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The occurrence of *Angiostrongylus vasorum* in terrestrial slugs from forests and parks in the Copenhagen area, Denmark

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Abstract

A total of 298 slugs belonging to four species, *Arion lusitanicus*, *A. ater*, *A. ater rufus* and *Limax maximus*, were collected from six different localities within a radius of 30 km from Copenhagen and examined for naturally acquired *Angiostrongylus vasorum* infection. Overall, 28 slugs (9%) were infected, but the prevalence varied among the studied localities: Rude Forest (26%), West Amager Forest (18%), Jaegersborg Forest and Deer Park (8%), Frederiksberg Park (4%), Assistens Cemetery Park (0%) and Frederiksberg Botanical Garden (0%). Only third-stage larvae (L3) were recovered from the slugs, in numbers ranging from 1 to 392 per slug. Overall 82% of the infected slugs harboured fewer than 10 larvae and only 14% harboured over 100 larvae.

Introduction

Angiostrongylus vasorum, commonly known as the 'French heartworm', is a metastrongylid nematode with an indirect life cycle. A wide range of gastropods serve as obligatory intermediate hosts for this parasitic nematode, and dogs and foxes are the typical definitive hosts (Rosen *et al.*, 1970; Guilhon & Cens, 1973; Bolt *et al.*, 1992). The gastropod hosts become infected by foraging on the carnivore faeces containing first-stage larvae (L1). Such larvae-infested gastropods may transmit the infection when ingested by a carnivore, where the adult worm resides in the pulmonary artery and the heart (Rosen *et al.*, 1970; Guilhon & Cens, 1973). In Denmark, recurrent epidemiological studies on fox and dog populations suggest that both the spatial distribution of *A. vasorum* and its prevalence are increasing (Willingham *et al.*, 1996; Saeed *et al.*, 2006; Koch & Willesen, 2007). However, information on the diversity of slug intermediate hosts and their distribution is lacking. Such information is central for the prevention of parasite transmission. The present study was therefore performed to investigate the spatial distribution of *A. vasorum* infected slugs in urban and peri-urban areas of Copenhagen.

Materials and methods

Study areas

Terrestrial slugs were collected from forests and parks in a 30 km radius of Copenhagen, Denmark, during the period from September to October 2007. Slugs were collected from six different localities: Frederiksberg Botanical Garden, Frederiksberg Park, Assistens Cemetery Park, Jaegersborg Forest and Deer Park, West Amager Forest and Rude Forest.

Among these study areas Frederiksberg Botanical Garden, Frederiksberg Park and Assistens Cemetery Park are regarded as public grassland parks with varieties of trees, herbs and ornamental vegetation. People use

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these areas for recreational purposes and to exercise their dogs and it is also possible that urban foxes visit these places at night in search for food. The peri-urban areas Jaegersborg Forest and Deer Park, West Amager Forest and Rude Forest are maintained as natural forest areas with open public access, and with a variety of trees and vegetation and a natural fauna. Jaegersborg Forest and Deer Park is populated by approximately 2000 semi-domesticated deer. All study areas have natural populations of foxes.

Collection and maintenance of slugs in the laboratory

Slugs collected by hand from the soil surface and vegetation were kept in plastic boxes according to locality, species and size. Species determination of slugs was done after Bondesen (1981), and sizes of the slugs were categorized, according to weight, into three groups (large, >10 g; medium, 5–10 g; and small, <5 g). Boxes were lined with moistened tissue paper and supplemented with cucumber and lettuce as a food source. Slugs were kept at 15°C until the parasitological examination took place.

Parasitological procedures

Isolation of *A. vasorum* larvae from slugs was conducted by tissue digestion. Individual slugs were cut into small pieces (1–2 mm) and incubated at 37°C in artificial digestion fluid (12 ml HCl (30%) and 30 ml liquid Pepsin (1:30,000 IU) in 1 litre 42°C tap water) under constant magnetic stirring at 300 rpm for 35 min, strained through a 180 μm sieve and subsequently allowed to sediment for 75 min. The sediment was washed twice with water until the suspension became clear and recovered larvae were counted in a stereomicroscope. The larvae were fixed on an object glass by a drop of Lugol's solution and examined under the microscope at 20× and 40× magnification to determine the larval stage, as described by Ash (1970) and Rosen *et al.* (1970). Larvae that could not be identified as *A. vasorum* or could not be identified to a specific larval stage were excluded from the count.

Statistical analysis

Descriptive analysis of the explanatory variables (weight and species of the slugs and locality) and outcome variable (presence or absence of *A. vasorum* larvae) was performed by Fisher's exact test. Statistical analysis to evaluate the effect of weight, locality and species on the outcome variable was tested by using the logistic analysis procedure for binomial distribution. In logistic analysis, taking all explanatory variables into account, the most significant variable was selected by excluding the non-significant and/or least significant variable in a backward elimination strategy. P values of <0.05 were considered to be statistically significant. All the statistical analyses were performed using SAS 9.1 package (SAS Institute Inc., Cary, North Carolina, USA) and Microsoft Excel 2000.

Results

From the six different study areas 48–50 slugs of up to four different species were examined for the presence of natural *A. vasorum* infection (table 1). *Arion lusitanicus* was the most common species in both park and forest areas, except in West Amager Forest. *Arion ater* and *A. ater rufus* were found exclusively in forest areas, and low numbers of *Limax maximus* were found at all localities.

Out of 298 examined slugs, 28 (9.40%) were infected with *A. vasorum*. Among infected localities the level of infection varied from 4% in Frederiksberg Park to 26% in Rude Forest. No naturally infected slugs were found in Frederiksberg Botanical Garden and Assistens Cemetery Park areas (table 1). *Angiostrongylus vasorum* was recovered from all of the four slug species but infection varied spatially. The highest overall prevalence was found in Rude Forest where all of the examined species, e.g. *A. ater*, *A. ater rufus*, *A. lusitanicus* and *L. maximus* were found to be positive for infection. In contrast only *A. lusitanicus* harboured the infection in Frederiksberg Park. In Jaegersborg Forest and Deer Park *A. ater* and *A. ater rufus* had comparable prevalence of infection. In West Amager Forest, two out of three *L. maximus* were infected (table 1). Only third-stage larvae (L3) were

Table 1. *Angiostrongylus vasorum* infection in naturally infected slugs in the Copenhagen area of Denmark.

Study area	Slug species	Number of infected (examined)	No. of recovered parasite L3 per slug	Infection (%) according to slug species	Infection (%) according to locality
Frederiksberg Botanical Garden	*Arion lusitanicus*	0 (50)	0	0	0
Assistens Cemetery Park	*A. lusitanicus*	0 (50)	0	0	0
Frederiksberg Park	*A. lusitanicus*	2 (49)	2, 392	4.1	4.0
	Limax maximus	0 (1)	0	0	
West Amager Forest	*Arion ater*	7 (47)	1, 1, 2, 2, 5, 5, 7	14.9	18
	L. maximus	2 (3)	1, 6	66.7	
Rude Forest	*A. ater*	9 (24)	1, 1, 2, 2, 4, 4, 39, 119, 121	37.5	26.0
	Arion ater rufus	2 (15)	3, 5	13.3	
	A. lusitanicus	1 (8)	2	12.5	
	L. maximus	1 (3)	8	33.3	
Jaegersborg Forest and Deer Park	*A. ater*	3 (28)	1, 3, 152	10.7	8.3
	A. ater rufus	1 (10)	4	10	
	A. lusitanicus	0 (10)	0	0	

recovered from the slugs (ranged from 1 to 392 per slug). Highest larval burdens were found in *A. lusitanicus* and *A. ater* slugs; about 82% of the total infected slugs harboured <10 larvae and only 14% harboured >100 larvae (table 1, fig. 1). No significant differences in larval burden could be demonstrated according to slug weight, although the highest burdens were found in larger specimens (figs 1 and 2). Logistic analysis for binomial distribution showed a significant association between the presence of natural *A. vasorum* infection and the locality from which slugs were collected ($P = 0.04$); however, the difference was not significant between species and size of the slug and presence of infection ($P = 0.11$ for species and $P = 0.17$ for size of the slug).

Discussion

Previous investigations have shown that *A. vasorum* can utilize a wide range of gastropods as intermediate hosts (Rosen *et al.*, 1970; Guilhon & Cens, 1973; Sauerlander & Eckert, 1974; Simpson & Neal, 1982; Barcante *et al.*, 2003). Accordingly, the present study demonstrated the occurrence of natural infection of *A. vasorum* in four slug species, i.e. *A. lusitanicus*, *A. ater*, *A. ater rufus* and *L. maximus*, which supports observations by Guilhon & Cens (1973) and Tonsberg (2006). Such lack of specificity in intermediate hosts has also been documented for other *Angiostrongylus* species, such as *A. cantonensis* (Wallace & Rosen, 1969; Yousif & Lammer, 1975b) and *A. costaricensis* (Rambo *et al.*, 1997; Laitano *et al.*, 2001).

The local variations in occurrence of slug species most likely rely on the diversity of the basic habitat, whereas the spatial differences in parasite prevalence in the slugs appears more complex and may depend on susceptibility, density and behaviour of the slug population, as well as on the quantity and infectivity of the larval population excreted by the local carnivore definitive hosts (Yousif & Lammer, 1975a; Bolt *et al.*, 1994; Saeed *et al.*, 2006; Weidema, 2006; Kozlowski, 2007). The most likely reasons for the absence of parasite-infected slugs in Frederiksberg Botanical Garden and in Assistens Cemetery Park are that the two areas are central urban parks with little faunal diversity and the low number of slugs examined. In spite of the observed local differences in larval burden among the slug species (table 1) a general pattern is not obvious due to the low numbers of specimens collected for some slug species, e.g. *L. maximus*. The few observations of a very high larval burden for *L. maximus* may reflect its long lifespan (up to 3 years, as compared to 1 year for *Arion* spp.) (Rollo, 1983) and the higher risk of exposure to larvae excreted in carnivore faeces. Although no significant association was found between slug weight and prevalence, the infection level tended to be higher in larger specimens than in the

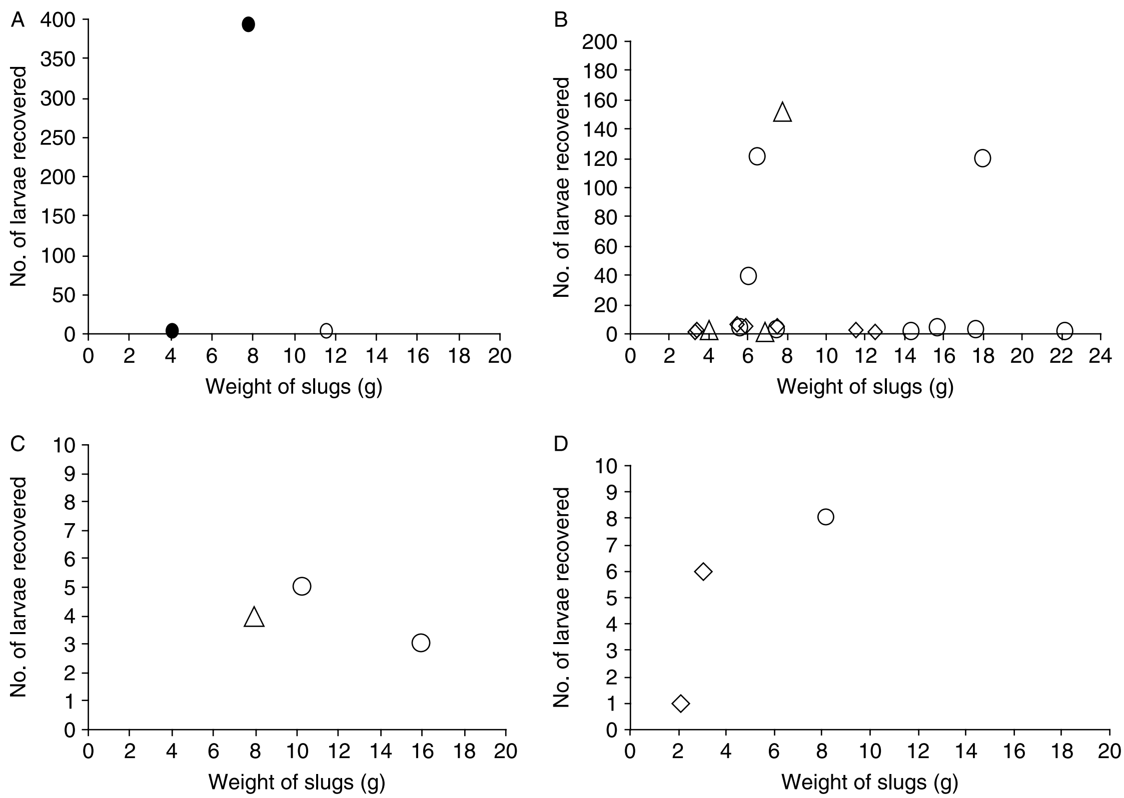

Fig. 1. Distribution of *Angiostrongylus vasorum* larvae in different slug species according to locality and body weight: (A) *Arion lusitanicus*, (B) *A. ater*, (C) *A. ater rufus*, (D) *Limax maximus* species. Frederiksberg Park (●), Rude Forest (○), Jaegersborg Forest and Deer Park (△), West Amager Forest (◇).

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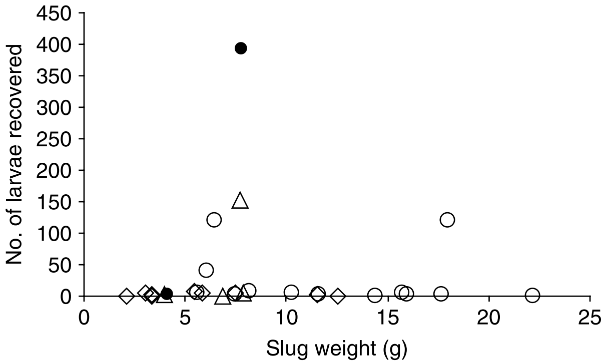

Fig. 2. Distribution of *Angiostrongylus vasorum* larvae in different localities according to body weight of the slugs. Frederiksberg Park (●), Rude Forest (○), Jaegersborg Forest and Deer Park (△), West Amager Forest (◇).

small slugs. A comparable association was also reported by Yousif & Lammer (1975a) for *A. cantonensis* in the snail *Biomphalaria glabrata*.

Overdispersion of parasites in their host populations, as described by Anderson & Gordon (1982), is evident from the present study. The majority of the slugs (270 out of 298) were not harbouring the infection, five slugs (14% of the infected slugs) had a high larval burden (>100 larvae), whereas 82% of the infected slugs (23 specimens) harboured <10 larvae. Similar findings were reported in a field survey in Brazil on *A. costaricensis* (Laitano *et al.*, 2001). Such overdispersion in the intermediate slug host may indicate that the definitive carnivore populations only rarely eat slugs with a high larval burden and thus many cases of dog angiostrongylosis may remain subclinical.

The recovery of exclusively third-stage *A. vasorum* larvae in the slugs supports previous observations (Tonsberg, 2006) where slugs collected over the summer (June–September) had an increasing proportion of *A. vasorum* L3 larvae (up to 100%). The collection period in the present study (September–October) is an extension of this period and it is therefore not surprising that all larvae recovered from the slugs had developed to the final, third stage. Overall, the present study illustrates that *A. vasorum* is widely distributed in several slug species in the forest and park areas of Copenhagen and that transmission of the infection is occurring via gastropods.

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References

Anderson, R.M. & Gordon, D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite induced host mortalities. *Parasitology* **85**, 373–398.

Ash, L.R. (1970) Diagnostic morphology of the third-stage larvae of *Angiostrongylus cantonensis*, *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus* and *Anafilaroides rostratus* (Nematoda: Metastongyloidea). *Journal of Parasitology* **56**, 249–253.

Barcante, T.A., Barcante, J.M., Costa Dias, S.R. & Lima, W.S. (2003) *Angiostrongylus vasorm* (Baillet, 1866) Kamensky, 1905. Emergence of third-stage larvae from infected *Biomphalaria glabrata* snails. *Parasitology Research* **91**, 471–475.

Bolt, G., Monrad, J., Henriksen, P., Dietz, H.H., Koch, J., Bindseil, E. & Jensen, A.L. (1992) The fox (*Vulpes vulpes*) as a reservoir for canine Angiostrongylosis in Denmark. Field survey and experimental infections. *Acta Veterinaria Scandinavica* **33**, 357–362.

Bolt, G., Monrad, J., Koch, J. & Jensen, A.L. (1994) Canine angiostrongylosis: a review. *Veterinary Record* **135**, 447–452.

Bondesen, P. (1981) Danske landsnegle. *Nature og Museum. Arhus Naturhistoriske Museum* **20**, 1–29.

Guilhon, J. & Cens, B. (1973) *Angiostrongylus vasorum* (Baillet, 1866). Etude biologique et morphologique [*Angiostrongylus vasorum* (Baillet, 1866). Morphological and biological study]. *Annales de Parasitologie Humaine et Comparee* **48**, 567–596 (abstract in English).

Koch, J. & Willesen, J. (2007) Canine pulmonary angiostrongylosis: an update. *Veterinary Journal* **179**, 348–359.

Kozlowski, J. (2007) The distribution, biology, population dynamics and harmfulness of *Arion lusitanicus* Mabile, 1868 (Gastropoda: Pulmonata:Arionidae) in Poland. *Journal of Plant Protection Research* **47**, 219–230.

Laitano, A.C., Genro, J.P., Fontoura, R., Branco, S., Siqueira, L., Maurer, R.L., Graeff-Teixeira, M.J., Chiaradia, L.A. & Thomé, J.W. (2001) Report on the occurrence of *Angiostrongylus costaricensis* in southern Brazil, in a new intermediate host from the genus Sarasinula (Veronicellidae, Gastropoda). *Revista da Sociedade Brasileira de Medicina Tropical* **34**, 95–97.

Rambo, P.R., Agostini, A.A. & Graeff-Teixeira, C. (1997) Abdominal angiostrongylosis in Southern Brazil prevalence and parasitic burden in mollusk intermediate hosts from eighteen endemic foci. *Memorias do Instituto Oswaldo Cruz, Rio de Jeneiro* **92**, 9–14.

Rollo, C.D. (1983) Consequences of competition on the reproduction and mortality of three species of terrestrial slugs. *Researches on Population Ecology* **25**, 20–43.

Rosen, L., Ash, L.R. & Wallace, G.D. (1970) Life history of the canine lung worm *Angiostrongylus vasorum* (Baillet). *American Journal of Veterinary Research* **31**, 131–143.

Saeed, I., Maddox-Hyttel, C., Monrad, J. & Kapel, C.M.O. (2006) Helminths of red foxes *(Vulpes vulpes*) in Denmark. *Veterinary Parasitology* **139**, 168–179.

Sauerlander, R. & Eckert, J. (1974) Die Achatschnecke (*Achatina fulica*) als experimenteller Zwischenwirt fur *Angiostrongylus vasorum* (Nematoda) [The African giant snail (*Achatina fulica*) as experimental intermediate host of *Angiostrongylus vasorum* (Nematoda)]. *Zeitschrift für Parasitenkunde* **44**, 59–72 (abstract in English).

Simpson, V.R. & Neal, C. (1982) *Angiostrongylus vasorum* infection in dogs and slugs. *Veterinary Record* **111**, 303–304.

https://doi.org/10.1017/S0022149X09377706 Published online by Cambridge University Press

Tonsberg, H. (2006) Epidemiological study of snail intermediate hosts in the life-cycle of *Angiostrongylus vasorum* in Denmark. Unpublished Masters Thesis, Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark.

Wallace, G.D. & Rosen, L. (1969) Studies on eosinophilic meningitis V. Molluscan hosts of *Angiostrongylus cantonensis* on Pacific Islands. *American Journal of Tropical Medicine and Hygiene* **18**, 206–216.

Weidema, I. (2006) NOBANIS – Invasive Alien Species Fact Sheet – *Arion lusitanicus*. From Online databases of the North European and Baltic Network on Invasive Alien Species – NOBANIS: www.novanis.org (accessed 23 January 2008).

Willingham, A.L., Ockens, N.W., Kapel, C.M.O. & Monrad, J. (1996) A helminthological survey of wild red foxes (*Vulpes vulpes*) from the metropoliton area of Copenhagen. *Journal of Helminthology* **10**, 259–263.

Yousif, F. & Lammer, G. (1975a) The effect of some biological and physical factors on infection of *Biomphalaria glabrata* with *Angiostrongylus cantonensis*. *Zeitschrift für Parasitenkunde* **47**, 191–201.

Yousif, F. & Lammer, G. (1975b) The suitability of several aquatic snails as intermediate hosts for *Angiostrongylus cantonensis*. *Zeitschrift für Parasitenkunde* **47**, 203–210.