

Original papers

Intestinal and external parasites of raccoon dogs (*Nyctereutes procyonoides*) in western Poland

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ABSTRACT. Parasites of an invasive species, the raccoon dog (*Nyctereutes procyonoides*) from western Poland were investigated to clarify poorly known ecological key aspects of the species. The research was conducted in two study areas: the Ujście Warty National Park and the Bogdaniec Forestry District. Intestinal samples were collected from the intestinal tracks of 39 dead animals and 51 faecal samples were collected in all seasons from latrines of raccoon dogs. Macro-parasites, their eggs and protozoan parasites were investigated to assess the taxonomic composition of parasites, the level of infection and the risk of potential transfer of dangerous parasites from raccoon dogs to people and native species. Among parasites potentially dangerous for human and native mammal species, *Toxocara canis* was found in the intestines and *T. canis* eggs, *Cryptosporidium* sp. oocysts and *Entamoeba* sp. cysts were identified in faecal samples. *Sarcoptic mange* was observed in the skin of two animals, whereas Diptera larvae (probably from the family Gasterophilidae) were found in the intestines of two other animals. This latter finding is very interesting, because Gasterophilidae are the typical parasites in horses and ungulates, but so far were never found in raccoon dogs.

Key words: intestinal parasites, external parasites, invasive species, *Nyctereutes procyonoides*, raccoon dogs

Introduction

The raccoon dog (*Nyctereutes procyonoides*) is one of those invasive species, which have been most successful in newly colonised areas and which may be responsible for disease transmission. While until recently its parasites were little known aspects of raccoon dog biology, it is known that these animals may suffer from various infections. As a relatively new and invasive species in the Polish and European fauna, it may transmit not only various known and unknown diseases, but also numerous ecto- and endoparasites, what showed the investigation in the north-eastern Poland from the year 2016 [1].

New species are frequently hosts of alien parasites and pathogens, which are specific to them

[2,3]. Although to date no new diseases or parasites have been identified in raccoon dogs, they may become a reservoir and vector for many pathogens found in Europe [4,5]. The role of the raccoon dog as a potential reservoir and vector requires further studies also because another invasive species, the raccoon, is a host for many nematodes, among them a parasite constituting a health hazard for humans, i.e. the raccoon roundworm *Baylisascaris procyonis* [6–8]. The spread of parasites and pathogens frequently changes with environmental conditions, as it is suggested e.g., by studies on the raccoon roundworm and canine distemper virus [9] as well as parasitic flatworms [10].

In Central Europe transmission of parasites and viruses by raccoon dogs into humans is relatively rare, but it may lead to serious consequences, as

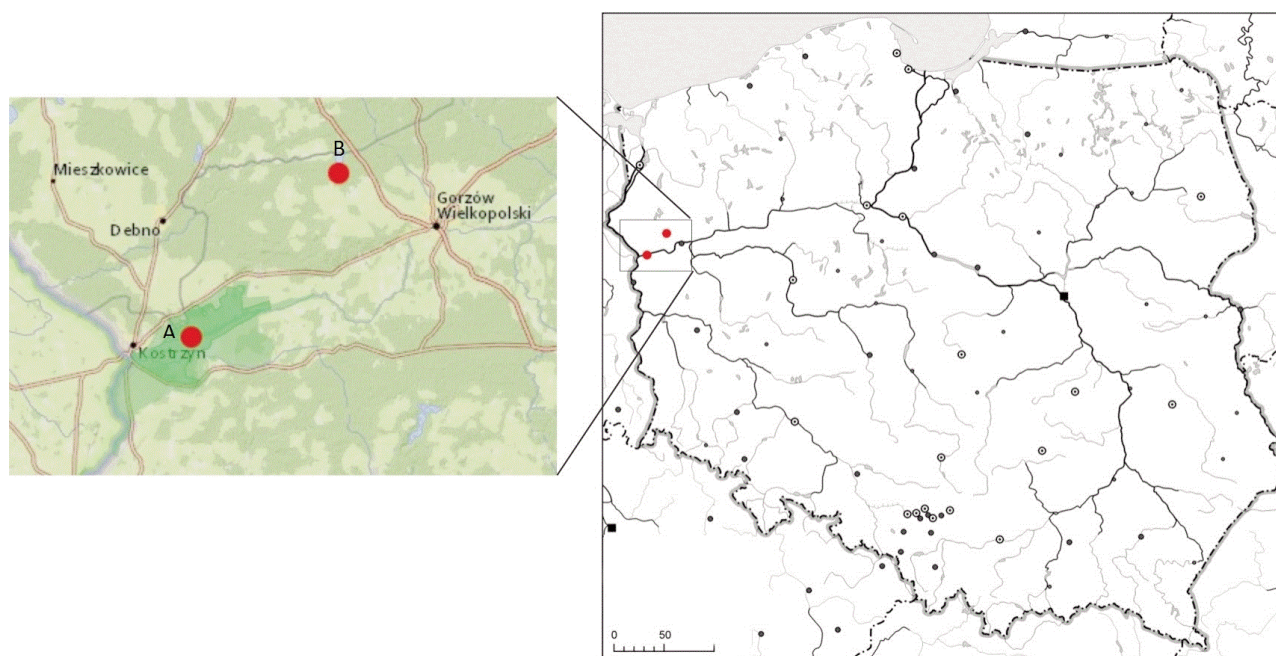


Fig. 1. Locations from which faecal samples of raccoon dogs were collected A: the Ujście Warty National Park; B: the Bogdaniec Forest District

racoon dogs may transmit zoonoses posing a serious health hazard for humans. Raccoon dogs are also frequently killed by large predators and wandering dogs, which may result in these animals and indirectly also humans becoming infected [11]. To date studies in Europe showed that the raccoon dog brought with it no new parasites, but rather became a new host for many parasites living in Europe. High percentages of foxes and raccoon dogs infected with *Echinococcus multilocularis* were recorded in Poland, Germany and Switzerland [12,13]. Due to the increasing population density of foxes and raccoon dogs environmental pollution with eggs of this parasite has been increasing. *Trichinella spiralis* is reported as one of the most frequent parasites in raccoon dogs, contributing to the further spread of this disease [14,15]. Studies in Poland indicate that *Trichinella* is found in various regions [16]. Moreover, *Toxoplasma* has also been detected in raccoon dogs [17]. In Belarus, Denmark and Germany the presence of dog roundworm *Toxocara canis* has been recorded [18,19]. *Listeria* is also frequently found among endoparasites of the raccoon dog [20]. The raccoon dog is also considered to be a reservoir of viruses from the family *Togaviridae*, transmitted to humans by ticks (*Ixodes* spp.) [21] and bacteria *Borrelia burgdorferi* [22]. Raccoon dogs may also be a source of infection with flatworms [23] as well as hookworms [24] in domestic and wild animals. Flatworms found

in raccoon dogs are primarily these species, which are transmitted by insects and amphibians, and to a lesser degree by rodents [25]. Raccoon dogs may suffer from brucellosis [26], Aujeszky's disease [27] and canine parvovirus [28]. Raccoon dogs may also transmit canine distemper to other animals [29]. What is more, the risk of various cross infections may increase as a result of raccoon dogs sharing burrows with badgers and foxes [30]. It was confirmed that raccoon dogs may also be carriers of ectoparasites, such as itch mites *Sarcoptes* spp., causing skin lesions [31]. Cases of mange were also reported in Poland [32].

The aim of this study was to identify intestinal parasitic fauna in raccoon dogs living in western Poland.

Materials and Methods

Material for analyses comprised contents of 39 intestinal tracts of raccoon dogs (20 from the Ujście Warty National Park and 19 from the Bogdaniec Forest District) as well as 51 faecal samples of raccoon dogs (38 samples collected from the Ujście Warty National Park and 13 from the Bogdaniec Forest District) (Fig. 1). The areas are located about 40 km from each other.

Intestinal tracts (the stomach, the small and the large intestines) were collected from animals, which were killed in road accidents, were shot by foresters

and hunters or were found dead in both study areas. These animals were frequently collected by hunters, foresters and employees of the National Park and frozen. After a larger number of animals were gathered their autopsies were conducted at the same time. Only dead raccoon dogs found in the study area by the first author of this study were autopsied immediately with no prior freezing. Isolated intestinal tracts together with their contents were placed in air-tight glass containers in 70% ethanol to preserve them until analyses. Droppings of raccoon dogs were collected from latrines in the vicinity of raccoon dog burrows and animal paths by the author or by the Ujście Warty NP employees as well as foresters and employees of the Bogdaniec Forest District. Faeces were placed in 2.5% potassium dichromate solution.

Parasites from intestinal tracts. After samples had been delivered to the laboratory the intestinal tracts of raccoon dogs were subjected to a thorough macroscopic examination. Parasites found in the intestinal tract contents of raccoon dogs were placed in air-tight glass containers in 70% ethanol. Collected materials were photographed under a SZx12 stereoscopic microscope (Olympus) with the 3.15×10 and 5×10 real image magnification. The camera used in taking images was an Astray model Artcam 500 MI, Series L 24407, while the applied software was Quick Photo Camera 2.3.

Taxonomic identification of parasites was performed based on the analysis of DNA marker sequences. DNA from 7 specimens representing one group of parasites was analysed, while 3 PCR products each were selected for sequencing.

Sections of 0.5 cm collected from parasites were cut into small pieces to facilitate tissue digestion. Total genomic DNA was isolated using the standard column method with an DNAeasy Blood & Tissue Kit (Qiagen) following the manufacturer's recommendations [33]. Isolated DNA was stored frozen at -20°C. The reaction mixture for the amplification of 18S rRNA and 28S rRNA gene fragments contained 1x concentrated Type-it Microsatellite PCR Master Mix (Qiagen), 0.25 µM concentration of each primer and 1 µl isolated DNA template. Amplification of the 28S rRNA gene fragment was performed using primers D3Brv (TCGGAAGGAACCAGCTACTA) and D2Afw (ACAAGTACCGTGA GGGAAAGTTG) [34], while the 18S rRNA gene fragment was amplified with primers 18SF01 (CTTGTCTCAAAGATTAA GCCATGCA) and rev930 (GACGGTCCAAGAAT

TTCAC) [35]. PCR amplification was run in a 2720 Thermal Cycler (Applied Biosystems). Following PCR the samples were diluted two-fold with water. Electrophoresis was run in 1% agarose gel. Unpurified PCR products were indirectly sequenced using the Sanger method with the D3Brv or 18SF01 primers. The PCR products were purified by precipitation with ethanol, next dissolved in formamide and separated in an automatic DNA ABI PRISM 3130XL analyser (Applied Biosystems). Chromatograms were edited using the Chromas Lite software and the resulting sequences were compared with the nucleotide database (nr/nt) GenBank NCBI (<http://blast.ncbi.nlm.nih.gov/>) using the BLAST programme and the *discontiguous megablast* algorithm.

Parasites from faeces. All faecal samples were condensed according to Meyer [36]. From the obtained precipitate 2 thin faecal smears were produced, a direct faecal smear in a drop of Lugol's iodine and a faecal smear stained according to the modified Ziehl-Neelsen method. In order to verify the analyses and isolate eggs and/or (oo)cysts from samples, additionally flotation was applied according to Fülleborn using a saturated NaCl solution as modified by Willis and the Fecalyzer method (Vetoquinol) with a saturated solution of sodium nitrite (NaNO₃). Preparations were examined under an Axioskop FL light microscope (Zeiss). Images of parasites were taken at a 100, 500 and 1000× magnification using a Sony 3CCD Color Video Camera and Ks300 and Hyper Snap4 computer software.

Results

Parasites found in the intestinal tracts. In the small intestine of two raccoon dogs (10%) from the Ujście Warty National Park and six animals (31.58%) from the Bogdaniec Forest District adult specimens of dog roundworm *Toxocara canis* were found. A comparison of the obtained sequences to those in the GenBank database showed 100% identity of nucleotides with the sequences deposited in the GenBank under accession numbers JN256997 and JN256976 [37]. Overall 34 specimens of this species were found in raccoon dogs.

In turn, in 2 raccoon dogs (10.53%) from the Bogdaniec Forest District parasites were found in the duodenum (a total of 119 larvae). Based on DNA analyses they were classified as *Brachycera* (Diptera) (98% identical nucleotides with the

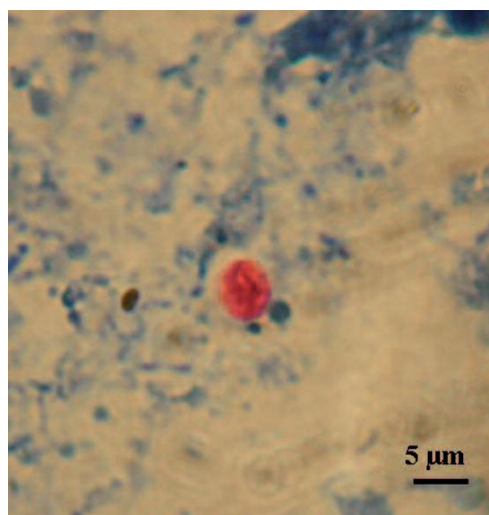


Fig. 2. An oocyst of *Cryptosporidium* sp., faecal smear stained by modified Ziehl-Neelsen method (photo Ł. Skrzypczak)

KC177300 sequence for the 18S rRNA gene fragment, 95% identical nucleotides with KC17746-8 sequences for the 28S rRNA gene fragment). Sequences from the GenBank database come from a study by Wiegmann [38] and a study by Caravas and Friedrich [39]. The GenBank database contains 18S and 28S sequences from the family Oestridae (*Cephenemyia*), including e.g. subfamily Gasterophylinae. Thus it may be stated that the retrieved parasites most probably belong to the family of parasitic flies sometimes classified as the family Gasterophilidae and the genus *Gasterophilus*.

Microscopic parasitological diagnostics

Among 51 faecal samples of raccoon dogs numerous oocysts of *Cryptosporidium* spp. were

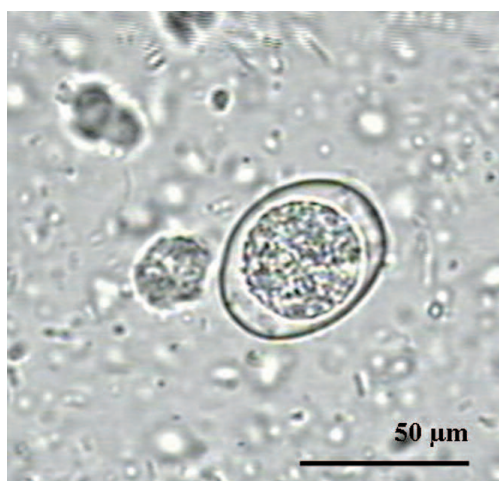


Fig. 4. An oocyst of *Cycloisospora* sp., direct faecal smear in a drop of Lugol's iodine (photo J. Pachoń)



Fig. 3. A cyst of *Entamoeba* sp., direct faecal smear in a drop of Lugol's iodine (photo Ł. Skrzypczak)

found in 11 samples (17.64%) (Fig. 2). The concentration of oocysts was 3.5×10^5 /g of faeces. Most positive samples (9 samples) came from the Bogdaniec Forest District, while in faeces of raccoon dogs from the Ujście Warty National Park oocysts were detected in only 2 samples. Oocyst size was 3–6×4–6 µm. Moreover, in one faecal sample (1.96%) from the Ujście Warty National Park numerous cysts of *Entamoeba* spp. were detected (Fig. 3). The concentration of cysts was 4×10^5 /g of faeces. Cyst size was 8×8 µm.

Using the Fecalyzer method (Vetoquinol) numerous oocysts of *Cystoisospora* spp. (33.3%), numerous eggs of *Trichuris vulpis* (9.8%) and *Toxocara canis* (15.7%) as well as eggs of nematodes most probably from the family Ancylostomatidae (7.8%) were found in faecal samples of raccoon dogs. Among 17 positive faecal

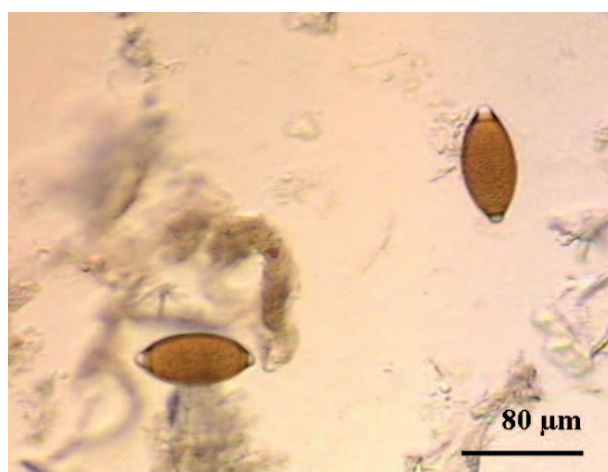


Fig. 5. Eggs of *Trichuris vulpis*, direct faecal smear in a drop of Lugol's iodine (photo J. Pachoń)

samples with *Cystoisospora* spp., 13 were collected from the Ujście Warty National Park. Oocysts of *Cystoisospora* spp. (Fig. 4) belong most probably to *Cystoisospora canis*. Eggs of *Trichuris vulpis* (Fig. 5) were detected in 5 faecal samples (9.8%). Three positive samples were collected from the Ujście Warty National Park, while one faecal sample was collected from the Bogdaniec Forest District. Moreover, in 8 faecal samples of raccoon dogs (15.7%) single eggs of *Toxocara canis* were found (Fig. 6). All positive samples were collected in the Ujście Warty National Park. Moreover, in 4 faecal samples of raccoon dogs (7.8%) nematode eggs were detected, most probably from the family Ancylostomatidae. Among the four positive samples, in three single eggs were found, while one sample contained numerous eggs. Eggs of unspecified nematodes were found only in samples collected from the Bogdaniec Forest District. Results provided by the Fecalyzer method (Vetoquinol) were confirmed using the Fülleborn method.

Among 39 dead raccoon dogs only on the skin of 2 animals (5.13%) scarce lesions were observed, caused by *Sarcoptic mange* infection. Both raccoon dogs came from the Bogdaniec Forest District.

Discussion

Recorded results, similarly as findings of showed no presence of *Echinococcus multilocularis*. No symptoms of canine distemper were observed in recently dead or captured live animals. However, it may not be assumed on this basis that these diseases and parasites are absent in western Poland. It should rather be assumed that the risk of their infection through forest fruits in the case of tapeworms or the direct contact of raccoon dogs with humans and with domestic animals is real and may lead to transmission of any of the above-mentioned diseases to humans. In turn, parasites belonging to flies Diptera probably from the genus *Gasterophilus* were detected in the intestinal tracts of 2 raccoon dogs (accounting for a small percentage of infected animals) coming from the Bogdaniec Forest District. No mention was found in available literature on the incidence of these parasites in raccoon dogs, while their presence was only reported in ungulates. The presence of flies Diptera in the presented studies was a surprising finding. However, they could not be identified to specific species, whereas from approx. 30 species the best known are those parasitizing in the intestinal tracts



Fig. 6. An egg of *Toxocara canis*, direct faecal smear in a drop of Lugol's iodine (photo J. Pachoń)

of horses [40], donkeys [41], as well as mules, cattle, sheep, goats and deer [42]. Accidental are found by small children [43]. It may be assumed that infestation of raccoon dogs was accidental and it is not a permanent parasite of this species. The presence of flies was recorded only in the Bogdaniec Forest District, with a larger deer population, thus indirectly more parasites of these animals are also found.

The reported presence of nematode eggs in fecal samples and intestinal tract contents is consistent with literature data, reporting that raccoon dogs are hosts for representatives of Nematoda [44]. In the intestines of eight raccoon dogs 34 specimens of dog roundworm *Toxocara canis* were recorded. They are found frequently both in wild and domestic animals [45,46]. *Toxocara canis* is a parasite easily spread among wild animals due to the easy infestation with eggs of this parasite found on the ground or in the ingested food contaminated with feces. *Toxocara canis* is a highly invasive parasite, which may also infect humans causing toxocariasis, characterised by the enteric larva migrans syndrome, burrowing in the host organisms in various internal organs as well as eyeballs. Roundworm infection occurs rather in children than in adults. Hosts for the representatives of the genus *Toxocara* may include foxes, dogs and raccoon dogs [47]. Obtained results showed that adult specimens of *Toxocara canis* were found over three times more frequently in the intestinal tracts of raccoon dogs from the Bogdaniec Forest District and in a large percentage of the tested feces the presence of invasive eggs and larvae was detected. This is most probably connected with the large numbers of foxes in this Forest District as well as farm and stray dogs.

Over 39% foxes in western Poland are infected with *Toxocara canis*.

In faeces of raccoon dogs numerous oocysts of *Cryptosporidium* spp. and cysts of *Entamoeba* spp. were identified. It is likely that they may be species potentially pathogenic for humans, with raccoon dogs serving as their potential reservoir [48–50]. In the case of *Entamoeba* spp. the main source of human infection may be connected with dog faeces [51].

Raccoon dog faeces found in the Ujście Warty National Park contained eggs and larvae probably belonging to Ancylostomatidae. They are parasites of the mammalian intestinal tract, which are potentially invasive for humans. They hook into the walls of the small intestine or the duodenum, damaging the mucosa and feeding on blood. Infection is caused by larva infecting the host in the active way. This is consistent with the results of studies in Japan [52] where *Ancylostoma kushimaense* was detected in raccoon dog faeces.

In the analysed faecal samples of raccoon dogs from both study areas numerous oocysts of *Cystoisospora* were found, which is consistent with the results from South Korea. They are organisms belonging to the subphylum Coccidia, which may be invasive also to humans. In approx. 10% raccoon dog faeces numerous eggs of *Trichuris vulpis* were detected. This parasite is mainly invasive for Canidae; however, in rare cases it may be invasive also for humans, particularly children [53].

A very small percentage of analysed animals from Bogdaniec were infested with the itch mite. However, they presented only local lesions, mainly on the heads of raccoon dogs, similarly as it was reported by Shibata and Kawarnihi [31] and Kowalczyk et al. [32].

The conducted analyses showed the presence of parasites, which may potentially be dangerous for humans and native fauna, with raccoon dogs considered as an important vector of various diseases and parasites. In conclusion, it may be assumed that the percentage of parasitic infection in raccoon dogs is probably connected with the type of food, lifestyle and environmental contamination by dispersion stages excreted by other animal species living in the same area.

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