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SHORT COMMUNICATION

Dogs are final hosts of *Sarcocystis morae* (Apicomplexa: Sarcocystidae): First report of this species in Hungary and its region – Short communication

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ABSTRACT

In this study, faecal samples of four American Staffordshire terrier dogs (used for illegal fighting) were analysed by DNA extraction, molecular-phylogenetic and parasitological methods, in order to examine the occurrence of protozoan, apicomplexan parasites. In one sample, the DNA of *Sarcocystis morae* was shown to be present. This species was identified based on 100% identity with already reported sequences of *S. morae* from cervids in Lithuania and Spain. The result was also confirmed by phylogenetic analysis. The sporocysts of the canine *S. morae* isolate measured 14.95 × 9.75 µm on average. This is the first molecular evidence in support of the final host role of domestic dogs in the life cycle of *S. morae*. The most likely source of the infection was raw meat given to the examined dog to increase its physical achievement. In conclusion, under similar circumstances dogs may participate in the life cycle of *S. morae* in a 'natural way', shedding sporocysts/oocysts when used for hunting or taken to walks in forested areas.

KEYWORDS

cystogenic coccidia, Canidae, Cervidae, 18S rRNA gene

Sarcocystis species (Apicomplexa: Sarcocystidae) are intracellular protozoan parasites, which are obligatorily heteroxenous, i.e., their development involves both an intermediate and a final host. While there are approximately 200 valid species in the genus *Sarcocystis*, until recently the complete life cycle was only known for 26 of them (Dubey et al., 2016a). *Sarcocystis* species usually have herbivorous/omnivorous animals as intermediate hosts, in which they undergo asexual multiplication in the endothelial cells of blood vessels and eventually establish themselves in the muscle or nerve cells (Dubey et al., 2016a). After infected tissues of the intermediate host are consumed by the final host, sexual reproduction takes place in its small intestinal wall, entailing passage of infective oocysts/sporocysts to the environment (Rommel et al., 1972; Dubey et al., 2016a). *Sarcocystis* infection may cause pathologic consequences in the intermediate host, while carnivorous final hosts usually remain unaffected even in case of copious sporocyst shedding (Dubey et al., 2016a).

Twenty-one valid species of *Sarcocystis* are known to infect dogs as final host, most of which develop in domestic and wild ruminants as intermediate hosts (Dubey et al., 2016b). In addition, several new *Sarcocystis* spp. have recently been described from cervids in Europe (Gjerde et al., 2017a, 2017b), for most of which the final hosts are unknown but are suspected to be canids based on phylogenetic properties. This study aimed at examining *Sarcocystis* sporocysts from dog faeces by molecular-phylogenetic and parasitological methods.

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All four dogs that provided samples for these analyses were American Staffordshire terriers (two males and two females, estimated age 5–6 years, body weight 16.7–23.2 kg), originating from the North Central region of Hungary. These dogs were found dead and were confiscated during a police operation against illegal dog fighting in December 2020. Their faecal samples, collected post mortem from the distal part of the large intestine, were analysed as part of a campaign to screen for *Babesia* infection, because recent data from Hungary support that dogs may be susceptible to a broader range of piroplasms than previously thought (Hornok et al., 2020). First, molecular screening was carried out to detect piroplasm and other apicomplexan DNA in the faeces. The remainder of the PCR-positive faecal samples were further analysed by flotation in Breza solution (specific gravity 1.3 g/mL) and concentrated sporocysts were examined by light microscopy, including the measurement of 50 sporocysts by morphometry.

DNA was extracted directly from the faecal samples (without concentration) using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA extracts were molecularly analysed by a conventional PCR modified from Casati et al. (2006), amplifying an approx. 500-bp-long part of the 18S rDNA with the primers BJ1 (forward: 5′-GTC TTG TAA TTG GAA TGA TGG-3′) and BN2 (reverse: 5′-TAG TTT ATG GTT AGG ACT ACG-3′). This method is also suitable for detecting *Babesia* DNA in faecal material (Hornok et al., 2015a), as well as for verifying the presence of other apicomplexan genera including *Sarcocystis*, as reported (Hornok et al., 2015b).

Purification and sequencing of the PCR product were done by Biomi Ltd. (Gödöllő, Hungary). The obtained sequence was manually edited using the BioEdit program, then aligned with GenBank sequences by the nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). The new sequence was submitted to GenBank (accession number: MW579603). This sequence and all sequences retrieved from GenBank were trimmed to the same length prior to phylogenetic analysis. This dataset was resampled 1,000 times to generate bootstrap values. Phylogenetic analysis was conducted with the Maximum Likelihood method (Jukes–Cantor model) by using MEGA 7.0.

In one faecal sample, the DNA of *Sarcocystis morae* was present, with 100% (491/491 bp) identity to GenBank sequences from fallow deer (MN443755), red deer (KY973375) and red fox (KT873775), reported from Lithuania, Spain and Germany, respectively. The phylogenetic analysis also supported the species identity of this canine isolate, because it clustered within the phylogenetic group of *S. morae* sequences deposited in GenBank from various parts of Europe. These formed a sister group to *Sarcocystis grueneri* (Fig. 1).

Following concentration with flotation, the only protozoan parasites seen in the PCR-positive faecal sample were *S. morae* sporocysts. These sporocysts were oval in shape and measured (12.5–17.5) × (7.5–12.5) µm, with mean values of 14.95 × 9.75 µm (Fig. 1, insert). This size range considerably overlaps with measurements of sporocysts of *Sarcocystis cervicanis* ([15.1–17.1] × [10.3–11.9] µm: Hernández Rodriguez et al., 1981b), an unidentified *Sarcocystis* sp. (15.4 × 8.8 µm: Poli et al., 1988) and *Sarcocystis gracilis* (15 × 10 µm: Dubey et al., 2016c). The sporocysts of these species were all reported from dogs after consuming the meat of relevant intermediate hosts, i.e., red deer, fallow deer and roe deer, respectively. Thus, *Sarcocystis* species that have cervids as intermediate hosts and canids as final hosts cannot be distinguished according to their sporocyst size in dog faeces.

This is the first molecular evidence of the final host role of domestic dogs in the life cycle of *S. morae*. Prior to this study, to the best of our knowledge, no experimental proof had been published in support of this, although sequences closely related to that of *S. morae* were reported from two wild living canids, the red fox (*Vulpes vulpes*) and the raccoon dog (*Nyctereutes procyonoides*) sampled in Germany (Moré et al., 2016).

Sarcocystis morae has recently been described as a new species, with red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) as typical intermediate hosts (Gjerde et al., 2017b). In a broader sense, *S. morae* belongs to the group of *Sarcocystis* species, of which canids are suspected to be the final hosts based on phylogenetic properties (Gjerde et al., 2017b).

In particular, it has long been postulated that red foxes and hunting dogs may play the final host role in the life cycle of *Sarcocystis* species affecting cervids, but for several species the exact final hosts are still unknown or are only suspected (Basso et al., 2020). In this context, based on molecular results and less consistent (98.8–99.1%) sequence identities, it was suggested that domestic dogs may be the final hosts of *Sarcocystis linearis* and/or *Sarcocystis taeniata* (Basso et al., 2020). Similarly, while tissue cysts of a *Sarcocystis* species with band-like protrusions were reported to be infective for the dog during experimental feeding (Poli et al., 1988), later the relevant species could not be identified with certainty (Gjerde et al., 2017b).

On the other hand, while the final host spectra of *Sarcocystis* species are frequently referred to at the family level, it is not necessarily true that all canids are natural hosts for the same *Sarcocystis* species. For instance, although hunting dogs may have regular access to mallards infected with *Sarcocystis rileyi*, they are not known to be susceptible to this species (Dubey et al., 2003), in contrast to red foxes (Szekeres et al., 2019).

In summary, *Sarcocystis* species which infect cervids as intermediate host and have a sarcocyst wall with ribbon-like or band-like protrusions (Gjerde et al., 2017b) cluster in the same phylogenetic group based on both the 18S rRNA (Gjerde et al., 2017b) and the cytochrome *c* oxidase subunit 1 (*cox*1) genes (Rudaitytė-Lukošienė et al., 2020). Among these five species (Fig. 1), only *S. cervicanis* (Hernández Rodriguez et al., 1981a, 1981b) and *S. grueneri* (Dubey et al., 2016c) have hitherto been known to infect dogs as final hosts. Based on the present results, *S. morae* is also added to this list.

In a geographical context, results of the molecular-phylogenetic analysis performed here are less conclusive,

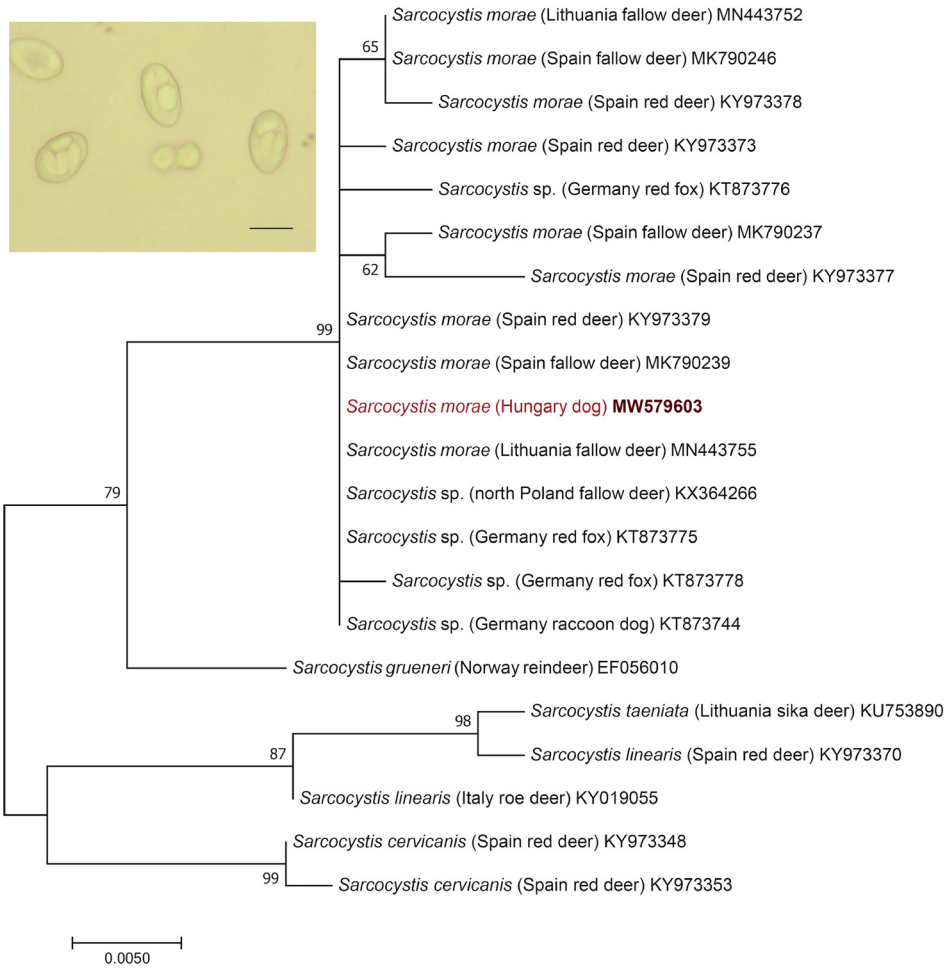

Fig. 1. Phylogenetic tree of species closely related to *Sarcocystis morae* based on the 18S rRNA gene. The tree was generated with the Maximum Likelihood method and the Jukes–Cantor model. The sequence obtained in this study is indicated with red colour and bold accession number. Branch lengths represent the number of substitutions per site inferred according to the scale shown. Insert: three sporocysts of *S. morae* from dog faeces (bar = 10 μm)

because identical or closely related 18S rRNA sequences of *S. morae* have been reported from northern, central and western Europe, as reflected by their clustering (Fig. 1). Nevertheless, *S. morae* has not been hitherto reported in Hungary and its region. At the same time, the occurrence of a closely related species, *S. grueneri* was already demonstrated in red deer in the country (Entzeroth et al., 1983), and *S. gracilis* (having dogs as final hosts) was originally described from roe deer in Hungary (Rátz, 1909).

The *Sarcocystis*-infected dog in this study could probably ingest meat from cervids as part of the raw meat-based diet (including game animals as its source) which is practised in Hungary among dog owners to increase physical achievement of their dog (Hornok et al., 2006). This mode of feeding is known as a potential source of transmission of *Sarcocystis* species (van Bree et al., 2018). Thus, giving raw meat of game animals to dogs allows the latter to participate in the life cycle of *S. morae* in a 'natural way', shedding sporocysts/oocysts when used for hunting or taken to walks in forested areas.

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