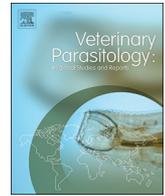




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Short communication

Efficacy of single topical treatment of Selamectin (Revolution®) against *Ancylostoma ceylanicum* in experimentally infected cats

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ABSTRACT

Ancylostoma ceylanicum, a species of hookworm, is a common parasite of dogs and cats in the Asia-Pacific region. The objective of this study was to determine the efficacy of a single topical treatment of selamectin (Revolution®) against *A. ceylanicum* in experimentally infected cats. 12 kittens were injected with 300 infective stage larvae (L3) of *A. ceylanicum* by the subcutaneous route. Faecal samples were examined daily (days 7–15) for the presence of hookworm eggs. On day 18 kittens were stratified by faecal egg count and randomly allocated in equal numbers to control (n = 6) and treatment (n = 6) groups. Faecal egg counts were then performed daily (days 14–27) or every 3 days (days 28–51) until the end of the study and compared between the treated and control groups. Kittens in the treatment group were administered a single topical dose of selamectin (Revolution®), per label recommendations (6 mg/kg), on day 19. Kittens in the control group were not treated. At 4 days post-treatment, no hookworm eggs were detected in the treated group. Faecal samples from this group remained negative throughout the study, the treatment efficacy was 100% egg reduction (P < .0001). Average faecal egg counts remained high (558 ± 231 eggs per gram) in the untreated control group until the end of the study period. In conclusion, a single topical treatment of selamectin (Revolution®) at the recommended dose was highly efficacious against infection with *A. ceylanicum* in cats.

1. Introduction

Ancylostoma ceylanicum, a species of hookworm, is a common parasite of dogs and cats in the Asia-Pacific region (Margono et al., 1979; Ngui et al., 2012; Setasuban et al., 1976; Yoshida et al., 1968). Infection in cats has also been reported in other areas including South America (Rep and Heinemann, 1976), the United Arab Emirates (Schuster et al., 2009) and South Africa (Baker et al., 1989). In the Asia-Pacific, *A. ceylanicum* is also a zoonotic concern, as it is considered the second most prevalent hookworm in humans, after *Necator americanus* (Inpankaew et al., 2014; Bradbury et al., 2017).

A. ceylanicum, similar to other hookworms, has a direct life cycle and resides in the small intestines of dogs, cats and humans (Traub et al., 2008). Third-stage larvae (L3) can infect through either the oral or percutaneous route from the environment. Paratenic hosts have not been found to play a major role in the transmission of *A. ceylanicum* (Ray et al., 1972), and no evidence of dormant larvae in the musculature of dogs has been observed (Yoshida et al., 1974). Description of experimental infections in cats are limited, however the pre-patent

period in cats is likely to reflect that of dogs (Taweethavonsawat et al., 2013). There are no reports regarding the clinical signs of experimental or natural infections with *A. ceylanicum* in cats, however anaemia resulting from blood loss may be a likely consequence. In dogs, the average blood loss has been reported to be 0.03–0.045 ml per worm per day (Carroll and Grove, 1984; Rep et al., 1971), which is comparable to a primary infection with *A. caninum*.

Successful treatment of patent infections with *A. ceylanicum* is essential for decreasing the risk of anaemia in the cat and zoonotic transmission to humans. Topical application of selamectin (Revolution®) was previously shown to be highly effective for treatment of gastrointestinal nematodes of cats with reported efficacies of 96.5–100% for ascarids and 98.9–99.9% for *Ancylostoma tubaeforme* (Six et al., 2000; McTier et al., 2000).

There are currently three anthelmintic formulations registered or approved for the treatment of cats infected with *A. ceylanicum*, namely a spot-on formulation containing emodepside and praziquantel (Profender®, Bayer Animal Health), praziquantel and pyrantel oral tablets (Drontal® for Cats, Bayer Animal Health) and a spot-on

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formulation containing imidacloprid 10% and moxidectin 1% (Advocate®/Advantage® Multi, Bayer Animal Health) (Taweethavonsawat et al., 2012; Taweethavonsawat et al., 2013). The objective of this study was to evaluate the efficacy of a single topical dose of selamectin (Revolution®, Zoetis) against *A. ceylanicum* in experimentally infected cats.

2. Materials and methods

2.1. Source of third stage larvae (L3) of *A. ceylanicum*

Hookworm eggs were collected from faecal samples and cultured to the L3 stage using the Harada-Mori technique (Taweethavonsawat et al., 2010). Then, to certify a pure infection, L3 were harvested after ten days and identified as *A. ceylanicum* using conventional PCR-RFLP, according to Palmer et al., 2007. Approximately 300 infective stage larvae were injected by the subcutaneous route into donor cats. Faeces of the donor cats were then collected on days 14 to 21 in order to isolate eggs that were ultimately used as the source of L3 for the experimental groups.

2.2. Study animals, clinical observations and efficacy study

The experiment was carried out as a blinded, randomised confirmation study in compliance with Chulalongkorn University Animal Care and Use Committee (Approval No. 13310044).

12 kittens were obtained from parasite-free queens. The kittens were weaned at 12 weeks of age and their faeces examined on a weekly basis for 4 weeks for gastrointestinal (GI) parasites by direct smear and zinc sulphate centrifugal floatation (ZSCF) to confirm freedom from such parasites prior to inclusion in the study at 16 weeks of age.

Each kitten was housed individually during the study period to facilitate faecal collection, was given commercial dry food and water *ad libitum*, and was vaccinated against feline panleukopenia virus and rabies virus.

On day 0 (16 weeks of age), 12 kittens were infected with 300 infective stage (L3) larvae. The number of infective stage larvae used to induce infection was based on guideline from the VICH GL20 recommendation for *A. tubaeforme* and *A. braziliense* in felines (VICH guideline: 20, 2001). The health of each kitten was assessed on a daily basis until the end of the study period by observing appetite, demeanour and faecal consistency, including presence or absence of blood. Mucous membrane colour was also recorded. After infection, faecal samples were examined daily from days 7–14 using ZSCF, until the presence of hookworm was confirmed. From day 15 until day 27, faecal eggs count in eggs per gram (epg) were performed using the McMaster technique. On day 18, kittens were stratified based on mean faecal egg count and randomly allocated in equal numbers into control and treatment groups. On day 19, the kittens were given with selamectin (Revolution®) at dose 6 mg/kg in the treatment group and kittens in the control group did not receive any anthelmintic drug. Faecal egg counts were carried out in all cats each 3 days from day 28 until the end of study (day 51).

2.3. Efficacy calculations/statistical analysis

Efficacy was calculated based on the arithmetic mean faecal egg counts from the treated and untreated control groups. Faecal egg count were summarized and compared at multiple time points using the powerful multivariate Wilcoxon–Mann–Whitney test with the simultaneous application of a one-sided Wei–Lachin procedure (Lachin, 1992) for superiority, with a significance level of $\alpha = 0.025$.

2.4. Molecular characterisation of feline hookworm larvae and DNA extraction

The presence of infective-stage larvae was detected by using light microscopy. The infective-stage larvae had filariform esophagous. Samples were cleaned with $1 \times$ PBS for three times. Then, DNA extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to manufacturer's instructions.

The PCR-RFLP was performed for hookworm species identification according to Taweethavonsawat et al. (2012). DNA sourced from adult worms confirmed as *A. braziliense*, *A. caninum* (Thailand), *A. ceylanicum* (Thailand), *A. tubaeforme* and *U. stenocephala* was used as the positive control for the PCR. Molecular grade water was used as the negative control.

3. Results

All kittens were infected with *A. ceylanicum*; hookworm eggs were first observed in faeces 15 days post-infection, with all kittens shedding eggs on day 16 post-infection. Larval samples collected from the original pool of adult worms and from all hookworm-infected cats on days 19 and 51 were successfully amplified by PCR, generating a single 545 bp product, which upon enzymatic digestion produced a restriction profile consistent with a single *A. ceylanicum* infection. None of the cats showed any clinical signs of ancylostomiasis and there were no adverse side effects following application of the recommended dose of selamectin (Revolution®) spot-on formulation in the treated group on day 19.

On day 18, mean EPG in control group and treatment group were 416 ± 326 and 608 ± 443 , respectively. Four days after treatment (Day 23), no hookworm eggs were detected in the treated group but mean EPG in control group was 675 ± 354 . Faecal samples from treatment group remained negative throughout the study, resulting in a treatment efficacy (egg reduction) of 100% ($P < .0001$). In contrast, average faecal egg counts remained high (558 ± 231 eggs per gram) in the untreated control group until the end of the study period at day 51.

4. Discussion

A. ceylanicum and *A. caninum* commonly infect cats in Thailand (Setasuban et al., 1976). It remains unascertained if seasonally and physiologically determined developmental arrest and pregnancy-induced transmammmary transmission of *A. caninum* from the queen to the kittens occur in a same pattern in dogs. Although this anthelmintic efficacy experiment was conducted according to recommended guidelines, molecular diagnostic tools provided additional assurance and unequivocal proof that the study cats harboured single species infections with *A. ceylanicum* at patency and at the end of the study period in the untreated control group.

Heavy infections with *A. ceylanicum* in dogs has been demonstrated to be associated with clinical signs such as diarrhoea and anaemia in experimental infection (Carroll and Grove, 1984) and while it is likely that cats would experience similar outcomes, there appears to be no studies reporting on the clinical signs or pathology of *A. ceylanicum* in either experimentally or naturally infected cats. In this study, although complete blood count profiles were not measured, the infected cats did not show any clinical signs of anaemia or diarrhoea, however this could be attributed to a nutritious diet and a light worm burden. The pre-patent period of *A. ceylanicum* in this study is similar to previous studies by Taweethavonsawat et al. (2012, 2013).

Egg counts in the treatment group still remained high until at least 3 days post-treatment. Therefore, vigilant environmental hygiene should be sustained for up to 4 days following selamectin (Revolution®) spot-on application to minimise environmental contamination with hookworm eggs.

A. ceylanicum is reported as the second most prevalent hookworm of

humans in the Asia-Pacific, and a One Health approach is strongly advocated for its sustainable control. In *A. ceylanicum*-endemic communities, the risk of transmission of this emerging zoonosis is likely associated with multiple factors, most importantly, the presence of poorly cared-for semi-domesticated free-roaming dogs and cats (Nguui et al., 2012; Inpankaew et al., 2014).

Strategic control programs of *A. ceylanicum* in dogs and cats through the use of efficacious anthelmintics and the maintenance of environmental hygiene is necessary to decrease the zoonotic risk of this hookworm. In addition to the control of hookworms, selamectin (Revolution®) spot-on formula has the advantage of ease of application and also targets a broad range of other feline intestinal nematodes, such as *Toxocara cati* (McTier et al., 2000), lungworms (Iannino et al., 2013), *Dirofilaria immitis* and fleas (Boy et al., 2000).

In conclusion, based on faecal egg count reduction, it is suggested that a single spot on application of selamectin (Revolution®) will not only benefit the feline patient, owner and veterinarian, but also address a public health concern through the reduction of faecal hookworm egg shedding into the environment.

Declaration of Competing Interest

The authors have declared that no conflict of interests exists.

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