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Strongyloides stercoralis infection and long-term follow-up in a privately-owned dog from north-west of Italy

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Introduction

Strongyloides stercoralis is a world-wide spread intestinal nematode that usually infects both primate and canids, less frequently cats.[1,2,3] It is typically found in tropical and subtropical areas, especially in kennels and intensive breeding facilities.[2,3] This parasite is characterized by a complex life cycle, which could be both a free-living and a host-mediated cycle. Most infections commonly occur through the penetration of third-stage larvae (L3) through the skin or mucous membranes, migrating to the lungs via the hematogenous path, and finally reaching the intestine after swallowing.[4] Both oral and lactogenic transmission are possible in puppies,[3,5] and autoinfection by first-stage larvae (L1), which subsequently develop through to infective L3 within the host, is reported.[4]

In human medicine, recent studies indicate that the infection is not an extinguished problem, and a prevalence up to 8% in some regions of the North of Italy has been reported.[1] In veterinary medicine, the number of canine strongyloidiasis reports has increased in the last years, with several cases reported in Southern Italy. So far, no cases have been reported in Piedmont region.[1-3,5-8] Although *S. stercoralis* is considered a zoonosis, recent studies revealed the existence of dog-specific genotype,[9,10] and so it is assumed that zoonotic transmission might occur less frequently.[11,12]

The infection is generally asymptomatic in adult healthy dogs. Clinical manifestations are typical for puppies and immunosuppressed dogs.[2,3,5] Nonspecific gastrointestinal, respiratory or dermatological symptoms may be present, with eosinophilia being less frequently detected than in humans.[13] In immunocompromised humans and dogs, complicated and potentially fatal clinical syndromes called hyperinfection and disseminated infection may develop.[7,13,14]

Baermann technique and agar plate culture of feces for detection of larvae are traditional techniques with the highest sensitivity.[15,16] Larvae observation on direct smear of fresh feces is also possible.[17,18] However, serological assays have been implemented to further increase the sensitivity.[17,18] In-house IFAT and real-time PCR techniques routinely used for the diagnosis in humans could also be useful for the diagnosis of the infection in dogs.[16]

The aim of this case report was to describe for the first time a case of strongyloidiasis in a privately-owned dog from north-west of Italy (Piedmont). Data recorded would clarify the spectrum of the clinical consequences, the importance of diagnostic methods and the need to regularly include this infection in the differential diagnosis list.

Case presentation

A 8-month-old, 7 kg, female intact French Bulldog living indoor with regularly taking anthelmintics, was presented for a second opinion to the internal medicine service of a Veterinary Hospital of the Piedmont region for evaluation of chronic hemorrhagic and mucous diarrhea (fecal score of 7; 7-point scale, Purina Fecal Scoring Chart®),[19] nasal discharge, cough, and emaciation. The dog was adopted from a local breeding-kennel at the age of 5 months. During the previous months before diagnosis, the dog received different hydrolyzed- and novel-protein diets and different empiric antibiotic trials with doxycycline, amoxicillin/clavulanic acid, and metronidazole (unknown therapeutic regimens), in order to control both gastrointestinal and respiratory signs. No improvement was achieved. In addition, a fecal examination by flotation test was performed and revealed coccidial parasites. Sulpametopyrazine (Vetkelfizina®, Ceva Vetem SpA, Italy) at unknown therapeutic regimen was given without improvement of hemorrhagic and mucous diarrhea, in addition to topical moxidectin/imidacloprid (Advocate® spot on, Bayer SpA, Italy) at unknown therapeutic regimen because of alopecia with desquamation and pustules, clinically suspected as demodicosis by a veterinarian.

At clinical examination, mild depression, several fits of cough and reverse sneezing episodes were noticed. A body condition score (BCS) of 3 was assigned to the dog (9-point scale, Nestlé Purina®).[20] A digital rectal exam revealed fresh blood and small amount of liquid fecal material. No other abnormalities were detected. Thoracic radiographs showed a mild to moderate diffuse bronchial pattern. Lesions found

on abdominal ultrasound included reactive changes of lymph nodes, small amount of free fluid, scattered hyperechoic small intestinal mucosal striations, thickening of the muscularis layer and abundant liquid content on the colon. Complete blood count revealed eosinophilia (2,670 cells/µl, reference range, RR: 0-1,200 cells/µl). Serum biochemical analysis revealed mild hypoalbuminemia (2.71 g/dl, RR: 2.8-4 g/dl), and mild hypoproteinemia (5.48 g/dL, RR: 5.5-7.2 g/dl). A mild increase of serum C-reactive protein (CRP) concentration (12.3 mg/l, RR: 0-10.7 mg/l) was also observed. Urinalysis and trypsine-like immunoreactivity test were normal. Cytological analysis of a rectal swab revealed the presence of many larvae compatible with *Strongyloides* sp. first-stage larvae (L1), columnar epithelial cells, occasional degenerated neutrophils, rare eosinophils, rare mast cells and numerous bacteria. A parasitic infection causing protein-losing enteropathy and bronchopneumonia was suspected.

The dog was then treated with a high digestible diet, an association of prebiotics and probiotics (Formalife®, Bayer, Italy,) 1 tablet, orally, once a day, for 15 days and fenbendazole (Panacur, MSD Animal Health Srl, Italy) 50 mg/kg, orally, once a day, for 15 days. Before starting anthelmintic therapy, the owner was asked to collect multiple fecal specimens on 3 consecutive days. Ten abundant fecal specimens were examined using two different techniques (sedimentation-zinc sulfate flotation and Baermann techniques). The analysis by the sedimentation-flotation method was negative. The Baermann technique, however, revealed a low number of rhabditoid larvae, which were morphologically identified as L1 of *S. stercoralis* (Fig. 1, a-b) and thin-shelled eggs (Fig. 1, c). A new coprological control 20 days after beginning fenbendazole therapy was performed on multiple fecal samples by the Baermann technique and was negative; however, diarrhea and cough were still present. Given the poor clinical response to the anthelmintic therapy and the persistence of clinical signs suggestive of strongyloidiasis, ivermectin (Ivomec®, Boehringer Ingelheim Animal Health SpA, Italy) 0.2 mg/kg, subcutaneously, repeated after two weeks, was administered (off-label) along with prednisolone (Prednicortone®, Dechra Veterinary Products Srl, Italy) 0.5 mg/kg, orally, once a day, for 10 days. At control 2 weeks after the second ivermectin administration, the dog was active and playful. A complete resolution of the cough and diarrhea with a final fecal score of 2 (7-point-scale, Purina Fecal Scoring Chart®),[19] and weight gain of 1 kg were reported by the owner. Physical and digital rectal examinations were normal and a BCS of 4 was assigned (9-point scale, Nestlé Purina®)[20] to the dog. Neither *S. stercoralis* larvae nor eggs were observed during fecal examinations (cytological analysis and Baermann technique). Eosinophilia disappeared, serum albumin (3.3 g/dl, RR: 2.8-4 g/dl), serum total protein (5.9 g/dL, RR: 5.5-7.2 g/dl) and serum CRP (2.3 mg/l, RR: 0-10.7 mg/l) concentrations turned normal.

During the following months, several fresh fecal samples were analysed by cytology and the Baermann technique, and all resulted negative for the presence of S. *stercoralis*. Six months after the second ivermectin administration, physical examination was normal, a body weight of 10.5 kg was recorded and a BCS of 6 was assigned to the dog. Neither gastrointestinal nor respiratory signs were reported by the owner. In addition to the coprological analyses (smear of fresh feces and Baermann technique), a fecal sample was shipped to the Centre for Tropical Disease of Negrar hospital, Verona (Italy) for execution of real-time PCR. Coprological analyses were negative for *S. stercoralis*, as well as real-time PCR.

Discussion

Herein, the authors describe the first report of *S. stercoralis* in a privately-owned dog from north-west of Italy (Piedmont) including pertinent details about its history, clinical, hematological, and parasitological findings, as well as specific anti-parasitic treatments and long-term follow-up.

In the last decade an increasing number of *S. stercoralis* infections have been reported in different European countries, as far west as the UK and as far north as Iceland and Scandinavia.[8,21-23] Although infection is more common in warm climate area, the parasite can survive and successfully complete its life cycle at lower environmental temperatures;[23] therefore, it is not surprising an infection in the Piedmont, one of the coldest regions of Italy.

S. stercoralis infections are often asymptomatic, and disease occurs mainly in massively challenged neonates and nurslings.[21,24] In dogs, severe infections cause bronchopneumonia and watery to mucous diarrhea,[21] and the condition can be easily confused with other generalized viral diseases common in puppies.[24] In this case, although the clinical features and the laboratory changes were unspecific, a generalized parasitic infection of the gut and lung was supported by the presence of gastrointestinal and respiratory impairment of some months duration, peripheral eosinophilia, panhypoproteinemia, and increase in CRP. Peripheral eosinophilia, although reported in both human and canine strongyloidiasis, is not a constant finding.[5,25] Eosinophils may play an important role in defense mechanisms against *S. stercoralis* larvae;[26] however, a defense collapse with low to normal number of eosinophils may explain the possibility of severe parasitosis in the absence of eosinophilia.[26] The inadequate weight gain and panhypoproteinemia of the dog during the infection, the weight gain and the resolution of the panhypoproteinemia after the ivermectin administration suggest the effects of *S. stercoralis* on the ability of the dog to absorb nutrients for essential metabolism and growth. Measurement of the levels of acute-phase proteins is of clinical importance in determining the presence and extent of inflammatory tissue damage as well as in providing diagnostic and prognostic information in infectious diseases and oncological disorders.[27,28] In human medicine, *S. stercoralis* infection is associated with elevated levels of acute-phase proteins and markers of inflammation.[29] In this case, serum concentration of CRP was increased before treatment, suggesting a systemic inflammatory reaction. After ivermectin administration, serum concentration of CRP normalized, indicating that *S. stercoralis* infection was directly associated with an enhance acute-phase response.[29]

In human medicine, the infection may also manifest with urticaria or larva currens.[14,21] To the authors' knowledge, cutaneous manifestations of *S. stercoralis* are seldom reported in dogs,[30] although it has been recently demonstrated that the infective larva of *S. stercoralis* is strongly attracted by a component of the dog's skin.[31] At the time of clinical examination no cutaneous signs were observed in this dog. However, alopecia with desquamation and pustules were noticed before diagnosis. A suspicion of demodicosis was raised by a veterinarian and a topical administration of moxidectin/imidacloprid (Advocate® spot on, Bayer SpA, Italy) was given with a complete resolution of the lesions. Since the demodicosis was never confirmed by a deep skin scraping, a dermatitis caused by *S. stercoralis* larvae cannot be excluded. Indeed, the topical therapy with moxidectin/imidacloprid is reported among the treatment protocols for S. stercoralis.[5]

Results from this case confirm that routine copromicroscopic methods may fail to diagnose *S. stercoralis* infection, as previously reported.[2,32] Indeed, even when present, larvae in feces become easily altered when saturated salt solutions are added.[21] Therefore, fresh fecal smears and the Baermann technique are required, although the sensitivity of these methods may be low because worms may be shed in low numbers and intermittently.[33] In this case, in addition to a fresh fecal smear, both the sedimentation-flotation and the Baermann techniques were performed on multiple and abundant fecal specimens collected on 3 consecutive days. Only the analyses by fresh fecal smear and Baermann technique turned positive for *S. stercoralis*. Two recent studies suggest that molecular (RT-PCR) techniques routinely used for *S. stercoralis* diagnosis in humans could be useful for the diagnosis of the infection in dogs.[16,34] However, only 4 fecal samples were tested in the former,[16] while a variable and inconstant positivity to pre-treatment fecal real-time PCR was observed in the latter.[34] Therefore, to date, the Baermann test remains the most frequently used test in vivo for the diagnosis.[16-18]

In humans, ivermectin is currently the most effective therapy at a dose of 200 µg/kg with cure rates up to 97% using a two-days course in asymptomatic cases.[29,31] In the veterinary counterpart, a standardized therapeutic protocol has not been defined yet and information on treatments is based on single cases or small case series.[2,6,26,32-35] Fenbendazole has been proposed alone or in combination with imidacloprid/moxidectin spot-on in naturally infected symptomatic dogs.[4] However, the treatment did not consistently result in the elimination of the infection. Some efficacious results have been achieved with off-label dosage of ivermectin given orally at different dosage schedules or subcutaneoulsy in single or repeated injections.[2,6,10,21,35] The dog of this report received topic moxidectin/imidacloprid for a clinical suspicion of demodicosis some months before diagnosis. This treatment led to resolution of only

dermatologic signs. Afterwards, fenbendazole was given for 15 days without significant clinical improvement. Therefore, ivermectin off-label was given subcutaneously two weeks apart with a progressive and complete resolution of clinical signs and the cease of larvae and eggs shedding, as confirmed by persistent fecal negative results (both at cytology and Baermann tests) throughout the post-treatment follow-up. Although imidacloprid/moxidectin and fenbendazole may have contributed to reduce the parasitic load and severity of symptoms, it seems that only the additional administration of ivermectin was highly effective in this dog.

Fecal real-time PCR was used at 6-month follow-up in this dog. Indeed, the addition of fecal monitoring by molecular tools might be a better choice when evaluating the efficacy of treatment in individual dogs.[35]

Conclusion

In conclusion, the authors describe for the first time a case of *S. stercoralis* infection in a privately-owned dog from north-west of Italy (Piedmont). From an epidemiological point of view, the present report demonstrates the presence of this parasite in a new region compared to those previously reported. Indeed, the dog lived in a regional breeding-kennel where he may have contracted the infestation. Since this infection is probably underestimated, especially in cold climate area, small animal clinicians should be aware of this disease among young dogs during the evaluation of differentials for gastrointestinal and/or respiratory signs. Moreover, since routine fecal flotation methods have low sensitivity for detection of L1, in case of gastrointestinal and/or respiratory signs, the authors suggest both direct fecal smear analysis and Baermann techniques on multiple and abundant fecal specimens for a correct diagnosis and monitoring, in addition to real-time PCR to evaluate the efficacy of treatment.