Strongyloides stercoralis in a Pomeranian Dog in Israel

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ABSTRACT

Strongyloidiasis, as a result of *Strongyloides stercoralis* (Rhabditida: Strongyloididae) infestation, is occasionally recognized in a range of vertebrate hosts, including humans and dogs and causes widespread clinical disease in infected individuals ranging from asymptomatic to fulminating respiratory or gastrointestinal disease. We describe a case of strongyloidiasis in a young Pomeranian puppy that presented after history of a seizure and gastrointestinal disease associated with inadequate weight gain. Blood and parasitological analyses, that included Baermann culture and fecal flotation, revealed severe leukocytosis, anemia and hypoglycemia, as well as the presence of *Strongyloides* spp. in feces. PCR targeting the nuclear ribosomal DNA and mitochondrial (cox1) genes followed by sequencing of the amplicons revealed 100% identity with *S. stercoralis* and HVR IV haplotype A, which is potentially zoonotic. After two repeated five-day treatments with oral fenbendazole, infection was cleared and the dog recovered. Small animal clinicians should be aware of this disease especially among the canine progeny of animal breeders and shelter dogs whereby ideal conditions for increased transmission cycles may likely take place. Moreover, due to its zoonotic potential, a correct diagnosis of *Strongyloides* is crucial in order to prevent infection of those people that are involved in the care of infected dogs.

Keywords: Canine; Israel; PCR; Strongyloidiasis; *S. stercoralis*.

BACKGROUND

Strongyloidiasis, due to *S. stercoralis* (Rhabditida: Strongyloididae) is occasionally recognized in a range of vertebrate hosts, including humans and dogs and is found primarily in tropical and subtropical areas of the world (e.g. Africa and South America) (1). The disease has been considered to be of zoonotic importance (2, 3) and comparative studies based on various targeted loci reveal the existence of two genetically different populations of *S. stercoralis* in dogs, one population that appears to be dog-specific, while another population is shared by dogs and humans (4, 5). The helminth has a complex life cycle consisting of parasitic and free-living generations. Infective third stage larvae (L3), invade a new host by skin penetration, and after migrating through the blood and the lungs, reach the oral cavity after coughing them up. When the infected host swallows, larvae eventually reach the small intestine and continue to reside there to develop only as adult females, which reproduce by parthenogenesis. The offspring of the parasitic females have various developmental options. They may develop into infective L3 within the host and re-infect the same individual (autoinfective cycle); leave the host as first-stage larvae and develop into infective L3 and search for a new host; or develop to non-infective free-living adult males and females. Free-living adults mate and reproduce in the environment, resulting in the development of infective L3 (2, 4, 6). Dogs are mainly infected via percutaneous penetration of L3, or via oral ingestion. However, lactogenic transmission may be

possible if the bitch is infected late in gestation or during lactation, even though it is considered only occasional (7). Although the infection is mostly asymptomatic, the parasites can disseminate in immunocompromised individuals to visceral organs and be potentially fatal (6). In dogs, clinical manifestations of strongyloidiasis include asymptomatic to severe clinical signs, mainly dermatological, gastrointestinal and/or respiratory, and mostly in young animals (1, 8, 9, 10). Specifically, diarrhea, malabsorption and bronchopneumonia may occur (9, 10, 11).

We hereby describe the first report of *S. stercoralis* in a puppy in Israel.

CASE REPORT

On the 28th May 2020, a female Pomeranian puppy, two months of age, was presented to a veterinary emergency clinic in central Israel after its owners complained that it had just experienced a "generalized seizure." In addition, the puppy had shown signs of weakness, intermittent vomiting and anorexia that had begun two days previously. On physical examination, the puppy was observed to be depressed with occasional episodes of sneezing accompanied by a serous bilateral nasal discharge. The dog was hypothermic (37.3^{0}C), and palpation of the abdomen revealed an empty intestinal tract. It weighed only 0.380 kg, a decrease of 0.070 kg (15.5%) compared to its weight as recorded on a routine check-up three days previously. Since hypoglycemia (43mg/dL) was noted after testing a venous blood sample, the dog was hospitalized and intravenous fluids supplemented with dextrose were administered. The puppy developed diarrhea whilst hospitalized. Two days later (30th May), after the pup's appetite had improved and glucose blood levels were normal (92mg/dL), it was discharged. However, two days after its discharge (1st June), the owners returned with the puppy complaining that its appetite had deteriorated once again and it displayed weakness. Since marked hypoglycemia was again recorded, the dog was hospitalized with intravenous fluids and dextrose, and 15 mg/kg amoxicillin was intramuscularly administered (Amoxy-LA, Norbrook Laboratories, UK; 150 mg/ml). In addition, venous blood was collected for analysis. Hematology results showed marked leukocytosis, specifically neutrophilia, lymphocytosis, and a low hematocrit (Table 1). In addition, ammonia levels in the blood were analyzed as the possibility of neurohepatopathy resulting from a portal shunt was suspected as an additional component that had triggered the seizure. Ammonia levels were found to be within normal limits (Table 1). The dog was administered 8mg/kg S.C. of a long acting cephalosporin, cefovecin sodium (Convenia, Zoetis, USA; 80mg/ml) and sent home two days, later (3rd June) when its appetite and glucose blood levels returned to normal. However, the dog's body score remained low (3/9) and its fecal contents loose. Thus, on the 16th June, a fresh fecal sample collected from the tiled floor of the owner's home was sent to the Kimron Veterinary Institute for parasitic and bacterial analysis. Following routine fecal flotation and Baermann culture, rhabditiform larvae, 285-310 µM long, showing a pronounced esophageal bulb and short, pointed tails that resembled *Strongyloides* spp. were observed under microscopy (Figs. 1a, 1b). In addition, hemolytic *E. coli*, sensitive to third-generation cephalosporins, was cultured.

Table 1: Hematological results of the *S. stercoralis* infected puppy.

Analyses	Reference interval	Infected animal
WBC, 10^3/µL	6-17	26.94
Lymphocytes, 10^3/µL	1-4.8	10.15
Monocytes, 10^3/µL	0.2-1.5	0.16
Neutrophils, 10^3/µL	3-12	16.60
Eosinophils, 10^3/µL	0.1-1	0.02
Basophils, 10^3/µL	0-0.5	0.01
Red Blood Cells, 10^6/µL	5.5-8.5	4.30
Hemoglobin, g/dL	12-18	9.3
Hematocrit, %	37-55	25.90
MCHC, g/dL	31-34	35.8
PLT, 10^3/µL	200-500	302
PCV, %	37-54	30
TS, g/dL	5.8-7.5	5.5
Ammonia, µmol/L	0-98	19

For the purpose of confirmation and identification of the *Strongyloides* spp., DNA was extracted from 200 µg of feces using a commercial kit (Presto Stool DNA Extraction Kit, Geneaid, New Taipei City, Taiwan) following the manufacturer's instructions. PCR amplified fragments of 320 bp of the nuclear ribosomal DNA using primers NEW_HVR_IV_F (5'-CGGGCCGGACACTATAAGG-3') and NEW_HVR_IV_R (5'-ATCTCTAAACAGGAACATAATGATCACTAC-3'), and 451 bp using primers NEW_HVR_I_F (5'-GCTCATTATAACAGCTATAGACTACACGGTA-3') and

NEW_HVR_I_R (5'-CCACAACAATCATTTT ATGCACTTGG-3'). Additional PCR amplified 229 bp of the mitochondrial cytochrome c oxidase subunit 1 (COX1) DNA with primers SSP_COX1_F (5'-TTTGATCCTAGTTCTGGTGGTAATCC-3') and SSP_COX1_R (5'-GTAGCAGCAGTAAAAT AAGCACGAGA-3'), as described by Beknazarova *et al.* (12). Amplicons were observed in 1.5% stained agarose gels (SMART GLOW nucleic acids stain, Benchmark Scientific, USA), and sequenced (HyLabs, Israel).

The sequences were evaluated using the Chromas Lite software (Technelysium Pty. Ltd., Brisbane, Australia) and compared to sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLASTs of sequences from all targeted loci showed 100% identity to sequences of *S. stercoralis*.

Using the nomenclature defined by Jaleta *et al.* (4) and expanded by Beknazarova *et al.* (12) and Basso *et al.* (11) according to the HVR IV sequence obtained in this study, the *S. stercoralis* strain detected was assigned to haplotype A. This haplotype has been reported in both humans and dogs, and therefore speculated to have zoonotic potential (4, 12). The HVR_I sequence was assigned to haplotype VI. This haplotype combination has been previously found in fecal samples from dogs in studies of both Beknazarova *et al.* (12) and Basso *et al.* (11). All sequences were deposited in GenBank (accession numbers MW857116, MW857130, and MW857133).

Following these findings, the puppy was treated with doramectin (Dectomex, Zoetis, USA; (10 mg/ml; 0.6 mg/kg SC) followed with five days of fenbendazole (fenbendazole, Vetmarket, Israel; 100 mg/ml; 50 mg/kg sid P.O.). Two days after the commencement of treatment, the owners returned to the clinic and complained that their pet was vomiting periodically and had passed soft stools with what appeared to be a "long white worm" even though the dog had remained vibrant during this period. Another subcutaneous injection of cefovecin sodium was administered according to the sensitivity profile of the fecal *E. coli* culture, and the dog discharged. On return visits to the clinic during the following months, the dog's body score steadily increased, and its demeanor as well as feces consistency consistently improved. However, on a repeat fecal analysis sampled from feces two months after the previous anthelmintic treatment, a single larva of *S. stercoralis* was still observed. Thus, another five-day treatment of fenbendazole was initiated. Parasitic analysis of a fecal sample performed six months after initial presentation was *Strongyloides* negative.

DISCUSSION

Herein, we describe in detail the first report and molecular identification of *S. stercoralis* in a dog in Israel, including pertinent details about its history, clinical, hematological and parasitological findings, as well as specific anti-parasitic treatment.

Similar to observations by others (1, 8, 9, 10, 11), the affected dog was young in age. It was born from a sire and

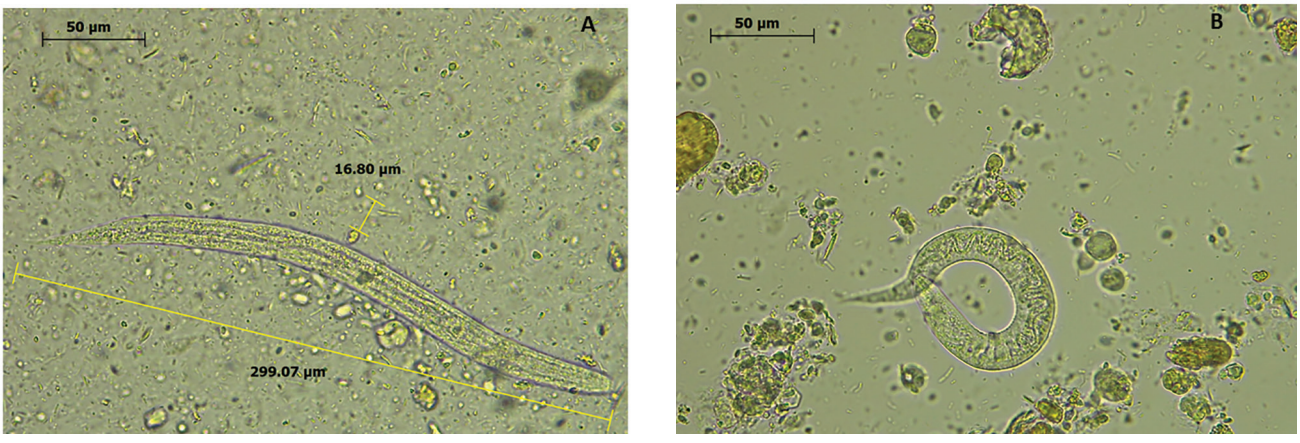

Figure 1 a and b: Low magnification (100x) of *S. stercoralis* rhabditoid larvae from a fecal filtrate of the puppy showing the typical shorter rhabdiform esophagus made up of the corpus, isthmus and bulbus components and its wider girth and shorter length compared to the infectious filiform L3 larvae.

dam raised entirely in Israel and had been dewormed once (anthelmintic not known) at two months of age concurrently with its first vaccination.

S. stercoralis infections are considered uncommon in Europe although it has been reported in countries as far west as the UK and as far north as Iceland and Scandinavia (reviewed in 11). The finding of *S. stercoralis* in Israel, which is characterized by a predominantly warm climate in which the helminth is more capable of sustaining infection cycles, is reasonable and it has previously been reported in a neighboring country, Turkey (9). However, *Strongyloides* can survive low environmental temperatures and successfully complete its life cycle and disseminate within dog shelters even in cold climates (13).

The affected canine was a young puppy that presented with gastrointestinal signs, commonly described by others with *S. stercoralis* infections in dogs (1, 8, 9, 10). Clearly, the fact that the puppy remained severely underweight and hypoglycemic during the helminth infection suggests the effect of *Strongyloides* infection on the ability of affected animals to absorb the necessary nutrients for essential metabolism and growth. Hypoglycemia has also been described by others in *S. stercoralis* affected dogs (11, 14) and a human (15), with some cases associated with immunosuppressive therapy and gastrointestinal signs. In addition, the condition has often been described in toy breeds like Pomeranians (14), Yorkshire Terriers and Chihuahuas (11). The clinical relevance of the hemolytic *E. coli* cultured *in vitro* from the dog's feces should be weighed with caution, in light of the fact that the dog had begun antibiotic treatment a week prior to this finding, with no apparent improvement.

Various parasitological techniques are available for the diagnosis of *S. stercoralis* in fecal samples, such as the formalin–ether concentration, nutrient agar plate culture, Harada-Mori culture and the Baermann method. Some of the former- mentioned methods require a few days to process before results are available and thus may prevent timely diagnosis and therapy of affected individuals. In addition, since worms may be shed in low numbers and intermittently, diagnosis of strongyloidiasis using parasitological methods is often difficult and multiple samples collected over a number of days are often required in order to increase the sensitivity of tests (16). Diagnosis of the disease was possible after fecal flotation and Baermann culture and microscopic preparations showed the typical morphological characteristics of *Strongyloid* rhabditoid larvae with a muscular esophagus and larval length that was relatively short (300 μm; Fig. 1) compared to the longer and more slender parasitic filiform larvae of the same species. Molecular confirmation of the worms was possible by conventional PCR targeting three different loci.

The rationale for the therapeutic use of fenbendazole to eliminate infection was based on the previous effective use of this drug against *Strongyloides* in six out of seven naturally infected dogs from Japan (17). However, similar to the experience of Paradies *et al.* (2017) (10), therapy with a five- day oral course with fenbendazole, combined with a single dose of doramectin, was not successful after one regime, and only the addition of another similar course eliminated infection in the dog. Other treatment protocols applied have either been the use of ivermectin (0.2 mg/kg sc) or oral fenbendazole (50 mg/kg for five days) on their own, or in combinations of the two drugs (11) with/without topical macrocyclic lactones, such as moxidectin plus imidacloprid spot-on (Advocate® spot-on, Bayer, Animal Health, Germany) (10).

Feces for parasitological analysis were collected from the ground and not rectally. Although the owners collected fresh feces from the tiled floor of their apartment, this method may impair the correct differentiation of *S. stercoralis* filiform larvae from larvae of free-living nematodes, which may also be present in fecal samples (10), especially when samples are contaminated with soil. Thus, it should be encouraged by clinicians to collect feces from the rectal ampulla rather than from the environment to avoid false positive detection of *Strongyloides* infection.

In conclusion, we describe the first report of *S. stercoralis* in a dog in Israel. Since infected dogs are often asymptomatic or only have gastrointestinal signs, and worms are frequently not detected on routine parasitological screening methods, strongyloidiasis is likely to be underestimated. Small animal clinicians should be aware of this disease especially among the canine progeny of animal breeders and shelter dogs whereby ideal conditions for increased transmission cycles are more likely to take place. Moreover, due to its zoonotic potential, a correct diagnosis of *Strongyloides* is crucial in order to prevent infection of people who are involved in the care of infected dogs.

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