Hystrix It. J. Mamm. (n.s.) 20(2) (2009): 121-126

ANGIOSTRONGYLUS VASORUM IN RED FOXES (*VULPES VULPES*) AND BADGERS (*MELES MELES*) FROM CENTRAL AND NORTHERN ITALY

MARTA MAGI[1*], LISA GUARDONE[1], MATTEO DELL'OMODARME[2], MARIA CRISTINA PRATI[2], WALTER MIGNONE[3], BEATRICE TORRACCA[1], GIANFRANCA MONNI[1], FABIO MACCHIONI[1]

[1]Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, Università degli Studi di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy
*corresponding author: E-mail: magim@vet.unipi.it
[2]Scuola Normale Superiore di Pisa, Piazza dei Cavalieri 7, 56126 Pisa, Italy
[3]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta, Sezione di Imperia, via Nizza 4, 18100, Imperia, Italy

Received 17 July 2009; accepted 10 November 2009

ABSTRACT - During 2004-2005 and 2007-2008, 189 foxes (*Vulpes vulpes*) and 6 badgers (*Meles meles*) were collected in different areas of Central Northern Italy (Piedmont, Liguria and Tuscany) and examined for *Angiostrongylus vasorum* infection. The prevalence of the infection was significantly different in the areas considered, with the highest values in the district of Imperia (80%, Liguria) and in Montezemolo (70%, southern Piedmont); the prevalence in Tuscany was 7%. One badger collected in the area of Imperia turned out to be infected, representing the first report of the parasite in this species in Italy. Further studies are needed to evaluate the role played by fox populations as reservoirs of infection and the probability of its spreading to domestic dogs.

Key words: red fox, badger, helminths, *Angiostrongylus vasorum*, Italy

RIASSUNTO - ***Angiostrongylus vasorum* nella volpe (*Vulpes vulpes*) e nel tasso (*Meles meles*) in Italia centro-settentrionale**. Nel 2004-2005 e 2007-2008, 189 volpi (*Vulpes vulpes*) e 6 tassi (*Meles meles*) provenienti da differenti aree dell'Italia settentrionale e centrale (Piemonte, Liguria Toscana), sono stati esaminati per la ricerca di *Angiostrongylus vasorum*. La prevalenza del nematode è risultata significativamente diversa nelle varie zone, con valori elevati nelle zone di Imperia (80%) e di Montezemolo (70%, provincia di Cuneo); la prevalenza in Toscana è risultata del 7%. Un tasso proveniente dall'area di Imperia è risultato positivo per *A. vasorum*; questa è la prima segnalazione del parassita in tale specie in Italia. Ulteriori studi sono necessari per valutare il potenziale della volpe come serbatoio e la possibilità di diffusione della parassitosi ai cani domestici.

Parole chiave: volpe, tasso, elminti, *Angiostrongylus vasorum*, Piemonte, Liguria, Toscana

INTRODUCTION

Angiostrongylus vasorum (Baillet, 1866) is a metastrongylid nematode affecting dogs and wild canids (e.g. red fox *Vulpes vulpes* and coyote *Canis latrans*), which can cause severe respiratory and circulatory distress and,

sometimes, sudden death.

Adult worms live in the heart and pulmonary arteries of canids. Eggs shed by female worms are transported to the pulmonary capillaries, where they hatch into larvae L1, break out into the airspace, are coughed up and swallowed to be passed in the faeces of infected animals. Various species of slugs and snails are exposed to L1 larvae by feeding on the faeces. Inside these intermediate hosts the L1 larvae develop into L3. Canids infect themselves by ingesting either snails and slugs or frogs (paratenic host) (Bolt *et al*., 1994).

A. vasorum is mainly transmitted in wildlife with the fox as its final host. Since its discovery in France, *A. vasorum* has been found in many countries in Europe, Africa and America. Recent reports suggest an expansion of the geographical range of the parasite that presently appears to be spreading from its traditional endemic foci with an increasing incidence of infection (Morgan *et al*., 2005).

Available information on *A. vasorum* in Italy is limited. In the dog, the parasite was found in one animal from Tuscany with respiratory distress, successfully treated with ivermectine (Della Santa *et al*., 2002), in two animals with severe cardio-respiratory signs from Abruzzo, which died shortly after admission (Traversa *et al*., 2008), in four animals examined post-mortem in Latium (Scaramozzino *et al*., 2007) and in one animal with clinical signs from Apulia, successfully treated with fenbendazole (Sasanelli *et al*., 2008).

Research on angiostrongylosis in the red fox in Italy showed an infection prevalence ranging from 6% to 40% (15%, Leoni *et al*. 1986; 6%, Iori *et al*. 1990; 24%, Poli *et al*. 1984; 39%, Poli *et al*., 1991), but all these surveys date back more than 15 years.

The aim of the present study was to investigate the presence of *A. vasorum* in red foxes and badgers in some areas of Central and Northern Italy (mainly Tuscany and Liguria).

METHODS

A total of 189 foxes were examined. The animals were killed during the hunting seasons 2004-2005 in Tuscany (Central Italy, within 42°30'-43°40'N and 10°25'-11°40'E; 0-900 m a.s.l.) and 2007-2008 in Liguria and Piedmont (Northern Italy, within 43°45'-44°40'N and 7°30'-8°15'E; 15-780 m a.s.l.). The foxes collected in Tuscany originated from plain and hilly areas around Grosseto (37), Cecina (50), Pisa (26) and Siena (16) (see Magi *et al.*, 2009 for details). The animals collected in Liguria originated from the district of Imperia (45), while those from Piedmont came from the municipalities of Borgo San Dalmazzo (5) and Montezemolo (10), both in the district of Cuneo (southern Piedmont). The sampling was opportunistic. Six road-killed badgers (*Meles meles*) were also collected (4 from Tuscany and 2 from Imperia). All carcasses were sexed and stored in sealed plastic bags at –20 °C, while the intestines were kept at –80 °C for at least seven days before necropsy, in order to inactivate potential sources of infection.

Larvae were detected by preparing smears of different pulmonary sections. Lungs, heart and main vessels were dissected and washed to search for

adult parasite and larvae. Nematodes were clarified with lactophenol and identified according to Anderson (1992).

The chi-squared test (χ^2) was used to compare the percentages of animals harbouring parasites, while parasite burdens in the foxes from Liguria and Piedmont were compared by the Kruskal-Wallis test. Multivariate analysis was carried out using logistic regression (Armitage *et al*., 2002). The software employed was R 2.8.1 (R Development Core Team, 2008).

RESULTS

A. vasorum was found in the cardiopulmonary system of 52 (27.5%) foxes. The infection prevalence in the seven areas was significantly different ($\chi^2 = 101$, d.f. = 6, $P < 0.001$; Tab. 1), with the highest values in the district of Imperia (80%) and in the municipality of Montezemolo (70%). The overall prevalence in Tuscany was 7% (95% confidence interval: 3.2% - 12.8%); there was no significant difference among the prevalences in the four areas examined in this region ($\chi^2 = 2.4$, d.f. = 3, $P = 0.50$). The prevalence of infection was significantly higher in male than in female foxes ($\chi^2 = 4.30$, d.f. = 1 , $P = 0.04$), but this effect is probably spurious and caused by sampling (many male foxes were gathered in the areas with higher prevalence). In the area of Imperia, where the sample was big enough to allow a statistical analysis, the prevalence of the infection was not different among sexes ($\chi^2 = 0.56$, d.f. = 1 , $P = 0.45$). Furthermore, the effects of area of collection and gender of the fox on the prevalence of *A. vasorum* were studied simultaneously by a multivariate logistic regression model. The analysis showed that the only significant effect was the one related to the sampling area ($P < 0.001$), while the effect of gender was not significant ($P = 0.43$).

The median burden of *A. vasorum* was of 3 nematodes per host (range: 0-40) in the municipality of Montezemolo and of 2 nematodes per host (range: 0-10) in the area of Imperia. The difference was not significant (Kruskal-Wallis $H = 0.98$, d.f = 1, $P = 0.32$). One badger from Imperia was found to be infected.

DISCUSSION

The prevalence of *A. vasorum* was highly variable between the areas of study. The foxes sampled in Liguria, a region never investigated before for *A. vasorum*, showed a prevalence much higher than in foxes from Tuscany. A similar patchy distribution of *A. vasorum* has already been reported in other countries (e.g. Morgan *et al*., 2008 and references therein). It is difficult to explain this high variability between neighbouring areas, lacking a precise knowledge of the biology of the parasite and of the relative importance of the various intermediate hosts in its transmission (Koch and Willesen, 2009). It is likely that interactions between the climate and intermediate and definitive host densities influence the chances of parasite establishment and persistence and therefore the local risk of infection for foxes and dogs (Morgan *et al*., 2008).

The infected badger from Liguria represents the first report of the presen-

Table 1 - Prevalence of *A. vasorum* in foxes recorded in the seven areas considered. N = number of foxes sampled in the area; F/M pos = number of female/male foxes infected by *A. vasorum*, F/M neg = number of female/male foxes not infected by *A. vasorum*.

Study area	N	F pos	F neg	M pos	M neg	Prevalence (%)
Grosseto	37	2	20	1	14	8.1
Cecina	50	2	29	3	16	10.0
Pisa	26	1	16	0	9	3.8
Siena	16	0	7	0	9	0.0
Imperia	45	15	5	21	4	80.0
Borgo San Dalmazzo	5	0	3	0	2	0.0
Montezemolo	10	2	1	5	2	70.0
Total	189	22	81	30	56	27.5

ce of *A. vasorum* in this mustelid in Italy. In Spain, 3/47 badgers from the western Mediterranean area (Torres *et al*., 2001), and 10/24 from the Basque country (Millan *et al*., 2004) were found positive for *A. vasorum*. As far as we know, these are the only cases recently recorded in Europe.

The prevalence recorded for Tuscany was much lower than that reported in both 1984 (24%; Poli *et al*., 1984) and 1991 (39%; Poli *et al*., 1991). *A. vasorum* is reported to be more frequent in the fox than in the dog (Morgan *et al*., 2005), canine angiostrongylosis representing an epiphenomenon of the parasite's wild cycle "Gasteropoda-fox", as found in other European countries (Bolt *et al*., 1994). However, it cannot be excluded that the infection in the dog could be underestimated, due to specific diagnostic deficiencies. The laboratory analysis of fresh faeces by Baermann's technique can give false negative results when applied to a single sample and thus it does not guarantee the absence of the infection. The analysis should be repeated at least on three samples, collected on different days, because the shedding of the larvae may be intermittent (Patteson *et al*., 1993; Oliveira-Júnior *et al*., 2006). It should be pointed out that angiostrongylosis can cause severe cardio-respiratory problems in the dog, resulting eventually in the death of the animal. Furthermore, ectopic locations of the parasite are reported in the dog, especially in the eye and in the CNS (see e.g. Oliveira-Júnior *et al*., 2004; Scaramozzino *et al*., 2007).

In the light of the present results, veterinary practitioners should be aware of the possibility of canine angiostrongylosis, since in the study area the prevalence of the parasite in the fox is relatively high. Transmission is possible (Bolt *et al*., 1992, 1994), although it is not fully proved that fox populations act effectively as a reservoir of the infection for dogs. The foxes sampled in the present study were from rural areas. Urban and sub-urban foxes have presumably greater opportunities of contacting domestic

dogs. In endemic regions, dog angiostrongylosis is reported to occur frequently in places where several dogs are confined into relatively small areas, such as kennels, and where fox density is high (Bolt *et al*., 1994). Further research is needed to estimate the importance of *Angiostrongylus* infection in dogs hosted in kennels in Tuscany and Liguria and to explore the relationship between the prevalence of the infection in foxes and dogs.

REFERENCES

Anderson R.C. 1992. Nematode Parasites of Vertebrates, CAB International, Wallingford, UK.

Armitage P., Berry G. and Matthews J.N.S. 2002. Statistical Methods in Medical Research, Blackwell Publishing, Oxford, UK.

Bolt G., Monrad J., Henriksen P., Dietz H.H., Kock J., Bindseil E. and Jensen A.L. 1992. The fox (*Vulpes vulpes*) as a reservoir for canine angiostrongylosis in Denmark. *Acta Vet. Scand.*, 33: 357-362.

Bolt G., Monrad J., Koch J. and Jensen A.L. 1994. Canine angiostrongylosis: a review. *Vet. Rec.,* 135: 447-452.

Della Santa D., Citi S., Marchetti V. and Tardoni S. 2002. Infestione da *Angiostrongylus vasorum* nel cane: review della letteratura e presentazione di un caso clinico. *Veterinaria*, 16: 9-14.

Iori A., Costantini R. and Cancrini G. 1990. Parassiti in volpi provenienti da alcune regioni italiane. *Parassitologia*, 32: 153-154.

Koch J. and Willesen J.L. 2009. Canine pulmonary angiostrongylosis: an update. *Vet J.*, 179(3): 348-359.

Leoni A., Garippa G. and Masala S. 1986. Osservazioni sull'elmintofauna della volpe (*Vulpes vulpes*) in Sardegna. *Parassitologia*, 28: 268-269.

Magi M., Macchioni F., Dell'Omodarme M., Prati M.C., Calderini P., Gabrielli S., Iori A. and Cancrini G. 2009. Endoparasites of *Vulpes vulpes* in Central Italy. *J. Wild. Dis*., 45(3): 881-885.

Millan J., Sevilla I., Gerrikagoitia X., Garcia-Perez A.L. and Barral M. 2004. Helminth parasites of the Eurasian badger (*Meles meles* L.) in the Basque Country (Spain). *Eur. J. Wildl Res.*, 50: 37-40.

Morgan E.R., Shaw S.E., Brennan S.F., De Waal T.D., Jones B.R. and Mulcahy G. 2005. *Angiostrongylus vasorum*: a real heartbreaker. *Trends in Parasit*., 21: 49-51.

Morgan E.R., Tomlinson A., Hunter S., Nichols T., Roberts E., Fox M.T. and Taylor M.A. 2008. *Angiostrongylus vasorum* and *Eucoleus aerophilus* in foxes (*Vulpes vulpes*) in Great Britain. *Vet Parasitol*., 154(1-2): 48-57.

Oliveira-Júnior S.D., Barçante J.M., Barçante T.A., Ribeiro V.M. and Lima W.S. 2004. Ectopic location of adult worms and first-stage larvae of *Angiostrongylus vasorum* in an infected dog. *Vet Parasitol*., 121(3-4): 293-296.

Oliveira-Júnior S.D., Barçante J.M.P., Barçante T.A., Dias S.R.C. and Lima W.S. 2006. Larval output of infected and re-infected dogs with *Angiostrongylus vasorum*. *Vet. Parasitol*., 141: 101-106.

Patteson M.W., Gibbs C., Wotton P.R. and Day M.J. 1993. *Angiostrongylus vasorum* infection in seven dogs. *Vet. Rec*., 133: 565-570.

Poli A., Arispici M., Marconcini A., Mancianti F. and de Monte D. 1984. *Angiostrongylus vasorum* (Baillet, 1866) in red foxes (*Vulpes vulpes* L.) in Italy. *J. Wild. Dis*., 20(4): 345-346.

Poli A., Arispici M., Mancianti F. and Abramo F. 1991. Pathology of naturally acquired *Angiostrongylus vasorum* infection in the red fox (*Vulpes vulpes*). *Angew Parasitol*., 32: 121-126.

R Development Core Team. 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sasanelli M., Paradies P., Otranto D., Lia R.P. and De Caprariis D. 2008. Haemothorax associated with *Angiostrongylus vasorum* infection in a dog. *J. Small Anim. Pract*., 49: 417-419.

Scaramozzino P., Eleni C., De Liberato C., Mastromattei A. and Terracciano G. 2007. Broncopolmoniti parassitarie da *Angiostrongylus vasorum* e *Aelurostrongylus abstrusus* nel cane e nel gatto. Atti IV Congr. Naz. AIPVet, 112-116.

Torres J., Miquel J. and Motje M. 2001. Helminth parasites of Eurasian badger (*Meles meles* L.) in Spain: a biogeographic approach. *Parasitol. Res*., 87: 259-263.

Traversa D., Torbidone A., Malatesta D. and Guglielmini C. 2008. Occurrence of fatal canine *Angiostrongylus vasorum* infection in Italy. *Vet. Parasit*., 152: 162-166.