

# The Fox (*Vulpes vulpes*) as a Reservoir for Canine Angiostrongylosis in Denmark

## Field survey and experimental infections

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**Bolt, G., J. Monrad, P. Henriksen, H. H. Dietz, J. Koch, E. Bindseil and A. L. Jensen: The fox (*Vulpes vulpes*) as a reservoir for canine angiostrongylosis in Denmark. Acta vet. scand. 1992, 33, 357-362.** – Until recently, *Angiostrongylus vasorum* was not considered to be an endogenous Danish parasite, since demonstration of this worm had been confined to necropsy findings in 2 dogs, both of which had visited France. During the last 2 years, however, clinical cases have been diagnosed among a considerable number of Danish dogs, none of which had ever been outside Denmark. All these cases have occurred north of Copenhagen, where an endemic focus seems to exist. In this field survey *A. vasorum* was found for the first time in wild Danish red foxes. Furthermore, experimental infections showed that the parasite can be transferred between foxes and dogs. Consequently, the wild fox population must be considered a potential reservoir for transfer of *A. vasorum* to domestic dogs.

*Angiostrongylus vasorum; experimental transfer.*

### Introduction

*Angiostrongylus vasorum* (French heartworm) is a metastrongyloid parasite of the domestic dog (*Canis familiaris*) and other carnivores belonging to *Canidae*. The adult worms parasitize in the right atrium of the heart and particularly in the pulmonary arteries. The life cycle is indirect with a number of mollusc species acting as obligatory intermediate hosts (Guilhon 1965, Guilhon 1967, Guilhon & Cens 1973.). The pathological and clinical manifestations of canine angiostrongylosis are multiple, as reported elsewhere (Dodd 1973, Guelfi 1976, Prestwood et al. 1981, Schelling et al. 1986). The major symptoms are respiratory distress and loss of condition (Cobb & Fisher 1990, Guelfi 1976, Koch & Bolt 1990). The parasitological

diagnosis is based on the demonstration of the characteristic first stage larvae (L1) in faecal samples analyzed by the Baermann method (Jacobs & Prole 1975, Jørgensen & Madsen 1982).

In Europe *A. vasorum* is known to be endemic among dogs in south-west France (Dorchies 1976, Guilhon & Cens 1973), Ireland (Dodd 1973, Jacobs & Prole 1975), and in south-west England (Jones et al. 1980, Simpson & Neal 1982). Sporadic cases have been reported from other parts of France (Guilhon 1969), south-east England (Cobb & Fisher 1990), former West Germany (Pal-laske 1967), Spain (Sanchez et al. 1979), Switzerland (Eckert & Lämmler 1972, Neff 1971), and from the European part of the former

Sovjet Union (Eckert & Lämmler 1972, Rosen et al. 1970). Outside Europe the parasite has been reported to be endemic among dogs in Uganda (Bwangamoi 1972), and sporadic cases have been reported from dogs in Brasil (Lima et al. 1985, Rosen et al. 1970), Columbia (Rosen et al. 1970), the United States (Williams 1985), and from the Asiatic part of the former Sovjet Union (Eckert & Lämmler 1972). All cases reported from the United States have occurred among imported dogs.

Among foxes (*Vulpes vulpes*) *A. vasorum* has been reported from France (Guilhon 1971), Ireland (Guilhon 1971), Italy (Poli et al. 1985), Spain (Tarazona 1974), the former Sovjet Union (Eckert & Lämmler 1972, Rosen et al. 1970), Germany (Rosen et al. 1970) and Canada (Smith & Threlfall 1973). In Italy and France the fox is considered to be a reservoir for transmission of *A. vasorum* to domestic dogs (Guilhon 1965, Guilhon 1971, Poli et al. 1985). Guilhon successfully transferred the parasite from dogs to 2 foxes and a jackal (*Canis aureus*). Patent infections were established in all 3 experimental animals (Guilhon 1965). According to our knowledge no experimental transfer from wild foxes to dogs has ever been reported.

In Denmark *A. vasorum* until recently had only been detected on necropsy of 2 dogs, both of which had visited France (Finnerup 1983, Monrad 1989). Over the last 2 years, however, clinical cases have been diagnosed in a considerable number of Danish dogs, none of which have ever been outside Denmark (Koch 1992, Koch & Bolt 1990, Rosenlund et al. 1991). Interestingly, all these cases have occurred north of Copenhagen, where an endemic focus of yet unknown, but possibly growing extension seems to exist. This suburban region is characterized by a dense population of red foxes living in the immedi-

ate vicinity of humans and their dogs. The present study was designed to examine the occurrence of *A. vasorum* in the red fox population in the locality north of Copenhagen, where the canine outbreaks had occurred. Furthermore, artificial infections were established in order to investigate, whether strains of *A. vasorum* isolated from Danish foxes and dogs might be transmissible to dogs and foxes, respectively.

### Materials and methods

#### *Examination of wild foxes*

During the period October 1990 to May 1991 a total of 15 adult red foxes (*V. vulpes*) from the above mentioned region were examined for *A. vasorum*. Of these 13 foxes were killed during hunting and can therefore be considered fairly representative of the fox population at the hunting ground, which is located in the northern outskirts of Copenhagen (Jægersborg Game Reserve). Faecal samples from all 15 foxes were subjected to the Baermann method. Hearts and lungs from some of the foxes were dissected. The right atrium and the pulmonary arteries were inspected for adult parasites, but neither gross pathological nor histopathological examinations were performed.

#### *Production of infective larvae*

L1 were isolated by the Baermann method from faeces of naturally infected foxes and dogs. The snails *Biomphalaria glabrata* and *Helix pomatia* (edible snail) were used as intermediate hosts. Experimental infection of *B. glabrata*, was performed by adding L1 to a beaker containing the snails according to the method described by Guilhon & de Gaalon (1969). Experimental infection of *H. pomatia* was performed in 2 steps. Firstly, each snail was fed a small piece of lettuce contaminated with fluid containing L1 as

described by *Guilhon & Afghabi* (1969), and secondly, each snail was placed on a water film containing the L1 larvae in a beaker.

In order to demonstrate the development of infective third-stage larvae (L3), the snails were comminuted, and a proportion (10%) of the snail tissue was digested in an HCl/pepsin solution at 30°C. Slightly modified versions of the techniques described by *Wallace & Rosen* (1969) were used. The L3 recovered from the snails by digestion were counted under a stereomicroscope.

#### *Infection of foxes and dogs*

The final hosts were infected by feeding them infective snail material mixed with a palatable feed. Two adult silver foxes (*V. vulpes*) originating from a fox farm were infected with L3 developed from L1, which had originally been isolated from the faeces of a dog suffering from angiostrongylosis. Fox No. 1 received approximately 250 larvae passed in *B. glabrata* and fox No. 2 approximately 100 larvae passed in *H. pomatia*. Another 2 uninfected foxes (No. 3 and No. 4), which were of the same origin as fox No. 1 and No. 2, served as non-infected controls. The faeces of all 4 foxes were examined by the Baermann method once a week.

Two 4 month old SPF beagle puppies (dog No. 1 and dog No. 2) were infected, as well. Dog No. 1 was infected with 100-200 L3 developed in *B. glabrata* from L1 isolated from the faeces of 2 wild red foxes. Dog No. 2 was infected with 300-500 L3 developed in *B. glabrata* from L1 isolated from the faeces of 2 dogs suffering from angiostrongylosis. Faeces from the dogs were examined by the Baermann method twice a week.

Gross pathological lesions were noticed at necropsy of foxes and dogs, while histopathological examination of lung tissue was carried out in the dogs only.

## **Results**

### *Wild foxes*

In 12 of the 15 foxes typical *A. vasorum* L1 were found in the faeces, though in moderate numbers (< 150 L1/g). Poor blood coagulation was observed in 4 of the infected foxes. Whenever infected foxes were necropsied, adult *A. vasorum* of both sexes were found in the right atrium of the heart and in the pulmonary arteries. Few worms were found in each fox, and all foxes appeared to be in good bodily condition, indicating that the infection was of minor significance to the foxes. Another 2 helminth species, *Crenosoma vulpis* and *Capillaria aerophila*, were found in 8 and 2 of the foxes, respectively.

### *Experimental infection of foxes*

In the faeces from fox No. 1 and fox No. 2 excretion of *A. vasorum* L1 started 52 and 55 days post infection, respectively. The foxes were euthanized 74 days post infection. At that time the faeces of fox No. 1 and fox No. 2 contained 153 L1/g and 1.5 L1/g, respectively. No clinical symptoms were noticed during the course of infection. At necropsy gross pathological lesions comparable to those described for naturally infected foxes (*Poli et al.* 1985) and experimentally infected dogs (*Neff* 1971, *Prestwood et al.* 1981) were found in the lungs of both foxes. A total of 52 adult worms (28 females and 24 males) were found in fox No. 1, whereas a total of 8 adult worms (5 females and 3 males) were found in fox No. 2. The faeces of fox No. 3 and fox No. 4 did at no time contain larvae, and no worms were found post mortem in these 2 foxes.

### *Experimental infection of dogs*

In the faeces from dog No. 1 and dog No. 2 excretion of *A. vasorum* L1 started 56 and 52 days post infection, respectively. The dogs

were euthanised 135 days post infection, as dog No. 2 was exhibiting mild symptoms of a circulatory disorder. At that time the faeces of dog No. 1 and dog No. 2 contained 5,760 L1/g and 12,480 L1/g, respectively. At necropsy gross pathological and histopathological lesions comparable to those described for other experimentally infected dogs (Neff 1971, Prestwood 1981) were found in both dogs. A total of 43 adult worms (24 females and 19 males) were found in dog No. 1, whereas a total of 437 adult worms (289 females and 148 males) were found in dog No. 2.

### Discussion

It appears that this was the first time for *A. vasorum* to be observed in wild Danish foxes, since this parasite was not detected in the only parasitological survey of wild foxes, previously reported from Denmark (Guildal & Clausen 1973). When and how the infection was introduced into the wild Danish fox population remains unknown, however. In Denmark even the first canine angiostrongylosis case was observed within recent years (Finnerup 1983). Being a conspicuous worm parasite, it seems most unlikely that *A. vasorum* would be overlooked in a country like Denmark for many decades.

However, the mere detection of *A. vasorum* in wild foxes originating from an area enzootic of canine angiostrongylosis would be of little value. Thus, certain nematode parasite strains isolated from wild deer have proved incompatible to cattle, although another natural strain of the same worm species is found in cattle (Korsholm 1988). Theoretically, something similar might apply to fox and dog strains of *A. vasorum*, for which reason, our successful experimental transfer of a fox strain of *A. vasorum* to dogs and *vice versa* was of great importance. Thus,

it was proved that this parasite may be transmitted between Danish foxes and dogs. In particular our experimental transfer from fox to dog must be considered the first proof of the fact that a fox population may serve as a reservoir for transmission of *A. vasorum* to domestic dogs.

No explanation can be given, so far, as to the uneven geographic distribution of *A. vasorum* among dogs, a distribution which is characterized by enzootic occurrence in some isolated regions and sporadic cases in others. Eckert & Lämmler (1972) proposed that *A. vasorum* is a common parasite among wild carnivores, from which it is only occasionally transmitted to the dog population. Here it can be maintained under favourable conditions, including a high density of the intermediate host population, resulting from mild winters and high air humidity. Indeed, the latest 3 winters have been exceptionally mild in Denmark.

The finding of a high incidence of low grade infections among wild foxes, which seemed unaffected by the infection, indicates a considerable degree of adaptation between *A. vasorum* and the fox. In combination with the finding that *A. vasorum* can be transmitted between foxes and dogs, and the fact that all known cases of canine angiostrongylosis in Denmark have occurred in a region with a dense suburban fox population, suggests that foxes could be a reservoir of epidemiological importance, particularly in such areas with close coexistence of foxes and dogs.

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#### Sammendrag

*Feltundersøgelser og eksperimentelle infektioner til belysning af vildråven (Vulpes vulpes) som reservoir for angiostrongylose hos hunde i Danmark.*

I Danmark havde *Angiostrongylus vasorum* indtil for få år siden kun været påvist ved sektion af to danske hunde, som begge havde været i Frankrig forinden. Inden for de sidste to år er imidlertid konstateret et betydeligt antal kliniske tilfælde hos danske hunde, som aldrig har været i udlandet. Alle disse tilfælde er forekommet nord for København, hvor et endemisk focus synes at eksistere. Ved feltundersøgelser påvist *A. vasorum* for første gang hos danske ræve. Ved eksperimentelle infektioner blev det bekræftet, at parasitten kan overføres mellem ræve og hunde. Rævebestanden i det endemiske focus må derfor betragtes som et potentielt reservoir for smitte til hunde.

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