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A pilot trial evaluating the efficacy of a 10% imidacloprid/2.5% moxidectin spot-on formulation in the treatment of natural nasal capillariosis in dogs

Fabrizia Veronesi[a], Giulia Morganti[a], Angela Di Cesare[b], Roland Schaper[c], Donato Traversa[b,*]

[a] Department of Biopathology and Hygiene in Animal and Food Production, Parasitology Section, Faculty of Veterinary Medicine, University of Perugia, Italy
[b] Faculty of Veterinary Medicine, University of Teramo, Italy
[c] Bayer Animal Health GmbH, Leverkusen, Germany

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ABSTRACT

The efficacy and safety of a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) were evaluated in a pilot trial for the treatment of canine nasal capillariosis caused by *Capillaria boehmi* (syn. *Eucoleus boehmi*). Sixteen dogs copromicroscopically positive for *C. boehmi* eggs were confirmed, either by rhinoscopy or species-specific PCR-coupled sequencing assays, as being affected by nasal capillariosis. The animals were randomly allocated to two different study groups, i.e. one (Group T) treated with Advocate® and one (Group C) left untreated, in a ratio of 1:1. The animals underwent clinical examination and quantitative copromicroscopy for *C. boehmi* eggs on Days -6 and -2 (baseline) and Day 28 ± 2 (post-baseline). Animals in Group T received Advocate® on Day 0. On Day 28 ± 2 the efficacy of the treatment (Group T) or the persistence of the infection (Group C) was confirmed by rhinoscopy or, alternatively, by molecular procedures. Seven of the eight dogs in Group T were negative on Day 28 ± 2 (reduction of baseline faecal egg counts by 99.14%), while for one dog a second treatment on Day 28 ± 2 was necessary to clear the infection, as demonstrated on Day 56 ± 2 (reduction of baseline faecal egg counts by 100% in Group T). Seven animals in Group C received a rescue dose of Advocate® on Day 28 ± 2 and scored microscopically and molecularly negative for the parasite on Day 56 ± 2, thus increasing the reduction of post-baseline egg counts to 99.57% after a single administration. These promising results show that Advocate® spot-on is an effective formulation for the treatment of canine nasal capillariosis under field conditions.

1. Introduction

Capillaria boehmi (syn. *Eucoleus boehmi*) is a capillarid nematode inhabiting the nasal turbinates and the frontal and paranasal sinuses of wild (e.g. foxes and wolves) and domestic canids. Since the 1980s, when cases of "nasal capillariosis" due to the closely related nematode *Capillaria aerophila* (syn. *Eucoleus aerophilus*) were published (Evinger et al., 1985; King et al., 1990), this parasite has been repeatedly described in the temperate regions of

* Corresponding author. Tel.: +39 861 266870; fax: +39 861 266873.
E-mail address: dtraversa@unite.it (D. Traversa).

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North America and Europe (Campbell and Little, 1991; Schoning et al., 1993; Sréter et al., 2003; Gajewska et al., 2004; Baan et al., 2011). Nonetheless, knowledge of the features of the disease, i.e. biological cycle, routes of transmission, epidemiology, clinical impact, remains scanty (Campbell and Little, 1991; Conboy, 2009). It is thought that animals acquire the infection by ingesting larvated eggs from the environment, and then larval stages migrate to the nasal cavities where they reach adulthood (Conboy, 2009). It has also been hypothesized, although never demonstrated, that larval *C. boehmi* might develop in earthworms acting as facultative intermediate or paratenic hosts (Campbell and Little, 1991), as has also been speculated for *C. aerophila* (Conboy, 2009; Traversa et al., 2010).

The infection caused by *C. boehmi* in dogs is either subclinical or clinically manifest when the damage in the epithelium of the nasal turbinates and sinuses induces rhinitis characterized by symptoms of varying severity, i.e. sneezing, reverse sneezing, nasal discharge and impairment of scenting ability (i.e. hypo- or anosmia) (Evinger et al., 1985; Campbell and Little, 1991; Piperisova et al., 2010; Baan et al., 2011; Veronesi et al., 2013). Furthermore, *C. boehmi* has recently been recognised as a potential cause of intracranial disease and meningoencephalitis in dogs as a result of aberrant migration in the cranial cavity (Clark et al., 2013).

Although *C. boehmi* is rarely detected in dogs, recent reports have suggested the spread of symptomatic infections in both the Americas and Europe (Piperisova et al., 2010; Baan et al., 2011; Di Cesare et al., 2012a; Magi et al., 2012; Clark et al., 2013; Veronesi et al., 2013). It is thus possible that *C. boehmi* is another non-intestinal nematode of dogs which is potentially emerging in several areas, as recently indicated for other respiratory parasites affecting dogs and/or cats (Traversa et al., 2010).

There is significant merit in evaluating effective therapeutic options for this neglected disease, in that no drug has been approved for the treatment of *C. boehmi* infection. The little information available is related to a few single clinical cases or small case series, most of which have evaluated macrocyclic lactones (MLs) with promising results (Evinger et al., 1985; Conboy, 2009; Veronesi et al., 2013; Conboy et al., 2013). In particular, moxidectin was recently shown to be effective in a single dog infected by *C. boehmi* (Veronesi et al., 2013) and in cats infected with the closely related *C. aerophila* (Traversa et al., 2012).

The pilot trial described here evaluated the efficacy and safety of a spot-on formulation containing 10% imidacloprid/2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) in the field treatment of canine nasal capillariosis.

2. Materials and methods

2.1. Pre-inclusion screening

The study was carried out from November, 2012 to June, 2013 in Italy following pre-inclusion screening of 287 dogs. The majority of the animals were kept in public or private kennels located in Central Italy and in particular in the municipalities of Latina and Rome (Latium region), Perugia (Umbria Region), Cesena (Emilia Romagna region) and Chiusi (Tuscany region), selected on the basis of previous history of suspected or diagnosed cases of nasal capillariosis. The others were dogs referred to veterinary hospitals for disorders of the upper respiratory tract or privately owned dogs whose faeces were examined by routine copromicroscopy at the Parasitological Unit of the Faculty of Veterinary Medicine in Perugia.

A faecal sample from each dog was collected and examined for *C. boehmi* eggs using a qualitative copromicroscopic concentration-flotation procedure with a sugar solution with 1.200 specific gravity (s.g.) (Sloss et al., 1994). The eggs of *C. boehmi* were identified on the basis of the following morphological and morphometric features: size $55.30 \pm 1.30 \times 32.40 \pm 2.60$ μm, a typical space between the embryo and the wall, asymmetry of the non-ringed plugs and the appearance of the egg shell characterized by several tiny pits (Di Cesare et al., 2012a).

All dogs which scored positive for eggs of *C. boehmi* (Fig. 1) at this qualitative copromicroscopical screening were submitted to confirmatory rhinoscopy to demonstrate the presence of the parasite *in situ* and/or to nasal flushing. If the owners did not consent the rhinoscopic procedure, a confirmatory species-specific PCR-coupled sequencing assay was used on the faecal samples. Briefly, the genomic DNA was extracted from each faecal sample and then subjected to a PCR assay specific for the mitochondrial *cox*1 gene Capillariinae Subfamily as described previously (Di Cesare et al., 2012b). Additionally, DNA extracted from three adult specimens of *C. boehmi* microscopically identified at the species level was subjected

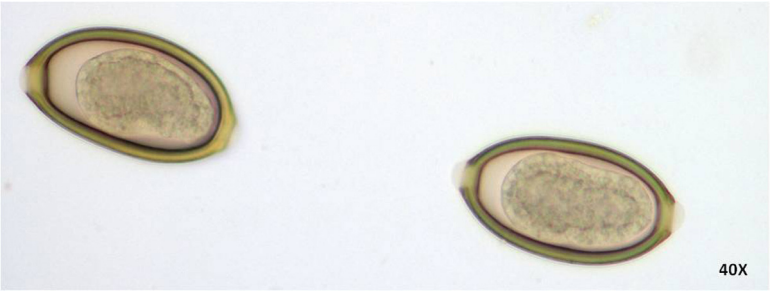

Fig. 1. Copromicroscopic examination: eggs of *Capillaria boehmi* showing the typical asymmetry of bipolar plugs and a small, clear to golden space between the embryo and the egg shell. Original magnification 40×. Scale bar: 20 μm.