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Canine angiostrongylosis in Sweden: a nationwide seroepidemiological survey by enzyme-linked immunosorbent assays and a summary of five-year diagnostic activity (2011–2015)

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Abstract

Background: For the first time in Sweden, Angiostrongylus vasorum was detected on the island of Sydkoster in foxes and dogs in 2003. After sporadic detection of the parasite in foxes in southern Sweden, the first positive canine faecal sample on the mainland was found in 2011. Since then a total of 2882 faecal samples have been analysed with the Baermann test at the National Veterinary Institute (SVA) during the years 2011–2015; 20 of them being positive. Contemporaneously, of over 525 fox necropsies, only three were found to be infected. To gather a more accurate knowledge of A. vasorum occurrence in Sweden, a large scale seroepidemiological survey was performed and totally 3885 serum samples from dogs were tested for both the presence of circulating antigens and of specific antibodies to A. vasorum.

Results: In total, 0.10% (n = 4, 95% Confidence Intervals, CI 0.03–0.26%) of the dogs were positive for both antigen and antibodies, whereas 0.51% (n = 20, CI 0.31–0.79%) of the tested dogs were only antigen positive and 0.88% (n = 34, CI 0.61–1.22%) only positive for specific antibodies. Seropositive animals, as well as the majority of A. vasorum-positive faecal samples tested during the same period, were spread over central and southern Sweden. Annual prevalence of positive faecal dog samples and of necropsied A. vasorum positive foxes (coming from southern Sweden) varied from 0.3 to 0.9% (overall: 0.7%, CI 0.4–1.1%) and 0.0 to 1.4% (overall: 0.3%, CI 0.1–0.9%), respectively.

Conclusions: The findings confirmed that A. vasorum has become established in various geographical areas of central and southern Sweden. Veterinarians and dog owners should be aware of the potential risks of infection in large areas of the country, since canine angiostrongylosis may be a fatal disease if left untreated.

Keywords: Angiostrongylus vasorum, Antigen, Antibodies, Serology, Dog
for a potentially fatal outcome of the disease in dogs. Clinical manifestations of coagulopathy can range from subcutaneous haematomas, epistaxis to neurological signs depending on the site of bleeding. The lung damage is related to microinjuries and inflammatory processes taking place both when eggs of *A. vasorum* are caught in the lung capillaries and when the hatched larvae migrate to the air passages. Tissue injuries intensify and may lead to the classical textbook clinical presentation of the disease characterized by dyspnoea, exercise intolerance, coughing, followed by lethargy and weakness [6].

A more accurate description of the geographical distribution of *A. vasorum* can be obtained using detection methods characterized by high sensitivity and specificity; such a description can be useful to promote better prevention measures and to increase disease awareness in areas where the risk of infection is present [7]. Traditionally the diagnosis relies on the detection of first stage larvae (L1) from faeces, but the excretion of L1 only takes place when lung lesions have already developed and furthermore, the Baermann technique has limitations in sensitivity and specificity [7]. For these reasons, other techniques such as Polymerase Chain Reaction (PCR) and serology have been developed and tested on individuals and—in the case of serology—in large scale studies. *A. vasorum* PCR protocols using specific primers targeting the ITS-2 region are usually able to detect a single L1 of *A. vasorum* [8–10]. Enzyme-Linked Immunosorbent Assays (ELISAs) have also developed to detect circulating parasite antigens [11] or antibodies against the parasite in serum samples [12, 13]. The diagnostic approach usually follows or completes a clinical assessment of individual patients, while large scale surveys are conducted on large numbers of randomly collected samples from individuals, distinguishing between infected and non-infected animals and therefore giving information on the occurrence and prevalence of *A. vasorum* in a given population.

During the past decades, the endemic foci of infection have expanded and new ones have been reported. The relative importance of different factors related to this apparent spread is still unclear. Increased urbanization of the red fox acting as wildlife reservoir [14] and increased movement of domestic dogs both within and between countries [15] are certainly involved. Climatic changes influencing the distribution of intermediate hosts may also play a role [16]. Beside historically endemic areas, like southern France, part of the British Isles and Denmark, there is a list of countries in South, Central and East Europe where the presence of the parasite has consistently been recorded. Recently an increased prevalence of the parasite in foxes [17, 18] and dogs [19, 20] has been reported in e.g. the United Kingdom (UK) and in Italy. Moreover, new autochthonous cases have been described in other countries such as Belgium [21], Slovakia [22] and Serbia [23]. During the past years, it has been possible to obtain a more complete picture of parasite prevalence in dog populations through large scale serological studies carried out in some countries, for example in Germany and UK [24], Italy [25], Switzerland [26], Poland [27], Hungary [7], Slovakia [28], Bulgaria [29], Belgium [30] and Portugal [31].

In Scandinavia, *A. vasorum* was initially detected in 1983 in Denmark in a dog that had travelled several times to southern France [32]. In Danish foxes, *A. vasorum* was first reported in 1992 [33], and the prevalence in foxes in the same area (northern Zeeland) rose from 48.7% in 1993 [34] to 90% in 2002 [35]. Despite a prevalence of 9.7% of *A. vasorum* infections was found in Danish dogs in 2004 [36], an overall prevalence of 2.2% was reported in hunting dogs in 2013 [37]. In Norway the parasite was identified for the first time in a red fox in March 2016 [38]. In Sweden, *A. vasorum* infection was first diagnosed in 2003, both in a dog and in a red fox (*Vulpes vulpes*) from the island of Sydkoster (Västra Göötland county) [39], and since then further individual cases of *A. vasorum* infections have been identified in dogs and red foxes on the mainland as well.

The aim of the present study was to determine the prevalence and geographical distribution of *A. vasorum* in Swedish dogs by using serological analyses and data from routine diagnostic activity performed at the National Veterinary Institute (SVA).

**Methods**

**Prevalence study using ELISAs**

Sera of 3885 dogs were collected from the Clinical Chemistry Laboratory (CCL) of the University Animal Hospital (UDS-SLU, Uppsala) and from the SVA. Sera from CCL (*n* = 1212) were collected from February to April 2014, either from dogs attending the University Animal Hospital (*n* = 495) or were sent to CCL from other veterinary clinics of the country (*n* = 717). Sera collected from the SVA (*n* = 2674) were submitted from veterinary clinics during 2013 for the analysis of tick-borne bacterial pathogens such as *Anaplasma phagocytophilum* and *Borrelia* spp. For each sample the postal code of the residence of the dog owner was recorded. No other data could be retained due to confidentiality issues.

An aliquot of the samples was sent to the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland, for analysis for the presence of circulating *A. vasorum* antigens using monoclonal (mAb Av 56/1/2) and polyclonal antibodies in a sandwich ELISA with a sensitivity of 95.7% and a specificity of 94.0%, as previously described [11]. In brief, tests were performed in 96-well microtiter plates (Maxisorp, Nunc Roskilde,
Test thresholds were regionally determined with 300 randomly selected samples based on the mean value of optical density (A405 nm) plus 3 standard deviations from uninfected dogs, as previously described [11, 13].

After anthelmintic treatment, antibodies may remain detectable until 9 weeks post treatment [13]. Additionally, a sandwich ELISA (sensitivity 81.0%, specificity 98.8%) using A. vasorum adult somatic antigen purified by monoclonal antibodies (mAb Av 5/5) was used for specific antibody detection [14]. Briefly, plates were coated overnight at 4 °C with A. vasorum adult somatic antigen (5 µg/mL), washed and saturated. Sera were used in a standard dilution of 1:200 in PBS–T and 10% foetal calf serum; 100 µL diluted sera/well were incubated at 37 °C in a humid chamber for 1 h.

After washing steps, specific affinity-purified, alkaline phosphatase conjugated goat anti-dog IgG (γ) antibodies were used at a dilution of 1:250 in PBS–T (100 µL/well). Substrate was added after the washing steps and absorbance values obtained as described above. The ELISA has been shown to detect circulating antigens starting at 5 weeks post infection (wpi) and at 10 wpi virtually all dogs are persistently positive. After anthelmintic treatment, antigens can be detectable until 9 weeks post treatment [11].

Data from routine diagnostic activity, 2011–2015

Dogs

Faecal samples (Table 2) submitted to the SVA by veterinary clinics or private dog owners were tested with a modified larval recovery method [41]. The SVA requires at least three samples from three different droppings collected over 3 days from each dog in order to perform the analysis. All samples originated from dogs showing clinical signs, either respiratory or other clinical signs compatible with A. vasorum infection, and therefore cannot be considered representative of the general canine population.

Red foxes

Red foxes (Table 2) received at the SVA for diagnostic necropsies or submitted by hunters for pathogen screening were examined specifically for A. vasorum and lungworms by visual inspection of the cardiovascular and respiratory systems. During 2011, 3000 hunted red foxes were submitted for the national Echinococcus multilocularis surveillance program. Over 500 heart and lung sets were examined for parasites that year. A joint database was created in order to produce Table 2.

Results

A total of 0.10% (n = 4, 95%, CI 0.03–0.26%) of the dogs were positive in both ELISAs, while 0.51% (n = 20, CI 0.31–0.79%) were antigen positive only and 0.88% (n = 34, CI 0.1–1.22%) were positive for specific antibodies only (Table 1). The geographical distribution of positive samples is shown in Fig. 1. Samples positive in both tests were found only in the counties of Gävleborg, Uppsala and Kronoberg (Fig. 1).

Endemic canine cases of A. vasorum were diagnosed once in 2011 and 2012, respectively. Subsequently, the parasite was sporadically found (4–9 times per year). A progressively increasing number of faecal samples was submitted to the SVA throughout this period.

Detailed figures on the number of examined faecal samples as well as the number of foxes necropsied at the SVA between 2011 and 2015 are provided in Table 2.
Table 1 Serological results of 3885 dog serum samples from Sweden tested for the presence of circulating antigens of *Angiostrongylus vasorum* and of specific antibodies to *A. vasorum*

| Positive samples (n) | %    | 95% confidence intervals |
|---------------------|------|-------------------------|
| Antibody-positive   | 38   | 0.98                    | 0.69–1.34               |
| Antibody-positive only | 34   | 0.88                    | 0.61–1.22               |
| Antigen positive    | 24   | 0.62                    | 0.40–0.92               |
| Antigen positive only | 20   | 0.51                    | 0.31–0.79               |
| Antibody and antigen positive | 4    | 0.10                    | 0.03–0.26               |

Discussion

The first Swedish fox being positive for *A. vasorum* was identified in 2003 on the island of Sydkoster, in the county of Västra Götaland (see Fig. 1). Six years later, in the beginning of 2009, the parasite was found in the lungs of four out of 269 foxes examined within an *E. multilocularis* surveillance program; again, the positive animals originated from Sydkoster. After this finding, a targeted survey aimed at finding other foci of *A. vasorum* was performed in 2009 on 137 foxes from south-western Sweden. Eight foxes were found to be infected, of which seven were from Sydkoster (64% of the foxes collected on this island within the survey) and one from the municipality of Osby (county of Skåne), which was the first *A. vasorum* case described on mainland Sweden [42]. Even though many red foxes have been necropsied thereafter, the parasite has been identified only three additional times, in 2011, 2013 and 2014 (Table 2). The fox that tested positive in 2011 is particularly interesting because it originated from the municipality of Kalvsvik (county of Kronoberg), in the same area where one of the four dogs being both positive for antibodies and antigen was found. No double positive samples were identified in the county where the majority of positive foxes were previously found (Västra Götaland).

The implications of positive results and the performance of the two assays used in the present study have been evaluated independently by determining sensitivities and specificities [11, 13]. In the present study, the number of sera positive for antibody or antigen detection only could indicate an apparently low correlation between the two ELISAs used. However, when combined, these test parameters change; using the OR rule (the combined results are considered positive if either individual test is positive), sensitivity increases by loss of specificity, while using the AND rule (the combined results are considered positive only if both individual tests are positive), specificity reaches 99.9%, while sensitivity decreases to 82.0% [24]. Importantly, these values are influenced by the prevalence of *A. vasorum* within a determined population: the positive predictive values of the serological tests, represented by the proportion of positive test results that indicate a truly positive sample, is highest with the AND rule and this may of particular value in study areas where little is known on the occurrence of the parasite: reliability of a positive sample is high. However, at low prevalence, such high positive predictive values are reached at the expense of low sensitivity [24].

Therefore, a combined positive result indicate a dog that harbours an active *A. vasorum* infection inducing the production of antibodies; it has higher specificity than that of a single positive test (for antigen or antibody detection), although sensitivity is lower. However, as such a test combination is reflected by high positive predictive values, its use is of particular interest in areas of expected low endemicity, as it may occur in countries with relatively new introduction of the parasite like in Sweden.

This is supported by large scale studies from Germany [24] and Poland [27]: applying similar methodologies, seroprevalences of *A. vasorum* were higher than the ones detected in Swedish dogs. Intermediate prevalence figures have been recorded in Italy [25] and in the UK [24], whereas the highest prevalence so far was recorded in Hungary [7]. The seroprevalence of antibody-positive dogs in Sweden was the lowest recorded so far. Dogs positive for antibodies only may have acquired the infection recently, i.e. 3 weeks before (while antigen is detected earliest 5 wpi), but they also could be parasite-free, i.e. after anthelmintic treatment or spontaneous clearance of infection. The data confirmed that the level of exposure to the parasite in Sweden is still quite low. This is in accordance with the extremely low number of infected foxes detected so far, which are considered as a reservoir contributing to the establishment of the parasite [26, 33], as supported by constantly higher prevalences in foxes than in dogs in corresponding areas [43]. In this context it needs to be added that the difficulty to identify positive foxes with low worm burdens combined with low disease awareness in an early phase of investigations may have contributed missing positive foxes, and that dissection may not be considered as a gold standard [44].

Another indirect demonstration of the low endemic presence of the parasite in Sweden is given by the results of faecal examinations performed at the SVA,
Fig. 1 Detection of *Angiostrongylus vasorum* circulating antigens and specific antibodies in serum samples from 3885 dogs from Sweden. Dark grey areas represent the origin of the tested dog sera. The first place of finding of *A. vasorum* in Sweden (island of Sydkoster) is marked with a yellow star.
Table 2 Results from the diagnostic activity concerning *A. vasorum* related methodologies carried out at the SVA between 2011 and 2015

| Year | Groups | Numbers |
|------|--------|---------|
|      | Dogs, Baermann tested samples | 298 | 355 | 504 | 693 | 1032 | 2882 |
|      | *A. vasorum* positive canine faecal samples (%) | 1 (0.3; 0.0–1.9) | 1 (0.3; 0.0–1.6) | 4 (0.8; 0.2–2) | 5 (0.7; 0.2–1.7) | 9 (0.9; 0.4–106) | 20 (0.7; 0.4–1.1) |
|      | County of origin* | M | O | M, S, K, Y | AB, C, D, H, M | AB, BD, E, O, M, Y, Z | – |
|      | Red foxes necropsied | 525 | 88 | 164 | 70 | 100 | 947 |
|      | *A. vasorum* positive foxes (%) | 1 (0.2; 0.0–1.1) | 0 (0.0; 0.0–0.3) | 1 (0.6; 0.0–3.4) | 1 (1.4; 0.0–7.7) | 0 (0; 0.0–3.0) | 3 (0.3; 0.1–0.9) |
|      | County of origin* | G | – | O | O | – | – |

**Notes:**
- Codes for Swedish counties: AB: Stockholm; BD: Norrbotten; C: Uppsala; D: Södermanland; E: Östergötland; G: Kronoberg; H: Kalmar; K: Blekinge; M: Skåne; O: Västra Götaland; S: Värmland; Y: Västernorrland; Z: Jämtland

Conclusions

The results confirmed that *A. vasorum* is endemically present in Sweden: not limited to restricted areas such as the Island of Sydkoster and the municipality of Osby in Scania, but also in foci in widespread areas of the country. Since suitable conditions for the establishment of *A. vasorum* apparently exist in southern Sweden and potentially also in other areas of Sweden, disease awareness among practitioners, dog owners and hunters, and an according strategic deworming management is recommended, despite the currently low prevalence. This may be particularly important in the case of dogs living or visiting highly endemic areas, in order to avoid further introduction of the parasite into new areas (with the potential to create new foci), and to prevent clinical disease due to angiostrongylosis. Regular deworming should be considered in dogs which belong to “high risk categories”, such as young dogs and/or dogs having free roaming activity that show risk behaviour like indiscriminate ingestion of items from their environment.

Authors’ contributions

GG, EOL and MS planned the study, GG collected the samples, MS analysed the samples and interpreted the results. RS provided graphical materials, GG and MS wrote the manuscript. EÅ provided data and wrote sections regarding wildlife surveillance programs and occurrence of *A. vasorum* in foxes. EOL, EÅ and RS commented on the manuscript. All authors read and approved the final manuscript.

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**Competing interests**
The authors declare that they have no competing interests. Roland Schaper is employee at Bayer Animal Health, Leverkusen, Germany.

**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**
Not applicable.

**Ethics approval and consent to participate**
Not applicable.

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