# Clinical evidence of nasal eucoleosis in dogs from central Italy

In the past few years the respiratory nematode *Eucoleus boehmi* has been increasingly reported in dogs from Europe, including Italy. Nonetheless, knowledge of the clinical importance of this parasitosis in canine practice is still poor. The present work describes 20 clinical cases of canine nasal eucoleosis (CNE) from central Italy in 2012-2013, to provide new insights into the clinical features of the infection and to promote awareness among veterinarians. Twenty dogs, scored positive for *E. boehmi* eggs at copromicroscopic examination, were selected and history details and clinical data were collected. Most of the infected animals (70%) were hunting dogs which lived in rural or suburban areas of central Italy, often in crowded conditions. The most frequently observed clinical sign was nasal discharge (16/20), followed by repeated sneezing (11/20) and cough (7/20). Other respiratory signs, e.g. epistaxis, and impaired sense of smell were also found, albeit less frequently. These results suggest that *E. boehmi* should be included in the differential diagnoses of upper respiratory tract disorders of the dog.

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## INTRODUCTION

*Eucoleus boehmi* (synonym *Capillaria boehmi*)[1] is a trichuroid nematode (Subfamily *capillarinae*) that infests the mucosa of the nasal cavities and paranasal sinuses of both domestic and wild canids (e.g., wolf, fox), causing a parasitosis known as canine nasal eucoleosis (CNE) or capillariasis.[2]

The biological life cycle and routes of transmission of this parasite are still unclear, although it is thought that *E. boehmi* has a direct life cycle similar to that of *Eucoleus aerophilus* (synonym *Capillaria aerophila*). The dog becomes infested by ingesting larvae-containing eggs, present in the ground; the eggs hatch in the intestines, releasing the larvae which migrate in the lympho-haematogenous system until they reach the mucosae of the upper airways, where they become sexually mature. After mating, the female parasites lay partially embryonated eggs which, once swallowed, are released into the external environment through the faeces, becoming infective in about 15-40 days.[3] The marked resistance of the eggs in the environment could explain the frequent infestation and re-infestation of animals, particularly in cases of crowding and of a high load of environmental faeces.[7] As hypothesized for *E. aerophilus,* earthworms could be intermediate facultative or paratenic hosts, although no studies are available providing conclusive information on this issue.[5,6]

In the last decade, an increasing number of clinical cases of CNE have been observed in the temperate regions of North America and Europe, Italy included.[2,7,8,9,10,11] Furthermore, recent epidemiological studies carried out in Italy have found an infestation rate between 1.3% and 5.6%,[12,13,14] in line with the prevalence of other parasites of the cardio-respiratory apparatus of small animals, including *E. aerophilus* and *Angiostrongylus vasorum.*[15,16]

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**CNE is a clinically underestimated disorder despite the considerable number of cases recorded in recent years; we describe 20 cases from central Italy which were selected on the basis of faecal positivity.**

Nevertheless, CNE is a neglected condition which is rarely included in the differential diagnoses of disorders of the upper airways in the dog, mainly because the knowledge about the real pathogenic role of *E. boehmi* is fragmentary.[2,6] The fact that subclinical forms prevail and the limitations of copromicroscopic diagnosis have probably contributed for a long time to the limited interest in this parasite, even though the increasing number of clinical cases, some of which are very severe,[17] justifies the need for new studies on the clinical impact of CNE.

The purpose of this study was to document 20 cases of CNE, thus contributing to the inclusion of this parasitosis among the differential diagnoses of upper respiratory tract disorders of the dog.

## MATERIALS AND METHODS

This study was performed between November 2012 and December 2013 and involved 20 dogs that had *E. boehmi* eggs in their faeces. The dogs were recruited from veterinary structures and public or private kennels in central Italy (Lazio, Umbria, Tuscany and Marche).

The cases collected for this study were based on the willingness of freelance colleagues, previously alerted, to select animals that presented with clinical pictures compatible with those described in the literature to be associated with CNE (Table 1), and for which the diagnostic work-up had not led to a certain diagnosis or to treatment that had produced a complete cure.

Faecal positivity for *E. boehmi* was determined by double sampling of faeces subjected to flotation in a sugar-based solution (specific gravity 1200) and McMaster's technique,[18] to establish egg excretion values in terms of eggs per gram of faeces (EPG). The eggs seen were identified by their typical morphometric features (dimensions 60.5-72 µm × 31-33 µm) and morphological characteristics, including the space between the wall and the embryo, asymmetry of the polar plugs and the punctuated appearance of the external surface of the wall.[19] The identity of the parasite egg was subsequently confirmed by a polymerase chain reaction based on amplification of a section of mitochondrial DNA encoding the *cox*1 gene of the Subfamily *Capillarinae*.[20]

Once the parasitic status had been determined, a detailed history form was completed, recording data concerning each animal. The following information was specifically collected: provenance, habitat and habits, travels, contact with wild animals, pica and geophagia, and antihelminthic treatments in the preceding 8 weeks. The animal's clinical signs were then assessed, including those of a general nature (e.g., poor overall condition, reluctance to work, weight loss), in order to correlate their frequencies with the parasitic status.

The dogs were then treated with a single dose of a spot-on antiparasitic product containing imidacloprid 10%/moxidectin 2.5% (Advocate® spot on, Bayer HealthCare, Milan, Italy).[4] Four weeks after treatment a second copromicroscopic examination was performed and, in the case of positivity, the animals were treated with Advocate® again.

**Table 1 - Main clinical signs seen in CNE according to literature reports**

| Signs present in CNE |
|---|
| Sero-mucosal/muco-purulent nasal discharge[4,10,11,26] |
| Repeated sneezing[4,11,26] |
| Reverse sneezing[4,11,24] |
| Epistaxis (single or recurrent episodes)[4,11] |
| Impaired sense of smell[2,4,10,11,27] |
| Muzzle scratching[4] |

## RESULTS

The 20 dogs with *E. boehmi* in their faeces had a mean excretory load of 581.1 EPG (range, 225 to 1525 EPG) (Figure 1). Ten animals also had *E. aerophilus* eggs in their faeces. Of the 20 dogs infested by *E. boehmi*, 13 were family dogs, whereas seven lived in two shelters in Lazio (n=5) and Umbria (n=2). The animals, of both


**Figure 1 -** Optical microscopy: eggs of *Eucoleus boehmi*. Magnification 20X/Scale bar: 30 µm.

**Tabella 2 - Summary of signalment data and history of the 20 dogs with nasal eucoleosis**

| Dog N. | Signalment | | | | Region | Type of dog | Aptitude | Habitat | Cohabitation with other dogs | Pica or geophagia | Travel with MLs | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Age* | *Sex* | *Breed* | *Weight* | | | | | | | | |
| 1 | 4 y | M | Mongrel | 25 kg | Tuscany | Owned | Guard dog | Rural | No | Yes | No | No |
| 2 | 3 y | F | Lagotto | 20 kg | Umbria | Owned | Hunting dog | Suburban | No | No | No | No |
| 3 | 2.5 y | F | Lagotto | 14 kg | Marche | Dog shelter | Hunting dog | Urban | Yes | No | No | No |
| 4 | 4 y | M | Mongrel | 15 kg | Marche | Dog shelter | Hunting dog | Urban | Yes | No | No | No |
| 5 | 3 y | M | Corso | 45 kg | Lazio | Dog shelter | Hunting dog | Suburban | Yes | Yes | No | No |
| 6 | 6 y | M | Mongrel | 25 kg | Marche | Owned | Hunting dog | Rural | No | No | No | No |
| 7 | 6.5 y | M | Breton | 17 kg | Marche | Owned | Hunting dog | Suburban | No | Yes | No | No |
| 8 | >10 y | F | Mongrel | 25 kg | Lazio | Dog shelter | Hunting dog | Suburban | Yes | No | No | No |
| 9 | 5 y | M | Rottweiler | 30 kg | Lazio | Dog shelter | Hunting dog | Suburban | Yes | Yes | No | No |
| 10 | >10 y | M | Mongrel | 35 kg | Lazio | Dog shelter | Hunting dog | Suburban | Yes | No | No | No |
| 11 | 2.5 y | F | Mongrel | 15 kg | Marche | Owned | Companion | Suburban | No | Yes | No | No |
| 12 | 1.5 y | M | Setter | 20 kg | Toscana | Owned | Hunting dog | Rural | No | No | No | No |
| 13 | 6 y | M | Mongrel | 22 kg | Marche | Owned | Companion | Suburban | No | Yes | No | No |
| 14 | 10 y | F | Mongrel | 15 kg | Lazio | Dog shelter | Companion | Suburban | Yes | No | No | No |
| 15 | 5 y | M | Mongrel | 28 kg | Lazio | Owned | Companion | Rural | No | No | No | No |
| 16 | 6 y | M | Setter | 18 kg | Lazio | Owned | Hunting dog | Suburban | Yes | No | No | No |
| 17 | 3 y | F | Setter | 20 kg | Lazio | Owned | Hunting dog | Suburban | Yes | No | No | No |
| 18 | 7 y | M | Setter | 20 kg | Lazio | Owned | Hunting dog | Suburban | Yes | No | No | No |
| 19 | 6 y | F | Setter | 17 kg | Lazio | Owned | Hunting dog | Suburban | Yes | No | No | No |
| 20 | 8 y | F | Pointer | 25 kg | Umbria | Owned | Hunting dog | Rural | Yes | No | No | No |

genders (8 females, 12 males), were mostly hunting breeds (n=14, 70%; 95% confidence interval (CI) 38.5-81.5%) and had a mean age of 5.5 years. The animals all lived outside in rural or suburban environments. The owners of six of the dogs (30%, 95% CI 9.9-50.1%) reported that the animals showed a tendency to pica or geophagia. None of the dogs included in the study had received anti-helminthic treatment in the 2 months preceding the investigation (Table 2).

The signs observed in the 20 dogs infested by *E. boehmi* are reported in Table 3. The most frequent clinical signs were bilateral nasal discharge (n=16), of a sero-mucosal (n=5) or muco-purulent (n=11) nature, repeated sneezing (n=11), and cough (n=7). Reverse sneezing (n=4), epistaxis (n=4), and impaired sense of smell (n=5) were also noted. No general clinical signs were present, except for weight loss, which was seen in four dogs.

In three animals (15%, 95% CI 1.5-30%), the nasal discharge was the only clinical sign detected, just as cough was the single sign in another animal (5%, 95% CI 0-17.5%). In the other animals, the clinical examination revealed a combination of clinical signs (from a minimum of 2 to a maximum of 5). Table 4 presents the different combinations of respiratory signs and their relative frequencies. Among the signs detected, a muco-

**After taking a careful history and examining the dogs, the animals were treated with a spot-on formulation containing moxidectin.**

**Table 3 - Clinical signs and their frequencies in dogs with nasal eucoleosis**

| Clinical signs | Dogs (n=20) | Frequency % (95% CI) |
|---|---|---|
| Bilateral nasal discharge | 16 | 80% (62.4-97.5%) |
| • sero-mucosal | 5 | 25% (6-44%) |
| • muco-purulent | 11 | 55% (33.2.-76.8%) |
| Repeated sneezing | 11 | 55% (33.2-76.8%) |
| Harsh cough | 7 | 35% (14-56%) |
| Altered breath sounds | 5 | 25% (6-44%) |
| Epistaxis | 4 | 20% (2.5-37.5%) |
| Impaired sense of smell | 5 | 25% (6-44%) |
| Reverse sneezing | 4 | 20% (2.5-37.5%) |
| Stertorous breathing | 3 | 15% (0-30.6%) |
| Tachypnoea | 2 | 10% (0-23.15%) |
| Muzzle scratching | 1 | 5% (0-17.5%) |
| Weight loss | 4 | 20% (2.5-37.5%) |
| Reluctance to work | 4 | 20% (2.5-37.5%) |

Table 4 - Different combinations of clinical signs and their relative frequencies in dogs with nasal eucoleosis

| Combination of clinical signs | Dogs (n=20) | Frequency % (95% CI) |
|---|---|---|
| Repeated sneezing, reverse sneezing, sero-mucosal nasal discharge, cough, tachypnoea | 2 | 10% (0-23.1%) |
| Repeated sneezing, muco-purulent nasal discharge | 3 | 15% (0-30.6%) |
| Repeated sneezing, muco-purulent nasal discharge, epistaxis, impaired sense of smell | 1 | 5% (0-14.5%) |
| Repeated sneezing, epistaxis, impaired sense of smell, cough, enhanced vesicular murmur | 1 | 5% (0-14.5%) |
| Repeated sneezing, muco-purulent nasal discharge, impaired sense of smell | 1 | 5% (0-14.5%) |
| Epistaxis, sero-mucosal nasal discharge | 1 | 5% (0-14.5%) |
| Muco-purulent nasal discharge, cough, enhanced vesicular murmur, weight loss | 2 | 10% (0-23%) |
| Repeated sneezing, scratching of nasal region, weight loss | 1 | 5% (0-14.5%) |
| Repeated sneezing, epistaxis, impaired sense of smell | 1 | 5% (0-14.5%) |
| Muco-purulent nasal discharge, cough, enhanced vesicular murmur | 1 | 5% (0-14.5%) |
| Sero-mucosal nasal discharge, cough, enhanced vesicular murmur, impaired sense of smell, weight loss | 1 | 5% (0-14.5%) |
| Cough | 2 | 10% (0-23%) |
| Nasal discharge | 3 | 15% (0-30.6%) |

purulent nasal discharge and repeated sneezing were those most frequently combined in CNE, being present together in five animals (25%; 95% CI 9.9-50.1%). Following treatment with Advocate®, 18 dogs were cured, as confirmed by complete faecal negativity for *E. boehmi* eggs.

In two animals which continued to have signs, albeit milder, the faecal examination confirmed the persistence of the infestation and these dogs were, therefore, given a second course of treatment. When tested again 28 days after this second treatment, the dogs had achieved faecal negativity together with complete remission of their symptoms.

## DISCUSSION

In the last decade there has been a worldwide increase in extra-intestinal nematode infestations of dogs,[21] whereas the literature reports of canine infestations by *E. boehmi* are rare.[10,11,13,17,19,20] For a long time this parasite was confused with another nematode with a closely related morpho-biological profile, *E. aerophilus*, which localises in the tracheo-bronchial tract of both wild and domestic carnivores, including the dog,[22,23,24,25] thus contributing to epidemiological and clinical underestimates of *E. boehmi* infections. The lack of specificity of the clinical presentation and the frequent paucity or complete absence of signs have together contributed to the underestimation of the presence and pathogenic role of the parasite.

Recently, however, there has been a fair number of reports on CNE,[10,11,20,26,27] associating the parasitosis with a variety of respiratory signs, of which the most common were bilateral sero-mucosal or muco-purulent nasal discharge, repeated sneezing, and cough of variable degree, just as observed in this study. There is also one recent description of meningoencephalitis caused by intracranial migration of *E. boehmi* eggs in a dog with seizures and loss of the sense of smell.[17]

In 2013 the first case of clinically manifest CNE in a dog from Italy was reported;[11] subsequently, an extensive epidemiological investigation in central Italy documented a far from negligible presence of the parasite (5.6%),[14] contributing to reviving interest in its pathogenic potential and impact on clinical and professional activity. This study is the first, numerically substantial, national survey of clinically manifest cases of CNE in Italy.

As emerged from this study, many of the clinical signs that occur during CNE are non-specific in that they occur in numerous other infectious, inflammatory, neoplastic and parasitic disorders of the upper respiratory tract. For example, cough is found more frequently in

**CNE is characterized by a variety of respiratory signs including bilateral sero-mucosal/mucopurulent nasal discharge, repeated sneezing and cough; the type and frequency of the signs observed in the present series matched those reported in the literature.**

subjects co-infected with *E. aerophilus*, a parasite which, by having a predominant pathogenic effect in the trachea and bronchi, makes it difficult to define the primary role of *E. boehmi*.

In contrast, the presence of an impaired sense of smell or reverse sneezing could be an important diagnostic indicator. The change in scenting ability, already documented in past cases,[10,20] is often the clinical feature most readily noted and reported by owners during an *E. boehmi* infestation. The important repercussions that this change could have on the activity of some types of dogs (mainly working and hunting dogs) makes thorough investigation of the pathogenic mechanisms involved necessary.

This study shows that a carefully taken history can play a relevant role in guiding the diagnosis.

An animal's usual habitat is information of great interest; on the basis of the clinical cases studied it was seen that the parasite tends to concentrate and spread in crowded environments such as kennels or in group situations such as in hunting packs. This is the consequence of the marked resistance of the eggs of *E. boehmi* in the environment and the tendency of collective housing to be reservoirs for the ground-borne spread of helminths because of the large amount of faeces in the environment.

Another diagnostic indicator that emerged from the history was the high number of subjects with CNE that lived or commonly frequented suburban or rural environments (18/20) and hunted (14/20). Both these characteristics, by facilitating interactions with wild habitats and synanthropic species such as foxes, which are parasite reservoirs, concur to increase the risk of infection.[28]

Six animals were also reported to practise geophagia or pica, which are additional, potential vehicles of transmission and direct spread of the parasite.

Although there is still no exact diagnostic algorithm for CNE, copromicroscopic examination is currently the fastest and most economic method of making the diagnosis; the flotation technique, performed using common flotation solutions, enables the eggs present in the faecal samples to be concentrated and then identified. The traditional coprological techniques have limited accuracy because of the intermittent excretion of eggs, but this problem is easily overcome by analysis of serial samples, such as those collected and analysed in this study.[4,7] The morphology of *E. boehmi* eggs, with their typical "barrel" shape, is similar to that of other much better known nematodes, including *Trichuris vulpis* and *E. aerophilus*. The risk of mistaken identification or missed detection increases when taking into consideration the frequent occurrence of mixed infestations,[19] a phenomenon documented in the current series.

A considerable improvement in diagnostic efficiency can be obtained by combining traditional faecal examination with polymerase chain reaction protocols able to discriminate *E. boehmi* from closely related species such as *E. aerophilus*. These molecular biology protocols are based on selective amplification of fragments of target genes such as *cox1* mitochondrial DNA which have pronounced interspecies sequence differences.[20] Given the high specificity of these protocols, in this study the parasitic status of the animals under investigation was determined mainly by faecal examination, and then confirmed by molecular biology studies, as suggested by recent publications on the subject.[4,20]

The use of rhinoscopy, an instrumental examination most frequently employed in endonasal disorders, has so far been described in a very small number of cases of CNE and there is absolutely no standardisation of the technique.[4,10,11]

Endoscopy is a very specific investigation, able to reveal infestation by *E. boehmi* through direct visualisation of the parasite *in situ*. However, in cases in which the adult parasites are located in the most caudal part of the nasal cavities, the complexity of the nasal structures, together with the presence of abundant mucus, could hamper good visualisation of the parasites, thus drastically decreasing the sensitivity of the investigation.

Endoscopic biopsy with histopathological examination, although described in most reports on the instrumental diagnosis of CNE,[10,11] is not able to make a decisive diagnostic contribution. The inflammatory reaction most often described in connection with cases of CNE is, in fact, characterized by a massive infiltrate of mainly lymphocytes and plasma cells, an expression of a probable activation of the local immune system following persistent antigenic stimulation, associated with a reduction of eosinophilic granulocytes;[10,11,24] by itself, this picture does little to indicate the true parasitic status of an animal in the absence of direct visualisation and sampling of the parasite *in situ*, which is an inconstant finding.[4]

As far as concerns therapy, there are currently no drugs registered for the treatment of CNE and the only strategies described are based on the off-label use of benzimidazoles (fenbendazole) and macrocyclic lactones (ivermectin, milbemycin, moxidectin).[2,11,12,24,25,26]

**In the absence of identifying signs, information from the animal's history, for example, provenance from a crowded environment such a dog shelter or a hunting pack, can play a significant role in the diagnostic work-up.**

Interesting future treatments are building on recent experiences with moxidectin; a pilot study of 16 animals with CNE showed that a single dose of a spot-on formulation containing 2.5 mg/kg of moxidectin was able to clear the faeces of eggs in 99.56% of cases and lead to complete resolution of the clinical signs.[4] The same compound was also shown to be highly effective (99.79% reduction of egg excretion) in the treatment of cats naturally infected with *E. aerophilus*,[29] suggesting that it could be useful in both nasal and pulmonary capillariasis, parasitic conditions that are often found concomitantly in dogs,[14,19] as indeed found in our series.
In this study, the moxidectin-based therapy (Advocate®) was safe and effective in treating the cases of CNE, leading to remission of symptoms together with faecal negativisation. The excellent therapeutic response encourages further "Good Clinical Practice" studies, with the aim of registering the drug, and also suggests that it would be interesting to evaluate the efficacy of the product not only for therapeutic purposes, but also in a prophylactic role, which would be extremely useful given the frequent occurrence of re-infestation.[10,11]

**The good therapeutic response to moxidectin, in terms of both faecal negativisation and resolution of clinical signs, support the use of this compound in the treatment of CNE.**

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### KEY POINTS

- Canine nasal eucoleosis is a neglected disease and is rarely included among the differential diagnoses of upper airway disorders in the dog, despite the increasing number of cases seen in Europe, including Italy.
- In the two years, 2013 and 2014, with the help of freelance veterinarians working in central Italy, 20 cases of clinically manifest canine nasal eucoleosis were collected.
- The cases of nasal eucoleosis were diagnosed by serial copromicroscopy and confirmed by molecular biology studies.
- The most frequently found clinical signs were nasal discharge, repeated sneezing, reverse sneezing and impaired sense of smell, often present together in various combinations.
- A fair number of dogs with nasal eucoleosis live in group circumstances, such as hunting packs or kennels, in which the high load of environmental faeces promotes the epidemiological cycle of geohelminths.
- The animals were successfully treated with a moxidectin-based spot-on formulation, supporting the use of this compound in the management of canine nasal eucoleosis.
- This study suggests that E. boehmi should be included among the differential diagnoses of disorders of the upper respiratory tract in dogs.

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