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Natural Infections of *Angiostrongylus vasorum* and *Crenosoma vulpis* in Dogs in Germany (2007–2009)

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Abstract

In order to assess the occurrence and regional geographical distribution of *Angiostrongylus vasorum* and *Crenosoma vulpis* in Germany, faecal samples of 810 dogs with clinical symptoms of respiratory and circulatory disease, bleeding disorder and/or neurological signs were collected from September 2007 to March 2009. The zinc chloride/sodium chloride flotation and Baermann funnel technique were used to examine the samples for presence of lungworm larvae. Infections with lungworms were diagnosed in 105 (13.0%) of the examined dogs. *A. vasorum* and *C. vulpis* were found in 60 (7.4%) and 49 (6.0%) faecal samples, respectively. 33 *A. vasorum*- and 12 *C. vulpis*-positive dogs were located in Baden-Württemberg, 13 and 12 in North Rhine-Westphalia, 3 and 4 in Bavaria, 1 and 7 in Rhineland-Palatinate, 7 and 4 in Saarland, 1 and 2 in Saxony, respectively. In Brandenburg only 2 dogs with *A. vasorum* and in Hesse a total of 5 dogs with *C. vulpis* were detected. In Mecklenburg-Western Pomerania, Lower Saxony and Thuringia only 1 dog with *C. vulpis* was detected at a time. 4 dogs were coinfected with *A. vasorum* and *C. vulpis*. These surprisingly high prevalence rates indicate that both parasites are endemic in Germany.

Introduction

Angiostrongylus vasorum is a highly pathogenic metastrongylid nematode which has been found in many countries in Europe, Africa, North and South America and sporadically in the Asian part of the former Soviet Union infecting dogs, foxes (*Vulpes vulpes*) and several other species. The adult *A. vasorum* resides in the right side of the heart and pulmonary arteries causing clinical signs varying from occasional cough and mild exercise intolerance to severe acute respiratory distress, haemorrhage, neurological symptoms and even death. This nematode commonly known as "French heartworm" appears to be endemic in distinct isolated areas in France, Denmark and Great Britain (Bolt et al. 1994; Chapman et al. 2004; Morgan et al. 2005). Within UK, *A. vasorum* is considered endemic in well-defined foci in South Wales, Cornwall and Southeast England with only sporadic cases occurring outside these areas. Adult *Crenosoma vulpis* resides in the distal aspects of the bronchial tree of dogs and wild canids. Data on the prevalence and distribution of *C. vulpis* and details of the clinical signs are rare. A few cases have been reported in dogs in Ireland (Reilly et al. 2000), Great Britain (McGarry et al. 1995), Switzerland (Unterer et al. 2002), Germany (Kriegleder and Barutzki 1988; Epe et al. 2004) and Italy (Rinaldi et al. 2007). By means of faecal examinations from 1999 to 2002, *A. vasorum* and *C. vulpis* were only accidentally found in dogs in Germany (Barutzki and Schaper 2003). However, recent studies from Denmark and Germany have shown that prevalence rates for both lungworm species in dogs might have been increased in the last years (Taubert et al. 2009). The aim of the study presented was to assess the prevalence and regional geographical distribution of natural infections with *A. vasorum* and *C. vulpis* in dogs in Germany.

Materials and methods

Between September 2007 and March 2009, veterinary practitioners in Germany were asked to look for dogs tentative for a lungworm infection with symptoms of respiratory and circulatory diseases, i.e. coughing, dyspnoea, depression, haemorrhage, exercise intolerance, anorexie, weight loss, vomiting, diarrhoea, subcutaneous swelling, clearing throat and/or neurological signs. Only dogs which had not been abroad within the last three months before developing clinical signs of a lungworm disease were included in the study. Faecal samples were collected on three consecutive days from 810 dogs, 388 male and 422 female, with a clinical tentative diagnosis of a lungworm infection, submitted to the private Veterinary Laboratory Freiburg and examined for lungworm larvae using a standard saturated salt solution flotation technique with zinc chloride and sodium chloride (specific gravity 1.3) and a modified Baermann funnel technique.

To perform the Baermann migration method, the collected three samples of each dog were mixed and about 5 g of the faeces were placed in a muslin bag (gauze) at the interior rim of a funnel which was sealed at the lower end by a rubber tube and a clip. The funnel was filled with tap water at room temperature until the half of the sample was submerged in water. Being heavier than water, the nematodes pass through the muslin and sink to the bottom. After about 12 hours, actively mobile living larvae could be collected by carefully opening the clip and gathering the first 2–3 drops of water from the bottom of the tube. The recovered first-stage larvae (L1) were transferred to a glass slide, shortly heated over a Bunsen burner until they were fully stretched and then determined by dis-

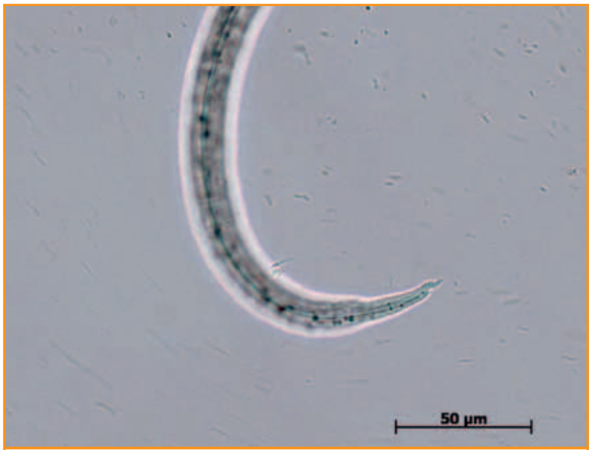

Fig. 1 Light micrograph of the posterior end of the first-stage larva (L1) of *Angiostrongylus vasorum* with a tip showing a sinus wave curve, immobilised by heat

Fig. 2 Light micrograph of the posterior end of the first-stage larva (L1) of *Crenosoma vulpis* with a very pointed tail, immobilised by heat

Fig. 3 Light micrograph of a dehydrated, damaged and morphologically altered first-stage larva (L1) of *Angiostrongylus vasorum* after flotation using a concentrated salt solution with zinc chloride and sodium chlorid (specific gravity 1.3)

tinctive morphological and morphometric characteristics according to the details described by Conboy (2000) and Bowman (2000). L1 with a tip at the posterior end showing a sinus wave curve and a dorsal spine were determined as *A. vasorum* (Fig. 1), and L1 with a very pointed and straight tail as *C. vulpis* (Fig. 2).

The collected data were analysed by a geographic information system (GIS) using the programme RegioGraph 10 (GfK GeoMarketing, Bruchsal) to visualise the regional distribution of *A. vasorum* and *C. vulpis*. Based on the five-digit postcodes as points of reference, the locations of the owners of lungworm-positive dogs were displayed on maps with administrative and postcode boundaries.

Results

The standard saturated salt solution flotation technique with zinc chloride and sodium chloride (specific gravity 1.3) was only marginal less sensitive compared to the Baermann method. Most of the examined lungworm-positive dogs showed larvae in the flotation as well as in the Baermann method. Due to the high specific gravity of the concentrated salt solution, larvae in the flotation were dehydrated, damaged, morphologically altered, considerably textural modified, hence difficult to identify and to determine and scarcely to distinguish from free-living nematode larvae (Fig. 3). Based on the Baermann method, the microscopic examination revealed infections with lungworms in 105 (13.0 %) of the 810 examined dogs (Table 1). *A. vasorum* and *C. vulpis* were found in 60 (7.4 %) and 49 (6.0 %) faecal samples, respectively. 33 *A. vasorum*- and 12 *C. vulpis*-positive dogs were located in Baden-Württemberg, 13 and 12 in North Rhine-Westphalia, 3 and 4 in Bavaria, 1 and 7 in Rhineland-Palatinate, 7 and 4 in Saarland, 1 and 2 in Saxony, respectively (Fig. 4). In Brandenburg only 2 dogs with *A. vasorum* and in Hesse a total of 5 dogs with *C. vulpis* were detected. In Mecklenburg-Western Pomerania, Lower Saxony and Thuringia only 1 dog with *C. vulpis* was detected at a time. 4 dogs were coinfected with *A. vasorum* and *C. vulpis*. A microgeographic analysis based on postcodes using the programme RegioGraph 10 provides a detailed profile of the regional geographical distribution of natural infections with lungworms in dogs in Germany. The area analysis shows most of

Table 1 Prevalence of *Angiostrongylus vasorum* and *Crenosoma vulpis* in dogs in Federal states of Germany

Federal state of Germany	Examined dogs	*Angiostrongylus vasorum* positve dogs		*Crenosoma vulpis* positve dogs		Lungworm[a] positive dogs	
	No. (n)	(n)	(%)	(n)	(%)	(n)	(%)
Baden-Württemberg	227	33	14.5	12	5.3	42	18.5
Bavaria	99	3	3.0	4	4.0	7	7.1
Berlin	9	0	0	0	0	0	0
Brandenburg	19	2	10.5	0	0	2	10.5
Bremen	2	0	0	0	0	0	0
Hamburg	1	0	0	0	0	0	0
Hesse	52	0	0	5	9.6	5	9.6
Mecklenburg-Western Pomerania	11	0	0	1	9.1	1	9.1
Lower Saxony	38	0	0	1	2.6	1	2.6
North Rhine-Westphalia	197	13	6.6	12	6.1	25	12.7
Rhineland-Palatinate	85	1	1.2	7	8.2	8	9.4
Saarland	40	7	17.5	4	10.0	10	25.0
Saxony	15	1	6.7	2	13.3	3	20.0
Saxony-Anhalt	2	0	0	0	0	0	0
Schleswig-Holstein	9	0	0	0	0	0	0
Thuringia	4	0	0	1	25.0	1	25.0
total	810	60	7.4	49	6.0	105	13.0

[a] Single infection with *Angiostrongylus vasorum* or *Crenosoma vulpis* or infection with both species

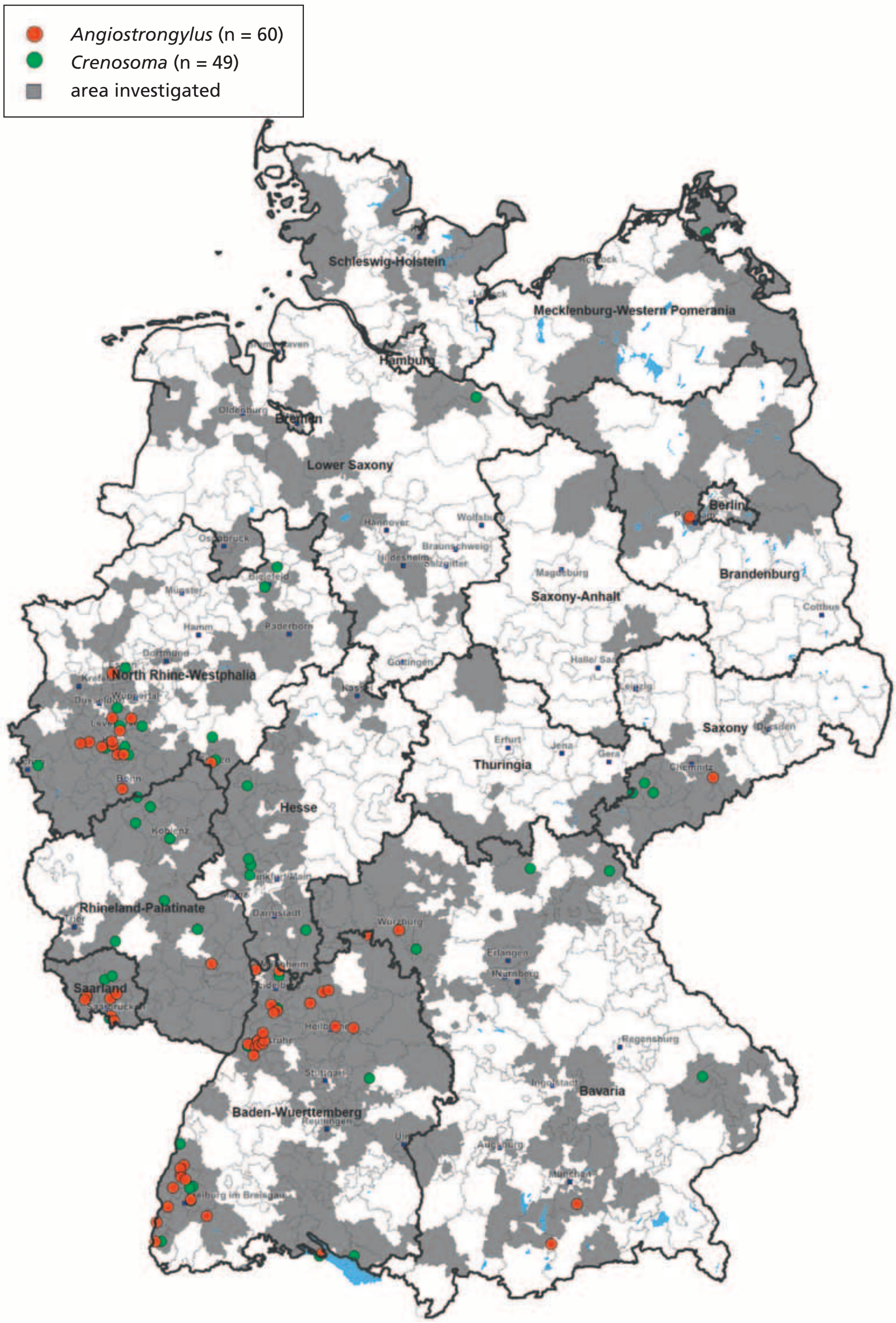

Fig. 4 Natural infections of *Angiostrongylus vasorum* and *Crenosoma vulpis* in dogs in Germany

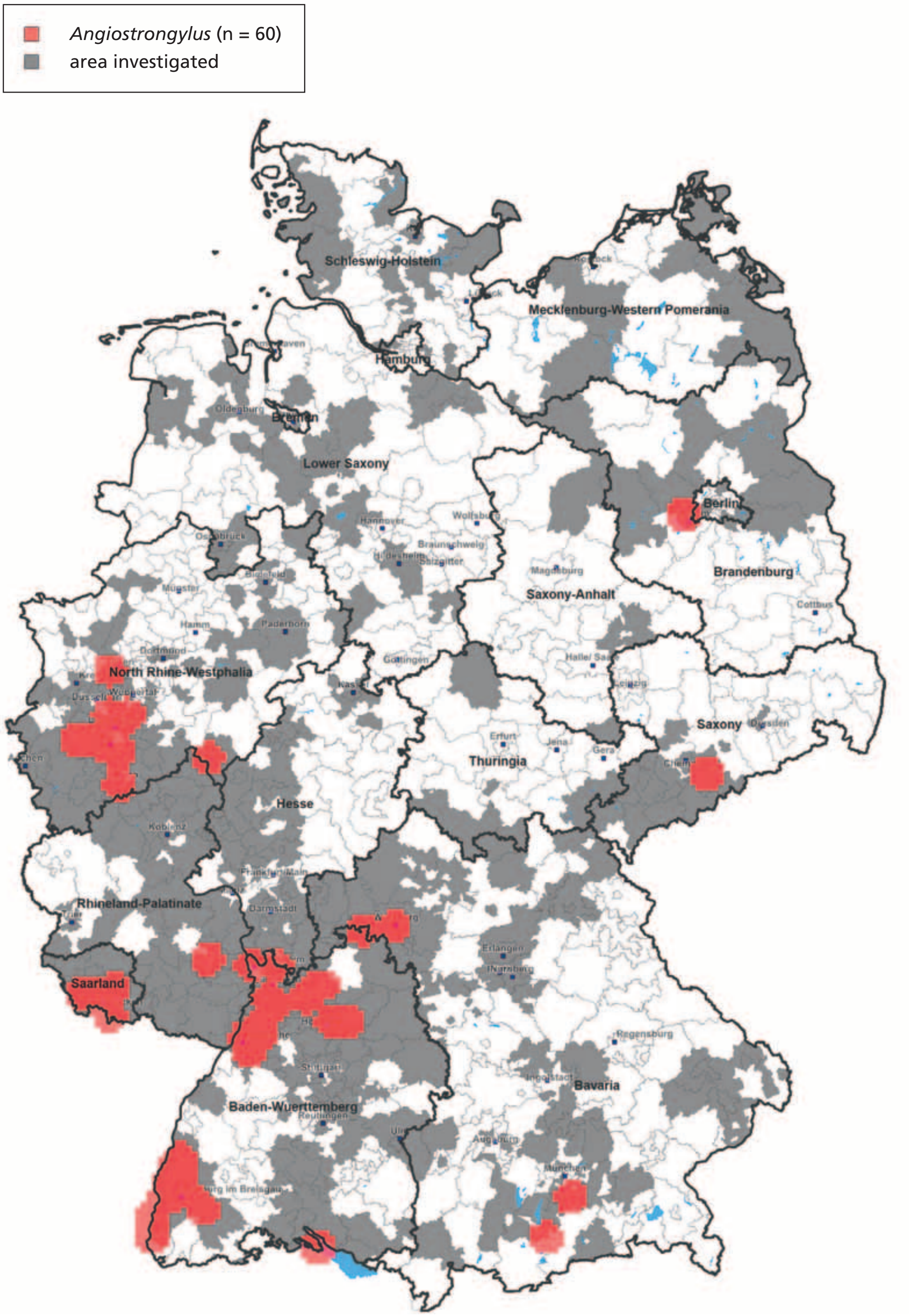

Fig. 5 Regional geographical distribution (cluster) of *Angiostrongylus vasorum* in dogs in Germany

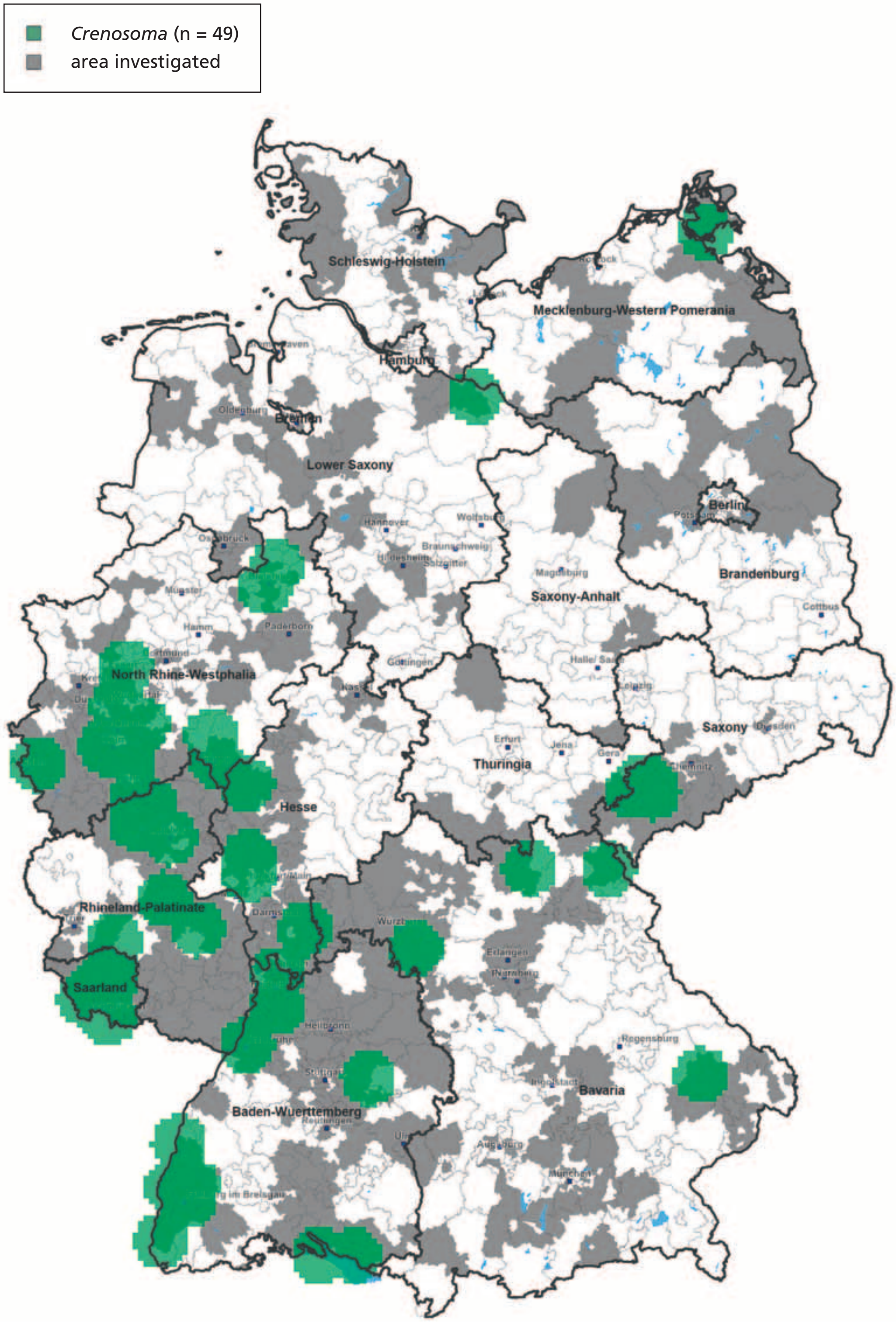

Fig. 6 Regional geographical distribution (cluster) of *Crenosoma vulpis* in dogs in Germany

the infections of lungworms in the southern parts of Germany with extensive clusters of *A. vasorum* in Baden-Württemberg, North Rhine-Westphalia, Rhineland-Palatinate and Saarland and defined epidemic foci in Bavaria, Saxony and Brandenburg (Fig. 5). Cluster of *C. vulpis* were more widely spread, spacious and converging mainly located in Baden-Württemberg, North Rhine-Westphalia, Bavaria, Rhineland-Palatinate, Saarland and Saxony (Fig. 6). The rate of infection with respect to the age of the animals showed distinctly higher values in young dogs until 1 year of age compared to older animals. The number of *A. vasorum*- and *C. vulpis*-positive dogs was 21 and 19, respectively, in dogs up to 1 year of age and 11 and 3, respectively, in 2-years-old dogs (Fig. 7). The number of infected dogs older than 2 years and up to 14 years ranged between 0 and 7 for *A. vasorum* and between 0 and 6 for *C. vulpis*.

Discussion

During the last decades, infections with *A. vasorum* and *C. vulpis* in dogs in Germany have been reported only sporadically (Pallaske 1967; Kriegleder and Barutzki 1988; Staebler et al. 2005; Pötz 2006). Analysis of the faecal examination of 8431 dogs between 1999 and 2002 in the Veterinary Laboratory Freiburg showed 0.1% *A. vasorum*- and 0.3% *C. vulpis*-positive dogs (Barutzki and Schaper 2003) and of 1281 dogs at the Institute for Parasitology of the School of Veterinary Medicine Hannover only 0.6% *C. vulpis*-positive dogs (Epe et al. 2004). In a new study from Germany including 958 dogs with clinical symptoms, larvae of *A. vasorum* were found in 1.2% and of *C. vulpis* in 2.4% of the examined faecal samples (Taubert et al. 2009). In the present study, *A. vasorum* and *C. vulpis* were found to a

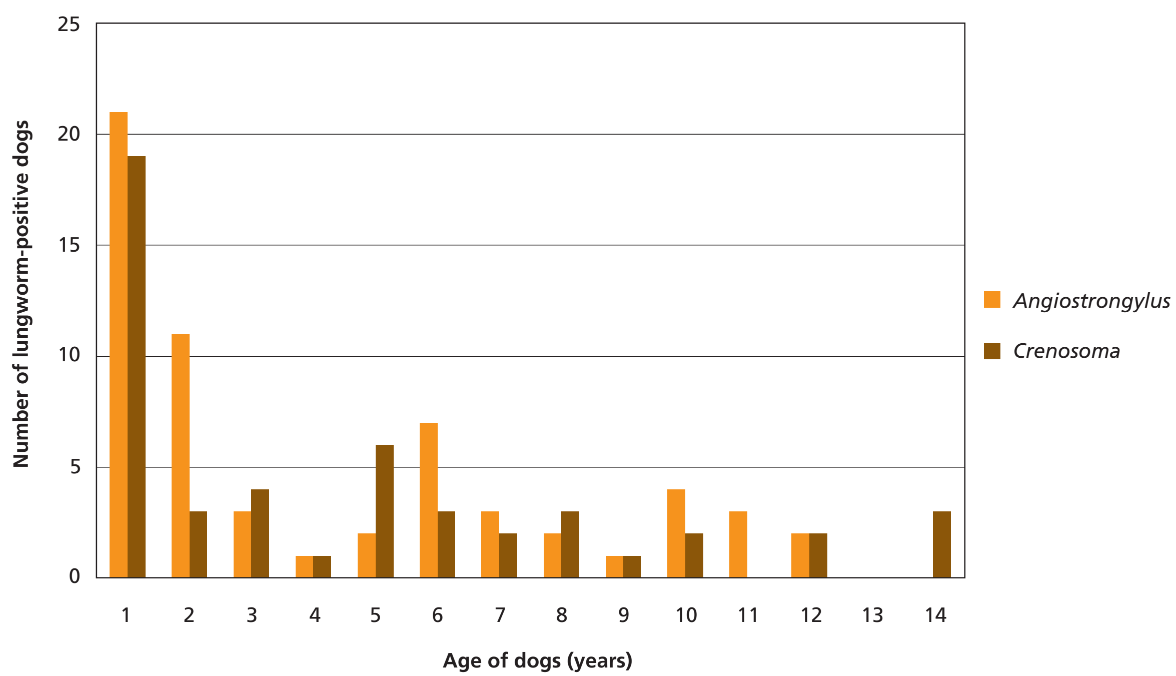

Fig. 7 Age structure of *Angiostrongylus vasorum*- and *Crenosoma vulpis*-positive dogs

greater extent in 7.4% and 6.0%, respectively, of the faecal samples of 810 dogs with clinical signs of lungworm infections. Even though these results are not indicative for the true prevalence of lungworms in dogs in Germany, the frequency of diagnosis of *A. vasorum* and *C. vulpis* shows an increasing number of positive dogs. This holds true for both parasites *A. vasorum* and *C. vulpis* which were formerly only rarely detected. If there would have been endemic foci in Germany in former times, infections with *Angiostrongylus* and *Crenosoma* would have been diagnosed even by conventional flotation method and standard coproscopical examination. In the study presented, most of the examined lungworm-positive dogs showed larvae in the standard saturated salt solution flotation technique with zinc chloride and sodium chloride (specific gravity 1.3) as well as in the Baermann method. The flotation technique used in this study was the same method proceeded for faecal examinations of the 8431 dogs in routine diagnostic (Barutzki and Schaper 2003). However, the Baermann migration method remains the gold standard to diagnose lungworm infections in dogs and to differentiate between members of the genera *Angiostrongylus* and *Crenosoma*. Due to the concentrated salt solution larvae in the flotation are dehydrated and morphologically altered, difficult to identify and to determine and scarcely to distinguish from free-living nematode larvae.

A. vasorum has been traditionally considered to be well-isolated in endemic geographic foci with occasional cases in other regions (Morgan et al. 2005). However, *A. vasorum* has increasingly been reported outside known endemic foci and appears to be spreading rapidly (Saeed et al. 2006). The collected data analysed by a geographic information system (GIS) indicate that *A. vasorum* and *C. vulpis* are endemic in Germany. Within the last years, *A. vasorum* and *C. vulpis* seemed to be sedentary in well-defined endemic foci. Today, both parasites are located in terms of extensive clusters mainly in southern and western parts of the country potentially expanding in northern and eastern parts of the country. In comparison with the known cases in Germany, up to 2003 both parasites have presumably been expanded into other areas of Germany.

Until now we only have several new data for dogs, but the role of wildlife in Germany is completely unknown. Foxes (*Vulpes vulpes*) are accepted as a significant reservoir for infection of dogs (Morgan et al. 2005). The prevalence of *A. vasorum* in foxes is commonly much higher compared to dogs (Koch and Willesen 2009). According to this, in Newfoundland infections with *A. vasorum* were found in foxes long before its diagnosis in dogs (Bourque et al. 2002). In contrast to the findings in Canada, the introduction of *A. vasorum* by dogs staying on holidays in endemic areas can lead to its establishment in foxes at home. In The Netherlands, autochthonous *A. vasorum* infections were first described in dogs (van Doorn et al. 2009). However, in Germany the population of susceptible foxes is expanding and due to the moderate temperature and humidity the climate is very suitable for gastropod mollusk responsible for transmission of the lungworms. Frequent trips of dogs into and from endemic areas as well as an inadequate screening and treatment of these parasites assist the spreading of the parasites. Due to the high number of lungworm-positive dogs, veterinarians should consider infections with *A. vasorum* and *C. vulpis* as a differential diagnosis in dogs with symptoms of respiratory diseases in Germany.

Acknowledgements

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