecific clinical signs such as *A vasorum*.

Dog 1 and dog 2 shared some similarities. Their owners were exemplary in taking the dogs to a veterinarian for a parasitic infection check-up. Both dogs were relatively young, and the diagnoses were made during the cold season, winter (February). These aspects are in line with the known risk factors for *A vasorum* infection in dogs, which include young age, winter and spring seasons and lack of a recent deworming history.²⁸ Dog 1 had the habit of coprophagy, and finding oocysts of *Eimeria* species of herbivores in one sample from dog 2 is suggestive of coprophagy, as the oocysts are shed in faeces. Coprophagy might predispose to ingesting other objects, including snails and slugs. An alternative explanation for the detected larvae could be their passage through the gastrointestinal tract of these dogs, after ingestion of faeces of, for example, an infected fox. This explanation is supported by the lack of clinical signs in both dogs and the negative result from the third faecal sample from dog 2. The findings nevertheless show the local presence of the parasite because the faeces eaten were likely from local animals. For one questionnaire-reported case, eating snails and slugs was specifically mentioned. In Finland, pet dogs are kept mainly indoors and under surveillance or in a leash when outdoors; preventing them from eating snails and slugs should be encouraged.

In severe cases, *A vasorum* can cause acute life-threatening manifestations in dogs.¹⁻³ For this reason, it is of clinically high importance to reach or rule out the diagnosis of angiostrongylosis quickly. Neither dog 1 nor dog 2 had obvious clinical signs attributable to angiostrongylosis at the time of diagnosis nor during follow-up, and *A vasorum* infection was originally a suspected diagnosis in neither of the dogs. The echocardiographic observation mentioned for dog 2 is not typical for *A vasorum*, and it could have been an artifact; *Dirofilaria immitis* was unlikely, as autochthonous *D immitis* infections have not been reported from Finland. Both diagnoses were based on copromicroscopic examination, and for example, antigen test could have been a good addition to support infection versus passage of larvae. That the findings from dog 2 were not confirmed by molecular methods that were applied directly to a new, single faecal sample could also support the explanation of earlier passage of ingested larvae, or it could have been due to low sensitivity of the approach. Collected larvae obtained by the Baermann method would have been better material for molecular confirmation of the species of the parasites detected.

Both dogs had larvae in their faeces which, assuming the dogs were indeed infected, exemplifies the potential contribution of dogs with undiagnosed, subclinical infection to the spread of *A vasorum*. *A vasorum* is yet another reason to encourage early diagnosis of parasitic infections as well as collection and appropriate disposal of canine faeces. This is of particular relevance in countries like Finland, where dogs do not generally receive any antiparasitic treatment routinely, for example, monthly.

The treatment selected for the two dogs was different: dog 1 was treated with imidacloprid and moxidectin spot-on,²⁹ and dog 2 was treated with milbemycin oxime tablets orally,³⁰ four times once a week. The products that were used were both licenced for treatment of *A. vasorum* infection in Finland.³¹ For another product that includes milbemycin oxime and praziquantel (Milbemax vet., Elanco Animal Health), two *A. vasorum* administration protocols are mentioned: administration four times once a week for treatment of *A. vasorum* infection, preferably changing to a product including only milbemycin oxime after the first dose, and administration repeatedly once in four weeks for preventative reduction of the number of parasites in areas where *A. vasorum* is common and treatment against tapeworms is also indicated.³¹ According to international guidelines, the combination of imidacloprid and moxidectin is currently considered the treatment of choice for *A. vasorum* infection, while milbemycin oxime can be used for reduction of the parasitic burden.³²

Because canine angiostrongylosis belongs to the list of differential diagnoses for dogs with a wide range of clinical signs, including aspecific clinical signs, raising awareness about it is important in regions where it is emerging. Veterinarians need to be aware of pathogens and diseases approaching or imported to their region and prepared to suspect, reach and confirm the diagnoses. The copromicroscopic approach used for dogs 1 and 2 cannot distinguish between infection and findings due to ingestion of faeces of infected animals, for example, red foxes. Antigen test in parallel could increase sensitivity and support the presence of infection,³² while molecular methods can be used to confirm the parasite species. It is worth emphasising that when suspecting an emerging infection, the diagnostic approach should rule out all relevant differential diagnoses and aim for a specific diagnosis that is preferably confirmed. This can be challenging if the internationally recommended tests are not locally available.

The distribution maps of diseases and pathogens are in constant change, which poses challenges to veterinarians.²⁷ The results of this study highlight that *A. vasorum* should be kept in mind also in the northernmost parts of Europe. Based on the results of this study, *A. vasorum* appears to be present at least in the southern and western parts of Finland, but this should be supported by more parasitological baseline data. It would be useful to investigate the extent of the geographical spread and estimate the prevalence, for example, by examining red foxes. However, already based on the results of this study, increasing awareness about *A. vasorum* is crucial in Finland, for early diagnosing of the infections in dogs and for preventing further spread.

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