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# Efficacy of Emodepside plus Praziquantel Tablets (Profender® Tablets for Dogs) against Mature and Immature Infections with *Toxocara canis* and *Toxascaris leonina* in Dogs

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## Abstract

The efficacy of emodepside plus praziquantel tablets (Profender® tablets for dogs) against mature adult, immature adult and larval stages of *Toxocara canis* and *Toxascaris leonina* was evaluated in ten randomised, blinded and placebo-controlled dose confirmation studies in naturally or experimentally infected dogs. The tablets were used at the proposed minimum dose of 1 mg emodepside and 5 mg praziquantel per kg body weight. Efficacy was calculated based on worm counts after necropsy. Five studies demonstrated >99% efficacy against mature adult, >92% efficacy against immature adult, >98% efficacy against L4 and >94% efficacy against L3 larval stages of *T. canis*. Another five studies demonstrated >99% efficacy against mature and immature adult and >95% efficacy against L4 larval stages of *T. leonina*. No side effects of the treatment were observed.

Emodepside plus praziquantel tablets thus provide a comprehensive new treatment option for ascarid infections in the dog.

## Introduction

*Toxocara canis* has a complex developmental cycle that can involve migration of larval stages through various organs including the lungs, liver and kidneys before either reaching the small intestine, where development to the adult stage is completed or entering a hypobiotic stage, e.g., in muscle tissue. The different pathways and times for development depend on multiple different factors including the route of infection (oral or prenatal), infection dose, age and immune status of the dog, so that prepatency can be as short as approximately 3 weeks after a prenatal infection and up to approximately 6 weeks after an oral infection (Parsons 1987).

Recent observations confirmed that not only young dogs are prone to infection with *T. canis*, but also adult dogs may be repeatedly infected with this parasite even under regular anthelmintic treatment (Fahrion et al. 2008; Sager et al. 2006). Sager et al. (2006) observed a yearly incidence of 32% in dogs that received anthelmintic treatment four times a year.

Patent infections with *T. canis* are not only of potential concern to the dog's health, but also lead to contamination of the environment with eggs that may survive for years and may be a source for human infection. Toxocarosis is a severe zoonosis where migrating larvae can cause lesions and granulomas in various organs (*larva migrans visceralis*). If the eye is affected, infection can lead to blindness (Parsons 1987).

Much research has been conducted on the occurrence of infective *T. canis* eggs in public places, but recently it was shown that dogs themselves may represent a source of infection by carrying infective eggs in their fur (Wolfe and Wright 2003; Roddie et al. 2008). Because of its zoonotic potential the risk posed by dogs infected with *T. canis* should therefore not be underestimated.

*Toxascaris leonina* has a more direct developmental cycle than *T. canis*. It has a lower prevalence and is not perceived to play a significant role as a zoonotic agent. After oral infection with infective eggs or a paratenic host, the larvae enter the small intestine and develop to the mature adult stage in the mucosa and lumen of the gastrointestinal tract within approximately 7 to 10 weeks. However, to a small extent, a somatic migration can also occur with this parasite and infection can cause enteritis in the dog (Parsons 1987).

Emodepside plus praziquantel tablets (Profender® tablets for dogs) are indicated for dogs suffering from, or at risk from, mixed parasitic infections caused by nematodes and cestodes, i.e., mature and immature *T. canis*, *T. leonina*, *Ancylostoma caninum, Uncinaria stenocephala, Trichuris vulpis, Echinococcus granulosus*, *Echinococcus multilocularis* and mature *Dipylidium caninum* and *Taenia* spp. This oral product is the second introduction of emodepside as a novel nematocide in veterinary medicine and the efficacy has been confirmed in a series of laboratory dose confirmation studies and a multicentre field study (Altreuther et al. 2009; Schimmel et al. 2009a,b; Schroeder et al. 2009).

This paper reports the findings of five studies (no. 1–5) that were conducted to investigate the efficacy of emodepside plus praziquantel tablets against mature and immature stages of *T. canis* and five studies (no. 6–10) that were conducted to investigate the efficacy of emodepside plus praziquantel tablets against mature and immature stages of *T. leonina* in dogs.

## Materials and methods

The investigations were performed as placebo-controlled, blinded and randomised dose confirmation studies, conducted in accordance with VICH guideline 9 "Good Clinical Practice" (July 2000), and followed the recommendations given in the VICH guidelines 7 "Efficacy requirements for anthelmintics: general requirements" (December 2000) and 19 "Efficacy of anthelmintics: Specific recommendations for canines" (July 2001) as well as the WAAVP guideline for evaluating the efficacy of

anthelmintics for dogs and cats (Jacobs et al. 1994). The study design is summarised in Table 1, 2.

### Study animals

The dogs used in the studies were either purpose bred individuals from different suppliers, animals owned by the CRO or animals obtained from commercial kennels according to the respective local regulations. The dogs were identified by subcutaneously implanted microchip, ear tattoo or numbered collar tags. They were housed single or in groups, however, at least on the day of treatment and the following day the dogs were housed individually (exception study no. 4, where dogs were housed in pairs from 4 h post treatment onwards). The dogs were fed with commercial dry dog food once or twice per day and water was available *ad libitum*. All dogs were acclimatised for at least 7 days prior to the start of the study.

General requirements for study inclusion were good health and no recent anthelmintic use that could interfere with the study. Dogs included in studies that used experimental infections were required to be negative for nematodes as examined by faecal egg counts during acclimation except for study no. 3, where dogs were required to be negative for nematodes expelled in the faeces after an anthelmintic treatment with pyrantel pamoate and febantel before study start. Dogs included in studies that investigated efficacy against patent infections (no. 1, 2, 6 and 7) were required to demonstrate a positive faecal egg count for *T. canis* or *T. leonina* at least once before treatment.

### Clinical observations

In all studies, dogs were physically examined at least once during acclimation and once before treatment. Additionally, all dogs were observed for their general health once daily. Clinical assessments with the aim to detect adverse events were conducted once before treatment and approximately 0.5, 1, 2, 3, 4 and 8 hours after treatment. Special attention was paid to vomitus or regurgitation of tablet matter at the assessments conducted post treatment. The assessments were continued twice daily for two days after treatment.

### Infection

Two studies (no. 1 and 6) were conducted with naturally infected dogs from the USA and the Republic of South Africa, respectively. In the other studies, dogs were orally infected with approximately 500 embryonated eggs of *T. canis* or *T. leonina*. The origin and age of the isolates that were used are shown in Table 1, 2.

### Treatment

Dogs of both sexes were randomly assigned to either treatment or control groups. In all studies, the dogs were treated once with emodepside plus praziquantel tablets or placebo tablets. For the studies with an experimental infection, the times of treatment in relation to the infection are shown in Table 1, 2.

In all studies, the dosage of emodepside and praziquantel was 1 mg emodepside and 5 mg praziquantel per kg body weight. The doses were based on the body weights taken one or two days before treatment. For exact dosing, excess tablet substance was filed off until the tablet weight corresponded to the target weight for the individual dog.

The emodepside plus praziquantel or placebo tablets were applied orally by forced dosing over the back of the tongue. Care was taken to ensure that all animals swallowed the full amount of the treatment without loss of product. The dogs were observed after dosing to determine whether any tablet matter was regurgitated.

### Faecal examination

Faecal egg counts were conducted to monitor presence or absence of helminths using a modified McMaster or a quantitative double centrifugation method.

As additional information, worms passed after treatment until necropsy were collected from the faeces in some studies (no. 2 for *T. canis*, no. 6, 7

**Table 1** Study design of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against mature and immature stages of *Toxocara canis* (p.i.: post infection)

| Study no. | Breeds | Age of dogs | Body weight (1 or 2 days before treatment) | No. of dogs (EP group/ control group | Infection | Origin of natural infection/ isolate (age of isolate) | Treatment day | Necropsy day | Faecal worm counts post treatment[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Beagle, cross-breeds | 2.5–4 months | 2.6–9.5 kg | 6/6 | Natural | Oklahoma, USA | 0 | 7 | – |
| 2 | Beagle | 12 weeks | 4.1–7.0 kg | 7/7 | Experimental (~ 500 eggs) | Bavaria, Germany (1.5 yrs) | 0 (41/47 p.i.[b]) | 7 | yes |
| 3 | Cross-breeds | 7–9 weeks | 0.9–6.0 kg | 7<br>10 /20 | Experimental (~ 500 eggs) | Bavaria, Germany (10 mths–2 yrs) | 21 p.i.<br>5 p.i. | 28 p.i. | – |
| 4 | Beagle | 10–12 weeks | 4.3–7.4 kg | 8/8 | Experimental (~ 500 eggs) | Lower Saxony, Germany (7 yrs) | 21 p.i. | 26 p.i. | – |
| 5 | Beagle | 11–12 weeks | 2.9–5.1 kg | 8/8 | Experimental (~ 500 eggs) | Lower Saxony, Germany (7.5 yrs) | 5 p.i. | 35 p.i. | – |

[a] additional information available for some studies, not required by VICH guidelines
[b] study was conducted in replicates due to availability of litters

**Table 2** Study design of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against mature and immature stages of *Toxascaris leonina* (p.i.: post infection)

| Study no. | Breeds | Age of dogs | Body weight (1 or 2 days before treatment) | No. of dogs (EP group/ control group) | Infection | Origin of natural infection/ isolate (age of isolate) | Treatment day | Necropsy day | Faecal worm counts post treatment[a] |
|---|---|---|---|---|---|---|---|---|---|
| 6 | Cross-breeds | 4 months – adult | 6.0–14.4 kg | 8/8 | Natural | Republic of South Africa | 0 | 7 | yes |
| 7 | Beagle | 5–6 weeks | 2.7–5.2 kg | 9/9 | Experimental (~ 500 eggs) | Spain (1 yr) | 0 (57 p.i.) | 7/8 | yes |
| 8 | Beagle | 5–6 weeks | 3.6–5.6 kg | 7/7 | Experimental (~ 470 eggs) | Spain (2 yrs) | 57 p.i. | 64 p.i. | yes |
| 9 | Beagle | 12–13 weeks | 4.4–7.7 kg | 7<br>8 /8 | Experimental (~ 500 eggs) | Spain (3 yrs) | 51 p.i.<br>35 p.i. | 56 p.i. | – |
| 10 | Beagle | 11 weeks | 5.9–9.6 kg | 8/8 | Experimental (~ 500 eggs) | Spain (4.5 yrs) | 35 p.i. | 50 p.i. | – |

[a] additional information available for some studies, not required by VICH guidelines

and 8 for *T. leonina*) using sieving techniques as described below for the necropsy worm counts.

### Necropsy

Five to 30 days post treatment the dogs were euthanised and subsequently necropsied (Table 1, 2). At necropsy, the digestive tract from stomach to rectum was removed. The intestinal content and the results of several mucosal strippings of the small intestine were washed over sieves with apertures of 50 µm to 150 µm (study no. 1: 425 µm). The same procedures were applied to the large intestine using sieves with apertures of 75 µm to 425 µm.

In studies no. 3, 4 and 9, the small intestines were additionally soaked in 0.9% saline at 37°C for approximately 3 hours to encourage release and sedimentation of adherent larvae. After soaking the saline solution was passed through a 36 µm or 38 µm aperture sieve and the small intestines were stripped and washed again using the same sieve.

All samples were analysed for mature and immature worms and the recovered specimens were counted and differentiated according to species, developmental stage and sex.

### Efficacy determination and statistical analysis

In all studies, adequacy of infection in the control group was assessed according to the methods suggested in VICH guidelines 7 and 19. A minimum of 6 control animals with at least five worms each was required. Additionally, the intensity of infection was considered adequate when the lower 95% confidence limit was greater than 10% of the central tendency (geometric mean if all worm counts in the control group > 0, or median if one or more worm counts in the control group = 0).

Percent efficacy for each treatment was calculated according to VICH guideline 7 recommendations and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) as follows:

$$\% \text{ Effectiveness (reduction)} = \frac{(N1 - N2)}{N1 \times 100}$$

N1: geometric mean nematode count for the control group
N2: geometric mean nematode count for the treatment group

Geometric means were calculated following transformation using a logarithmic method (averaging the transformed values, and converting the average using antilog to represent a geometric mean). Because neither the actual worm counts nor the logarithmically transformed counts were distributed normally, the non-parametric Wilcoxon rank sum test (two-tailed, using $\alpha = 0.05$) was used to test for both gender and treatment group (emodepside plus praziquantel tablet vs. placebo) effects. The analyses were performed using SAS software (SAS® Institute, Cary, NC, USA).

Calculations were performed for each parasite stage that occurred in adequate numbers in the control group as described above. In studies where treatment and euthanasia of dogs were more than 7 days apart, calculations were performed on total parasite counts as the stage distribution at necropsy was not considered to be representative for the distribution at treatment. In these cases, the calculated efficacies were attributed to the specific larval stage based on the time of treatment after infection according to VICH GL 19 (i.e., 5 days post infection for migrating L3/early L4 larvae of *T. canis*, 35 days post infection for L4 larvae of *T. leonina*).

## Results

None of the dogs from the ten studies showed signs of adverse reactions after treatment until necropsy. The requirements for the adequacy of infection were fulfilled in all studies except for the numbers of mature adult *T. leonina* in study no. 6, where only four of eight control dogs had the required min-

**Table 3** Results of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against mature adult *Toxocara canis* in dogs

| Study no. | No. of dogs in control group with ≥ 5 mature worms/total no. of dogs in control group | Mature worms per group at necropsy (EP/control) | | | Efficacy | p-value |
|---|---|---|---|---|---|---|
| | | Total no. | Range | Geometric mean | | |
| 1 | 6/6 | 0/61 | 0/5–21 | 0/8.9 | 100 % | 0.012 |
| 2 | 7/7 | 1/119 | 0–1/8–36 | 0.1/15 | 99.3 % | 0.0071 |

**Table 4** Results of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against immature adults, L4 and L3 larvae of *Toxocara canis* in dogs (p.i.: post infection)

| Developmental stage | Study no. | Treatment day | No. of dogs in control group with ≥ 5 worms[a]/ total no. of dogs in control group | Worms[a] per group at necropsy (EP/control) | | | Efficacy | p-value |
|---|---|---|---|---|---|---|---|---|
| | | | | Total no. | Range | Geometric mean | | |
| Immature adults | 3 | 21 p.i. | 15/20 | 0/757 | 0/0–222 | 0/15.4 | 100 % | 0.0015 |
| | 4 | 21 p.i. | 6/8 | 10/95 | 0–4/4–20 | 0.8/10.4 | 92.1 % | 0.0055 |
| L4 larvae | 3 | 21 p.i. | 13/20 | 0/594 | 0/0–120 | 0/13.8 | 100 % | 0.0010 |
| | 4 | 21 p.i. | 8/8 | 7/358 | 0–3/13–78 | 0.6/38.3 | 98.4 % | 0.0045 |
| L3 larvae | 3 | 5 p.i. | 20/20 | 52/1,420 | 0–21/6–257 | 1.9/41.4 | 95.3 % | 0.0007 |
| | 5 | 5 p.i. | 8/8 | 55/455 | 0–34/9–82 | 2.9/49.7 | 94.2 % | 0.0065 |

[a] only immature adult worms or L4 larvae for evaluation of the respective stage, all stages combined for evaluation of L3 larvae (see Materials and methods)

imum of 5 worms at necropsy and three further control dogs had three adult worms. However, in this study the faecal worm counts of the group treated with emodepside plus praziquantel tablets demonstrated an adequate infection with 6 of 8 dogs having expelled 6 to 30 mature adult *T. leonina* post treatment and the other two dogs in the group having expelled 2 and 4 mature adult *T. leonina*. Therefore efficacy was calculated despite of the failure to meet one of the criteria for adequacy of infection.

Efficacy of emodepside plus praziquantel tablets against mature adult *T. canis* was > 99%, efficacy against immature adults was > 92%, efficacy against L4 larvae was > 98% and efficacy against L3 larvae was > 94% (Table 3, 4). The *T. leonina* studies demonstrated > 99% efficacy against the mature adult and immature adult and > 95% efficacy against the L4 larval stage (Table 5, 6). All differences between treatment and control groups were statistically significant and no gender effect was found. The results are presented in detail in Table 3–6.

Worms of dogs treated with emodepside plus praziquantel tablets were expelled mostly within 2 days after treatment as demonstrated by the faecal worm counts that were conducted in some of the studies (Table 1, 2).

**Table 5** Results of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against mature adult *Toxascaris leonina* in dogs

| Study no. | No. of dogs in control group with ≥ 5 mature worms/total no. of dogs in control group | Mature worms per group at necropsy (EP/control) | | | Efficacy | p-value |
|---|---|---|---|---|---|---|
| | | Total no. | Range | Geometric mean | | |
| 6 | 4[a]/8 | 0/87 | 0/1–36 | 0/6.5 | 100 % | 0.0015 |
| 7 | 9/9 | 4/237 | 0–4/7–49 | 0.2/21.7 | 99.1 % | 0.0018 |
| 8 | 7/7 | 0/135 | 0/9–33 | 0/16.9 | 100 % | 0.0060 |
| 9 | 8/8 | 0/148 | 0/8–26 | 0/16.9 | 100 % | 0.0046 |

[a] at least 6 dogs in the EP group were adequately infected as demonstrated by faecal worm counts post treatment

**Table 6** Results of controlled studies on the efficacy of emodepside (E) plus praziquantel (P) tablets against immature adults and L4 larvae of *Toxascaris leonina* in dogs (p.i.: post infection)

| Developmental stage | Study no. | Treatment day | No. of dogs in control group with ≥ 5 worms[a]/total no. of dogs in control group | Worms[a] per group at necropsy (EP/control) | | | Efficacy | p-value |
|---|---|---|---|---|---|---|---|---|
| | | | | Total no. | Range | Geometric mean | | |
| Immature adults | 8 | 57 p.i. | 7/7 | 1/283 | 0–1/8–74 | 0.1/30.9 | 99.7 % | 0.0071 |
| | 9 | 51 p.i. | 8/8 | 0/326 | 0/24–64 | 0/39.5 | 100 % | 0.0044 |
| L4 larvae | 9 | 35 p.i. | 8/8 | 30/513 | 0–11/34–88 | 2.6/61.9 | 95.8 % | 0.0047 |
| | 10 | 35 p.i. | 8/8 | 2/312 | 0–2/16–87 | 0.1/34.4 | 99.6 % | 0.0035 |

[a] only immature adult worms for evaluation of immature adult stage, all stages combined for evaluation of L4 larvae (see Materials and methods)

## Discussion

In the studies presented in this paper a high efficacy of emodepside plus praziquantel tablets against *T. leonina* and *T. canis* including their developmental stages was observed. The developmental cycle of *T. canis* is complex, but the data demonstrate that the product is able to act on different levels of development. Treatment was effective as early as 5 days after the infection, the time when L3/early L4 larvae are expected to be migrating through tissues. Efficacy was then confirmed 21 days after infection when the parasite population consisted mainly of L4 larvae and immature adults. Finally, efficacy against a patent *T. canis* infection was demonstrated. The efficacy on different levels of development can be regarded as particularly important in the light of the zoonotic potential and the possibility of frequent reinfection with this parasite. As outlined in the introduction of this paper, not only young pups but also adult dogs may get repeatedly infected (Fahrion et al. 2008) and 32 % of dogs that were under quarterly anthelmintic treatment turned positive for *T. canis* at least once during a one-year period (Sager et al. 2006). The studies on *T. leonina* complement

the picture of the efficacy of emodepside plus praziquantel tablets against ascarids in the dog. No side effects of the treatment were observed in the studies.

The data presented in this paper were confirmed by the results of a multicentre field study in 44 dogs with a patent ascarid infection, where faecal egg count reduction after treatment with emodepside plus praziquantel tablets was 99.9% against *T. canis* and 100% against *T. leonina* (Altreuther et al. 2009).

It can therefore be concluded that emodepside plus praziquantel tablets (Profender® tablets for dogs) are an effective and safe treatment against *T. canis* and *T. leonina* including their developmental stages and thus provide a comprehensive new treatment option for ascarid infections in the dog.

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