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A coprological investigation of gastrointestinal and cardiopulmonary parasites in hunting dogs in Denmark

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ABSTRACT

A coprological survey was conducted to investigate the prevalence of parasites infecting hunting dogs with no history of recent anthelmintic treatments and with no overt clinical manifestations of cardiopulmonary or gastrointestinal illness. The hunting dogs were recruited from four different areas in Denmark, and fecal samples were obtained in October and November, 2007. For detecting gastrointestinal parasites, samples ($N = 178$) were examined by a commercial flotation kit (Fecalyzer® EVSCO, USA). For detection of cardiopulmonary parasites, samples ($N = 181$) were collected on three consecutive days and examined using the Baermann method. Parasites were recovered from 22.1% of the hunting dogs: *Angiostrongylus vasorum* (2.2%), *Toxocara canis* (12.4%), *Uncinaria stenocephala* (7.3%), *Taenia* spp. (1.7%), *Toxascaris leonina* (0.6%), Coccidia (0.6%) and unidentified trematode eggs (1.1%). Infection with only one species of parasite was more common (89.5%) than infection with two species (10.5%). A multiple logistic regression model showed that prevalence of intestinal parasites was not influenced by age, gender or breed in adult dogs. There was a significantly higher prevalence of intestinal parasites in the densely populated area of the island Zealand compared with the less populated regions of the peninsular Jutland. The present study reports the first case of *A. vasorum* in a dog from Jutland. The dog had been visiting the endemic area of western Zealand, suggesting that translocation of sub-clinically infected dogs may contribute to introduction of *A. vasorum* into non-endemic areas.

1. Introduction

Several parasite species infect dogs with significant interspecies transmission and zoonotic potential. Over the last years surveys were conducted to investigate the prevalence of gastrointestinal (GI) parasites among dogs (Fok, 2001; Papazahariadou et al., 2007; Savilla et al., 2011). In Denmark, prevalence studies of GI parasites have involved companion dogs (Pelle, 1999) and hunting dogs (Tønsberg et al., 2004). The later study was restricted to hunting dogs from one area while no previous studies of GI parasites included hunting dogs from the largest region in Denmark; Jutland. Baseline prevalence studies of pet and hunting dogs are essential for providing data for developing protocols for controlling canine parasitic infections, which could eventually not only benefit the dog's health but also reduce the risks of zoonotic infections (Robertson and Thompson, 2002).

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Dog infections with *Angiostrongylus vasorum* can cause severe illness including pulmonary distress, hemorrhagic diathesis, depression, anorexia and even sudden death (Chapman et al., 2004). For detecting cardio-pulmonary helminth infections, first stage larvae (L1) of the parasites are usually isolated using the Baermann method, and sampling fecal material from three consecutive days increases the sensitivity due to intermittent excretion (Willesen et al., 2004). In case of absence of larvae in the examined fecal material, other methods such as radiology, tracheal lavage and blood work may be used to detect canine angiostrongylosis (Koch and Willesen, 2009).

In Denmark, *A. vasorum* was first reported in a dog in 1983 (Finnerup, 1983). Since then, the annual numbers of cases of *A. vasorum* infections have increased (Tønsberg et al., 2004). The fox (*Vulpes vulpes*) is a wildlife reservoir host for *A. vasorum* (Tønsberg et al., 2004; Saeed et al., 2006). The well documented high prevalence *A. vasorum* among foxes from the city of Copenhagen together with the high fox population density in that area (Saeed et al., 2006), might favor the transmission to the domestic dog (Morgan et al., 2005). The main aim of this study was to screen for the possible presence of subclinical infections with *A. vasorum* in clinically healthy hunting dogs, and secondary aims were to investigate the prevalence of GI and cardiopulmonary (CP) parasites in these dogs within the different regions of Denmark using coprological examination.

2. Materials and methods

2.1. Study area

The study area comprised of the two major regions of Denmark; Zealand and Jutland. The sparsely populated region of Jutland is part of the European mainland, and politically divided into three major areas: Northern (NJ), Central (CJ) and Southern Jutland (SJ) (Fig. 1). Within Zealand, samples were only collected from the densely populated area of Northern Zealand, from which previous studies showed high prevalence of *A. vasorum* in dogs.

2.2. Study population

Sampling of dogs was dependent upon the willingness of dog owners to participate after announcement in the Danish hunting dog organizations and on the Danish Kennel Club's homepage. Only dogs that could meet both of the following criteria were included in this study: (1) Dogs whose owners noticed no pulmonary symptoms; (2) Active hunting dogs which had not been treated with anthelmintics or macrocyclic lactone within one month prior sampling. Additional information about the dogs was obtained including address of owner, age of dog, sex, breed, id number, date of last anthelmintics treatment and trade name of used anthelmintics drug. The maximum number of participating dogs from the same household was set to four. Of the 181 examined dogs, 67 were males and 114 females. Nineteen different breeds were represented and the most common breeds were Labrador retriever ($N = 57$), German Wirehaired Pointer ($N = 39$), Small Munsterlander ($N = 17$), Flat Coated Retriever ($N = 15$) and German Shorthaired Pointer ($N = 10$). The median age of the sampled animals was four years and four months ranging from six months to 13 years (25% quartile: 2 years, and 75% quartile: 6 years).

2.3. Sampling of fecal material

Fecal samples from hunting dogs were collected in October and November, 2007. Feces was collected by the owner from the ground on three consecutive days and kept at 4 °C until submission for examination to the parasitological laboratory at the University of Copenhagen. To minimize the risk of contamination with free-living nematodes the owners were informed that only fresh stools should be collected with as little grass or ground material contamination as possible.

2.4. Examination of fecal samples

For recovery of larvae of *A. vasorum* and *Crenosoma vulpis*, a total of 543 fecal samples from 181 dogs were collected on three consecutive days (~10 g/dog). The three samples were examined individually using the Baermann technique described by Hardon (2001), with slight modifications: three layers of gauze (Gauze swabs, 10 cm × 10 cm, type 17, Kruuse, China) surrounding the sample, were submerged in tap water (20–22 °C) in a plastic bag for 24 h. The plastic bags were then opened, and the fecal mass was removed. The sediment material, minimum 1.5 ml was aspirated by glass pipettes (long form, 230 mm, Cat. No. 567/2, Assistant™, Sondheim/Rhön, Germany) and placed in 1.5 ml micro-centrifuge tubes, maximum three tubes, and centrifuged at 3000 × *g* for approximately 30 s. 2–3 drops from the sediment were transferred to a glass slide with a drop of Lugol's fluid and mounted with a cover slip and thereafter examined under light microscope (200×–400× magnifications). The larvae were identified according to the morphologic description given by Rosen et al. (1970). If positive larvae were detected, they were transferred to a 3.5 ml petri dish containing deionized sterile water and examined under a dissection stereomicroscope (40×–100× magnifications). Larvae were then collected, using a glass pipette and transferred into a 200 μl tube containing 5 μl distilled water or PBS and kept frozen at −20 °C for later molecular verification of *A. vasorum* using PCR (Al-Sabi et al., 2010).

Additional fecal samples (2 g) from 178 dogs were examined for GI parasites within one to three days of being submitted. There was insufficient material for examination for GI parasites in the samples from three dogs. Eggs of GI parasites were isolated by a standard flotation kit (Fecalyzer® EVSCO, USA), which uses sodium nitrate as a flotation fluid (specific gravity: 1.22), and the duration of parasite egg floatation was around 15 min. The parasite eggs were differentiated according to morphologic characteristics described by Thienpont et al. (1986). Quantitative measurement of helminth infection (EPG) was not implemented.