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Efficacy in cats of a novel topical combination of fipronil, (S)-methoprene, eprinomectin, praziquantel, against induced infestations of *Echinococcus multilocularis*

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ABSTRACT

Although foxes are the main reservoir of *Echinococcus multilocularis*, it is recognized that dogs and cats also may become infected. In cats the infection and egg production rates are usually low. Nevertheless, cats are a potential source of transmission of *E. multilocularis*. Due to the high human medical significance of *E. multilocularis* infection, it is important in endemic areas that owned cats are dewormed regularly. This paper presents the efficacy results of a new topical formulation, Broadline® (Merial) tested against *E. multilocularis* infection in cats. Two blinded laboratory studies were conducted to evaluate this novel topical combination of fipronil, (S)-methoprene, eprinomectin, and praziquantel against *E. multilocularis*. In each study, purpose-bred cats were assigned randomly to two treatment groups of 10 cats each: one untreated control group and one group treated at the minimum therapeutic dose of 0.12 mL/kg bodyweight to deliver 10 mg fipronil, 12 mg (S)-methoprene, 0.5 mg eprinomectin and 10 mg praziquantel/kg bodyweight. The cats were inoculated orally with *E. multilocularis* protoscolices, 22 or 23 days before treatment. Based on necropsy and intestinal worm count, 8 or 11 days after treatment, the two studies confirmed 100% efficacy of Broadline® against adult *E. multilocularis*.

1. Introduction

Echinococcus multilocularis is distributed widely in the Northern Hemisphere and is endemic in central Europe and in various regions of North America and Asia (Gottstein et al., 1996; Romig, 2003; Romig et al., 2005; Davidson et al., 2012). The definitive host of *E. multilocularis* is the fox (red fox and arctic fox), but other carnivores, such as wild canids (e.g. wolves, coyotes), wild felids, raccoons, or domestic dogs and cats, may serve as hosts. Intermediate hosts are small rodents, mainly *Microtidae* (e.g. voles, muskrats, lemmings, hamsters, gerbils and related species) (Eckert and Deplazes, 2004; Burlet et al., 2011). The prevalence of the parasite is considered to be increasing and it is spreading to new countries through the movement of wildlife. The parasite also has spread from rural to urban areas, because of the increase in urban fox populations (Romig, 2003; Deplazes et al., 2004; Jenkins et al., 2005; Fischer et al., 2005; Deplazes, 2006; Takumi et al., 2008; Dakkak, 2010; Siko et al., 2011; Davidson et al., 2012; Takumi et al., 2012). Human exposure is becoming more common in endemic area and may be exacerbated by contact with infested domesticated carnivores (Dyachenko et al., 2008). *E. multilocularis* has a high human medical significance by causing

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alveolar echinococcosis, which can be fatal (Vuitton et al., 2003; Deplazes and Eckert, 2001).

Transmission of *E. multilocularis* usually occurs via a sylvatic cycle, with the possibility of infection of domestic dogs and cats via infected small mammals. The definitive host harbours the adult cestode, and the infestation typically is asymptomatic. Eggs are passed in the faeces of the definitive host and contaminate the environment. Eggs have a patency of 2–4 months and may remain infective in the environment for many months or years, in cool and damp conditions. Eggs are ingested by an intermediate host and develop into alveolar metacestode cysts. Metacestode cysts contain numerous vesicles, each containing high numbers of protoscolices, the infective form. In the intermediate host, the metacestode larva is characterized by an exogenous tumour-like tissue proliferation, which progressively infiltrates the infected organ and may lead to severe disease and death. Intermediate hosts are preyed upon by carnivores. When ingested by the definitive host, protoscolices evolve into adult *E. multilocularis* in the small intestine, and egg production can begin as early as 25 days after infestation. Eggs also may be ingested by aberrant hosts, such as pigs, horses, monkeys and humans. Such ingestion may cause alveolar echinococcosis, one of the most lethal helminthic infection in humans (Deplazes and Eckert, 2001; Bowman et al., 2003; Eckert and Deplazes, 2004; Davidson et al., 2012).

In comparison to dogs and wild canids, cats shed fewer *E. multilocularis* eggs, for a shorter duration. Therefore, cats appear to have a lesser role in the maintenance of *E. multilocularis* in endemic area, and infestations in cats may be of more limited public health significance (Thompson et al., 2003, 2006; Kapel et al., 2006; Learmount et al., 2012). Nevertheless, cats are regarded as a potential source of contamination for intermediate hosts including humans (Thompson et al., 2003; Dyachenko et al., 2008) and should be treated on a regular basis when they have regular outdoor access in endemic areas. Praziquantel, administered by topical and oral routes, has long been established as an efficient treatment against *E. multilocularis* infestations in cats and dogs (Jenkins and Romig, 2000; Charles et al., 2005; Schroeder et al., 2009; Knaus et al., 2014; Rehbein et al., 2014). Merial has developed Broadline®,[1] a topical endo- and ectoparasiticide drug for cats combining fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v), and praziquantel 8.3% (w/v). The present investigations were carried out to evaluate the efficacy of a single topical treatment with that novel formulation against *E. multilocularis* infestations in cats.

2. Materials and methods

The two study designs used were in accordance with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products VICH GL7, "Efficacy of Anthelmintics: General Requirements" (Vercruysse et al., 2001) and VICH GL20 "Efficacy of Anthelmintics: Specific Recommendations for Felines" (Vercruysse et al., 2002), and the "World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats" (Jacobs et al., 1994); and the studies were conducted in compliance to VICH GL9 entitled *Good Clinical Practice*. Animals were managed similarly and with due regard for their well-being in compliance with the local Ethics Committee approvals and other local applicable regulations and requirements.

Table 1
Characteristics of experimental animals.

Study	Sex[a]	Age[b] (months)	Pre-treatment[c] body weight (kg)
Study 1	10M, 10 F	7	2.4–5.8
Study 2	10M, 10 F	7–11	2.3–6.1

[a] M, male; F, female.
[b] Age on the day of treatment (=Day 0).
[c] Day −2, prior to treatment.

The studies described here were blinded, laboratory-based, single centre, clinical efficacy studies, with an untreated control group and used a randomized block design based on bodyweight. Healthy laboratory purpose-bred European Short Hair cats were included (see Table 1). None of the cats had been treated with an endoparasiticide within 3 months of acclimatization start.

In the two studies, cats were housed individually in a controlled environment with an air conditioning system. They were identified individually with microchips. They were observed at least once daily for general health during the entire in-life phase and had a veterinary examination for suitability before *E. multilocularis* inoculation. The cats were also observed hourly four times after application of the treatment.

After acclimatization of at least 8 days to the study environment and conditions, cats were sedated and orally inoculated with protoscolices. Cats were fasted overnight before the inoculation. A viability assessment, based on motility of protoscolices seen under the microscope, was performed shortly before the inoculation. For each inoculation, the container of *E. multilocularis* was shaken lightly to ensure uniform suspension and the volume loaded in a disposable syringe. The syringe was connected to an appropriate-sized flexible tube, the tube was inserted into the oesophagus, and the inoculum expelled. The tube was flushed with ~2 mL of saline before removal. Each cat was inoculated with approximately 30,000 (Study 1) and 38,000 protoscolices (Study 2) at Day −23 (Study 1) or Day −22 (Study 2). Protoscolices of *E. multilocularis* for both studies originated from common voles infected with recent field isolates (<10 years) that were obtained from red foxes from central Europe (Germany).

Cats were ranked by body weights and then assigned to 10 blocks of 2 cats each. Within blocks cats were allocated randomly to the untreated control group or the treated group. All cats were weighed on study Day −2 for treatment dose calculation. Treatments were applied on study Day 0. The test product was applied at the minimum therapeutic dose of 0.12 mL/kg body weight directly on the skin in the midline of the neck between

[1] Broadline® is a trademark of Merial; all other marks are the property of their respective owners.