Efficacy of Procox® Oral Suspension for Dogs (0.1 % Emodepside and 2 % Toltrazuril) against Experimental Nematode (*Toxocara cati* and *Ancylostoma tubaeforme*) Infections in Cats

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Abstract

Two exploratory studies were performed to determine the optimum therapeutic dose of Procox® for the removal of experimental infection with mature adult *Toxocara (T.) cati* and *Ancylostoma (A.) tubaeforme* in kittens. Procox® is a new oral suspension containing a combination of the nematocidal and coccidiocidal active principles emodepside (0.1 %) and toltrazuril (2 %).

In the first study, 18 eight-weeks-old kittens were inoculated with 450 L3 larvae of *T. cati*. 56 days after infection, the kittens were allocated to three treatment groups and were treated with 0.5 mg emodepside/kg body weight (group 1), 0.25 mg emodepside/kg body weight (group 2) and 0.1 mg emodepside/kg body weight (group 3), respectively. In the second study, 10 eight-weeks-old kittens were inoculated with 350 L3 larvae of *A. tubaeforme*. Four weeks after infection, the kittens were allocated to two treatment groups and were treated

with 0.1 mg emodepside/kg body weight (group 1) or 0.25 mg emodepside/kg body weight (group 2).

In both studies, all kittens received a reference treatment with Drontal® (230 mg pyrantel embonate and 20 mg praziquantel per tablet) at the recommended dose of one tablet/4 kg body weight 5 days after treatment with Procox®. Anthelmintic efficacy was calculated by reduction in worm numbers expelled with the faeces following treatment with Procox® as compared with faecal worm numbers after reference treatment with Drontal®, by thus avoiding necropsy of the animals.

In the *T. cati* study, emodepside was at 99.9 %, 100 % and 96.5 % effective at a dosage of 0.5 mg, 0.25 mg and 0.1 mg per kg body weight, respectively. Against *A. tubaeforme* emodepside was at 95.7 % and 100 % effective at a dosage of 0.1 mg and 0.25 mg per kg body weight. No adverse events were seen during either study.

It can be concluded that Procox® is efficacious for the control of mature adult *T. cati* and *A. tubaeforme*

infections in cats at a single-dose rate of 0.25 mg emodepside/kg body weight.

Introduction

Infections with ascarids and hookworms are still the most common nematode infections in kittens and young adult cats worldwide as demonstrated in numerous current surveys (Beck and Pantchev 2008), with *T. cati* being the most common round worm in cats. Epe et al. (2004) detected *T. cati* (Zeder 1800) eggs in 3.9 % of feline faecal samples in Germany, while Barutzki and Schaper (2003) found ascarid eggs in 6.4 % of faecal samples. In Italy, Perrucci et al. (2001) found ascarid eggs in 28 % and hookworm eggs in 3 % of feline faecal samples. In Spain, Gracenea et al. (2009) found *T. cati* eggs in 22 % and *A. tubaeforme* eggs in 4 % of feline faecal samples with distinct higher prevalences in kittens.

Cats can become infected with *T. cati* by one of the three routes: (1) direct ingestion of larvated eggs; (2) ingestion of a vertebrate host that harbours *T. cati* larvae in its tissues; or (3) by lactogenic transmission, in which larvae are passed in milk from a queen to her kittens (Sprent 1956). Accordingly, most infections in kittens are probably acquired lactogenically, whereas predation is the likely source for adult cats (Dubinsky et al. 1995). Depending on the severity of infestation, clinical signs that can be seen in kittens are variable appetite, vomiting after feed, diarrhoea alternating with constipation, and developmental disturbances (Eckert 2000). Particularly in kittens, *T. cati* produce large numbers of eggs which are extremely resistant and long-lived in the environment. The kitten rather than the adult cat is thus responsible for increasing and spreading the danger of parasitism. A cat's living area which has become contaminated remains source of infection for a period of years.

Feline faeces also pose significant zoonotic threat because humans who ingest larvated *T. cati* eggs can develop visceral or ocular larva migrans

(Overgaauw 1997). Tharaldsen (1982) found *Toxocara* spp. eggs in 5 of 13 sandpits from Oslo municipal nursery schools, which indicated a high faecal contamination from dogs and cats. Although epidemiological studies carried out have concentrated on the canine *T. canis* as the main ethiological agent of the disease in man, also *T. cati* presents a high risk for man of acquiring toxocarosis (Dubinsky et al. 1995).

Prevention and immediate therapy of patent infections are therefore key strategies for minimising the risk of zoonotic transmission with the kitten being the main target.

The hookworm *A. tubaeforme* can be detected in domestic cats worldwide. Prevalence rates from Europe and the US during recent years ranged between 0–75 % with highest rates of infection in feral or stray cats (Anderson 1992; Barutzki and Schaper 2003; Labarthe et al. 2004). Cats become infected by skin penetration or ingestion of infective third-stage (L3) larvae of *A. tubaeforme* with a prepatent period between 18 and 28 days depending on the infection route (Bowman et al. 2002). The adult parasites are present in the small intestine, where they feed on host blood. While low to moderate infections cause low-grade enteritis, heavy infections can lead to severe anaemia and possibly death (Hendrix 1995; Onwuliri et al. 1981). Procox® oral suspension for dogs is a new oral suspension containing a combination of the nematocidal and coccidiocidal active principles emodepside (0.1 %) and toltrazuril (2 %). It is approved for the treatment of puppies and young dogs with suspected or confirmed mixed infections caused by roundworms (*T. canis*, *Uncinaria stenocephala*, *A. caninum*) and *Isospora* (*Isospora canis*, *Isospora ohioensis*-complex). The nematocidal activity of emodepside has been established for dogs and cats (Altreuther et al. 2005, 2009; Schimmel et al. 2009; Reinemeyer et al. 2005) and is already approved for its veterinary use in combination with praziquantel as Profender® spot-on for cats and Profender® tablets for dogs. So far, emodepside for the treatment of cats is formulated for dermal spot-on

Tab. 1 Study design of the two exploratory studies on the efficacy of Procox® oral suspension for dogs (emodepside (E)/toltrazuril (T) oral suspension) against mature adult stages of *T. cati* and *A. tubaeforme*

Study	Cat breed	Age of kittens at the day of infection	Age of kittens at the day of treatment	Body weight of cats	Number of kittens per treatment group	Nematode (Infection)	Treatment Procox® on day	Treatment Drontal® on day	Faecal worms counts
A	Domestic Shorthair	8 weeks	16 weeks	1.2–2.2 kg	6/6/6	mature <i>T. cati</i> (experimental infection with 450 L3 larvae 56 days before study start)	0	5	day 0 to 8
B	Domestic Shorthair	8 weeks	12 weeks	1.2–1.7 kg	5/5	mature <i>A. tubaeforme</i> (experimental infection with 350 L3 larvae 26 days before study start)	0	5	day 1 to 8

administration only with proven efficacy against *T. cati*, *Toxascaris leonina* and *A. tubaeforme*.

The availability of the new oral combination product for puppies and the present use of emodepside as nematocidal active principle in Profender® spot-on for cats was a justification to test Procox® under experimental conditions in kittens. The purpose of the present studies was to titrate the dosage of emodepside in the combination product Procox® against the two most common feline nematodes *T. cati* and *A. tubaeforme* in kittens.

Materials and methods

Study design

Two exploratory studies were performed as randomised, laboratory studies to evaluate the efficacy of Procox® oral suspension for dogs against experimental *T. cati* and *A. tubaeforme* infections in kittens. Both studies were conducted as dose determination studies using a test procedure described by von Samson-Himmelstjerna et al. (2000). This test procedure uses experimentally infected animals which act as their own control. It provides reliable results at an early stage of screening of new drugs. Necropsy which is usually required for

the controlled test and the critical test (Jacobs et al. 1994) can be avoided by using a reference product with proven high anthelmintic efficacy. All animal procedures were approved by the responsible authorities and the animal welfare officer. Husbandry of animals complied with the European Commission guidelines for the accommodation of animals used for experimental and other scientific purposes (June 18 2007/526/EC). The design of both studies is summarised in Tab. 1.

Study animals

In both studies, healthy purpose-bred kittens were used that had not been treated previously with anthelmintic or anticoccidial drugs. All kittens were acclimatised to the study facilities for at least seven days before the experimental infection. They were identified by ear tattoo number. Kittens were eight weeks of age at the day of experimental infection in both studies. The body weight (b.w.) ranged between 1.2 and 2.2 kg. Kittens were housed in groups during the prepatent period and were then transferred to single housing during the patent period to facilitate individual faecal sampling. Kittens were fed once daily a commercial dry cat food and had *ad libitum* access to water. General requirement for study inclusion was good health as

Tab. 2 Procox® oral suspension doses and Drontal® pyrantel embonate/praziquantel doses used against mature *T. cati* (study A) and mature *A. tubaeforme* (study B) in kittens

Study	Number of kittens per group	Emodepside (E) + Toltrazuril (T) doses (mg/kg b.w.) on day 0	Volume of Procox® (ml)	Pyrantel embonate + Praziquantel dose (mg/kg b.w.) (Drontal® tablets for cats) on day 5
A	6/6/6	E: 0.5 + T: 10.0	0.5	57.5 + 5.0
		E: 0.25 + T: 5.0	0.25	
		E : 0.1 + T: 2.0	0.1	
B	5/5	E: 0.1 + T: 2.0	0.1	57.5 + 5.0
		E: 0.25 + T: 5.0	0.25	

determined by a veterinary examination before the start of the study.

Clinical observations

The general health of all kittens was assessed once before the experimental infection, once before treatment and once daily after treatment. Kittens were evaluated for general attitude and behaviour, and faecal consistency was assessed. Following treatment, cats were also observed every hour for 6 hours after dosing for any potential adverse reactions to the test product.

Infection

In both studies, kittens were experimentally infected. In study A, the *T. cati* infection was induced by an oral administration of approximately 450 L3 larvae eight weeks before the start of the study. In study B, the *A. tubaeforme* infection was induced by an oral administration of approximately 350 L3 larvae four weeks before the start of the study. The strains had been isolated from naturally infected cats from a northern region in Germany and thereafter had been passaged in cats several times and in this way kept in culture at the study facility.

Treatment

In both studies, kittens were ranked according to their faecal egg counts from day 0 and were randomly allocated to treatment groups. Kittens were weighed prior to treatment, and body weights were

used to determine individual doses of the Procox® oral suspension to be administered once at day 0. In study A (*T. cati*), three study groups with 6 kittens each were treated at day 56 (8 weeks) post infection. In study B (*A. tubaeforme*), two study groups with 5 kittens each were treated at day 26 post infection. Applied doses of emodepside and toltrazuril are given in Tab. 2.

As a reference treatment, all kittens were treated at day 5 after Procox® treatment with the anthelmintic product Drontal® tablets for cats containing 20 mg praziquantel and 230 mg pyrantel embonate per tablet. Each kitten received the recommended dosage of 5 mg praziquantel + 57.5 mg pyrantel embonate per kg body weight as single oral application.

Parasitological techniques

A faecal egg count (FEC) using a modified McMaster method was performed for each kitten before treatment with Procox® on day 0 to determine whether a patent infection was established. Total 24-hour faeces were collected daily from each kitten for 5 days after treatment with Procox® in both the *T. cati* study (study A) and the *A. tubaeforme* study (study B) for the determination of adult worm numbers excreted with faeces. Faecal worm counts were continued for 3 days after treatment with Drontal®. Faeces were processed by soaking the material over night. The total amount of faeces was washed gently over a 75 µm sieve to recover all nematodes eliminated from the gastrointestinal

Tab. 3 Efficacy of emodepside (E) plus toltrazuril (T) oral suspension against mature adult *T. cati* (study A) and *A. tubaeforme* (study B) in kittens

Study	Treatment group (doses of E + T in mg/kg b.w.)	No. of kittens per group	Total individual worm counts day 0–5	Total individual worm counts day 0–8	Worms shed after treatment with reference product Drontal®	Efficacy (%)
A	E: 0.5 + T: 10.0	6	97/164/15/140/100/196	97/165/15/140/100/196	0/1/0/0/0/0	99.9
	E: 0.25 + T: 5.0	6	63/54/46/20/26/71	63/54/46/20/26/71	0/0/0/0/0/0	100
	E : 0.1 + T: 2.0	6	60/19/45/67/7/73	60/19/45/80/7/73	0/0/0/13/0/0	96.5
B	E: 0.1 + T: 2.0	5	19/19/10/29/56	19/19/10/37/56	0/0/0/8/0	95.7
	E: 0.25 + T: 5.0	5	11/75/88/15/38	11/75/88/15/38	0/0/0/0/0	100

tract by treatment. All adult *T. cati* or *A. tubaeforme* found were counted under the microscope.

Efficacy calculation

Efficacy of the different emodepside doses was calculated by comparing the total faecal worm counts of each kitten from day 0 to day 8 and the worm counts from day 0 to day 5 according to von Samson-Himmelstjerna et al. (2000).

The percentage reduction of worm numbers was calculated by the following formula:

$$\text{Efficacy (\%)} = \frac{\text{number of worms excreted from day 0–5}}{\text{total number of excreted worms from day 0–8}} \times 100$$

Results

The presence of a patent infection of *T. cati* and *A. tubaeforme* was demonstrated by positive egg counts in faecal samples from all kittens on day 0 before treatment with Procox®.

Study A (*T. cati*)

All three emodepside doses were highly effective against mature *T. cati* infections (Tab. 3). Full anthelmintic efficacy was obtained in study group 1 (99.9 %) and 2 (100 %) with 0.5 and 0.25 mg emodepside per kg b.w., respectively. Worm counts in group 1 and 2 were negative starting on day

3 post treatment. After reference treatment with Drontal®, no further worms were shed by any of the kittens in group 2 and one worm was shed by one kitten in group 1. In group 3, 96.5 % worm count reduction was achieved at an emodepside dose of 0.1 mg/kg b.w. with five out of six kittens shedding no further worms and one kitten shedding 13 worms after the reference treatment.

Study B (*A. tubaeforme*)

Procox® was 95.7 % effective against mature *A. tubaeforme* infections at an emodepside dose of 0.1 mg per kg b.w. in group 1. Four out of five kittens did not shed any further worm after reference treatment, whereas one kitten shed 8 worms.

In group 2, 100 % efficacy was achieved at an emodepside dose of 0.25 mg per kg b.w. (Tab. 3). At day 3 post treatment, worm counts were negative. No worms were shed after reference treatment with Drontal®.

As demonstrated in both studies by an easy and uncomplicated uptake, the Procox® oral suspension was highly palatable. In all dose groups, no adverse reactions due to Procox® treatment were observed during the post treatment interval.

Discussion

Both studies were designed as preliminary dose finding studies to determine the optimum therapeutic dose of Procox® for the removal of adult mature *T. cati* and *A. tubaeforme* in kittens. To avoid necropsy, which is usually required for critical and controlled tests in later stage of drug development, in this study a method described by von Samson-Himmelstjerna et al. (2000) was used. The test procedure uses experimentally infected animals which act as their own control and gives reliable results at an early stage of screening.

In both studies, experimental infections were induced with L3 larvae, and the presence of a patent infection of *T. cati* and *A. tubaeforme* was demonstrated by positive egg counts in faecal samples from all kittens on day 0 before treatment with the test product.

In the *T. cati* study, a single treatment with the emodepside/toltrazuril combination produced a worm reduction of 99.9 %, 100 % and 96.5 % using 0.5 mg, 0.25 mg and 0.1 mg emodepside per kg b.w., respectively. In the *A. tubaeforme* study, a single treatment with the emodepside/toltrazuril combination produced a worm reduction of 100 % and 95.7 % using 0.25 mg and 0.1 mg emodepside per kg b.w., respectively.

It can be concluded that in both experimentally induced infections, Procox® was highly efficacious in the control of mature adult *T. cati* and *A. tubaeforme*

at a single oral dose rate corresponding to 0.25 mg emodepside per kg b.w.

The Procox® application was well tolerated and no adverse events were noted at any dosages of emodepside/toltrazuril oral suspension in the studies reported here.

Oral administration of the suspension to kittens was easy because palatability was high. This supports for a high degree of owner and breeder compliance within a nematode control programme.

Within the context that certain feline nematodes still occur with high prevalences, the effective control of feline ascarids and hookworms is important because of their potential pathogenicity in both cats and humans.

The efficacy, safety and high palatability of the Procox® oral suspension offers a suitable treatment option for kittens affected by ascarid and hookworm infections.

Compliance statement

All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations.

Disclosure statement

The authors were employed by Bayer Animal Health GmbH, Germany, during the conduct of the studies. The studies were sponsored by Bayer Animal Health GmbH.

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