Parasitol Res (2005) 97:S33–S40
DOI 10.1007/s00436-005-1442-3

Evaluation of the efficacy of emodepside+praziquantel topical solution against cestode (*Dipylidium caninum*, *Taenia taeniaeformis*, and *Echinococcus multilocularis*) infections in cats

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Abstract

Emodepside+praziquantel topical solution was developed to provide broad-spectrum anthelmintic activity against gastrointestinal parasites in cats. Eight controlled studies were conducted to evaluate the efficacy of a topical solution of emodepside (3 mg/kg) and praziquantel (12 mg/kg) (Profender®, Bayer AG, Leverkusen, Germany) against feline infections with three species of cestodes. Studies featured naturally acquired infections of *Dipylidium caninum* or *Taenia taeniaeformis*, or experimental infections with *Echinococcus multilocularis* that were placebo-controlled, randomized and blinded. Cats were euthanatized and necropsied between 2 and 11 days after treatment, depending on the target parasite. The efficacy of emodepside+praziquantel topical solution was 100% against *D. caninum* and *T. taeniaeformis*, and 98.5– 100% against *E. multilocularis*. No significant systemic or local adverse reactions to treatment were noted in cats that received the combination. Topical treatment of cats with emodepside+praziquantel topical solution was safe and highly effective against cestode infections.

Introduction

Control of parasitic infections of companion animals is an important aspect of public health, as well as of animal welfare. In view of the wide variability in the prepatent periods of gastrointestinal parasites of cats, the limited sensitivity of some diagnostic techniques, and the constant threat of reinfection from the environment, regular deworming of companion animals has become a routine recommendation of many veterinary practitioners. However, fewer cat owners are likely to comply with these recommendations than dog owners, due to the inherent difficulty of administering oral medications to felines.

Cestodes and nematodes are the major gastrointestinal parasites of cats. In one coprological survey in the United States, 2.4% of 452 feline fecal samples were positive for cestode eggs (Kirkpatrick 1988). A similar study determined that 4% of 2,000 feline fecal samples were positive for eggs of *Taenia taeniaeformis*, *Dipylidium caninum* or *Mesocestoides* spp. (Nolan and Smith 1995). In Europe, an Austrian survey found that 33% of cats were infected with *T. taeniaeformis* (Hinaidy 1991), as were 20–28% of cats sampled in Belgium (Vanparijs et al 1991).

Although *D. caninum* and *T. taeniaeformis* are common endoparasites of felids, cats can also be infected by other cestodes such as *Echinococcus multilocularis* (Eckert 1996). Awareness of the zoonotic significance of this parasite has increased over the last decade, and more information is sought about the potential role of cats in natural transmission. In a previous study it was

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shown that the well known cestocide, praziquantel, applied topically, is effective in rapidly removing *E. multilocularis* from cats (Jenins and Romig 2000).

Bayer has developed a combination anthelmintic product containing praziquantel plus a novel nematocide, emodepside. Emodepside is a member of the cyclooctadepsipeptide class of anthelmintics, and is a semisynthetic product derived from the fungus *Mycelia sterilia* that grows on the leaves of the camellia, *Camellia japonica* (Harder et al 2003; Harder and von Samson-Himmelstjerna 2002).

Emodepside+praziquantel is formulated as a solution for topical application. It is intended for use in cats as a single treatment for adult and immature nematodes (*Toxocara cati*, *Toxascaris leonina, Ancylostoma tubaeforme*) and cestodes (*D. caninum*, *T. taeniaeformis*, *E. multilocularis*).

Eight controlled studies were conducted in compliance with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines 7, 9 and 20 (VICH 2000a, 2000b, 2001) to evaluate the efficacy of emodepside+praziquantel topical solution against cestode infections in cats.

Materials and methods

Many elements of the experimental design and certain technical procedures were common to all eight studies, so those features are described together. Aspects that were unique to each trial are presented in the latter part of this section.

Common features

Study Design

All studies featured placebo-treated control animals, random allocation of healthy, qualified cats to treatment groups, and assessment of outcome measures by masked personnel who had no knowledge of treatment assignments.

Acclimation

All cats were acclimated to study conditions prior to treatment. Cats were housed individually in cages (except study 7), had ad libitum access to water, and were fed daily a commercial diet in quantities sufficient for growth and maintenance.

Health observations

A veterinarian who had no knowledge of treatment assignments conducted physical examinations at the beginning of acclimation, on the day before or the day of treatment (i.e., day 0), and on the day of or the day prior to euthanasia and necropsy.

In addition, trained personnel who were unaware of treatment assignments observed the study animals once daily during the acclimation period for changes in behavioral attitude, eyes, feces, respiration, locomotion/musculature, and skin. On day 0, enrolled cats were observed for at least 30 min, 1, 2, 4, and 6 or 8 h post-treatment. Animals were observed again 24 h after treatment. Thereafter, the general health of enrolled cats was assessed at least once daily, until termination of the study. The skin and hair coat at the treatment application site were examined once daily.

Emodepside+praziquantel and placebo

Emodepside+praziquantel topical solution is a combination of emodepside (2.14% w/v) and praziquantel (8.58% w/v) (Profender®, Bayer AG, Leverkusen, Germany) formulated as a solution intended for topical administration to cats. The placebo was formulated from the same excipients as the combination solution, but contained no emodepside or praziquantel.

Allocation and treatment

Cats were weighed prior to treatment and body weights were used to determine individual doses of emodepside+praziquantel topical solution or placebo to be

administered on day 0. Body weights also were used to rank cats within sex and to block them into replicates. Within a replicate, animals were assigned randomly to treatment groups.

Doses were administered on the dorsal midline, at the base of the skull. The hair was parted and each dose was applied directly to the skin.

Parasitologic techniques

Fecal examination

In certain studies, feces were collected from individual cats on study days indicated by the protocol. Approximately 1 g of feces was processed by means of a modified sucrose centrifugation technique (Cox and Todd 1962), and cestode eggs were counted and expressed as eggs per gram of feces.

Total worm counts

At the end of the observation period, enrolled cats were euthanatized, necropsied, and recovered worms were preserved. The preserved worms were examined microscopically, identified to genus and species, and counted.

Calculations and statistical analyses

Total worm counts of individual animals were transformed by the formula $\log_e(\text{count}+1)$, and geometric means were calculated for each treatment group. If adequacy of infection was demonstrated (i.e., at least six placebo-treated cats were infected), group means were compared by the Wilcoxon rank sum test. In addition, efficacy of each treatment was calculated using the formula:

$$\% \text{ Efficacy (reduction)} = [(N_2 - N_1)/N_2] \times 100$$

where N_1=geometric mean worm count of the treatment group, and N_2=geometric mean worm count of the control group.

A treatment was considered effective if treated and control group means were significantly different ($P<0.05$), and if worm count reduction was >90%.

Study-specific features

Separate studies were conducted with three species of cestodes that utilize domestic felids as the definitive host, i.e., *D. caninum*, *T. taeniaeformis*, and *E. multilocularis*.

D. caninum

Study 1 was a dose-determination trial conducted with 40 cats that were naturally infected with *D. caninum*, as confirmed by fecal examination or recovery of proglottids. Cats were randomized by gender and body weight to four treatment groups. The emodepside+praziquantel topical solution was evaluated at three dosages (see below). Cats were treated topically on day 0 and necropsied on day 10 or 11. Due to the unavailability of 40 infected cats at one time, the study was conducted in replicates.

Study 2 was a dose-confirmation and non-interference study. Forty cats with naturally acquired infections of *D. caninum* confirmed by fecal examination, were randomized by gender and body weight to four treatment groups. One group (T4) received the recommended dosages of emodepside+praziquantel topical solution; group T2 was treated only with emodepside (3 mg/kg); group T3 was dosed only with praziquantel (12 mg/kg); and group T1 was treated with a topical placebo. This study was conducted in replicates. Animals in all groups were treated on study day 0 and necropsied 10 days post-treatment.

T. taeniaeformis

Study 3 employed 20 cats that were naturally infected with *T. taeniaeformis*, as confirmed by fecal examination or recovery of proglottids. Cats were randomized by gender and body weight to two treatment groups, treated topically on day 0, and necropsied on day 11 for worm recovery. Due to the unavailabhility of 20 infected cats at one time, the study was conducted in replicates.

Study 4 featured ten adult cats that were naturally infected with *T. taeniaeformis*, as confirmed by fecal examination. Cats were randomized by gender and body weight to two treatment groups, treated on day 0 and necropsied on day 10 for worm recovery.

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Study 5 used 20 cats with naturally acquired *T. taeniaeformis* infections that were confirmed by fecal examination. Animals were randomized by gender and body weight to two treatment groups, treated topically on day 0 and necropsied on day 10. Due to the unavailability of 20 infected cats at one time, the study was conducted in replicates.

E. multilocularis

In study 6, twenty-two cats were infected orally with ~39,600 protoscolices of *E. multilocularis*. Animals were randomized by gender and body weight into two groups. On day 21, cats in groups 1 and 2 were treated with either emodepside+praziquantel topical solution or placebo, respectively. All cats were euthanized and necropsied 23 days post-infection, and tapeworms were recovered and counted.

In study 7, eighteen cats infected orally with ~10,000 *E. multilocularis* protoscolices were randomized by gender and body weight into two groups: cats in groups 1 and 2 were treated topically with emodepside+praziquantel topical solution or topical placebo 21 days after infection, respectively. Animals were euthanized and necropsied 23 days post-infection for worm recovery.

In study 8, twenty cats were infected orally with ~20,000 *E. multilocularis* protoscolices. Animals were randomized by gender and body weight into two groups. Cats in group 1 were treated topically with emodepside+praziquantel topical solution 21 days post-infection to evaluate efficacy against mature cestodes, and group 2 received topical placebo. Cats in both groups were euthanized and necropsied 23 days after infection, and cestodes were collected and counted.

Results

D. caninum

Study 1

Emodepside+praziquantel topical solution was 100% effective (geometric mean of 0.0, $P<0.05$) against *D. caninum* at praziquantel dosages of 12 and 24 mg/kg (Table 1). In the placebo group, nine of ten animals (geometric mean of 8.5) had *D. caninum* scolices. Three of ten cats in the group receiving the lowest dosage of praziquantel (6.0 mg/kg) still harboured tapeworm scolices at necropsy. The geometric mean cestode count for this group was 1.3, an 84.4% reduction in *D. caninum* numbers compared to placebo-treated control animals (Table 1). No systemic or local abnormalities in response to treatment were observed in any of the cats receiving the emodepside+praziquantel topical solution.

Study 2

The efficacy of the emodepside+praziquantel topical solution was 100% (geometric mean of 0.0, $P<0.05$) against *D. caninum* (Table 1). In comparison, the cestocidal component (praziquantel; 12 mg/kg) removed 98.7% of target tapeworms ($P<0.05$) when used alone (Table 1). Emodepside is exclusively nematocidal, and predictably exhibited no activity against cestodes at a dosage of 3 mg/kg. All ten cats treated with emodepside were infected with the target tapeworm. In the placebo group, eight of ten cats (geometric mean of 5.5) were positive for *D. caninum*. This study demonstrated that concurrent administration of emodepside did not diminish the cestocidal efficacy of praziquantel. No abnormal systemic or local observations in response to treatment were recorded for any of the cats that received the emodepside+praziquantel topical solution.

The distribution of worms among the placebo groups of both *D. caninum* studies met adequacy-of-infection standards suggested in the VICH guidelines.

T. taeniaeformis

Study 3

Emodepside+praziquantel topical solution was 100% effective ($P<0.05$) against naturally acquired infections of *T. taeniaeformis* (Table 2). The geometric mean count of worms for the placebo-treated groups was 2.6

Table 1. Results of controlled evaluations of the efficacy of emodepside (Emo)+praziquantel (Prz) topical solution against infections with *Dipylidium caninum* in domestic cats

Study no.	Treatment group	No. infected cats/total no. of cats	Geometric mean worm nos.	Adequacy of infection	Percentage efficacy
1	Placebo	9/10	8.5	Yes[a]	N/A
	Emo 1.5 mg/kg; Prz 6.0 mg/kg	3/10	1.3		84.4
	Emo 3 mg/k; Prz 12 mg/kg	0/10	0.0		100
	Emo 6 mg/kg; Prz 24 mg/kg	0/10	0.0		100
2	Placebo	8/10	5.5	Yes[a]	N/A
	Emo 3 mg/kg	10/10	5.3		2.7
	Prz 12 mg/kg	1/10	0.1		98.7
	Emo 3 mg/kg; Prz 12 mg/kg	0/10	0.0		100

[a]Adequacy of infection calculated as suggested by International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines
N/A Not applicable

Table 2. Results of controlled evaluations of the efficacy of Emo+Prz topical solution against infections with *Taenia taeniaeformis* in domestic cats. *Emo+Prz* Combination of 3 mg/kg Emo+12 mg/kg Prz administered topically; for other abbreviations, see Table 1

Study no.	Treatment group[a]	No. infected cats/total no. of cats	Geometric mean worm nos.	Adequacy of infection	Percentage efficacy
3	Placebo	8/10	2.6	Yes[a]	N/A
	Emo+Prz	0/10	0.0		100
4	Placebo	5/5	2.1	Yes[b]	N/A
	Emo+Prz	0/5	0.0		100
5	Placebo	10/10	4.5	Yes[a]	N/A
	Emo+Prz	0/10	0.0		100

[a]Adequacy of infection calculated as suggested by VICH guidelines
[b]Only five controls were infected, but infection was adequate because the lower 95% confidence interval exceeded 10% of the geometric mean (VICH guidelines)

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with eight of ten animals positive for *T. taeniaeformis* scolices. One cat treated with emodepside+praziquantel topical solution vomited 2 h post treatment; however the cause of this condition could not be established.

Study 4

Emodepside+praziquantel topical solution removed 100% ($P<0.05$) of *T. taeniaeformis* from five treated cats (Table 2). The geometric mean number of adult worm in animals that received the placebo was 2.1 as compared to 0.0 in those that received emodepside+praziquantel topical solution. Although the placebo group contained only five cats, all were infected with cestodes at necropsy, and adequacy of infection was demonstrated by statistical calculations recommended in the VICH guidelines. No abnormal systemic or local health observations to treatment were observed.

Study 5

Efficacy of the emodepside+praziquantel topical solution was 100% ($P<0.05$) against *T. taeniaeformis* in ten cats treated with the product. The geometric mean number of adult worms in animals that received the placebo was 4.5 as compared to 0.0 in those that received the product (Table 2). No abnormal systemic or local health observations to treatment were recorded in cats that received the emodepside+praziquantel topical solution.

The distribution of worms in placebo groups employed in studies 3 and 5 met adequacy-of-infection standards suggested in the VICH guidelines.

E. multilocularis

In study 6, emodepside+praziquantel topical solution was effective (98.5%) against *E. multilocularis* that had been exposed to the product 21 days after inoculation (Table 3). The geometric mean of worms in animals treated with emodepside+praziquantel topical solution at 21 days post infection was 1.2 as compared to 79.8 in animals that received the placebo. The worms recovered in this study were noted to be small in comparison with those recovered from dogs. No treatment-related abnormal systemic or local observations were recorded in cats that received emodepside+praziquantel topical solution.

In study 7, no specimens of *E. multilocularis* were recovered from groups of cats treated with emodep-

Table 3. Results of controlled evaluations of the efficacy of Emo+Prz topical solution against infections with *Echinococcus multilocularis* in domestic cats. For abbreviations, see Tables 1 and 2

Study no.	Treatment group[a]	No. infected cats/total no. of cats	Geometric mean worm nos.	Adequacy of infection	Percentage efficacy
6	Placebo	11/11	79.8	Yes[a]	N/A
	Emo+Prz	2/11	1.2		98.5
7	Placebo	8/9	76.4	Yes[a]	N/A
	Emo+Prz	0/9	0.0		100
8	Placebo	9/10	226.5	Yes[a]	N/A
	Emo+Prz	0/10	0.0		100

[a]Adequacy of infection calculated as suggested by VICH guidelines

side+praziquantel topical solution 21 days after inoculation with protoscolices. The geometric mean numbers of worms in animals treated with emodepside+praziquantel at 21 days post infection were 0.0 as compared to 76.4 (eight of nine) in animals that received placebo. Accordingly, efficacy against *E. multilocularis* was 100% ($P<0.05$) (Table 3). In this study, no abnormal, treatment-related systemic or local observations were reported for any cats that received the emodepside+praziquantel topical solution.

In study 8, 100% efficacy against infections with *E. multilocularis* was observed in cats that received emodepside+praziquantel topical solution. The geometric mean of worms in placebo-treated animals was 226.5 compared to 0.0 in animals that received the product (Table 3). No abnormal systemic or local observations were recorded for cats treated with emodepside+praziquantel, except for one cat that exhibited a single drop of saliva 2 h post treatment. The cause of this condition could not be established.

The distribution of cestodes within the placebo groups of all three studies with *E. multilocularis* met adequacy-of-infection standards suggested in the VICH guidelines.

Discussion

Study 1 (dose determination) demonstrated a reduction in cestocidal efficacy of the anthelmintic combination at the lowest dosages of emodepside+praziquantel (1.5 and 6 mg/kg, respectively). This confirmed the results of preliminary studies conducted for this formulation. Accordingly, the 12 mg/kg regimen for praziquantel was selected as the label dosage for the emodepside+praziquantel topical solution (G. Altreuther, personal communication 2005). As demonstrated in a non-interference trial (study 2), the cestocidal efficacy of praziquantel against *D. caninum* was not diminished when it was combined with emodepside.

In Studies 1–5, the topical application of emodepside+praziquantel topical solution was 100% effective against naturally acquired infections with *D. caninum* or *T. taeniaeformis*. Because naturally infected cats had been used in these five studies on the basis of patent infections (i.e., observation of proglottids or detection of eggs via fecal examination), adult cestodes were the presumed target stages.

Adult tapeworm infections generally do not cause clinical signs in the cat, but some of these infections may represent a risk to human health. *D. caninum*, for instance, can cause a zoonotic infection in a human that inadvertently ingests a flea containing a cysticercoid (Bowman et al. 2002). Infection usually occurs in children living and interacting closely with domestic pets (Shane et al. 1986). Human infection with *D. caninum* tapeworms is mostly apathogenic but may lead to anorexia and weight loss in infected children (Bowman et al. 2002).

A far more important zoonotic agent, however, is *E. multilocularis*, which causes alveolar echinococcosis in man (Vuitton et al. 2003). Alveoloar echinococcosis develops in humans who accidentally ingest eggs of *E. multilocularis*, with subsequent development and amplification of the metacestode stage (alveolar hydatid) in various visceral tissues. Human infections are fortunately rare, but commonly fatal without extensive chemotherapy and/or surgery (Amman and Eckert 1995). The majority of cases appear to have resulted from exposure to infected foxes. Although cats can become infected by *E. multilocularis*, felines are less favorable hosts than foxes or dogs (Crellin et al 1981).

In three studies conducted to evaluate the efficacy of emodepside+praziquantel topical solution against *E. multilocularis* in cats, efficacy was 100% in two studies and 98.5% in the third. Thus, the efficacy of topical emodepside+praziquantel solution in the control of *E. multilocularis* infections was comparable to conventional oral treatments.

In the eight studies discussed in this paper, a single topical treatment of cats with emodepside+praziquantel topical solution was found to be safe and highly efficacious against *D. caninum, T. taeniaeformis*, and *E. multilocularis* infections and will be a practical and useful treatment for cats against intestinal helminths.

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Acknowledgements

The authors thank Dr. Thomas Romig (Department of Parasitology, University of Hohenheim, Stuttgart, Germany) for providing the *E. multilocularis* protoscoleces used in studies 7 and 8. All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations.

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