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SHORT COMMUNICATION

First record of *Giardia* assemblage D infection in farmed raccoon dogs (*Nyctereutes procyonoides*)

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Abstract

The presence of *Giardia* genotypes was investigated in 18 raccoon dogs (*Nyctereutes procyonoides*) and 80 red foxes (*Vulpes vulpes*) on one farm. To demonstrate *Giardia* cysts, fresh and trichrome stained smears were microscopically screened. Two molecular markers were used for *Giardia* genotyping: a fragment of the beta-giardin gene and a fragment of the glutamate dehydrogenase gene. All faecal samples obtained from red foxes were negative. *Giardia* cysts were identified only in 2 of the 18 raccoon dogs. The result of genotyping and phylogenetic analysis showed that the *G. duodenalis* from both raccoon dogs belonged to the D assemblage. This finding of a new animal reservoir of *G. duodenalis* canids-specific genotypes is important in order to eliminate the risk of infecting other animals bred for fur. Further molecular analyses of *Giardia* isolates in raccoon dogs are required. The present study represents the first contribution to knowledge of *G. duodenalis* genotypes in raccoon dogs.

Key words

Giardia, molecular genotyping, raccoon dog

INTRODUCTION

Giardia duodenalis (syn. *G. intestinalis*, *G. lamblia*) is an intestinal protozoan parasite that infects humans and a wide range of animals including wild and domestic canids. This species exhibits great genetic diversity, and 8 major assemblages (A–H) have been defined [1]. These assemblages differ in host specificity: assemblages A and B are found in both humans and animals, whereas the rest of the assemblages are more host-adapted. There is increasing molecular evidence that canids may be infected with host-specific genotypes (C and D) as well as zoonotic ones (A and B); therefore, they may play a role as a potential source of *Giardia* infection impacting on humans and other Canidae. Although giardiasis in canids may be associated with various gastrointestinal abnormalities, most infected animals are asymptomatic [2].

The raccoon dog belongs to the Canidae family, and is native to Siberia and the Far East. The animals were introduced to Europe in the early 20th century, and now raccoon dogs are becoming more widespread throughout Europe and are considered as a potentially hazardous invasive species. The raccoon dog is the one of the animals used in the fur trade because of the quality of the pelt [3]. Diseases of animals are one of the indicators which provide information about farmed animal welfare. Although much research has been conducted on helminth fauna in raccoon dogs, there are limited data on their intestinal protozoan parasites [4, 5, 6, 7, 8, 9, 10]. To our knowledge, there is one report of *Giardia* infection in raccoon dogs; the parasite cysts were only microscopically observed in the feces of 3 out of 5 examined animals, and the *Giardia* isolates were not genotyped [7]. Therefore, the main aim of the present study was to determine the prevalence of *Giardia* genotypes in breeding raccoon dogs.

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MATERIALS AND METHOD

Sample collection. In the presented study, a total of 18 fresh faecal samples were collected from farmed raccoon dogs on one small farm located in the west-central region of Poland. Since this farm specializes in breeding raccoon dogs and red foxes (*Vulpes vulpes*), faecal specimens sampled from 80 foxes were also examined. Each animal was kept in a separate cage. Fresh faecal specimens were taken from the ground below the bottom of the cage and placed into sterile plastic tubes, preserved in 2.5% potassium dichromate solution, transported to the laboratory in a cooler, and stored at 4 °C until they were analyzed. Two thin smears were made from each faecal specimen. One of them was examined as a fresh smear with a drop of Lugol's iodine solution and the second as a permanent trichrome stain. To demonstrate *Giardia* cysts and/or trophozoites, wet mounts and trichrome stained smears were microscopically screened using x 600 and x 1,000 magnification, respectively. In addition, part (2 g) of each faecal sample was concentrated using the 0.85 M sucrose gradient centrifugation technique, with the final sediment being examined using a light microscope.

Molecular methods. Total genomic DNA was directly extracted from *Giardia* positive faecal samples. The FastDNA kit (BIO101, Vista, USA) was used for extraction of the *Giardia* DNA based on a protocol described earlier [11]. The eluted

DNA was purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the kit instructions. Two molecular markers were used for *Giardia* genotyping: a 753 bp fragment of the beta-giardin gene (*bg*) was amplified using the G7 forward PCR primer and the G759 reverse primer, as previously described [12], and a 430 bp fragment of the glutamate dehydrogenase gene (*gdh*) was amplified using 2 forward primers (GDHeF, GDHiF) with one reverse primer (GDHiR) [13]. PCR amplification was performed under conditions previously reported [12, 13]. *Giardia* DNA originating from cultured trophozoites of the Portland-1 reference strain was used as a positive control, whereas the negative control was a reaction mixture without the DNA template. Spike control was provided to rule out inhibitors. The PCR products were sequenced using the ABI Prism 3130 XL BigDye v3.1, Terminator Cycle Sequencing in both directions with the same set of primers.

Phylogenetic analysis. Both sequences were analyzed using Chromas and MEGA version 4.0 programmes. A phylogenetic tree was constructed by the neighbour-joining algorithm. Distance-based analyses were conducted using Kimura 2-parameter distance estimates using alignments obtained from ClustalW. Bootstrap proportions were calculated by analysis of the 1,000 replicates of the phylogenetic tree.

RESULTS

All faecal samples obtained from red foxes were negative for *Giardia*. *Giardia* cysts were identified only in 2 (NP1 and NP2) of the 18 raccoon dogs (11%). Low infection intensity ranging from 12,000 – 13,000 cysts per gram of faeces was detected. Both *Giardia* infected animals were asymptomatic.

The partial *bg* and *gdh* sequences were successfully amplified in the NP1 and NP2 *Giardia* isolates. The sequences at both molecular markers from both NP1 and NP2 *Giardia* isolates were the same; it was also found that both *Giardia* isolates from the raccoon dogs belonged to the D assemblage. No double peaks in the chromatograms occurred in the tested loci.

The sequence of the *bg* gene fragment of *Giardia* isolates obtained from the raccoon dogs (753 bp) shared 100% identity (D1–001, FJ009205) and 99% similarity (A21, AY545647 and F42, HM061152) to the sequences from the reference isolates of *G. duodenalis* from dogs. The sequence of the *bg* gene from both raccoon dog *Giardia* isolates differed only by one nucleotide substitution (1 SNP) from the A21 and F42 genotype D isolates. Comparison of the sequence of the *gdh* gene fragment of the *Giardia* isolate from the raccoon dogs showed 100% (NY24, JF958083) and 99% (differed at this marker from 1 SNP to 4 SNPs) similarity to the sequences of this gene of the parasite isolated from dogs in various parts of the world (C29, EF507629; gi-dog2, DQ840535; NLDE3, AY827498; Dd4, AB569389). All mutations were synonymous. The sequences of the *bg* and *gdh* gene fragments from the *G. duodenalis* obtained from the raccoon dogs were deposited in GenBank (NCBI) under Accession Nos. HQ538708, HQ538710, HQ538709 and HQ538711, respectively. The phylogenetic analysis of the *gdh* nucleotide sequences obtained from the NP1 and NP2 *Giardia* isolates placed them within clade D (99% bootstrap support). Bootstrap analysis indicated strong statistical support for this grouping (Fig. 1).

CONCLUSIONS

Although *G. duodenalis* is the most prevalent parasitic intestinal protozoan to be found in wild, domestic and breeding *Canidae* in different regions of the world, this study has demonstrated a low rate of *Giardia* prevalence in raccoon dogs. This is probably due to the good hygienic conditions on the examined farm. Based on the molecular characteristics of *Giardia* isolates obtained from 2 raccoon dogs, it was determined that these animals were infected

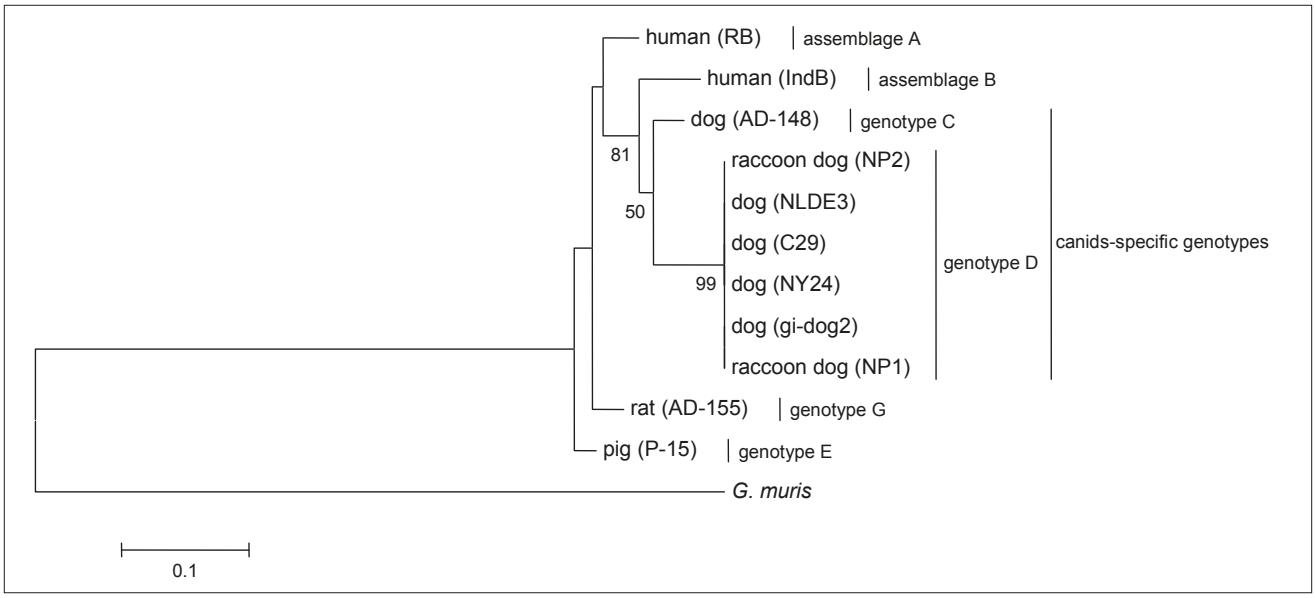

Figure 1. Phylogenetic relationship of 12 *Giardia* isolates inferred by the neighbour-joining analysis of glutamate dehydrogenase nucleotide sequences. Evolutionary distances computed using the Kimura 2-parameter method in the units of the number of base substitutions per site. Isolates from this study: NP1 and NP2 - from raccoon dogs. Reference isolates: RB (EF685702) and IndB (AB569386) represent assemblage A and assemblage B, respectively; AD-148 (U60982), NY24 (JF958083), C29 (EF507629), gi-dog2 (DQ840535), NLDE3 (AY827498) – reference dogs isolates; P-15–reference pig isolate (AY178741); AD-155 – reference rat isolate (AY178745). *G. muris* (AY258618) represents an outgroup.

with the D genotype, which is adapted only to *Canidae*. However, recently it was found that 16 out of 17 *Giardia*-positive patients were infected with assemblage C which, like assemblage D, is found in canids [14]. Therefore, it is also possible that *Giardia* assemblage D has zoonotic potential. Nevertheless, the finding of a new animal reservoir of *G. duodenalis* genotype D, infectious to other animals bred for fur, is also important in order to eliminate the risk of infecting farmed raccoon dogs and red foxes. The rapid detection of *Giardia* infection and the treatment of fur breeding animals is necessary in order to provide good development conditions for these animals [15].

The presented study represents the first contribution to knowledge about the *G. duodenalis* genotypes in raccoon dogs. Further molecular analyses of *Giardia* isolates in raccoon dogs are required.

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