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Alien species and their zoonotic parasites in native and introduced ranges: The raccoon dog example

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ABSTRACT

The raccoon dog (*Nyctereutes procyonoides*) is a canid that is indigenous in East Asia and alien in Europe, where it was introduced more than half a century ago. The aim of this study was to compare the parasite faunas associated with raccoon dogs in their native and introduced ranges, and to identify zoonotic parasite species. We examined 255 carcasses of hunted raccoon dogs from Estonia and recorded a total of 17 helminth species: 4 trematodes, 4 cestodes and 9 nematodes. The most prevalent parasite species were *Uncinaria stenocephala* (97.6%) and *Alaria alata* (68.3%). Average parasite species richness was 2.86 (the highest was 9) and only two animals were not parasitized at all. Although the infection intensity was determined by weight and not by sex, all animals infected with more than five helminth species were males. We also found that animals infected with higher numbers of helminth species fed significantly more on natural plants. Intentional consumption of grass may represent a self-medicating behaviour among raccoon dogs. We included the Estonian data into a wider comparison of raccoon dog parasite faunas and found a total of 54 helminth taxa, including 28 of zoonotic potential. In Europe, raccoon dogs are infected with a minimum of 32 helminth species of which 19 are zoonotic; in the native range they are infected with 26 species of which 17 are zoonotic. Most species were nematodes or trematodes, with fewer cestodes described. The recent increase in the number and range of raccoon dogs in Europe and the relatively high number of zoonotic parasite taxa that it harbours suggests that this species should be considered an important source of environmental contamination with zoonotic agents in Europe.

1. Introduction

The raccoon dog (*Nyctereutes procyonoides*) is an alien canid, introduced to Europe in 1929–1958 from the Far-East of Russia (Heptner and Naumov, 1998). Its native distribution area includes south-eastern Russia, eastern provinces of China, northern Vietnam, and Japan (Nowak, 1984; Pitra et al., 2010). There are six raccoon dog subspecies recognized in East Asia (Kauhala and Kowalczyk, 2011) and the precise origin and subspecies identity of the introduced animals is largely unknown. However, only one subspecies (*N. p. ussuriensis)* is believed to have been present in the eastern part of the former Soviet Union (Heptner and Naumov, 1998) at the beginning of the twentieth century. Hence, translocation of raccoon dogs to the western part of the former Soviet Union during that period involved this subspecies.

In Europe the raccoon dog is known to be an important vector of multiple zoonotic agents, of which some, e.g., the rabies virus, the fox tapeworm *Echinococcus multilocularis* and *Trichinella* spp., are highly hazardous to human health (Kauhala and Kowalczyk, 2011). Characteristics including omnivorous diet, high reproductive potential and the ability to hibernate at high latitudes have allowed the raccoon dog to successfully colonise new areas (Kauhala and Kowalczyk, 2011). As a result, the raccoon dog is now well-established in northern, eastern and central parts of Europe and continues to expand its range towards the west and south.

An outstanding question in invasion ecology is to understand what happens to parasite faunas when host species become established in a new territory: (1) which parasites are prevalent in native ranges but absent in new territories, and *vice versa*; (2) and do some parasites pose a particular threat to local fauna and to human health in invasive ranges. Epidemiological studies describing the endoparasite fauna of the raccoon dog in Europe have been conducted in several countries, e.g., Belarus (Shimalov and Shimalov, 2002), Lithuania (Bružinskaite-Schmidhalter et al., 2012), Denmark (Al-Sabi et al., 2013) and Germany (Thiess et al., 2001). A number of smaller studies targeting the parasites that infect humans have also been conducted in Europe, e.g., on *E. multilocularis* and nematodes from the genus *Trichinella* (Machnicka-Rowinska et al., 2002; Oivanen et al., 2002; Pannwitz et al., 2010; Schwarz et al., 2011).

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The only parasitological study of the subspecies *N. p. ussuriensis* in its native range that we could find originates from the 1970s (Judin, 1977). In that study, a total of 26 endoparasite species were reported, many of which, including *E. multilocularis,* are of considerable zoonotic potential (Knapp et al., 2015; Vuitton et al., 2015). By comparison, the first case of *E. multilocularis* infection in raccoon dogs in Europe was only reported in 2001 (Thiess et al., 2001). To date, no studies have compared the parasite fauna of the alien *N. p. ussuriensis* – the subspecies introduced to Europe – in its native and introduced distribution areas. Sutor et al. (2014) described the raccoon dog parasites in Far-East and Europe, however, that comparison was made with *N. p. koreensis*.

Raccoon dogs were first introduced to Estonia from the Russian Far East in 1950 (Aul et al., 1957). In recent decades the population has been regulated by hunting, but also by diseases, such as rabies and sarcoptic mange (Süld et al., 2014). However, following a successful vaccination campaign initiated in 2005, the rabies virus has been eradicated from Estonia (Pärtel, 2013), and judging by hunting bags recorded during the period 2005–2012 (growth from approximately 4000 to more than 12,000 individuals), the number of raccoon dogs has increased considerably (Veeroja and Männil, 2014). The parasite fauna of Estonian raccoon dogs was investigated about a decade ago, when a pilot study based on examination of 21 animals revealed six endoparasite species (Moks, 2004). Since the known endoparasite fauna of raccoon dogs in Europe is significantly larger, e.g., consisting of 25 species in Belarus (Shimalov and Shimalov, 2002), it seems likely that raccoon dogs in Estonia harbour more parasite species than indicated by the pilot study.

The aim of this study was to examine the raccoon dog parasite fauna in Estonia to determine the potential for environmental contamination with zoonotic agents, and to compare the parasite fauna of the subspecies in its native and introduced ranges.

2. Material and methods

2.1. Sample collection

255 raccoon dog carcasses were collected from animals legally harvested by hunters for purposes other than this project, and examined for internal parasites. Samples were collected between autumn 2010 and spring 2012 from different parts of Estonia, covering 9 of 15 counties.

All animals collected with fur ($n = 227$) were examined for sores and patches of thick crusty skin as signs of sarcoptic mange. After weighing the carcasses, intestinal organs were removed and kept at −80° C for at least 5 days before parasitological examination as a safety precaution (Eckert et al., 2001), since this kills the eggs of the highly dangerous tapeworms *E. multilocularis* and *Echinococcus granulosus* which have been recorded in Estonia (Moks et al., 2005, 2006, 2008; Laurimaa et al., 2015a,b). Lungs, gall bladder and urinary bladder were studied using established washing and sieving techniques for helminth detection (Parre, 1985). The small and large intestines were separated and examined by the sedimentation and counting technique (Hofer et al., 2000). Up to 200 specimens were counted per helminth species. Parasites were stored in 95% ethanol.

2.2. Morphological identification

Trematodes, cestodes and nematodes were identified according to their morphology after Kozlov (1977). Cestodes from the genera *Echinococcus, Taenia* and *Mesocestoides* were further identified after Abuladze (1964), Loos-Frank (2000) and Hrčkova et al. (2011), respectively.

2.3. Genetic analysis

As the scoleces of tapeworms from the genus *Taenia* were deformed and lacking some of the features required for morphological identification (e.g., hooks), these samples were submitted to genetic identification. Genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's instructions. A 506 bp fragment of *cox1* gene of tapeworm mitochondrial DNA was amplified with primers CesCox1F (5′–TGATCCGTTAGGTGGTGGTGA) and CesCox2R (5′–GACCCTAACGACATAACATAATGAAAATG). 20–80 ng of purified genomic DNA and 5 pmol of primers were used in the PCRs performed in a total volume of 20 μL also containing 1× Advantage-2 PCR buffer, 1U Advantage-2 Polymerase mix (BD Biosciences, USA), 0.2 mM dNTP (Fermentas, Lithuania). Thermocycling was performed using a touchdown protocol: a 1 min-denaturing step at 95 °C, followed by 10 cycles of 20 s at 95 °C, 30 s at 55 °C and 45 s at 68 °C, but with the annealing temperature reduced by 0.5 °C in each step (touchdown), followed by 25 cycles of 20 s at 95 °C, 30 s at 45 °C and 45 s at 68 °C. Samples were purified and sequenced as in Saarma et al. (2009), with sequencing performed using the same primers as used in the primary PCR.

2.4. Statistics

For statistical analysis, collected animals were divided into two seasons reflecting the availability of natural food resources: 1) autumn (August–October); and 2) winter and early spring (November–April). As few animals originated from the summer period, these were omitted from the statistical analysis. We used the Mann–Whitney *U* test to reveal significant associations between the season and number of identified helminth species. We also tested whether infestation with some helminth species, the number of helminths or animal weight depended on host sex (Mann–Whitney *U* test); if the number of parasite specimens (<200 or >200) depended on animal weight (Logistic regression); and whether animals infected with different numbers of helminth species consumed some food items significantly more than others (ANOVA). Statistical tests were performed using software STATISTICA 7.

For comparative analysis of parasites and consumed food items, we used the raccoon dog dietary data from Süld et al. (2014), which originate from the same animals used in this study. To assess the co-occurrence of parasite species and food categories, we calculated the C-score (Stone and Roberts, 1990) for all pairs of parasite species, and for parasite species and food types. To generate a distribution of C-scores that could be expected if parasites were distributed randomly with respect to one another, we generated 999 random matrices and recalculated all pairwise C-scores for each matrix. Analyses were carried out using software R (package vegan) on parasite species that were represented in at least 10 animals.

3. Results

3.1. Parasite fauna in Estonia

Due to decomposition or severe carcass damage some samples were excluded from the analyses. In total, 249 small intestine, 240 lung and 223 urinary bladder samples were examined. Gall bladders were also investigated, but only for 41 animals, and parasites detected in this organ were not included in statistical analyses due to the low sample size.

We identified a total of 17 helminth species (Table 1), 12 from small intestine, 3 from lungs, 1 from urinary bladder and gall bladder (Table S1). Raccoon dogs were most often infected with