International Journal for Parasitology: Parasites and Wildlife 2 (2013) 144–151

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Endoparasites of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in Denmark 2009–2012 – A comparative study

Mohammad Nafi Solaiman Al-Sabi [a,*], Mariann Chriél [a], Trine Hammer Jensen [b], Heidi Larsen Enemark [a]

[a] Section for Bacteriology, Pathology and Parasitology, National Veterinary Institute, Technical University of Denmark, DK-1870 Frederiksberg C, Denmark
[b] Department of Biotechnology, Chemistry and Environmental Engineering Aalborg University/Aalborg Zoo, DK-9000 Aalborg, Denmark

ARTICLE INFO

Article history:
Received 21 December 2012
Revised 26 March 2013
Accepted 2 April 2013

Keywords:
Gastrointestinal helminthes
Invasive species
Nyctereutes procyonoides
Vulpes vulpes
Echinococcus multilocularis
Alaria alata
Intestinal distribution
Zoonosis

ABSTRACT

Invasive species negatively influence the biodiversity of the ecosystems they invade and may introduce pathogens to native species. Raccoon dogs have very successfully invaded Europe, including, recently, Denmark. This study included analyses of gastrointestinal helminths and *Trichinella* spp. from 99 raccoon dogs and 384 native red foxes collected from October 2009 to March 2012. The sedimentation and counting method used revealed that raccoon dogs and foxes harboured 9 and 13 different helminth species, respectively, of which several known to be zoonotic. Significantly more nematode and cestode species were found in foxes while raccoon dogs had more trematode species. Rodent transmitted parasites were more prevalent in foxes, while amphibian transmitted parasites were more prevalent in raccoon dogs. One fox was infected with *Echinococcus multilocularis* (0.3%), while no *Trichinella* spp. were detected in raccoon dogs or foxes. The trematode *Brachylaima tokudai* was detected for the first time in Denmark in five of 384 foxes (1.3%). Prevalences of *Pygidiopsis summa* (3.0% and 3.4%) and *Cryptocotyle* spp. (15.2% and 15.4%) were comparable in raccoon dogs and foxes, respectively. Four helminth species were more prevalent in foxes than in raccoon dogs: *Toxocara canis* (60.9% and 13.1%); *Uncinaria stenocephala* (84.1% and 48.5%); *Mesocestoides* spp. (42.7% and 23.2%); and *Taenia* spp. (30.7% and 2.0%), respectively. Three helminth species were more prevalent in raccoon dogs than in foxes: *Dipylidium caninum* (5.1% and 0.3%); *Mesorchis denticulatus* (38.4% and 4.2%); and *Alaria alata* (69.7% and 34.4%), respectively. *T. canis* was more abundant in foxes while *A. alata* was more abundant in raccoon dogs. The intestinal distribution of a number of helminth species was comparable between hosts, but highly variable between parasite species. Inherent biological factors and host invasion of new areas might have shaped these marked differences in helminth fauna between the invasive raccoon dog and the native red fox.

1. Introduction

The raccoon dog (*Nyctereutes procyonoides*) was deliberately introduced into the Western part of the former Soviet Union in the 1920s for use in fur production, and later migrated to numerous European countries. In Denmark, only one raccoon dog was observed prior to 1995. Throughout the period 1995–2003, 25 raccoon dogs were reported (Baagøe and Ujvári, 2007). However, in 2008 five raccoon dogs were shot or road-killed. Subsequently, and coinciding with increased public awareness, from July 2008 to December 2010, a total of 91 raccoon dogs were shot or road-killed (Danish Nature Agency, 2010), all in the peninsula of Jutland which is the only part of Denmark connected to mainland Europe, via the border to Germany.

In addition to its negative effects on biodiversity, the introduction of raccoon dogs is associated with the risk of introducing parasites to new areas (Ivanov and Semenova, 2000). Numerous field surveys on raccoon dogs have documented the presence of several parasite species of zoonotic potential (Zablotskii, 1970; Sato et al., 1999a, b; Ivanov and Semenova, 2000; Thiess et al., 2001; Shimalov and Shimalov, 2002; Anisimova, 2008; Kornyushin et al., 2011; Bruzinskaite-Schmidhalter et al., 2012). Among the most important zoonotic parasites transmitted by raccoon dogs in Europe are nematodes of the genus *Trichinella*. Wild carnivores are major hosts contributing to the transmission of the infection to pigs and subsequently to humans (Malakauskas et al., 2007). A field study in Finland concluded that raccoon dogs together with red foxes were the most important reservoir hosts for *Trichinella* spp. (Airas et al., 2010). This is of particular interest in non-endemic countries, for example Denmark (Enemark et al., 2000), where increasing numbers of invading raccoon dogs may affect the pig industry negatively, and therefore the *Trichinella* prevalence is

* Corresponding author. Tel.: +45 35886196.
E-mail address: moals@vet.dtu.dk (M.N.S. Al-Sabi).

2213-2244 © 2013 Australian Society for Parasitology Published by Elsevier Ltd. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.ijppaw.2013.04.001

continuously monitored in wild carnivores (Danish Zoonosis Centre, 2012).

Another important zoonotic parasite of the raccoon dog is *Echinococcus multilocularis*. Experimental infections showed that the raccoon dog excrete infective eggs in quantities similar to that of foxes (Kapel et al., 2006). In Europe, adult *E. multilocularis* were recovered from naturally infected raccoon dogs in Germany (Thiess et al., 2001; Wolf and Weber, 2009), Poland (Machnicka-Rowińska et al., 2002), and Lithuania (Bruzinskaite-Schmidhalter et al., 2012). However, the role of raccoon dogs in the life cycle of *E. multilocularis* is still questionable because of lower prevalence and abundance within natural infection, possibly because rodents are less common in their diet than in the diet of foxes (Bruzinskaite-Schmidhalter et al., 2012). In addition, raccoon dogs are shy animals and maintain a long distance from human activities. They are also nocturnal, settle in wet habitats, and hibernate in winter (Kauhala and Kowalczyk, 2011), and these characteristics may limit their role in *E. multilocularis* transmission. In Denmark, *E. multilocularis* was detected for the first time in three of 646 foxes from the island of Zealand in 2000 (Saeed et al., 2006), but nothing is known about the current prevalence, and the present study is the first for a decade to estimate the prevalence of this important zoonotic parasite among foxes and raccoon dogs.

In 2009 the Danish Ministry of the Environment initiated a plan for eradication of raccoon dogs primarily because of the expected negative effects on biodiversity (Danish Nature Agency, 2010). This provided an opportunity to study intestinal helminths and *Trichinella* spp. in raccoon dogs and to compare their parasite fauna with that of the red foxes (*Vulpes vulpes*).

2. Materials and methods

2.1. Study area and animal sampling and processing

Animals were collected from two major regions in Denmark, the mainland and the islands. The mainland is a peninsula named Jutland and is connected to mainland Europe, whereas the islands (Zealand, Funen, Møn and Lolland) that are separated from the mainland. Both regions are relatively flat (maximum elevation above sea level: 173 m) and are rich in woodlands and agricultural areas, as well as villages, small towns and cities (Statistics Denmark, 2012). During the period October 2009–March 2012 raccoon dogs that had been road-killed, hunted or caught in traps and euthanized as part of the eradication plan in mainland were examined. Foxes that had been hunted were collected from throughout Denmark. All animals were necropsied, and animal age (young < 1 year; adult > 1 year; Kauhala and Helle, 1990), season of sampling (only given for raccoon dogs since foxes were hunted only in winter), gender, weight and general health condition were recorded.

Viscera of animals sampled were frozen for at least 4 days at −80 °C to deactivate viable eggs of *E. multilocularis* (Eckert et al., 2001). Subsequently, the viscera were thawed and dissected using the intestinal sedimentation and counting technique (Hofer et al., 2000). Briefly, the small intestine was divided into three equal sections. Solid pieces of intestinal contents were removed before transferring the sections into a 1 L glass bottle containing physiological saline solution. After vigorous shaking for approximately 20 s, the intestinal sections were removed after scraping the mucosa from the underlying tissue by running the intestine between two fingers. The intestinal contents were then left to sediment for at least 15 min, after which the supernatant was decanted and the bottle was refilled with saline. This washing procedure was repeated until a clear supernatant was obtained. The sediment was stored in jars with 70% ethanol for identification and counting of parasites. The entire sediment was examined in Petri dishes using a stereomicroscope at 100× magnification, and the helminths recovered were identified according to Euzeby (1982) and Christensen and Roth (1949). When helminth counts exceeded 100 before the entire sediment had been examined, one sub-sample was examined following a 20:1 dilution with physiological saline. If *E. multilocularis* was detected, the 12S rRNA gene was sequenced (Stefanic et al., 2004). Larvae of *Trichinella* spp. were detected and recovered using the magnetic stirrer method according to Commission Regulation No 2075/2005/EC (European Commission, 2005).

2.2. Statistics

Statistical analyses were performed using SAS statistical package (SAS Enterprise Guide Version 4.3®; SAS institute Inc. Cary, NC). Summaries of parasites prevalence, range of intensity of infection, mean intensity and abundance were assessed according to Bush et al. (1997), and data were presented as mean values ± 95% confidence intervals (95% CI) fitted for binomial distribution. The prevalence of parasite infections among raccoon dogs and foxes was evaluated for association with several ecological and biological factors in a negative binomial and zero inflated model. The dependent variable was the parasite infection, and the independent variables included the region sampled (two levels), season of collection (four levels), animal gender and age (two levels). Seasons were defined as follows: spring: February–April; summer: May–July: autumn: August–October; and winter: November–January. Backward and forward selection was used, and variables ($p > 0.05$) were removed based on Wald statistics. The difference of incidence of the different parasite groups in raccoon dogs and foxes was evaluated using the chi square statistics.

3. Results

In general, all animals were in good health and had body condition scores within normal ranges. During the study period, 99 raccoon dogs were collected only from mainland, and 384 foxes were collected throughout Denmark, although mainly from the mainland ($N = 209$, Table 1, Fig. 1). Complete data were not available for all animals included, for example because of decapitation and/or removal of the animal's fur for conservation purposes

Table 1
Demographic information for foxes and raccoon dogs collected for parasitological examination in Denmark 2009–2012.

Variable	Category	Animal species	
		Fox ($N = 384$)	Raccoon dog ($N = 99$)
Gender	Male	153 (39.8%)	48 (48.5%)
	Female	183 (47.7%)	47 (47.5%)
	Not recorded	48 (12.5%)	4 (4%)
Age	<1 year old	107 (27.9%)	29 (29.3%)
	>1 year old	224 (58.3%)	55 (55.6%)
	Not recorded	53 (13.8)	15 (15.2%)
Weight	<3 kg	0	13 (13.1%)
	3–4.9 kg	14 (3.6%)	22 (22.2%)
	5–6.9 kg	186 (48.4%)	29 (29.3)
	>7 kg	121 (31.5%)	20 (20.2%)
	Not recorded	63 (16.4%)	15 (15.2%)
Region	Mainland	218 (56.8%)	99 (100%)
	Islands	162 (24.2%)	0
	Not recorded	4 (1%)	0
Season*	Autumn	–	21 (21.2%)
	Spring	–	10 (10.1%)
	Summer	–	11 (11.1%)
	Winter	–	43 (43.4%)
	Not recorded	–	14 (14.1%)

* Reported only for raccoon dogs as foxes were solely hunted during winter.