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Efficacy of a topically administered combination of emodepside and praziquantel against mature and immature *Ancylostoma tubaeforme* in domestic cats

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Abstract

This paper reports the efficacy of emodepside/praziquantel spot-on (Profender®, Bayer AG, Leverkusen, Germany), a novel broadspectrum anthelmintic for dermal application, against L4 larvae and immature adult and adult stages of *Ancylostoma tubaeforme* in cats. The formulation contains 2.14% (w/w) emodepside and 8.58% (w/v) praziquantel, with emodepside being active against gastrointestinal nematodes and praziquantel against cestodes. Five randomized, blinded and controlled laboratory studies demonstrated 100% efficacy of emodepside/praziquantel spot-on against mature *A. tubaeforme* and an efficacy of >95% and >97%, respectively, against L4 larvae and immature adults (based on worm counts after necropsy) at approximately the minimum proposed dose rate in cats of 3.0 mg emodepside and 12.0 mg praziquantel/kg body weight. No adverse reactions to the treatment were observed. It is concluded that emodepside/praziquantel spot-on is an effective and safe treatment against infections with mature and immature *A. tubaeforme*. Emodepside/praziquantel spot-on will considerably facilitate the treatment of cats against nematodes and cestodes compared with orally administered preparations.

Introduction

The hookworm *Ancylostoma tubaeforme* is endemic in domestic cats throughout the world. Cats are infected by skin penetration or ingestion of infective third-stage (L3) larvae. The prepatent period of *A. tubaeforme* ranges 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 blood. While low to moderate infections cause mild enteritis, heavy infections can lead to anemia and may be fatal. Reported prevalence of *A. tubaeforme* in cats from Europe and the Americas during recent years ranges between 0 and 75%, with highest rates of infection in feral/stray cats (Table 1).

Little is known about the life cycle of *A. tubaeforme* (Anderson 1992). Data from the literature and recent findings indicate a zoonotic potential comparable to that of the canine hookworm *Ancylostoma caninum* (Prociv 1998; F. H. M. Borgsteede, personal communication).

Emodepside/praziquantel spot-on (Profender®) is a novel broadspectrum anthelmintic product intended for the topical treatment of cats with gastrointestinal parasites, i.e., mature and immature nematodes (*Toxo-*

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Table 1. Results of recent studies on the prevalence of *Ancylostoma tubaeforme* in cats from Europe and the Americas

Study location	Study period	No. of cats studied	Prevalence (%) of *A. tubaeforme*	Comments	Reference
France, Germany	—	3,500	0.4	—	Coati et al. (2003)
Germany	—	441	1.6	—	Coati et al. (2003)
Germany	1999–2002	3,167	0.3	—	Barutzki and Schaper (2003)
Netherlands	1993–1994	236	0	Cats from households	Overgaauw (1997)
Netherlands	—	305	3.0	Cats from animal shelters	Robben et al. (2004)
Spain (Ebro Valley)	1989–1992	58	29.3	Stray cats	Calvete et al. (1998)
Brazil (Rio de Janeiro)	—	135	8.9	Feral/stray cats (n=99) and cats from animal shelters (n=36)	Labarthe et al. (2004)
USA (Florida)	—	60	75.0	Feral cats	Anderson et al. (2003)
USA (Connecticut)	—	450	0.4	—	Rembiesa and Richardson (2003)

cara cati, *Toxascaris leonina*, *A. tubaeforme*) and mature cestodes (*Dipylidium caninum*, *Taenia taeniaeformis*, *Echinococcus multilocularis*). Emodepside is a semisynthetic derivative of PF1022A and belongs to a new class of anthelmintic compounds, the cyclooctadepsipeptides. PF 1022A was isolated from the mycelial cake of a fungus (*Mycelia sterilia*) that was found in the microflora on the leaves of the plant *Camellia japonica* (Harder and von Samson-Himmelstjerna 2002). Emodepside is active against gastrointestinal nematodes, and in emodepside/praziquantel spot-on is responsible for the efficacy against *T. cati*, *T. leonina*, and *A. tubaeforme*. Emodepside acts presynaptically at the neuromuscular junction of nematodes, leading to an inhibition of pharyngeal pumping and locomotion (Harder et al. 2003, 2005; Willson et al. 2003).

The present paper reports the findings of five laboratory studies that evaluated the efficacy of emodepside/praziquantel spot-on against mature and immature stages of *A. tubaeforme* in naturally and experimentally infected cats.

Materials and methods

Three of the studies (A1–A3) were conducted to investigate the efficacy of emodepside/praziquantel spot-on against mature *A. tubaeforme* in cats, and two studies (B1, B2) that of emodepside/praziquantel spot-on against immature (fourth stage larvae and immature adults) *A. tubaeforme* in cats. The results of study B1 also allowed us to evaluate the efficacy of emodepside/praziquantel spot-on against mature *A. tubaeforme*.

The investigations were performed as placebo-controlled, blinded and randomized studies, conducted in accordance with the VICH guideline 9 (VICH 2000b), and followed the recommendations given in the VICH guidelines 7 (VICH 2000a) and 20 (VICH 2001) as well as the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994).

The design of the studies is summarized in Tables 2 and 3.

Table 2. Design of controlled studies on the efficacy of emodepside (E)/praziquantel (P) spot-on (EP) against mature *A. tubaeforme* in cats. p.i. Post-infection, p.t. post treatment

Study	Cat breed	Age of cats	Body weight of cats (kg)	No. of cats (EP group/ control group	Infection	EP dosage (mg/kg body weight)	Treatment day	Necropsy day
A1	Domestic shorthair	13-14 Weeks (at day-34)	1.6-2.5	7/7	Experimental (~300 L3 larvae)[a]	3.1–4.6 (E); 12.2–18.4 (P)	0 (34 Days p.i.)	7 p.t.
A2	Domestic shorthair/ longhair	~10 Months-3 years	1.8-5.5	10/10	Natural	3.0–3.1 (E); 11.9–12.2 (P)	0	10 p.t.
A3	Domestic shorthair/ longhair, and Siamese	<6 Months-3 years	2.1-4.5	10/10	Natural	3.0 (E); 11.8–12.1 (P)	0	10 p.t.

[a]Strain isolated in 1995 in Australia

Table 3. Design of controlled studies investigating the efficacy of emodepside/praziquantel spot on (EP) against immature *A. tubaeforme* in cats. Study B1 also provided data on the efficacy of EP against mature *A. tubaeforme*. For abbreviations, see Table 2

Study	Cat breed	Age of cats	Body weight of cats (kg)	No. of cats (EP group/ control group	Infection	EP dosage (mg/kg body weight)	Treatment day	Necropsy day
B1	Domestic shorthair	11-16 Weeks	0.8-2.2	8/8	Experimental (~560 L3 larvae)[a]	2.9–3.2 (E); 11.4–12.6 (P)	7 p.i.	12 p.i.
				8/8			14 p.i.	19 p.i.
B2	Domestic shorthair	~3-4 Months	1.1-1.7	8/8	Experimental (~200 L3 larvae)[b]	2.9–3.1 (E); 11.5–12.4 (P)	7 p.i.	12 p.i.
				8/8			11 p.i.	17 p.i.

[a]Strain isolated in 2000 in the Republic of South Africa
[b]Strain isolated in 1995 in the USA

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Study animals

The cats used in the studies were either purposely bred individuals from different breeding facilities or animals obtained from commercial kennels. The cats were identified by transponder and/or ear tattoo and were individually housed in cages. They were fed a commercial cat food meeting dietary requirements, and were provided with water *ad libitum*. All cats were acclimatized for a period of at least 7 days prior to the start of the study.

None of the experimentally infected cats had received any anthelmintic before and were negative for nematode eggs prior to infection with *A. tubaeforme*. Cats in studies A1–A3 were confirmed to be positive for *A. tubaeforme* on the basis of fecal egg counts prior to treatment.

Clinical observations

In all studies, cats were physically examined at least twice before treatment and once after treatment. Additionally, all cats were observed for signs of impaired health at least once daily. On the day of treatment, cats were observed at approximately 0.5, 1, 2, 4, and 8 h after treatment. Special attention was paid to the local tolerance of the product at the observations conducted post-treatment.

Infection

In two studies (A2, A3), cats naturally infected with *A. tubaeforme* were used. The cats in these studies originated from the USA.

In studies A1, B1, and B2, cats were orally infected with L3 larvae of *A. tubaeforme* (for details see Tables 2, 3). The strains used in the studies had been isolated from cats in Australia in 1995 (study A1), the Republic of South Africa in 2000 (study B1), and the USA in 1995 (study B2).

Treatment

Cats of both sexes were randomly assigned to either treatment or control groups. In all studies the cats were treated once with either emodepside/praziquantel spot-on or placebo.

In studies A1–A3 cats were treated after their infection with *A. tubaeforme* had been confirmed. In study A1 where infection had been produced experimentally, the cats were treated on day 34 post-infection.

In studies B1 and B2 the cats were treated either 7 or 14 days post-infection (study B1), or 7 or 11 days post-infection (study B2). The earlier of the two administrations in each study was aimed at the fourth-stage larvae of *A. tubaeforme*, while the later one was applied to evaluate the efficacy against immature adults.

In all studies, the concentration of emodepside in the topically administered emodepside/praziquantel spot-on was 2.14% (w/v), that of praziquantel 8.58% (w/v). The composition of the test product was identical to that of the product intended for use in the market. Dose ranges in the cats on a per kilogram body weight basis are given in Tables 2 and 3. Except for one study (A1) using a somewhat higher dosage, the cats were treated with approximately the proposed minimum dose of emodepside/praziquantel spot-on of 3 mg emodepside and 12 mg praziquantel/kg body weight.

For application of emodepside/praziquantel spot-on or placebo, the hair on the cat's neck at the base of the skull was parted until the skin was visible. The tip of a pipette containing the appropriate amount of emodepside/praziquantel spot-on or the placebo was then placed on the skin and the pipette's content expelled directly onto the cat's skin.

Necropsy

Five to 10 days post treatment, the cats were euthanized and subsequently necropsied (Tables 2, 3). At necropsy, the digestive tract from stomach to rectum was removed. The intestinal content and the results of several mucosal strippings were washed over sieves with apertures of 100 or 425 µm (studies A1–A3) or of

50 or 75 µm (studies B1 and B2). Additionally, in studies B1 and B2, the intestines were soaked in prewarmed PBS or 0.9% saline for 3 h after the initial mucosal stripping to enhance the release of worms attached to the intestinal wall. All samples were analyzed for mature and immature worms and the recovered specimens were counted and differentiated according to 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 20 (VICH 2000a, 2001). A minimum of six animals with at least five worms each in the control group was required. Additionally, the intensity of infection was considered adequate when the lower 95% confidence limit was >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 (VICH 2000a) and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) as follows:

$$\% \text{ Effectiveness (reduction)} = (N_2 - N_1)/N_2 \times 100;$$

where N_1=geometric mean nematode count for the treatment group, and N_2=geometric mean nematode count for the control group.

Geometric means were calculated following transformation using a logarithmic method (averaging the transformed values, and converting the average using the antilog to represent a geometric mean). Because of the occurrence of zero worm counts, 1 was added to each count prior to transformation. Accordingly, 1 was subtracted from the antilog value to represent the geometric mean for each group. Because neither the actual worm counts nor the logarithmically transformed counts were distributed normally, the nonparametric Wilcoxon rank sum test (two-tailed, using α=0.05) was used to test for both gender and treatment group (emodepside/praziquantel spot-on vs. placebo) effects.

Results

None of the cats from the five studies showed signs of a local or systemic adverse reaction after treatment until necropsy. The requirements for the adequacy of infection with *A. tubaeforme* were fulfilled in all studies except study A2. In this study only four cats in the control group had five or more worms, but two further cats had four worms each. Thus the control group was short of two worms. Since this result was very close to the requirements and the lower 95% confidence limit was >10% of the geometric mean the infection was considered to be adequate.

In the studies (A1–A3, B1) investigating the efficacy of emodepside/praziquantel spot-on against mature *A. tubaeforme*, the treatment was totally effective in all treated cats, removing 100% of the worms in the treatment group (Table 4).

Table 4. Results of controlled studies on the efficacy of emodepside/praziquantel spot on (EP) against mature *A. tubaeforme* in cats. For abbreviations, see Table 2

Study	Total no. of mature worms per group at necropsy (EP/control)	No. of cats in control group with ≥ 5 mature worms/total no. of cats in control group	Geometric mean no. (range) of mature worms at necropsy (EP/control)	P-value	Efficacy of treatment
A1	0/145	6/7	0/14.2 (0/12-37)	0.0124	100%
A2	0/104	4 (2 Further cats with 4 worms each)/10	0/4.1 (0/1-53)	0.0032	100%
A3	0/431	7/10	0/16.8 (0/2-186)	0.0008	100%
B1	0/734	6/8	0/41.7 (0/0-147)	0.0062	100%

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One of the two studies (B1) investigating the efficacy of emodepside/praziquantel spot-on against immature *A. tubaeforme*, demonstrated an efficacy of >95% against L4 larvae and of >97% against immature adults. The second study (B2) demonstrated 100% efficacy against both stages (Tables 5, 6).

All differences between treatment and control groups were statistically significant (for *P*-values see Tables 4–6).

Discussion

Four studies demonstrated 100% efficacy of emodepside/praziquantel spot-on against mature *A. tubaeforme* at approximately the minimum proposed dose rate of 3.0 mg emodepside and 12.0 mg praziquantel/kg body weight (studies A2, A3, B1), or a somewhat higher dose rate between 3.1–4.6 mg emodepside and 12.2–18.4 praziquantel/kg body weight (study A1).

Additionally, two studies (B1, B2) demonstrated a high efficacy of emodepside/praziquantel spot-on at approximately the minimum proposed dose rate against L4 larvae (efficacy >95%) and immature adults (efficacy >97%) of *A. tubaeforme*. No adverse reactions to the treatment were observed in the cats. It can therefore be concluded that emodepside/praziquantel spot-on is an effective and safe treatment against L4 larvae, immature adult and adult stages of *A. tubaeforme*.

It has become common veterinary practice to simultaneously treat cats for infections with gastrointestinal cestodes and nematodes. Several combination products for such treatment are available for oral administration but this formulation type can be difficult to apply, particularly in non-cooperative cats. Therefore, dermal administration of anthelmintics, as a more con-

Table 5. Results of controlled studies on the efficacy of emodepside/praziquantel spot on (EP) against L4 larvae of *A. tubaeforme* in cats. For abbreviations, see Table 2

Study	Treatment day	Total no. of L4 larvae per group at necropsy (EP/control)	No. of cats in control group with 5 L4 larvae/ total no. of cats in control group	Geometric mean no. (range) of L4 larvae at necropsy (EP/control)	P-value	Efficacy of treatment
B1	7 p.i.	45/1152	8/8	1.8/137.8 (0-20/85-228)	0.0043	98.7%
	14 p.i.	55/255	8/8	1.4/29.4 (0-43/14-58)	0.0196	95.3%
B2	7 p.i.	0/127	8/8	0/14.0 (0/6-32)	0.003	100%
	11 p.i.	0/119	8/8	0/13.5 (0/5-22)	0.003	100%

Table 6. Results of controlled studies on the efficacy of emodepside/praziquantel spot on (EP) against immature adults of *A. tubaeforme* in cats. For abbreviations, see Table 2

Study	Treatment day	Total no. of immature adults per group at necropsy (EP/control)	No. of cats in control group with 5 immature adults/total no. of cats in control group	Geometric mean no. (range) of immature adults at necropsy (EP/control)	P-value	Efficacy of treatment
B1	7 p.i.	0/0	0/8	0/0 (0/0)	—	—
	14 p.i.	31/710	8/8	1.9/76.1 (0-13/31-179)	0.0045	97.6%
B2	7 p.i.	0/86	8/8	0/8.7 (0/1-24)	0.003	100%
	11 p.i.	0/222	8/8	0/27.1 (0/20-44)	0.003	100%

venient and less stressful way of application, may help to increase the compliance of owners and veterinarians to deworm cats. Use of emodepside/praziquantel spot-on will considerably facilitate the anthelmintic treatment of cats and should thus increase the likelihood of successful treatment taking place.

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