Abstract

The adulticidal efficacy of a topical combination of emodepside 2.1% (w/v) plus praziquantel 8.6% (w/v) (Profender® spot-on for cats, Bayer) against adult Aelurostrongylus abstrusus nematodes was evaluated in two randomised, placebo-controlled laboratory efficacy studies. Each study involved 16 cats experimentally inoculated with L3 (800 and 600 each in studies no. 1 and 2, respectively) and randomised into two study groups of 8 cats each after onset of patency. While cats in the treatment group in study no. 1 received a single spot-on application at the minimum therapeutic dose (3 mg/kg emodepside and 12 mg/kg praziquantel), cats in study no. 2 were treated twice with an interval of 14 days. The faecal output of first stage larvae was monitored throughout the study. Necropsy was conducted 4 or 5 weeks after the (first) treatment and the worm counts were used for efficacy calculations. The control groups showed a geometric mean of the total worm count (live and dead worms) of 28.8 (study no. 1) and 17.6 (study no. 2), respectively. All control animals were infected. While the single treatment in study no. 1 resulted in a reduction of the total worm burden by 73.0% (p = 0.0070), the treatment protocol in study no. 2 was 99.2% effective (p = 0.0035). Based on live worm counts, the efficacy in study no. 2 was 100% (p = 0.0030).

It is concluded that two applications of Profender® spot-on given two weeks apart represent a safe and highly efficacious treatment regime against feline aelurostrongylosis.
Introduction

In the past few years, increased attention has been paid to nematode infections of the cardiopulmonary system of cats and dogs due to their spread in several geographic areas (Traversa et al. 2010). In cats, *Aelurostrongylus (A.) abstrusus* is deemed to be the main agent causing verminous pneumonia. Adults of this metastrongylid nematode inhabit the alveolar ducts and lung parenchyma of domestic cats and wild felids. The females produce eggs that embryonate and hatch in the lung tissue. Aided by the mucociliary clearance mechanisms, the first stage larvae (L1) migrate up the tracheobronchial tree, are swallowed and are released with the cat’s faeces into the environment (Scott 1973). The indirect life cycle involves various terrestrial or aquatic slugs and snails as intermediate hosts (Hobmeier and Hobmaier 1935a, Ash 1970). After ingestion of an infected snail, rodents, birds, amphibians and reptiles may serve as paratenic hosts (Hobmeier and Hobmaier 1935b). Cats become infected by ingesting intermediate or paratenic hosts. Depending on the worm burden, age and immune response of the infected animal, the clinical presentation varies from asymptomatic to fatal. Symptoms include respiratory signs such as dyspnoea, coughing, sneezing and nasal discharge and unspecific signs such as lethargy, depression and weight loss (Hamilton 1967, Scott 1973, Schnyder et al. 2014). *Aelurostrongylus abstrusus* has a worldwide distribution and, although the infection is considered sporadic in many countries, prevalences can reach around 20% in endemic areas, e.g. Portugal (Payo-Puente et al. 2008) and Italy (Traversa et al. 2008).

Anthelmintics used in recent years against *A. abstrusus* include the benzimidazole fenbendazole and various macrocyclic lactones. In the United Kingdom, various formulations of fenbendazole (paste, suspension, granules) are licensed for the treatment of *A. abstrusus* at a dosage of 50 mg/kg daily for three consecutive days. While some authors confirm the efficacy of this treatment protocol (Schmid and Duewel 1990, Barrs et al. 1999), others experienced only partial activity against adult parasites, and reappearance of first stage larvae (L1) in the faeces of 5 of 15 treated cats (Roberson and Burke 1980). Grandi et al. (2005) stated that a 15-day treatment course at 50 mg/kg was necessary with a tablet formulation of fenbendazole, and treatment recommendations for a duration of up to 21 days can also be found in the literature (Norsworthy 2011, Vig and Murray 1986). In contrast, Hamilton et al. (1984) successfully treated experimentally infected cats with only five daily doses of 20 mg/kg of a 2.5% suspension of fenbendazole. Among the macrocyclic lactones, a single administration of 0.4 mg/kg ivermectin s.c. was successful in one of two cats, while the other needed a second treatment to be cleared of L1 in the faeces (Burgu and Sarimehmetoğlu 2004). Incomplete efficacy of a single treatment with ivermectin has been confirmed by other authors (Grandi et al. 2005, Kirkpatrick and Megella 1987). Similarly variable results were obtained with a selamectin spot-on. Two treatments given five weeks apart were successful in one cat (Reinhardt et al. 2004), while the same treatment regimen failed in another case (Grandi et al. 2005).

In the same report by Grandi et al. (2005), one cat was successfully treated with a single topical treatment. In a field study, 9 of 10 cats showed a negative faecal larval count after two treatments given 23 days apart (Iannino et al. 2013). Traversa et al. (2009a) evaluated the efficacy of a single application of imidacloprid/moxidectin spot-on in a positive controlled field efficacy study. While the faeces of all 12 imidacloprid/moxidectin-treated cats were free of *A. abstrusus* L1 four weeks after treatment, one cat in the control group that had been treated with 50 mg/kg fenbendazole for three consecutive days still showed a positive faecal L1 count. The efficacy of eprinomectin in a topical combination of eprinomectin/fipronil/(S)-methoprene and praziquantel has been evaluated in an experimental study. After a single treatment 32 days post inoculation (dpi), faecal larval counts were reduced by 99.6% compared to a negative control group (Knaus et al. 2014).
Profender® spot-on for cats is a combination of emodepside and praziquantel. Emodepside belongs to the cyclooctadepsipeptide class and is effective against a wide variety of gastrointestinal nematodes in cats and dogs. In a field efficacy study conducted by Traversa et al. (2009b) emodepside/praziquantel spot-on showed promising results in the treatment of feline aelurostrongylosis. Four weeks after a single application, 11 of 12 treated cats were negative at the faecal examination and symptomatic cats had clinically recovered. The aim of the studies presented here, was to evaluate the efficacy of the topical emodepside/praziquantel solution in a controlled laboratory test situation.

**Materials and methods**

Two studies were conducted to evaluate the efficacy of emodepside/praziquantel spot-on against experimental *A. abstrusus* infections in cats. Both studies were performed as placebo-controlled, randomised and blinded laboratory efficacy studies. While study no. 1 evaluated the efficacy of a single treatment, cats in study no. 2 were treated twice with an interval of two weeks. Investigations were conducted in accordance with VICH guideline 9 “Good Clinical Practice” (June 2000) and the recommendations given in VICH Guideline 7 “Efficacy of anthelmintics: general requirements” (November 2000), VICH Guideline 20 “Efficacy of anthelmintics: Specific Recommendations for Felines” (June 2001), and the WAAVP guidelines for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) were followed.

As these guidelines do not include specific information on *A. abstrusus*, the study design was based on scientific knowledge and experience gained in a preliminary study (Schnyder et al. 2014). The study designs are summarised in Table 1.

**Study animals**

Sixteen purpose-bred European shorthair cats (8 male/8 female) were included in each study. They were either owned by the study facility or purchased from commercial breeders. On the day of inoculation they were less than 10 months old and had been vaccinated against major feline infectious diseases. Before study start, all cats were dewormed once with pyrantel at the dose recommended by the manufacturer. Treatment with drugs that could influence the study results, especially macrocyclic lactones, was not allowed. All cats were acclimatised for at least 5 days prior to the experimental inoculation. They were either housed individually in cages or pens or group-housed within their respective study group. Individual housing was provided at least on the day of treatment, the two consecutive days and for collection of individual faecal samples. Toys and scratching places were offered for environmental enrichment. The cats received standard commercial wet and/or dry food and water was available *ad libitum*. Cats were handled with due regard for their well-being and the protocols were approved by the site-specific ethics committee.

**Health observations**

General health observations were conducted daily and the cats were physically examined at least before infection and before treatment(s). Only

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**Table 1: Summary of study designs (dpi: days post inoculation)**

| Study no. | No. of cats (treatment / control) | Age at inoculation (months) | Inoculation dose | Study day / treatment | Study day / necropsy |
|-----------|----------------------------------|----------------------------|------------------|----------------------|---------------------|
| 1         | 8/8                              | 3.9–9.2                    | 800 L3           | 0 (36 dpi)           | 26–30               |
| 2         |                                  | 6–7.7                      | 600 L3           | 0 and 14 (43 and 57 spi) | 33–36               |
healthy cats were included in the study. On treat-
ment day(s) clinical assessments were performed
before treatment and approximately 1 and 4 hours
after treatment for the detection of adverse effects.
Body weights were determined in conjunction with
the physical examinations.

**Experimental infections**

Cats were inoculated with approximately 800
(study no. 1) or 600 (study no. 2) infective third stage
larvae (L3) of *A. abstrusus* isolated from snails
(*Biomphalaria glabrata* and *Helix (H.) aspersa*).
The snails had been experimentally infected with
L1 from the faeces of an *A. abstrusus*-positive
cat originating from Italy. The snail tissue was
minced and then digested for 20–30 minutes at
approximately 41 °C. The solution for digestion
was prepared by mixing 0.4 g pepsin (800–2500 U/
mg protein) in 250 mL of 1% hydrochloric acid
(HCl) solution. The digested material was passed
through a 180 μm sieve and centrifuged at 500 g
for 5 minutes. The supernatant was discarded and
the number of L3 per milliliter was determined
in a sub-sample taken from the well-stirred lar-
vae suspension. Individual inoculation doses were
prepared and applied intragastrically via stomach
tube. Before inoculation, the cats were anaesthe-
tised with a combination of medetomidine and
ketamine (with or without premedication with
acepromacine) and metoclopramide was adminis-
tered intramuscularly to avoid vomiting or regur-
gitation. After inoculation, the cats were carefully
observed for vomiting/regurgitation and re-inocu-
lated if loss of inoculation material was observed
within 60 minutes post inoculation.

**Faecal examination**

To demonstrate the parasitological status of the
individual cat, one faecal sample was examined by
a combined sedimentation/flotation method and by
the Baermann technique (Baermann 1917) during
the acclimatization period. Faecal samples of all
cats had to be negative for nematode eggs and lar-
vae before inoculation with *A. abstrusus*. Starting
30 dpi, pooled faecal samples were examined by the
Baermann technique for L1. As soon as a pooled
sample was positive, individual faecal samples
were collected every other day until randomisation.
Post-treatment faecal examinations were contin-
ued until necropsy. Individual samples were col-
lected every other day in study no. 1 and twice per
week in study no. 2.

**Allocation and treatment**

After onset of patency, cats were randomly allo-
cated to two study groups based on sex and their
highest faecal L1 count. After randomisation each
group in the two studies consisted of 8 cats with
equal sex distribution. Treatment was performed
on study day (SD) 0, which was 4 to 5 days after
all cats had shown a positive faecal larval count.
In study no. 1 this date corresponded to 36 dpi and
in study no. 2 to 43 dpi. In both studies, cats in
the treatment group were dosed with the recom-
ended minimum therapeutic dose of emodepside
(3 mg/kg) and praziquantel (12 mg/kg), correspond-
ing to 0.14 mL spot-on formulation per kilogram
body weight. Cats in the control group received a
placebo spot-on formulation to mimic the appear-
ance of the emodepside/praziquantel spot-on. In
study no. 1 treatments were performed once on
SD 0. Cats in study no. 2 were treated twice with a
two-week interval (SD 0 and 14).

**Necropsy**

Necropsy was performed on several consecutive
days starting on SD 26 in study no. 1 and SD 33 in
study no. 2. The lungs, heart and trachea were
removed. The lung tissue was cut into pieces of
approximately 1 cm³ and checked for lungworms
by dissecting piece by piece under a stereo micro-
scope. Lungworms were identified to species and
counted. In study no. 2 the examination method
for worms was modified to take the complicated
worm count procedure into account and to avoid
false negative results. As *A. abstrusus* is embedded
very deeply in the lung parenchyma, it had to be
considered that viable worms could be destroyed
and cut into pieces during isolation from the lung tissue. Additionally, worms might die during the examination due to the fact that the method is very time-consuming. Therefore each lungworm or part of a worm present was carefully examined under a stereo microscope and checked for viability, general appearance and structure of the integument. All worms which were obviously dead before necropsy started (i.e. lytic or necrotic worms with distinct changes of the integument) were counted as dead worms. For worm fragments the number of heads and tails present was determined. If the number of heads was greater than the number of tails, the heads were used to calculate the total number of worms and vice versa.

**Efficacy determination and statistical analysis**

The adequacy of infection was determined according to VICH guidelines 7 and 20. In general, these guidelines consider a minimum of 6 animals in the control group with at least 5 worms each to be adequate. However, as the guidelines do not include specific recommendations for studies with *A. abstrusus* and lower infection rates were observed in a preliminary study (Schnyder et al. 2014), an infection with at least one worm in each of 6 control animals was deemed to be adequate. Additionally, the lower limit of the 95% confidence interval was required to be greater than 10% of the geometric mean.

The efficacy was based on worm counts at necropsy and calculated according to VICH guideline 7 and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) as follows:

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

N1: geometric mean (GM) of *A. abstrusus* counts in the control group  
N2: geometric mean (GM) of *A. abstrusus* counts in the treated group

Due to the presence of worm count values of “0”, all counts were modified by adding 1 prior to log transformation and subtracting 1 from the antilog value. In study no. 1 efficacy calculations were based on total worm counts (live + dead). As the data were normally distributed, a parametric analysis of variance was used to test for a treatment group effect (emodepside/praziquantel vs. placebo). In study no. 2 calculations were performed on the live and total worm counts. The data were not normally distributed, therefore the non-parametric Wilcoxon rank sum test (two tailed, \( \alpha = 0.05 \)) was used to test for a treatment group effect.

At necropsy it became apparent that in both studies some control animals were infected with a second lungworm species identified as *Troglostrongylus* sp. Due to the fact that the L1 of this parasite is morphologically extremely similar to the L1 of *A. abstrusus*, the contamination was not detected at the faecal examinations. Therefore no valid data could be generated for the faecal larval reduction post treatment of *A. abstrusus* alone. Rather the faecal larval counts had to be considered as combined *Aelurostrongylus* and *Troglostrongylus* L1 counts. For informational purposes, descriptive statistics were calculated but no inferential analysis was performed.

All analyses were performed using SAS software 9.2 (SAS Institute, Cary, North Carolina, USA, 2001).

**Results**

**Faecal examinations**

In study no. 1 pooled faecal samples were positive on 30 dpi. All individual samples were positive 32 dpi, therefore the onset of patency could not be clearly determined. In study no. 2 one group sample was positive 30 dpi, and by 38 dpi lungworm larvae were present in all individual faecal samples. Faecal larval counts in the control group of both studies continuously increased towards necropsy. Larval counts in emodepside/praziquantel-treated animals were reduced by ≥ 99.6% at necropsy.
Results of the faecal L1 counts are presented in Fig. 1. As explained above, the data represent the combined faecal larval counts of *A. abstrusus* and *Troglostrongylus* sp.

**Efficacy evaluation**

The requirements for an adequate infection were fulfilled in both studies. All control cats were infected. While 11–68 worms (geometric mean: 28.8) were found at necropsy of control cats in study no. 1, the control group in study no. 2 harboured 3–45 live worms (geometric mean: 8.9) and in total (live + dead) 5–95 worms (geometric mean: 17.6). Total worm counts in the treated group in study no. 1 were reduced by 73 % (p = 0.0070). In study no. 2 no live *A. abstrusus* were isolated from cats treated twice with emodepside/praziquantel. Thus, the efficacy based on live worm counts was 100 % (p = 0.0030). One cat harboured two dead worms. Based on total worm counts (live + dead) the efficacy was 99.2 % (p = 0.0035). Results are summarised in Table 2.

Table 2: Results of controlled studies on the efficacy of emodepside/praziquantel spot-on against adult *Aelurostrongylus abstrusus* nematodes.

| Study no. | Group                | Live worm counts | Total worm counts (live + dead) |
|-----------|----------------------|------------------|---------------------------------|
|           |                      | Range            | Geometric mean | Reduction | Range | Geometric mean | Reduction |
| 1         | placebo              | Not evaluated*   |                   |           | 11–68 | 28.8           |          |
|           | emodepside/praziquantel |                 |                   |           | 1–40  | 7.8            | 73.0%     |
| 2         | placebo              | 3–45             | 8.9              | –         | 5–95  | 17.6           |          |
|           | emodepside/praziquantel | 0               | 0.0              | 100%      | 0–2   | 0.1            | 99.2%     |

*Differentiation between live and dead nematodes was not applicable.
General health and safety evaluation
Apart from one cat in the treatment group, all cats in study no. 1 showed respiratory signs on at least one evaluation date. These were abnormal respiratory sounds, an abnormal breathing character and rate and coughing. Furthermore, signs probably related to lungworm were enlarged lymph nodes, lethargy, a rough coat and in rare cases cardiovascular signs. Due to pneumonia, one cat in the treatment group received concomitant treatment with meloxicam once on SD 22. The only abnormal clinical signs in study no. 2 were vomiting in three cats on a single occasion 10 or 11 dpi. Apart from that, all cats stayed clinically healthy throughout the study period. In both studies treatments were well tolerated. No adverse effects were observed.

Infection with Troglostrongylus sp.
Six control cats in study no. 1 harboured Troglostrongylus nematodes (range 2–23). In study no. 2 four cats in the control group were infected. One cat harboured a single worm, in another cat three worms were present and two cats were infected with two Troglostrongylus sp. each. No Troglostrongylus worms were detected in any of the emodepside/praziquantel-treated cats.

Discussion
VICH Guidelines 7 and 20 require a reduction in worm burdens of at least 90% in treated animals compared to a control group in order to claim efficacy of an anthelmintic substance. While this requirement was not met by a single treatment with emodepside/praziquantel spot-on, two treatments given at an interval of two weeks showed complete efficacy (100%) based on live worm counts, and only two dead worms were isolated from a single cat in the treatment group. These results demonstrate that the combination of emodepside and praziquantel is highly effective not only against a wide variety of gastrointestinal nematodes and cestodes, but also against a nematode that resides in the lung tissue such as A. abstrusus. Spot-on treatment is a convenient application method, especially for cats. Oral application can become highly challenging in these patients and is even more problematic in sick animals that refuse food or exhibit respiratory problems, as with clinical aelurostrongylosis. Thus, the topical formulation Profender® for cats has major advantages in the treatment of feline aelurostrongylosis compared to oral formulations of fenbendazole.

In both studies it became evident at necropsy that cats in the control group were infected with a second lungworm species identified as Troglostrongylus sp. While adults of this species are clearly distinguishable from A. abstrusus, the L1 shed through faeces are extremely similar. In the past, nematodes of the genus Troglostrongylus have been considered to be associated only with wild felids (Di Cesare et al. 2015a) and prior to 2010, when the nematode was identified in cats from Ibiza, Spain (Jefferies et al. 2010), the parasitological community was not aware of Troglostrongylus spp. infestations in domestic cats. Various authors speculated that especially T. brevior infections might have been confused with A. abstrusus in the past due to the extremely similar morphological features of the L1 and the fact that diagnosis is routinely based on faecal examinations (Brianti et al. 2012, Otranto et al. 2013, Traversa and Di Cesare 2013). In fact, this is what has happened in the studies presented here. The contamination with Troglostrongylus sp. was not identified until necropsy. Although measures were taken in the second study to avoid this contamination by checking the inoculation material very carefully, some Troglostrongylus worms were again present in control animals.

There was a marked difference in clinical signs between the two studies. While in study no. 1 almost all cats showed signs of lung disease at least on one evaluation time point, cats in study no. 2 appeared to be healthy throughout the study. This difference was probably due to the lower challenge dose used...
in study no. 2. Hamilton (1967) infected cats with doses ranging from 50 to 3200 L3. While minor clinical signs were present in kittens that received low infective doses, cats receiving 800 and more L3 displayed pronounced clinical signs of lung disease. These findings correlated with severe and widespread alterations of the lung tissue. The basis for study no. 1 was an experimental infection by Schnyder et al. (2014) that used infective doses of 100 and 800 L3. As the differences in clinical and post mortem examinations between the infective doses were not as distinct as those described by Hamilton (1967), and an adequate infection rate was observed at the higher dose, it was decided to use 800 L3 in study no. 1. Due to the clinical signs observed in study no. 1, the infective dose was reduced in the second study.

Although there is no proof that emodepside/praziquantel-treated cats and the control groups were infected with *Troglostrongylus* sp. to a similar degree, it might be speculated that the treatment was also effective against this species. No *Troglostrongylus* nematodes were recovered from treated animals, and from a statistical point of view it seems unlikely that the treated groups were not infected while contemporaneously the control animals showed a *Troglostrongylus* worm burden. Recently, Profender® spot-on was used to treat two cats with mixed infections with *T. brevior* and *A. abstrusus* in the one case and *T. brevior* and *Capillaria aerophila* co-infection in the other (Di Cesare et al. 2015b). After one or two administrations (given two weeks apart) faeces were free of lungworm larvae/eggs and clinical signs resolved, providing further evidence of the anthelmintic efficacy of emodepside against *Troglostrongylus*.

It can be concluded that two treatments with Profender® spot-on at an interval of two weeks are highly efficacious against adult *A. abstrusus*. The treatment regime is safe and the ease of application of the topical formulation is advantageous, especially in cases where oral dosing is problematic.

**Ethical standards**

The study was performed in compliance with current national laws and regulations.

**Funding**

The study was funded by Bayer Animal Health GmbH, Germany.

**Conflict of interest**

At the time the studies were conducted, Claudia Böhm was employed by the Institute for Parasitology, University of Veterinary Medicine Hannover, and is currently an employee of Bayer Animal Health GmbH. Manuela Schnyder, Walter Basso and Peter Deplazes are employed by the Institute of Parasitology, University of Zurich. Katrin Deuster and Roland Schaper are employees of Bayer Animal Health GmbH. Sonja Wolken is a self-employed consultant. At the time the studies were conducted Angela Di Cesare held a PhD fellowship supported by Bayer Animal Health at the Faculty of Veterinary Medicine of Teramo, Italy.
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