Short communication

Nasal eucoleosis in a symptomatic dog from Italy

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ABSTRACT

A dog with chronic muco-purulent nasal discharge, sneezing, reverse sneezing and impaired scenting ability was diagnosed as being affected by nasal eucoleosis based upon rhinoscopic evidence of *Eucoleus boehmi* in situ, identification of the adult parasites in nasal biopsies, and eggs in the faeces by light and scanning electron microscopy. The dog was successfully treated with a single administration of moxidectin. A second course of moxidectin was required for about 10 weeks after the first treatment, because clinical signs recurred due to a likely re-infection. This second administration, along with measures undertaken to prevent geo- and coprophagic pica, resolved the parasitism, as demonstrated by negative copromicroscopic and rhinoscopic examinations, and prevented reinfestation for the next 4 months. To the best of the authors' knowledge, this represents the first report describing a clinical case of nasal eucoleosis with a demonstration of the adult parasites in situ in a dog from Italy where, until recently, infestation of *E. boehmi* was only detected by a coprological examination. Veterinarians and parasitologists should be aware of the occurrence of canine infection with *E. boehmi*. They should include this parasite in the differential diagnoses for animals suffering from upper airway distress and look systematically for it during rhinoscopic and copromicroscopic examinations.

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1. Introduction

Eucoleus boehmi (previously: *Capillaria boehmi*) is a little known, trichuroid, parasitic nematode that lives embedded in the epithelium of the nasal turbinates, frontal and paranasal sinuses of wild canids, e.g. foxes and wolves, although infection in domestic dogs is also described. The life cycle and routes of transmission of this parasite are almost unknown (Conboy, 2009). The occurrence of infection following coprophagia has been reported in some cases (Baan et al., 2011) but generally speaking, this route is most unlikely, because it would imply the ingestion of infectious eggs present in environmental faecal matter over a certain period, as yet unknown. Nonetheless, a direct life cycle, in which environmental infectious eggs are ingested by canids and larval stages migrate to the nasal cavities where they reach adulthood, is likely for this parasite (Conboy, 2009). Alternative development in earthworms that could act as facultative intermediate or paratenic hosts (Campbell, 1991), similar to that hypothesised for the closely related species *Eucoleus aerophilus*

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(previously: *Capillaria aerophila*) (Traversa et al., 2011), cannot be excluded.

Most canine infections are sub-clinical, even though dogs may show distress of the upper respiratory tract with varying degrees of severity.

Although the interest in extra-intestinal nematodes affecting dogs (e.g. *Dirofilaria immitis*, *Angiostrongylus vasorum*, *E. aerophilus*) is increasing due to the apparent emergence of these pathogens in several countries, including Italy (Traversa et al., 2010), infection by *E. boehmi* is poorly documented and considered to be sporadic throughout the world. Few cases have been reported in Europe (Zarnowski and Patyk, 1960; Sréter et al., 2003; Gajewska et al., 2004) and in North America (Campbell and Little, 1991; Schoning et al., 1993; Baan et al., 2011) and as regards Italy, the presence and distribution data on *E. boehmi* infection in dogs is scarce and only supported by copromicroscopic investigations (De Liberato et al., 2009; Di Cesare et al., 2012; Guardone et al., 2012).

The emergence of extra-intestinal canine nematodes and practitioners' experiences anectodically referred to by the authors suggest that some drivers could also nurture the dispersion of this parasite throughout Italy, as well as in other areas. Indeed, there is significant merit in improving basic knowledge of the distribution of this "new" parasite affecting dogs and in describing, from a practical standpoint, the potential impact it may have in causing respiratory distress in canine patients. Therefore, this article describes a case of nasal eucoleosis in a dog living in Central Italy, and includes a presentation of practical features in a clinical setting.

2. Case report

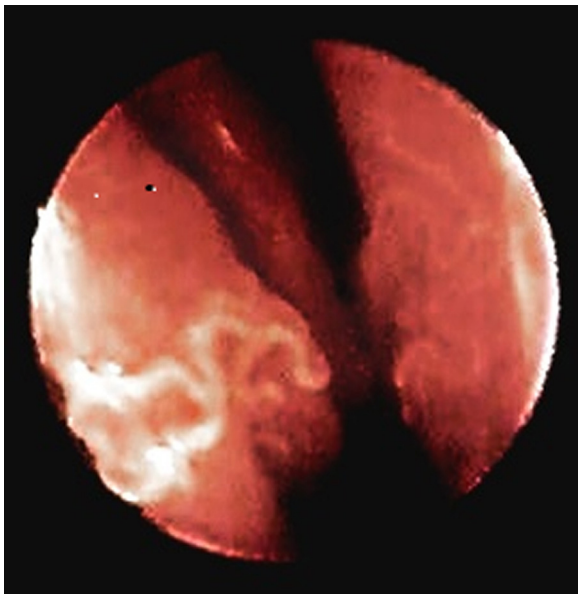A male, 4-year-old, crossbred dog was presented to the referring veterinary surgeon in February 2012 with a chronic, year-old history of muco-purulent nasal discharge, sneezing and reverse sneezing, which had worsened in the last month before being referred to the clinic.

The dog lived outdoors in a rural area of Tuscany (Central Italy) and the owners reported that the dog was frequently affected by copro- and geophagic pica.

At the clinical examination, the patient showed severe distress of the upper respiratory tract, including repeated sneezing, reverse sneezing, tachypnea, bilateral nasal discharge and discomfort on nose inspection. Trachea palpation and chest auscultation did not show any alteration. Neither a superficial inspection of the nostrils nor radiographic examinations of the cervical tract and nasal *planum* showed any significant alterations.

The patient underwent antegrade and retrograde rhinoscopy using a flexible fibroendoscope (VFS/2A, VetVu®, USA – 6.0 mm in diameter and 100 cm in length). The rhinoscopy showed mild, bilateral and diffuse mucosal hyperaemia with oedema and on antegrade examination, several white, linear, serpentine-shaped, motile nematodes, mostly located on the surface of the turbinates
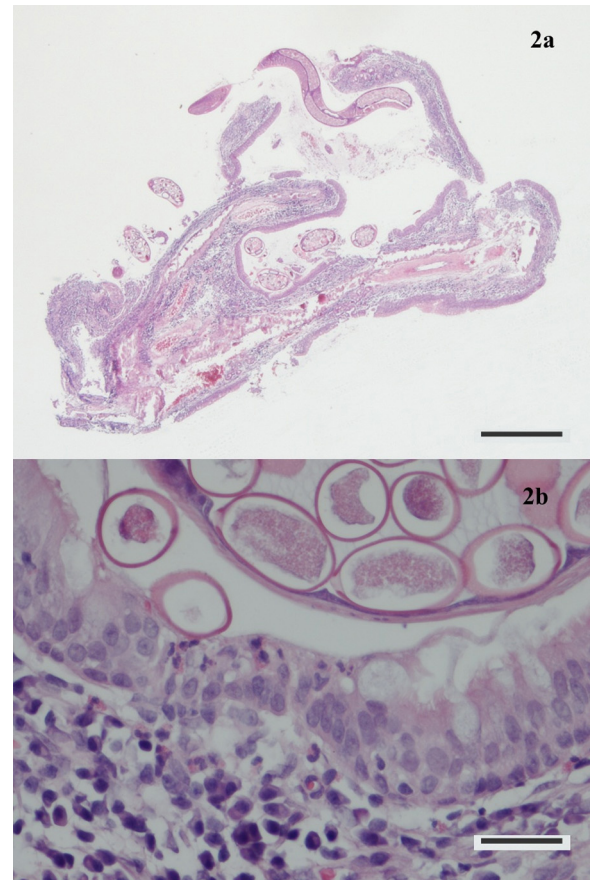
Fig. 2. Nasal cavity of a dog infected by *Eucoleus boehmi*, histological examination. (a) Fragment of nasal turbinates with multiple section of an adult nematode embedded in catarrhal exudate. Hematoxilin and eosin. Scale bar: 400 μm. (b) Nematode section with embryonated eggs laying on a moderately hyperplastic respiratory epithelium and a *lamina propria* infiltrated by lymphocytes, plasma cells and eosinophils with focal exocytosis. Hematoxilin and eosin. Scale bar: 20 μm.

Fig. 1. Rhinoscopic evidence of *Eucoleus boehmi* adult worms embedded in the mucosa of medium meatus of a naturally infected dog from Italy.

delimiting the medium meatus (Fig. 1). There were multiple attempts to recover the nematodes using an endoscopic biopsy forceps, but the organisms were friable and difficult to collect whole. Therefore, a biopsy including nematode specimens was performed, as well as a touch imprint cytology of the biopsy. At the cytological examination, the sample showed chronic inflammation, with the presence of lymphocytes, eosinophils and mast cells. A histopathological evaluation of the biopsy revealed multiple sections of a nematode parasite approximately 150–200 μm in width, with the internal genital tract full of oval eggs containing an undifferentiated embryo, embedded in a catarrhal exudate with few neutrophils (Fig. 2a). Respiratory mucosa was diffusely congested with marked dilation of *lamina propria* due to the presence of a severe lymphocytic and plasmacytic infiltration and fewer eosinophils. The latter were mainly lodged in the superficial subepithelial layers with transepithelial exocytosis (Fig. 2b). Respiratory epithelium was moderately hyperplastic, although flattened in the areas in contact with the parasite.

On the basis of the clinical symptoms, rhinoscopic findings and cytological evidence, a presumptive diagnosis of infection by *E. boehmi* was made. To confirm such a suspected diagnosis, two consecutive faecal samples were collected and examined at the Parasitological Laboratory of the Department of Biopathological Sciences and Hygiene of Animal and Food Productions of the University of Perugia. Two different flotation procedures were followed using a sugar solution with a specific gravity (s.g.) of 1200 and a zinc sulphate solution ($ZnSO_4$) with a 1350 s.g. barrel-shape eggs found during the copromicroscopic examination with both solutions were regarded as those of *E. boehmi* on the basis of key morphometric and morphological characteristics (Di Cesare et al., 2012), i.e. size (50–60 μm long and 30–35 μm wide), asymmetry of bipolar plugs, a small, clear to golden space between the embryo and the wall, and tiny pits on the surface of the wall giving them a porous appearance (Fig. 3a and b).

For additional confirmation of the light microscopic findings, eggs isolated by flotation in $ZnSO_4$ solution were sieved using a modified technique, as previously described (Al-Sabi et al., 2010), mounted on aluminium stubs, air dried, gold coated with the sputtering technique and submitted to scanning electron microscopy (SEM) analysis using a Philips XL30 SEM (Philips Electron Optics BV, Eindhoven, Netherlands). The SEM observation demonstrated
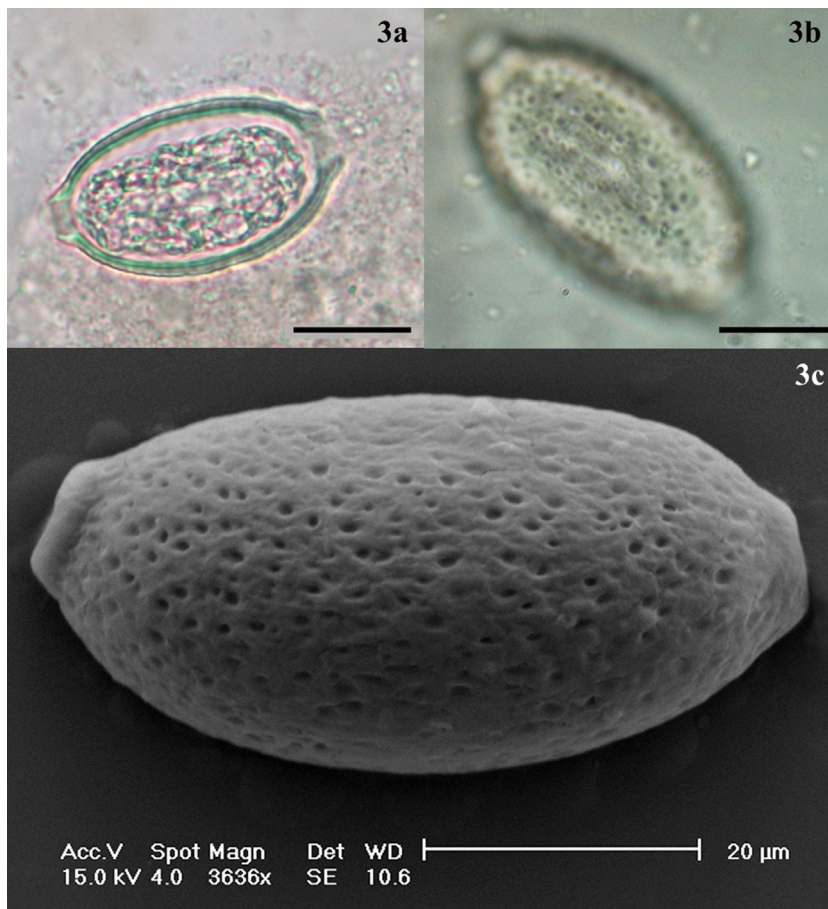

Fig. 3. Eggs of *Eucoleus boehmi*. (a) Light microscopy: eggs showing the asymmetry of bipolar plugs and a small, clear to golden, space between the embryo and the egg shell. Original magnification 40×/scale bar: 20 μm; (b) light microscopy: outer shell of a *Eucoleus boehmi* egg showing the typical tiny pits. Original magnification 100×/scale bar: 20 μm; (c): scanning electron microscopy: egg of *Eucoleus boehmi* showing the characteristic outer densely network surface with irregularly distributed small pits. Scale bar: 20 μm.

an external surface with a dense surface with irregularly distributed small pits characteristic of *E. boehmi* eggs (Fig. 3c).

The dog was subsequently treated with a single dose of the spot-on formulation containing 10% imidacloprid/1% moxidectin (Advocate®, Bayer Animal Health) according to the dog's body weight. Clinical signs disappeared completely within 1 week and on day 28 after the treatment, two further, consecutive faecal examinations and an additional rhinoscopy were performed. The dog showed a complete recovery, with absence of eggs in the faeces and adult nematodes in the nasal mucosa, respectively. Nonetheless, clinical signs including sneezing and reverse sneezing recurred approximately 6 weeks after the first control (i.e. 10 weeks after the anthelmintic treatment), probably due to a re-infection caused by persisting geophagic pica, and again reported by the owners. In addition to the prompt removal of faeces from the environment and moving the animal to a concrete-floored kennel for geo- or coprophagia prevention, a second application of Advocate® resolved the parasitism, as demonstrated by subsequent copromicroscopic and rhinoscopic examinations conducted 4 weeks after the treatment, and prevented the recurring of clinical signs in the next 4 months of follow-up.

3. Discussion and conclusion

This article describes the first case of canine nasal eucoleosis together with evidence of adult parasites in situ in a symptomatic dog from Italy.

The vast majority of data available on the recovery of this nematode from the nasal cavity of dogs come from necropsy (Campbell and Little, 1991; Schoning et al., 1993), because only limited reports have demonstrated the presence of adult stages of *E. boehmi* in living, symptomatic dogs by means of endoscopic or histological examinations (Evinger et al., 1985; Baan et al., 2011).

The infection may occur asymptomatically or with mild symptoms which might pass un-noted and, therefore, not referred by the owners or undetected by veterinarians (Baan et al., 2011). Indeed, the parasite may trigger evident clinical signs, usually consisting of not only chronic rhinitis with coughing, sneezing and a catarrhal blood-stained or muco-purulent nasal discharge, especially when bacterial infections intervene, but also the impairment of the scenting ability (hypo- or anosmia), with a severe reduction in the ability to work (Evinger et al., 1985; King et al., 1990; Campbell and Little, 1991; Piperisova et al., 2010; Baan et al., 2011), as shown also by the clinical case presented herein. Moreover, an additional sign, consisting of reverse sneezing also previously observed in a dog from the USA (Evinger et al., 1985), is also reported here.

The inflammatory response described in nasal eucoleosis is a marked plasmacytic rhinitis with a variable number of eosinophils, probably due to the activation of the local immune system in response to chronic, antigenic and mechanical stimulation (Evinger et al., 1985; Baan et al., 2011). Eosinophilic inflammation is frequently reported in human beings as a manifestation of allergic inflammation (Ahmadiafshar et al., 2012), but it has also been found

in other nasal parasitoses, i.e. ovine oestrosis, for which an immune or allergic reaction to parasitic antigens has been postulated (Silva et al., 2012). In the present case, the inflammatory infiltrate was mainly composed of lymphocytes and plasma cells, reflecting a chronic Th2 response. Although eosinophils were scarce, a common finding in chronic inflammations, they were concentrated around the parasites, thus suggesting a direct parasite-induced chemotaxis, rather than an immune mediated activation. Given that eosinophils and mast-cell mediated destruction of the parasites is thought to occur in ovine oestrosis (Angulo-Valadez et al., 2012), a similar mechanism could also be hypothesised for other nasal parasitic diseases, including eucoleosis, thus warranting further investigations.

Nasal eucoleosis is an uncommonly diagnosed, parasitic infection and may currently be underestimated worldwide. Due to the low specificity of clinical signs and the absence of characteristic findings, the infection is neglected and often misdiagnosed and confused with other several upper respiratory distresses caused by other parasites e.g. *E. aerophilus*, or by infectious and mycotic causative agents, foreign bodies and tumours.

A support for the diagnosis can be represented by anamnestic data, e.g. living in crowded environments including kennels, shelters or breeding facilities, where the parasite may concentrate and spread due to its biology and the high resistance of the eggs in soil even in harsh conditions. Indeed, the habit of copro- and geophagic pica in dogs could represent a potential risk factor supporting a clinical suspicion of nasal eucoleosis and the recurrence of symptoms after therapy. On the other hand, the ingestion of eggs in faecal matter would require a certain period of time to allow the eggs to reach the infectious stage in the environment. Thus, from a practical standpoint, coprophagia is unlikely to have played a role in any potential re-infection in the case described herein, whereas it is more probable that the major route of infection was via geophagia.

A definitive diagnosis of nasal eucoleosis cannot be achieved without specific, diagnostic procedures, including the detection of eggs during the cytologic evaluation of nasal flushes or faecal examinations, or via direct, endoscopic observation of adult nematodes in the nasal cavities.

Detecting *E. boehmi* eggs in stool samples is easy, quick and cost-effective. Flotation using both the $ZnSO_4$ solution and sugar solution, which are the most popular in current use in veterinary clinics and hospitals, is able to concentrate and detect the eggs. Moreover, the most important key features of *E. boehmi* eggs, i.e. the porous shell, the size and plug aspects, are easy to observe with a conventional, light microscope, as demonstrated in the case described herein.

However, a classic, copromicroscopic diagnosis of canine eucoleosis may be hampered by different drawbacks. Egg shedding has been hypothesised as being cyclic, thus multiple faecal examinations might be required to diagnose infection (Schoning et al., 1993; Baan et al., 2011). Furthermore, veterinary practitioners do not generally take into account the eggs of *E. boehmi* when barrel-shaped eggs are found in faecal samples, because in most cases they are probably mistaken for the better known eggs of the

intestinal whipworm, *T. vulpis*, and the lungworm, *E. aerophilus*, which has been frequently reported in dogs over the past few years and can induce similar respiratory symptoms (Traversa et al., 2010, 2011).

The direct observation of adult nematodes in situ by rhinoscopy represents a powerful and specific diagnostic approach which is, however, both expensive and time consuming. Moreover, the rhinoscopic procedure may also have some limitations due to the sometimes inaccessible location adopted by the worms in the caudal part of the nasal cavity, within the epithelial lining of the nasal turbinates, or in the frontal and paranasal sinuses, associated with the presence of abundant mucus which impairs an accurate visualisation of the airway. The sensitivity of rhinoscopic procedures could be increased by performing contemporaneous anterograde and retrograde access to the nasal cavity, especially if during these procedures a flushing of nasal cavity is performed to collect mucus and exudates are examined for egg presence (Evinger et al., 1985; Schoning et al., 1993; Baan et al., 2011).

At present, there is no drug licensed for the treatment of *E. boehmi* infection in dogs even though, in the past, different therapeutic regimens have been attempted (Evinger et al., 1985; Conboy, 2009; Baan et al., 2011). Macrocyclic lactones have been reported to be clinically effective in the treatment of the infection, although only in single clinical cases. For instance, a single dose of ivermectin (0.2 mg/kg PO) was able to eliminate clinical signs in a dog suffering from nasal eucoleosis within 7 days and faecal shedding of the eggs within 14 days (Evinger et al., 1985). A similar success was reported with the use of milbemycin oxime (2.0 mg/kg PO) (Conboy, 2009). However, no clinical, experimental studies have been published so far to confirm the efficacy of these drugs in large case series. In this work, a single administration of moxidectin (2.5 mg/kg) was able to treat the infection, as demonstrated by clinical recovery and negative results at both faecal and rhinoscopic examinations carried out 4 weeks after the treatment. The clinical signs recurred 6 weeks after the first control, probably as a consequence of a re-infection, thus a new treatment was required. A second treatment with Moxidectin, associated with pica prevention and faeces removal from the environment, gave long-term success, supporting the potential usefulness of such anthelmintic in the treatment of canine infection with *E. boehmi*.

In conclusion, this report and the similar course of the case reported by Baan et al. (2011), suggest that the key strategy to control *E. boehmi* is represented by a combination of direct prevention measures minimising the re-infection and a specific therapy, also observed in other trichuroid-caused infections (Traversa, 2011). However, no definitive conclusions can be drawn from single clinical cases, thus further studies on the efficacy of Moxidectin against *E. boehmi* infection in dogs are warranted.

Furthermore, veterinarians and parasitologists should be aware of the occurrence of *E. boehmi* in dogs and should include this parasite in the differential diagnosis in those animals suffering from superficial airway distress, by using specific procedures such as rhinoscopy and faecal examinations.

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