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A case of a dog refractory to different treatments for pulmonary capillariasis

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Abstract

Pulmonary capillariasis is a parasitic disease caused by the nematode *Eucoleus aerophilus* which affects wild and domestic carnivores. Currently, there are no anthelmintics approved for use in the treatment of dogs infected with *E. aerophilus*. The use of several anthelmintics has been reported in a few case reports and field efficacy studies in cats, much less is known on the treatment of dogs infected with *E. aerophilus*. The paper describes a case of a 4-month-old, mixed breed intact male referred to the Veterinary Teaching Hospital (VTH) of the Department of Veterinary Medical Science of the University of Bologna for a routine vaccination and tested positive for *E. aerophilus*. The dog has not been responding to three different administered treatments, such as moxidectin, fenbendazole and milbemycin oxime. Eighteen months after the first fecal examination, owner has brought in the dog for a routine visit, a coprological examination was requested and performed resulting negative for parasites. Veterinary practitioners, parasitologists, diagnostic laboratories and dog owners need to be aware of the increased danger of possible treatment failure when attempting to control parasitic infections for which there are no approved anthelmintics with established efficacies available for use.

Keywords: *Eucoleus aerophilus*; Dog; Treatments; Moxidectin; Fenbendazole; Milbemycin oxime; Veterinary Teaching Hospital

28 **Introduction**

29 *Eucoleus aerophilus* (Dujardin, 1845) also called fox lungworm, is a parasitic nematode belonging to the
30 *Trichuridae* family, which affects wild and domestic carnivores (Levine 1980). Occasionally, humans can
31 act as an accidental host, with 11 cases reported to date (Lalošević et al. 2008). *Eucoleus aerophilus* has a
32 worldwide distribution. In Italy, different rates of prevalence have been reported, ranging from 0.5% to
33 6.1%, based on the region involved (Traversa et al., 2019) or 8% if hunting dogs are involved (Veronesi pers
34 comm). There is confusion concerning the life cycle due to the contradictory results reported from older
35 studies indicating transmission maybe direct from ingestion of larvated eggs or indirect through the ingestion
36 of earthworm intermediate hosts (reviewed by Anderson 2000). Although based on only a few animals,
37 infection in dogs was not achieved by administering larvated eggs directly but was successful by feeding
38 earthworms exposed to larvated eggs (Radman et al. 1986). Adult stages live beneath the tracheal, bronchial
39 and bronchiolar epithelium (Anderson 2000), producing non-specific respiratory clinical signs (Di Cesare et
40 al. 2012). Depending upon the burden of infection, clinical signs may range from intense respiratory distress
41 that may be complicated by secondary bacterial infection (Traversa et al. 2011), to a complete absence of
42 clinical signs (Vieson et al. 2012). *E. aerophilus* represents a neglected parasite among the nematodes
43 causing respiratory disease in pets (Traversa et al. 2011). Capillariasis can be diagnosed by bronchoscopy
44 and bronchoalveolar lavage (Elhamiani Khatat et al. 2016) or suspected by performing radiographical
45 examination (Crisi et al., 2017). The prepatent period is thought to be about 3-5 weeks and infected animals
46 shed eggs in the feces for 8–11 months (Pechman 1995). The eggs, maintained at low temperature, high
47 humidity, in the shade, are seen to survive for over a year. Specifically, they are able to bear temperatures as
48 low as -26° C (Christenson 1938).

49 Detection of the distinctive bipolar plugged eggs by fecal flotation examination is the diagnostic method of
50 choice (Conboy 2009). In order to maximize detection sensitivity for parasitic infections examination of at
51 least two or three independently collected stool samples is recommended (Cartwright 1999). The eggs are
52 typically trichuroid-shaped and tend to be longitudinally asymmetric. The eggs are 58-79 × 29-40 µm in size
53 and have a shell wall surface pattern consisting of a series of anastomosing ridges (Conboy 2009). Capillarid
54 eggs can be differentiated from those of *Trichuris* spp. based on size and morphology (Zajac and Conboy

55 2012). Due to the similarities in egg size and morphology, misidentification of the various capillarids and
56 confusion with *Trichuris* spp are probably common (Guardone et al. 2013).

57 Currently there are no anthelmintics approved for use in the treatment of dogs infected with *E. aerophilus*.
58 The use of a number of anthelmintics has been cited in case reports and field efficacy studies in cats (Endres
59 1976; Traversa et al. 2012; Knaus et al. 2015; Di Cesare et al. 2017). Much less is known on the treatment of
60 dogs infected with *E. aerophilus*; fenbendazole, 50 mg/kg BW, PO, daily for 10 days (Burgess et al. 2008)
61 and milbemycin oxime 0,5-1,07 mg/kg BW, PO, monthly (Abbate et al. 2018) have both been reported.

62 This paper aims to describe a case of *E. aerophilus* infection in a dog which was refractory to treatment
63 using multiple anthelmintics. In addition, the final diagnosis has also been confirmed by using a PCR
64 method.

65

66 **Case history and discussion**

67 In January 2019 a 4-month-old, mixed breed intact male was referred to the Veterinary Teaching Hospital
68 (VTH) of the Department of Veterinary Medical Science of the University of Bologna for a routine
69 vaccination. The dog had been adopted at the age of 3-months from a shelter in the Viterbo province and was
70 immediately moved to Bologna. The owner reported that the dog lived indoor but was taken to a local dog
71 park area twice *per* day. The dog appeared normal on physical examination. Since there was no previous
72 veterinary medical history, a complete blood count and urine examination was done to establish baseline data
73 on the dog. Additionally, a fecal examination using the Di Felice and Ferretti's (1962) floatation solution
74 (specific gravity = 1.3) in a standardized centrifugal flotation method was performed (Euzeby 1981). No
75 abnormalities were detected on blood and urine examinations. Fecal examination results were positive for *E.*
76 *aerophilus* eggs (Figure 1) and *Cystoisospora canis* oocysts. The dog was treated topically with moxidectin
77 (Advocate) at a dosage of 2.5 mg/kg once a month for two months. No specific treatment was administered
78 for *C. canis* due to the self-limiting nature of coccidian infections in asymptomatic animals (Hall and
79 German 2005). One month after the second administration of Advocate, the dog, still asymptomatic, was
80 returned to the VTH for reassessment. A follow-up fecal examination at this time was still positive for *E.*
81 *aerophilus* and was also positive for *Giardia duodenalis* cysts. The treatment protocol was changed to oral
82 fenbendazole (Panacur) in tablets, 50 mg/kg daily for 21 days as suggested by Burgess et al. (2008). Thirty

83 days later the owner brought in a three consecutive-day fecal sample collection for examination. At this time,
84 the dog tested negative for *Giardia*, but was still positive for eggs of *E. aerophilus* (Figure 1). Following the
85 protocol used by Conboy et al. (2013) to treat canine nasal capillariasis due to *Eucoleus boehmi* infection, the
86 dog was re-treated once again with an elevated oral dose of milbemycin oxime (2 mg/kg). Post-treatment
87 centrifugal fecal flotation examinations remained positive at 7 days, 14 days, 21 days, and 28 days post
88 treatment. General hematological and biochemical parameters were evaluated during the observational
89 period and no abnormalities were detected. Radiographic images showed a mild and diffuse bronchial pattern
90 (Figure 2). Additionally, the dog was also examined by supplementary rhinoscopy and bronchoscopy
91 examinations. On bronchoscopy, a mild diffuse edema of the bronchial wall was noted but no adult worms
92 were detected, and no eggs were recovered from bronchoalveolar lavage. A concentrated pool of eggs
93 obtained from the various fecal sample collections were processed for genomic DNA extraction using the
94 QIAmp DNA Stool Mini Kit (Qiagen® GmbH, Hilden, Germany) according to the manufacturer's
95 instructions. A semi-nested PCR protocol was applied to amplify a specific 299-bp-long fragment of the *E.*
96 *aerophilus cox1* gene (Di Cesare et al. 2012). DNA extracts produced amplicons of the expected size
97 according to the PCR protocol applied.

98 In view of the complete absence of respiratory disease signs and in consultation with the owner, it was
99 decided to forgo further anthelmintic treatment and conduct further fecal examination testing at a later date.
100 The owner was advised to maintain proper hygiene with respect to prompt removal and proper disposal of
101 pet feces in order to avoid environmental contamination by their dog. Fecal flotation examination performed
102 5 months after the milbemycin oxime treatment was still positive for *E. aerophilus* eggs. Eighteen months
103 after the first fecal examination (1-year after the last treatment with milbemycin oxime), the owner brought
104 the dog in for a routine visit and a coprological examination conducted at this time was negative for
105 parasites.

106 The manuscript reports the failure of three different anthelmintics to control an *Eucoleus aerophilus*
107 infection in a dog. *E. aerophilus* is a nematode commonly found in various wildlife hosts particularly wild
108 canids and only sporadically in dogs and cats. In Italy the prevalence of infection ranges from 0.5 to 6.1%
109 both in dogs and cats (Traversa et al. 2019). However, prevalence in pets is likely underreported because
110 infection most often results in mild or no clinical signs of disease (Traversa et al. 2009). In the present case,

111 no clinical signs of respiratory disease were observed at any time by the owner and none were detected on
112 any of the physical examinations conducted at the VTH during the course of the management of this case.
113 Bronchoscopy revealed mild abnormality (bronchial wall edema) consistent with *E. aerophilus* infection but
114 no adult worms were observed. As reported by Elhamiani Khatat et al. (2016), clinical signs and
115 hematological, x-ray and endoscopy exams are suggestive but nonspecific for the diagnosis of capillariasis.
116 When clinical signs are present, they may appear as generalized respiratory distress, dry or moist cough and
117 sneezing (Traversa et al. 2009). The difficulties involved in visualizing adult worms by diagnostic imaging
118 highlights the importance of fecal examination which remains the method of choice for the diagnosis of
119 capillariasis in dogs and cats (Conboy 2009).

120 Numerous protocols have been reported in the scientific literature for the treatment of both nasal and
121 tracheobronchial capillariasis in dogs, including the use of fenbendazole, ivermectin, milbemycin oxime and
122 moxidectin (see Table 1). Treatment with fenbendazole at a dosage of 50mg/kg BW, PO once *per* day for
123 two weeks was reported to be effective in a dog infected with *E. aerophilus* and also in a dog infected with
124 *E. boehmi* (Burgess et al. 2008; Baan et al. 2011). Treatment success and failure have both been reported in
125 the use of elevated orally administered dosages (2 mg/kg) of milbemycin oxime in dogs infected with *E.*
126 *boehmi* (Alho et al. 2016; Conboy et al. 2013). Topical applications of imidacloprid 10% / moxidectin 2.5%
127 has been reported as an effective treatment on dogs infected with *E. boehmi* (Veronesi et al. 2014; Alho et al.
128 2016; Veronesi et al. 2017). Despite receiving multiple anthelmintics including moxidectin, fenbendazole
129 and milbemycin oxime the dog described in the present study continued to shed eggs of *E. aerophilus*
130 throughout the entire treatment period and beyond. It could not be determined what role if any re-infection
131 may have played in the difficulty in controlling the infection in this case. Furthermore, coprophagia could be
132 another important source of egg shedding in dogs as suggested by Nijssen et al. (2015). However, the
133 frequency of fecal examinations which at times occurred weekly, make it unlikely that re-exposure leading to
134 re-infection or coprophagia could have been the explanation for the consistently positive test results. The
135 eighteen-month follow up suggests that the patent period for *E. aerophilus* may last longer than 11 months as
136 proposed by Pechman (1995).

137 This report details the many unsuccessful attempts to treat this dog for the *E. aerophilus* infection using a
138 variety of commonly used anthelmintics. Veterinary practitioners, parasitologists, diagnostic laboratories and

139 dog owners need to be aware of the increased danger of possible treatment failure when attempting to control
140 parasitic infections for which there are no approved anthelmintics with established efficacies available for
141 use. Further epidemiological studies are needed to assess the real threat posed by this underestimated
142 infection. Additionally, further work to establish an effective treatment protocol based on a larger number of
143 dogs either through an efficacy study or a larger case series of naturally infected animals is required.

144

145 **Conflict of interest**

146 Benedetto Morandi, Maria Chiara Sabetti, Fabrizia Veronesi, Giulia Morganti, Marco Pietra, Giovanni
147 Poglayen, Nikolina Linta, Gary Conboy, Roberta Galuppi, all together, declare that they have no conflict of
148 interest.

149

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232

Authors	Species	Treatment	Dosage	Efficacy
Abbate et al. 2018	<i>Capillaria aerophila</i>	afoxolaner 1.9% / milbemyacin oxime 0.4%	2,50-5,36 mg/kg/0.5-1.07mg/kg PO; once	✓
Alho et al. 2016	<i>E. boehmi</i>	milbemyacin oxime	2.0mg/kg PO, once	✗
Alho et al. 2016	<i>E. boehmi</i>	fenbendazole	100mg/kg PO, q24h, 2-wk	✗
Alho et al. 2016	<i>E. boehmi</i>	imidacloprid 10 % / moxidectin 2.5 %	10 mg/kg/2.5%mg/kg spot-on; once	✓
Baan et al. 2011	<i>E. boehmi</i>	fenbendazole	50mg/kg PO, q24h, 2-wk	✓
Burgess et al. 2008	<i>E. aerophilus</i>	fenbendazole	50mg/kg PO, q24h, 10 d	✓
Conboy et al. 2013	<i>E. boehmi</i>	milbemyacin oxime	0.5mg/kg PO, once	✗
Conboy et al. 2013	<i>E. boehmi</i>	milbemyacin oxime	1.0mg/kg PO, twice	✗
Conboy et al. 2013	<i>E. boehmi</i>	milbemyacin oxime	2.0mg/kg PO, once	✓
Evinger et al. 1985	<i>Capillaria aerophila</i> *	ivermectin	0.2mg/kg PO, once	✓
Veronesi et al. 2014	<i>E. boehmi</i>	imidacloprid 10 % / moxidectin 2.5 %	10 mg/kg/2.5%mg/kg spot-on; once	✓
Veronesi et al. 2017	<i>E. boehmi</i>	imidacloprid 10 % / moxidectin 2.5 %	10 mg/kg/2.5%mg/kg spot-on; once	✓

234 **Table 1.** Alphabetically listed treatments adopted for dogs by different Authors and their efficacy. ✓= occurred
 235 therapeutic effect; ✗ = treatment failure. * Authors report the diagnosis as *Capillaria aerophila* but due to its
 236 localization it is most likely *E. boehmi*.

238 **Figure captions**

239

240 **Figure 1.** *Eucoleus aerophilus* eggs detected at the time of the fecal examination. Top left corner 100x
241 magnification and 20x on the right.

242 **Figure 2.** Right lateral (**A**) and dorsoventral (**B**) thoracic radiograph of the dog. There is a mild bronchial
243 pattern evidenced by ring shadows (white arrows) and tram lines (black arrows). The entire lung is abnormal,
244 and only the most obvious ring shadows and tram lines have been pointed out. Human radiographic system
245 (VILLA GENIUS HF, Italy) and Digital Radiography (DRX-Transportable, Carestream).