

***Angiostrongylus vasorum*, “The French Heartworm”: a Serological Survey in Dogs from France Introduced by a Brief Historical Review**

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Abstract

Angiostrongylus vasorum was first described in dogs from south-western France in the nineteenth century. The life cycle of this nematode living in the heart and pulmonary arteries was also elucidated in France, leading to the byname “the French heartworm”. Since then, its occurrence has been increasingly reported from various European countries, but little is known about its distribution in France. In this first large scale survey, 2289 sera from French dogs were collected for various reasons and tested using two distinct ELISAs for the detection of *A. vasorum* circulating antigen and of specific antibodies, respectively. As much as 1.14 % of the animals (n=26, 95 % confidence intervals, CI: 0.74–1.66 %) were positive by

both ELISAs, while 0.61 % of the tested dogs (n=14, CI: 0.33–1.02 %) were antigen-positive and 2.01 % (n=46, CI: 1.47–2.67 %) were positive for specific antibodies. Regions where antigen- and antibody-positive animals came from were overlapping and distributed over the northern and southern parts of the country, while in central France relatively low numbers of positive dogs were detected. These results confirm the occurrence of *A. vasorum* in dogs originating from different parts of France. Early diagnosis with appropriate tools is essential to ensure adequate anthelmintic treatment, before the onset of fatal canine angiostrongylosis. It is therefore of great importance to improve our knowledge of the occurrence of *A. vasorum* and to maintain disease awareness.

Introduction

Angiostrongylus vasorum is a metastrongylid nematode of dogs and other wild carnivores. Canine angiostrongylosis is associated with respiratory distress, bleeding, neurological signs or several other manifestations. Although it is also present in South America, Africa and Atlantic Canada, there are different reasons why *A. vasorum* is referred to as “the French heartworm”. Its first certified description was most probably made by Serres in 1853 at the veterinary school in Toulouse, in south-western France: he described a hunting dog with nonspecific signs of apathy, loss of appetite and signs of a potential gastroenteritis, for which the animal was treated. Four days later, the dog was considered cured. At the time of discharge, for unknown reasons, a part of the tail was cut off and the dog started bleeding copiously. The injury stopped bleeding after cauterisation and no further abnormalities were observed. Nevertheless, the dog was found dead the next morning. The autopsy revealed an increased heart volume due to an increased volume of the right ventricle, among others. A huge number of worms (“entozoaires”) of approximately 15 mm in length were found in the right ventricle and the right auriculum (Serres 1854). Only two older reports are known, describing worms found in the heart of animals, from 1813 (in a dog that died in Paris) and from 1831, both summarised by Cuillé and Darraspen (1930a). Twelve years after Serres, Baillet described nematodes occurring again in the vessels and the heart of a dog and named them *Haemostrongylus vasorum* (Baillet 1866). Later, Lafosse (1868) observed cardiopulmonary and other clinical signs in chronically infected dogs, followed by studies by Laulanié (1884), in which pathological similarities between the lung lesions due to parasites and the lesions caused by the “Koch bacillus” (actually *Mycobacterium tuberculosis*) were described. Around 1900, further cases of cardiopulmonary strongyloses in dogs (reviewed by Cuillé and Darraspen 1930a) were detected. Guilhon (1963) refers

to morphological studies performed by Kamensky in 1905 and Railliet and Henry in 1907 being at the origin of the currently used binominal name *A. vasorum*, which has been used synonymously with *Haemostrongylus* and *Strongylus vasorum* for some time (Guilhon 1963).

In 1911 two students of the veterinary school in Toulouse described a fatal case of *Strongylus vasorum* in a female foxterrier presenting with a history of epileptic episodes. The autopsy revealed a haemorrhagic nodule in the left hemisphere containing a live nematode, in addition to heavily affected lungs (Capdebelle and Hussenet 1911). Interestingly, the authors already affirmed that this exceptional case represented a complication of a disease frequently observed in their area. Approximately twenty years later, two above mentioned professors of the veterinary school of Toulouse (Cuillé and Darraspen 1930 a, b) reviewed cardiopulmonary strongylosis in French dogs using both terms, *Haemostrongylus* and *Strongylus vasorum*. They mentioned then that the occurrence of this parasite was not limited to the south-west of France, as cases had been observed also in dogs from the meridional centre (departments of Aveyron and Hérault, see Fig. 1) and from further north (departments of Indre-et-Loire and Meuse and around Paris), in overall nineteen departments. Nevertheless, the life cycle was still unknown at this point: experimental transmission of the parasite by feeding of finely minced infected dog pulmonary tissues containing larvae was conducted in 1884 already and apparently successfully (Laulanié 1884). Attempts to reproduce these experiments failed: researchers from the veterinary school of Maisons-Alfort, close to Paris, were not able to transmit the infection following the same procedure. They suspected that the previously claimed successful results by Laulanié could have been due to natural infections and recommended that such trials be repeated in non-endemic areas (Railliet and Cadiot 1892). Subsequently, Neumann (1914) proposed that, in view of the restricted geographical occurrence of canine cardiopulmonary

strongylosis, larvae excreted by dogs (Fig. 2) may be transmitted through an invertebrate host distinctly occurring in the area of Toulouse. Cuillé and Darraspen (1930b), after failing to infect animals with fed infected tissue, also hypothesised the involvement of a potential intermediate host. It is only with the work performed by Guilhon and co-researchers in the 1960s that the intermediate hosts were finally identified. They first suspected slugs acting as intermediate hosts when they were asked to help eliminating or reducing the occurrence of cardiopulmonary strongyloses on two dog breeding sites in the Pyrénées Atlantiques. There, they noticed that the difference in the prevalence of infected dogs correlated with a striking slug abundance. Following experiments confirmed that the oral ingestion of *Arion* sp. from this highly endemic spot induced patent infections and severe disease in dogs from a non-endemic area (Guilhon and Bressou 1960), and that larval stages excreted by infected dogs may develop to infectious stages in local slugs given enough time and temperatures above 18 °C (Guilhon 1963). Experimental infection of slugs (Guilhon 1965a), dogs (Guilhon and Cens, 1969) and wild canids such as foxes (*Vulpes vulpes*) and the golden jackal (*Canis aureus*) (Guilhon 1965b) delivered at once highly valuable insights into the life cycle of *A. vasorum*, that had remained obscure for so long.

After France, *A. vasorum* has also been considered endemic in distinct isolated foci in Ireland (Roche and Kelliher 1968), Switzerland (Wolff et al. 1969),

south-east England and Wales (Jacobs and Prole 1975, Simpson and Neal 1982) and in Denmark (Bolt et al. 1992, Rosenlund et al. 1991). The combination of new diagnostic tools, increased disease awareness and an effective spread of the parasite with foxes acting as reservoir hosts, contributed to the publication of an exponential number of reports from several European countries in the last two decades. Recent papers from France (all from the surroundings of Paris) illustrated particular manifestations of *A. vasorum* in dogs: pulmonary hypertension was described in two dogs (Esteves et al. 2004, Nicolle et al. 2006), another one displayed ocular problems after a specimen lodged itself in the anterior eye chamber (Colella et al. 2016), while a very unusual dermatitis due to *A. vasorum* infection was observed in another animal (Cavana et al. 2015). From the veterinary school in Maisons-Alfort as well, a retrospective study evaluating the occurrence of *A. vasorum* from 2005 to 2014 identified 1-5 positive dogs per year (Bedel et al. 2016).

The aim of the present study was to illustrate the distribution of the parasite in France using serological methods.

Materials and methods

Sera of 2289 dogs from France were collected from animals coming to the veterinary clinics for different reasons. All samples were shipped to a private

Table 1 Serological results of 2289 dog serum samples from France tested for the presence of circulating antigens of *A. vasorum* (Schnyder et al. 2011) and of specific antibodies against *A. vasorum* (Schucan et al. 2012).

	Positive samples (n)	%	95 % Confidence Intervals
Antibody positive	72	3.15	2.47–3.94
Antibody positive only	46	2.01	1.47–2.67
Antigen positive	40	1.75	1.25–2.37
Antigen positive only	14	0.61	0.33–1.02
Antibody and antigen positive	26	1.14	0.74–1.66

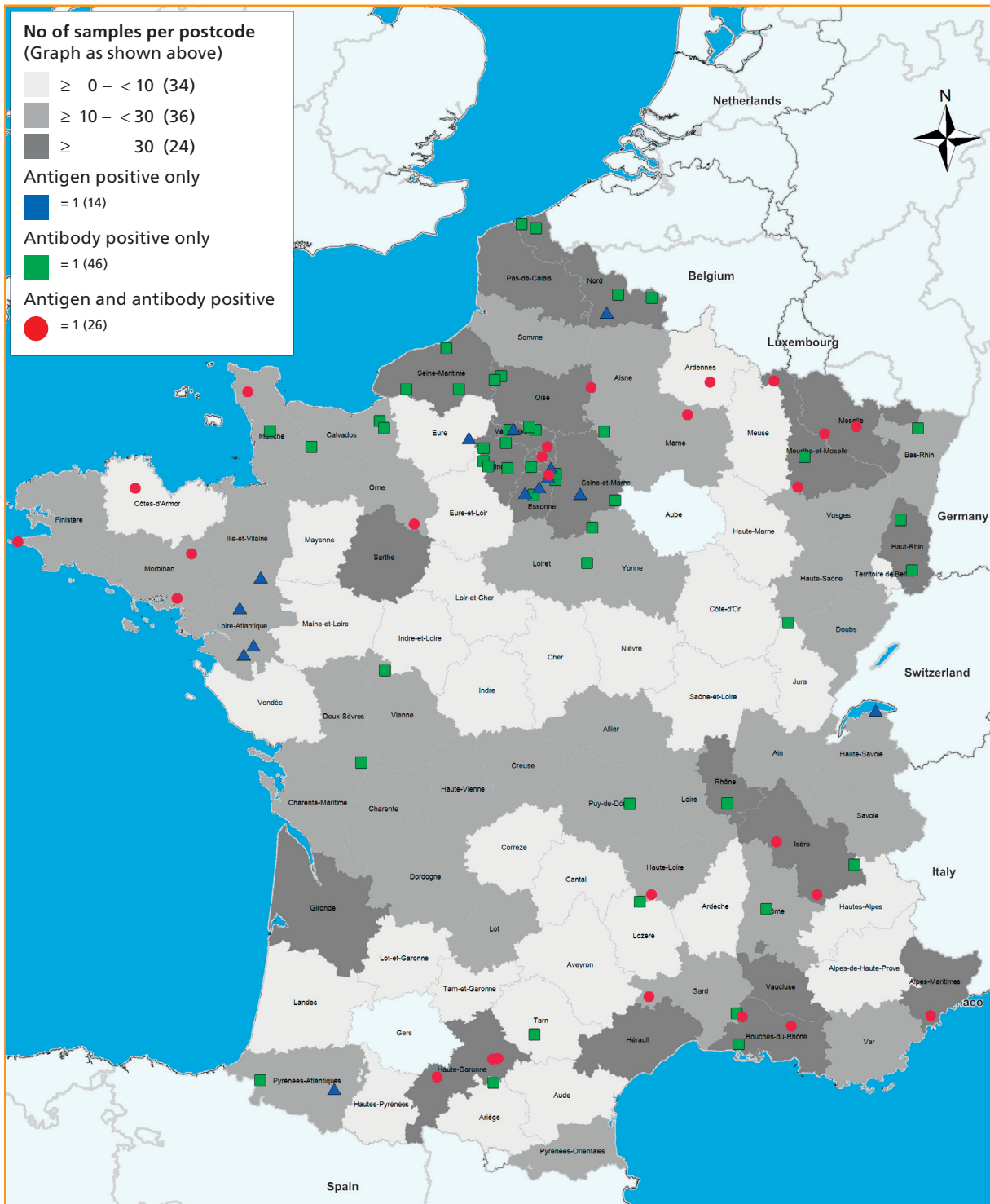


Fig. 1 Occurrence of *Angiostrongylus vasorum* in 2289 dogs from France detected by ELISA. Grey areas represent the origin of the tested dog sera. Dogs positive for detection of specific antibodies against *A. vasorum* (Schucan et al., 2012) are pictured by green squares (n=46), dogs positive for detection of circulating antigen of *A. vasorum* (Schnyder et al., 2011) by blue triangles (n=14) and dogs positive by both ELISAs are pictured by red circles (n=26).

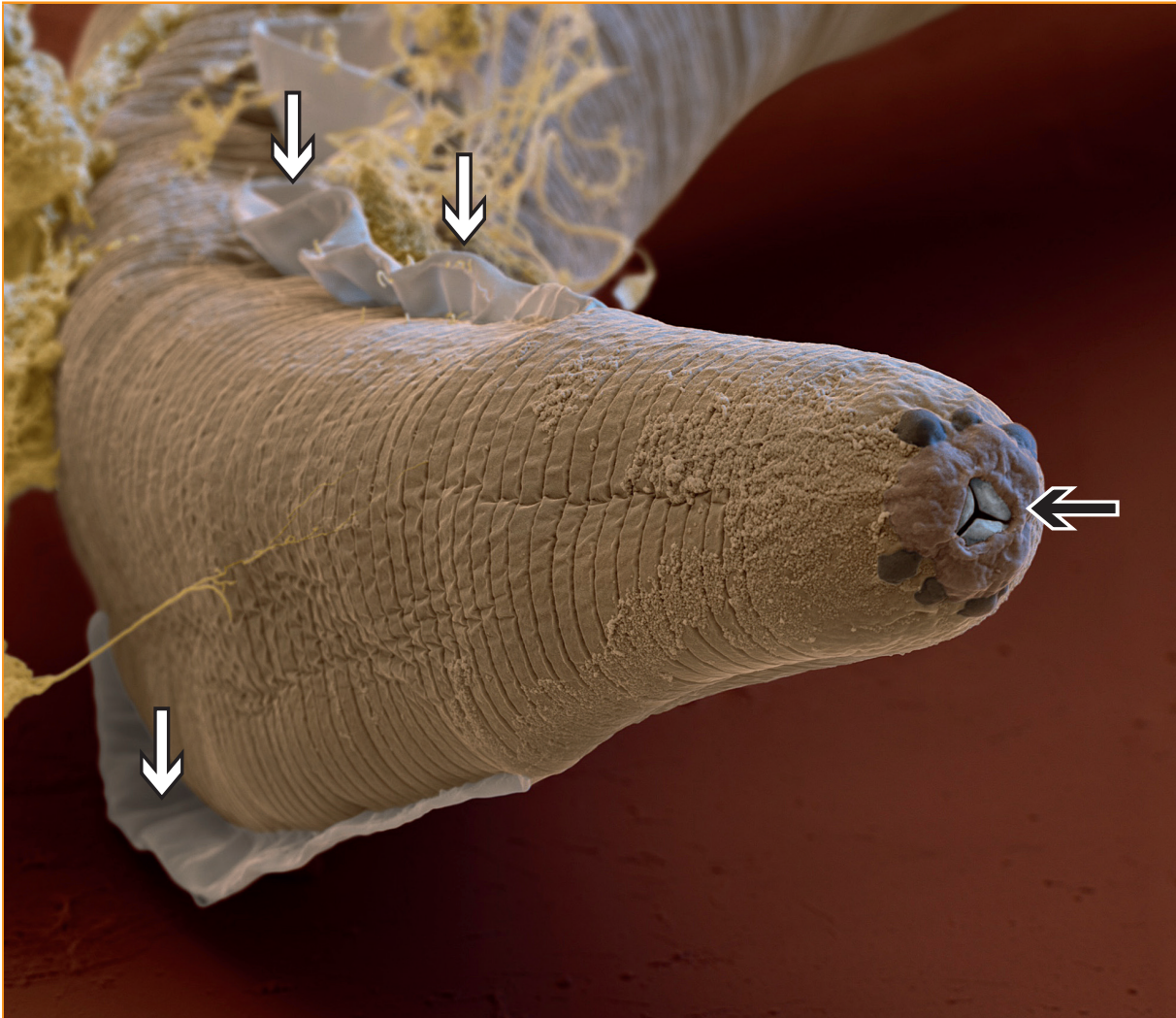


Fig. 2 Anterior part of a first stage larva of *Angiostrongylus vasorum* showing the mouth opening with 3 cutting plates (black arrow) and lateral papillae and lateral alae (white arrows) SEM 8000 x

veterinary diagnostic laboratory (IDEXX Vet Med Lab, Ludwigsburg, Germany) for different analyses. Subsamples were sent to the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland and were analysed for the presence of circulating *A. vasorum* antigens using monoclonal and polyclonal antibodies in a sandwich-ELISA with a sensitivity of 95.7% and a specificity of 94.0%, as previously described (Schnyder et al. 2011). 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 (Schucan et al. 2012). Test thresholds were regionally determined with 300 randomly selected samples based on the mean value of optical density ($A_{405\text{ nm}}$) plus 3 (antigen detection) or 4 (antibody detection) standard deviations. All test runs included a background control, a conjugate control, three positive control sera from three experimentally infected dogs and two negative control sera from uninfected dogs.

The collected data were analyzed by a geographic information system (GIS) using the program

RegioGraph 10 (GfK GeoMarketing, Bruchsal, Germany) to visualize the regional distribution of collected and analyzed serum samples and *A. vasorum* antigen and/or antibody positive samples. Based on postal codes, the locations of positive samples were displayed on maps with administrative and postcode boundaries.

Excel 2007 for Windows (Microsoft Corporation, Redmond, USA) was used to calculate the prevalence values and the 95% confidence interval (CI) of prevalence values.

Results

The seropositivity of all tested samples is resumed in [Tab. 1](#). In summary, 1.14% (n=26, CI: 0.74–1.66%) of the animals were positive in both ELISAs, while 0.61% of the tested dogs (n=14, CI: 0.33–1.02%) were only antigen-positive and 2.01% (n=46, CI: 1.47–2.67%) were positive for specific antibodies only. The geographical locations corresponding to positive dog sera are shown in [Fig. 1](#). Out of the 96 European departments of France, the tested samples originated from 94 departments (no samples tested from Gers and Aube), Regions where antigen- and antibody-positive animals came from were overlapping and distributed over the northern and southern parts of the country, while in central France, relatively low numbers of positive dogs were detected. More in detail, samples positive in both ELISAs originated from 15 departments, mostly located in northern and southern parts of France. Samples positive for antibody detection only originated from seven corresponding and additional 20 departments, including 5 departments from central France. Samples for antigen detection only originated from overlapping and from three further departments (Loire-Atlantique, Eure and Haute-Savoie). A consistent number of positive samples were detected from Paris and surroundings (Essonne, Yvelines, Val-d'Oise, Seine-et-Marne, Oise, Seine-Maritime), where also more than 30 samples per departments were tested.

Also in the south, in five departments more than thirty samples per department were investigated, including the one with the city of Toulouse (Haute-Garonne). However, in two of them (Vaucluse and Hérault) no positive samples were detected.

Discussion

The use of ELISA techniques allowed a large seroepidemiological survey, showing that this parasite is present in large areas of France. Both tests, for circulating parasite antigen and specific antibody detection, have been previously validated and compared to the copromicroscopic Baermann method and to PCR performed with tracheal swabs, blood or faeces. ELISAs were shown to deliver the earliest and most consistent results (Schnyder et al. 2015). However, the two adopted tests measure different aspects of the infection: while specific antibody detection is useful for the determination of parasite exposure, antigen detection indicates an actual infection. This explains the higher number of positive dogs for antibody detection compared with antigen detection. Dogs positive in both tests can be assumed to host an active *A. vasorum* infection inducing an immunological reaction with production of antibodies. Cross reactions and false positive results may occur, even though the specificity has been shown to be as high as 94.0% for the antigen test (Schnyder et al. 2011) and 98.8% for the antibody test (Schucan et al. 2012). Combining both ELISAs enhances their individual performance: specificity is increased to 99.9% when only samples positive in both tests are considered, increasing the positive predictive value consequently, which is of great advantage especially in areas with low prevalence (Schnyder et al. 2013b). They represent a useful and reliable tool for both population studies and individual clinical diagnosis, requiring a single serum sample instead of several faecal samples. The overall prevalence for dogs positive by both ELISAs was 1.14% (CI: 0.74–1.66%) and

therefore higher than e.g. in Poland (0.51%, CI: 0.30–0.81), where *A. vasorum* cases were discovered only very recently (Schnyder et al. 2013b).

In 24 out of 96 French departments more than thirty samples per department were investigated; in particular, from the surroundings of the capital Paris a corresponding high number of positive samples was detected. The occurrence of *A. vasorum* in the surroundings of Paris was confirmed by recent clinically confirmed positive cases (Bedel et al. 2016, Cavana et al. 2015, Colella et al. 2016, Esteves et al. 2004, Nicolle et al. 2006). Some serologically positive animals were also identified in the traditionally considered *A. vasorum* endemic areas in south-western France, but interestingly by far not in all: for instance, in the departments of Gironde and Hérault, where also more than thirty samples were tested and where cases had been described already by Cuillé and Darraspen (1930a), none of the samples was positive. In the department of Haute-Garonne, including Toulouse as the city of the main discoveries about *A. vasorum* (Serres 1854, Bailliet 1866, Cuillé and Darraspen 1930a, b, Guilhon and Bressou 1960, Guilhon 1963) positive dogs were identified. Furthermore, seropositive dogs were identified in south-eastern France and in most of the northern boundaries, from where little or nothing is known about the presence of *A. vasorum*.

Using a climatic model and based on known *A. vasorum* prevalence in dogs and foxes, a European map indicating areas potentially suitable for the presence of the parasite was built (Morgan et al. 2009): for France, the whole country was deemed suitable, but interestingly, the model indicated a higher suitability for *A. vasorum* establishment in areas of the northern coast and western Atlantic coast, compared to south-western regions (traditionally more notorious for the parasite's transmission). Although south-western regions represent the "cradle" of the French heartworm and the source of most data collected in France, our results show that cases of canine angiostrongylosis may occur

in several other departments. In the central areas of the country, climate and fauna may be different and potentially fewer adequate intermediate and end hosts may be present, explaining the absence of positive results.

Unlike in France, having the longest history of *A. vasorum* occurrence, cases of *A. vasorum* in neighbouring countries were described only in the last decade. For instance, in north-western regions of Italy (Liguria and Piedmont) at the south-eastern border of France, the prevalence of *A. vasorum* positive foxes was as high as 78.2% (Magi et al. 2015), probably putting local dogs at high risk of infection, too. Further north, the prevalence of dogs positive by both ELISAs in a comparable serological survey was 2.2% in the area around Geneva (Switzerland), bordering to the central-eastern area of France (Lurati et al. 2015). In German regions neighbouring to France, based on a study with more than 12,000 dogs analysed between 2003–2015, the prevalence of *A. vasorum* was between 0 and 8.7% (Maksimov et al. 2017). Also in Belgium, the presence of *A. vasorum* has been increasingly reported (Canonne et al., 2015), including areas bordering France (Lempereur et al. 2016). Limited information is available from areas bordering the western part of France: a prevalence of 3.4% was observed in foxes from the Spanish Pyrenees and Catalonia (Garrido-Castane et al. 2015). The localisation of the here presented positive results suggests that *A. vasorum* has been spreading within France, and/or has been introduced from neighbouring countries in parallel. Different reasons for the apparent spread of lung worms have been discussed. Although *A. vasorum* has been focally present in France for a long time, movement of dogs from endemic to non-endemic areas, movement of and growing fox populations (Deplazes et al. 2004), as well as regional climate causing changes in intermediate host epidemiology (Morgan et al. 2009, Traversa et al. 2010) have to be taken into account.

Due to the high variability of clinical signs (Bedel et al. 2016, Koch and Willesen 2009) and the often chronic course of the infection, clinical diagnosis is challenging and may be missed. Early diagnosis is essential to ensure adequate anthelmintic treatment, before the onset of fatal canine angiostrongylosis (Chapman et al. 2004, Staebler et al. 2005). It is therefore of great importance to improve our knowledge of the occurrence of *A. vasorum* and maintain disease awareness.

Ethical standards

All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of interest

Graham Bilbrough and Carola Hafner were full-time employees of IDEXX Laboratories. Roland Schaper was employed by Bayer Animal Health. The study was financially supported by Bayer Animal Health Germany.

Acknowledgements

We highly acknowledge Kathrina Stebler and Katja Huggel from the Institute of Parasitology in Zurich and Doerte Schuepbach and further staff from IDEXX Vet Med Lab in Ludwigsburg for their very precious support.

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