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Crenosoma vulpis in dog: first case report in Italy and use of the FLOTAC technique for copromicroscopic diagnosis

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Abstract *Crenosoma vulpis* is a metastrongylid nematode that infects the bronchi, bronchioles, and trachea of wild and domestic canids and various other carnivores. It is endemic in the red fox population in the north-eastern parts of North America and in Europe, including Italy. Dogs are susceptible to infection with clinical signs consisting primarily in a chronic cough. The present paper reports— to the authors' knowledge—the first case of spontaneous *C. vulpis* infection in a dog in Italy. In addition, it also reports, for the first time, the use of the FLOTAC technique for *C. vulpis* diagnosis in canine fecal samples, with results compared to the following four standard copromicroscopic techniques: the Baermann technique, the McMaster technique, the simple flotation technique, and the Wisconsin technique. The results showed that the FLOTAC technique produced mean larvae per gram of feces greater than that produced by the other more widely used diagnostic tools. After the treatment of the *C. vulpis* infected dog with a single oral dose of 0.5mg/kg milbemycin oxime, the clinical signs resolved and the shedding of larvae ceased.

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In conclusion, the discovery of *C. vulpis* for the first time in a dog in Italy indicates that the fox lungworm should be considered in the differential diagnosis of respiratory disease in dogs; in addition, the findings of the comparison study showed that the FLOTAC technique may improve the ability to accurately diagnose canine lungworm infections.

Introduction

Crenosoma vulpis Dujardin 1945, the fox lungworm, is a metastrongylid nematode that infects the bronchi, bronchioles, and trachea of wild and domestic canids and various other carnivores (Bihr and Conboy 1999). It is endemic in red fox (*Vulpes vulpes*) populations in the north-eastern parts of North America and in Europe (Sreter et al. 2003; Nevarez et al. 2005; Saaed et al. 2006), including Italy (Iori et al. 1990; Manfredi et al. 2003). Since the first report in a domestic dog in the UK (Cobb and Fisher 1992), very few cases in dogs in Europe have been reported in literature, e.g., in Ireland (Reilly et al. 2000), Switzerland (Unterer et al. 2002), and Germany (Barutzki and Schaper 2003).

Infection in dogs appears to be non-lethal with clinical signs consisting mainly of chronic cough; diagnosis is based on detecting first-stage larvae in fecal samples using the Baermann technique or fecal flotation techniques (Bihr and Conboy 1999; Conboy 2004).

The present paper reports—to the authors' knowledge— the first case of spontaneous *C. vulpis* infection in a dog in Italy. In addition, it also reports, for the first time, the use of the FLOTAC technique (Cringoli 2006) for *C. vulpis* diagnosis in canine fecal samples, with results compared to other four standard copromicroscopic techniques.

Materials and methods

Case report

A 2-year-old male English Setter from the Campania region of southern Italy was presented to the referring clinician with a productive cough, a dribbled saliva, and dyspnea. On auscultation, the dog had increased lung sounds over the entire lung fields. Bronchoscopic examination revealed a hyperemic trachea and a mucopurulent exudate affecting the lower bronchi. Nematode parasites were visible grossly, and the examination of fluid collected by bronchoalveolar lavage revealed the presence of adults of *C. vulpis*, easily identified by microscopic examination, as the presence of their distinctive horizontal cuticolar ridges at their anterior ends (Fig. 1; Craig and Anderson 1972; Georgi and Georgi 1992).

Fecal examination by the FLOTAC technique (Cringoli 2006; see next section) revealed the presence of *C. vulpis* larvae (95 larvae per gram of feces, LPG; Fig. 2) and the presence of eggs of *Toxocara canis* (4,428 eggs per gram of feces, EPG), *Ancylostoma caninum* (26 EPG), and *Trichuris vulpis* (76 EPG).

The dog was treated with a single oral dose of 0.5mg/kg milbemycin oxime (Interceptor®, Novartis Animal Health), as suggested by Conboy (2004).

Comparison of copromicroscopic techniques

For the study on the copromicroscopic diagnosis of *C. vulpis*, the following five techniques were compared:

1. The Baermann technique, considered as choice technique for the lungworm diagnosis in canids (Bihr and Conboy 1999);

Fig. 1 Anterior end of an adult of *C. vulpis* at 400× magnification. Note the presence of the distinctive horizontal cuticolar ridges

Fig. 2 *C. vulpis* first stage larvae at 400× magnification. The larvae have a straight, pointed tail and their length ranges from 246 and 308 μm (Wetzel 1940)

2. The FLOTAC technique (Cringoli 2006), a new multivalent copromicroscopic technique in both human and veterinary field;
3. The McMaster technique (MAFF 1986), the most universally used technique for estimating the number of helminth eggs/larvae in animal feces (Cringoli et al. 2004);
4. The simple flotation technique (MAFF 1986), utilized as copromicroscopic technique at most veterinary clinics; and
5. The Wisconsin technique (Cox and Todd 1962; Egwand and Slocombe 1982), which consists in a flotation in centrifuge.

A 1-day fecal sample (≈130g) was collected from the *C. vulpis* infected dog and accurately homogenized. First, ten replicates of 10g-based Baermann technique (technique 1) were performed and analyzed 24h later. Second, the remaining 30g were suspended in tap water (dilution ratio = 1:10). The suspension was then poured through a wire mesh screen having an aperture of 350μm and, after discarding debris and homogenizing, was divided into 40 aliquots of 6ml to have ten replicates of each of the four flotation-based methods (techniques 2, 3, 4, and 5). All tubes were centrifuged for 2min at 1,500rpm, and the supernatant was poured off and discarded (MAFF 1986), leaving only a pellet in the tube, thus containing 1/6 of gram of feces. Each tube was then randomly assigned to a technique. A zinc sulfate solution (specific gravity = 1.200) was used for techniques 2, 3, 4, and 5. This solution was chosen from a battery of 14 solutions with specific gravity ranging between 1.200 and 1.450 (Cringoli et al. 2004) after a pre-testing study performed on the canine fecal sample containing *C. vulpis* larvae (data not shown).

When the FLOTAC or the McMaster technique was used (techniques 2 and 3), the tube was filled with the solution to the previous 6-ml level and slowly agitated. The resulting agitated suspension was then taken up by a pipette to load the two chambers of either the McMaster slide (Weber Scientific International, England, volume = 1.0ml) or one chamber of the FLOTAC® apparatus (volume = 5ml).

When the simple flotation or the Wisconsin technique was used (techniques 4 and 5), the tube was filled with the solution to 15ml, covered with a coverslip, and left for 15min (technique 4) or centrifuged at 1,500rpm for 10min (technique 5).

Statistical analysis

Data were double-entered and cross-checked, and statistical analyses were performed using version 13 of the SPSS software for Windows (SPSS; Chicago, USA). Mean, standard error (SE) and 25th, 50th, and 75th percentiles of LPG values were calculated for the five different techniques. The statistical differences between the mean LPG were analysed using analysis of variance (ANOVA; GLM for repeated measures) in conjunction with the Bonferroni test for post hoc comparison.

Results

After treatment, the C. vulpis infected dog made a rapid recovery, with resolution of all clinical symptoms within 14days; in addition, shedding of larvae in feces ceased as revealed by the copromicroscopic examination performed with the FLOTAC technique, performed at follow-up examination 2weeks after treatment. Further, no more eggs of T. canis and T. vulpis were found in the dog's feces.

Table 1 summarizes C. vulpis LPG values (mean, SE, and percentiles) according to the copromicroscopic technique used and the multiplication factors utilized for each technique to obtain LPG values. The mean LPG revealed by the FLOTAC technique was significantly higher ($P < 0.05$) than that obtained by all the other four techniques. Statistical differences were observed neither between the mean LPG produced by the Baermann technique and the flotation technique ($P = 0.793$) nor between the mean LPG produced by the McMaster technique and the Wisconsin technique ($P = 1.000$).

Discussion

European literature reports of C. vulpis in dogs are quite scant. Spontaneous infections have been recently reported in dogs from UK (Cobb and Fisher 1992), Ireland (Reilly et al. 2000), and Switzerland (Unterer et al. 2002); in addition, data from 8,438 dogs from Germany revealed a C. vulpis prevalence of 0.9% (Barutzki and Schaper 2003). The present paper reports, according to the author's knowledge, the first case report of C. vulpis infection in Italy, a country where this lungworm has been already reported in red foxes (Iori et al. 1990; Manfredi et al. 2003). The infected dog had a typical respiratory disease caused by metastrongylidae. The treatment with milbemycin oxime showed full efficacy against C. vulpis—as previously reported by Conboy (2004)—as well as against T. canis and T. vulpis. Besides mylbemicyn oxime, an anthelmintic currently approved for use in dogs, although there is no label claim for C. vulpis, other successful treatment options against the fox lungworm in dogs include febandazole, febantel, levamisole, diethyl-carbamazine, and ivermectin (Bihr and Conboy 1999).

The findings of the present paper also provide important new information on the performance of available methods for the in vivo diagnosis of C. vulpis infection in domestic dogs, an infection that might be more widespread than the literature reports suggest (Reilly et al. 2000). In fact, larvae are not generally detected using the standard fecal flotation techniques utilized at most veterinary clinics; thus, a huge number of C. vulpis infected dogs could be misdiagnosed as having allergic respiratory disease (Bihr and Conboy

Table 1 LPG values (mean, standard error, and percentiles) of *Crenosoma vulpis* detected by the five copromicroscopic techniques

Copromicroscopic techniques	Multiplication factor	LPG (n=10 replicates)				
		Mean*	SE	Percentiles		
				25th	50th	75th
FLOTAC	2	91.3[a]	5.1	82.0	88.0	104.0
McMaster	10	36.7[b]	6.1	27.5	30.0	52.5
Baermann	1/10=0.10	0.7[c]	0.05	0.6	0.7	0.8
Flotation	1/6=0.17	11.1[c]	0.5	10.0	10.8	12.8
Wisconsin	1/6=0.17	36.7[b]	6.7	31.6	36.7	43.3

*Significant differences for different letters ($P<0.05$).

1999). In addition, also the Baermann technique, considered as choice technique for this lungworm infection, had proven negative in a dog with spontaneous infection (Reilly et al. 2000).

In the present study, the FLOTAC technique produced mean LPG greater than that produced by the other more widely used diagnostic tools, i.e., the Baermann, the McMaster, the simple flotation, and the Wisconsin technique. For the flotation of the *C. vulpis* larvae, the zinc sulfate solution is recommended as reported by Bihr and Conboy (1999) and as shown in our pre-testing study on 14 flotation solutions. It is important to note that flotation solutions utilized at most veterinary clinics, e.g., sodium chloride (s.g. = 1.200) and sodium nitrate (s.g. = 1.200), produced false negative results or produced very few *C. vulpis* larvae (data not shown). This demonstrates once again that the type of solution used in the flotation-based copromicroscopic techniques significantly influences LPG/EPG values; solutions having the same density may give different mean LPG/EPG in the helminth egg/larva counts (Cringoli et al. 2004).

The findings of the present paper showed that the FLOTAC technique can be utilized for quantifying lungworm larva burdens in canine fecal samples because of its higher sensitivity compared to the other more widely used diagnostic tools; these results thus underscore previous observations made for various parasites of veterinary and human importance (Cringoli 2006; Rinaldi et al. 2007; Utzinger et al. 2007).

In conclusion, the discovery of *C. vulpis* for the first time in a dog in Italy indicates that the fox lungworm should be considered in the differential diagnosis of respiratory disease in dogs; in addition, the findings of the comparison study showed that the FLOTAC technique may improve the ability to accurately diagnose canine lungworm infections.

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