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Efficacy of Emodepside/Toltrazuril Suspension (Procox® Oral Suspension for Dogs) against Mixed Experimental *Isospora felis*/ *Isospora rivolta* Infection in Cats

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Abstract

The coccidia *Isospora felis* and *Isospora rivolta* are intestinal parasites occurring worldwide in domestic cats. In young cats, they can be detected with higher prevalence.

The effects of toltrazuril in the new combination product Procox® oral suspension for dogs containing 0.1 % emodepside and 2 % toltrazuril (0.9 mg emodepside + 18 mg toltrazuril per ml) were studied in eighteen kittens experimentally infected each with a total of 1 x 10^5 oocysts of a mixture of *Isospora felis* and *Isospora rivolta*. In the infectious material, the quantitative relation of *I. felis* and *I. rivolta* was about 1:5. Following a three-days period after infection, two groups of 6 kittens were treated during the prepatent period with either a single dose of 0.45 mg emodepside + 9 mg toltrazuril/kg body weight or 0.9 mg emodepside + 18 mg toltrazuril/kg body weight. A group of six kittens without any treatment served as a control. On day 5 post infection, the untreated kittens started the excretion of oocysts. Treatment with both toltrazuril doses significantly reduced oocyst excretion. Following the single higher dose, the reduction of oocysts of both *Isospora* spp. was more pronounced (96.7 % to 100 %) in comparison to the lower dose (57.2 % to 100 %). The Procox® application was well tolerated and no adverse events were seen with any of the applied dosages.

When administered to kittens and as a single treatment during the prepatent period, Procox® is suitable to control the number of oocysts excreted in the faeces in case of an *Isospora felis* and *Isospora rivolta* infection.

Introduction

Domestic cats can be the hosts of a number of coccidia species representing various genera, but only *Toxoplasma gondii* and certain species of the genus *Isospora* are associated with clinical disease. The enteropathogenic coccidia of the cat are *Isospora* (*Cystoisospora*) *felis* (Wenyon 1923) and *Isospora* (*Cystoisospora*) *rivolta* (Grassi 1879). Prevalence rates from faecal screenings vary significantly depending on the *Isospora* species, the age group and the living conditions of the cats (Kirkpatrick 1987). Araujo (1996) detected *Isospora* oocysts in 10 % of feline faecal samples in Brazil. In Germany, Barutzki and Schaper (2003) found *Isospora* oocysts in 21.9 %, and Epe et al. (2004) in 10.7 % of feline faecal samples. Particularly heavy infections can occur in kittens kept in commercial breeding colonies when hygiene is poor and this may result in diarrhoea (Battersby and Harvey 2006). A comparison of the prevalence of gastrointestinal parasites from well-cared and animal shelter cats in Australia showed that *I. felis* was present in 1.7 % of the well-cared cats and in 10.2 % of the animal shelter cats (Palmer et al. 2008). Young kittens less than 6 months of age had higher rates of oocyst shedding (Lopez et al. 2006).

Pathogenicity of the two feline *Isospora* species is discussed controversely. There is agreement about the fact that most infections are mild or subclinical, especially in adult cats. However, in some cases, the parasitosis can be more severe, particularly when complicated by other disease-causing agents, or in very young kittens and in debilitated animals. In these cases, haemorrhagic enteritis, dehydration, anaemia, anorexia, weight loss and emesis can occur (Dubey 1993; Tzannes et al. 2008). In young kittens, clinical cases often manifest after the kitten has been moved, for instance, to a new home, suggesting that stress factors may be necessary for disease outbreak (Lindsay et al. 1997). In older cats, the infection can also occur when the cats are immunocompromised (Levine 1973).

There are two routes of infection. The oral ingestion of *Isospora* cysts in tissues of intermediate hosts will result in intestinal infection in cats. The oral ingestion of sporulated (but not unsporulated) *Isospora* oocysts by cats from faeces of other cats will also establish infection. There is no evidence for congenital transmission, but kittens may become infected early in life from faecal shedding by lactating queens (Dubey 1977).

Isospora rivolta (*I. rivolta*) develops in the enterocytes of the small intestine (Dubey 1979). Under experimental conditions, *I. rivolta* caused disease in newborn kittens (Dubey 1979). Diarrhoea occurred 3 – 4 days after administration of 1 x 10^5 or 1 x 10^6 sporulated oocysts. No disease was observed in 10- to 13-weeks-old kittens inoculated with up to 10^5 oocysts (Dubey 1979). Cats developed immunity to infection, but it was not complete as some oocysts were shed after challenge (Dubey 1979).

Isospora felis (*I. felis*) develops in the enterocytes of the small intestine and occasionally in the caecum (Shah 1971). Experimental studies indicate that *I. felis* is non-pathogenic for cats over 1 month of age (Shah 1971). Few signs of disease were seen in 6- to 13-weeks-old kittens when given 1 x 10^5 to 1.5 x 10^5 oocysts. Four-weeks-old kittens seemed to be the most susceptible, as enteritis and emaciation occurred after inoculation of 1 x 10^5 oocysts (Andrews 1926). A comparison of feline *Isospora* species is shown in Tab. 1.

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 (*Toxocara canis*, *Uncinaria stenocephala*, *Ancylostoma caninum*) and *Isospora (Isospora canis*, *Isospora ohioensis*-complex).

Beside the indications listed for the combination product Procox®, the coccidiocidal active toltrazuril has been proven to be effective against *Eimeria* infections in poultry and, more recently, against coccidiosis in sheep, rabbits, piglets and calves (Haberkorn 1996; Mundt et al. 2006; Gjerde et al.

Tab. 1 Comparison of the life cycles of feline *Isospora* species and description of oocysts (Shah 1971; Dubey 1979, Lindsay 1990)

	I. rivolta **(Grassi 1879)**	*I. felis* **(Wenyon 1923)**
Location	Small intestine (jejunum, ileum)	Small intestine (ileum) and caecum
Site	Enterocytes	Enterocytes
Prepatent period	4 to 7 days	7 to 11 days
Patent period	> 14 (to 23) days	10 to 11 days
Number of asexual types (schizont generations)	3	3
Peak oocyst production	*	6th day of patent period
Oocyst description (size µm)	20–30 x 21–27	39–48 x 23–27
Sporocyst description (size µm)	12–16 x 9–12	20–27 x 17–22

* no literature found

2009). For these animal species, toltrazuril is available as oral solution to be mixed with drinking water or oral suspension for direct oral application under the brand Baycox®.

Besides, there have been some reports that toltrazuril reduces *Isospora* species oocyst counts in man and cats (Kayembe 1989; Cieslicki et al. 1993; Lloyd and Smith 2001; O'Brien 2002). Both, Lloyd and Smith (2001) and O'Brien (2002) used treatment with toltrazuril (Baycox®) against natural *Isosopora* infections in kittens suffering from vomiting and diarrhoea as an empiric therapy. In both cases, rapid improvement of clinical signs and suppression of oocyst excretion was observed using different dose regimens and treatment durations.

The availability of the new combination product for puppies and the successful treatment described by Lloyd and Smith (2001) and O'Brien (2002) was a justification to test Procox® under experimental conditions in kittens against the two enteropathogenic cat coccidia *Isospora felis* and *Isospora rivolta*, respectively.

Materials and methods

Study design

This study was performed as a negative controlled, randomised and non-blinded efficacy study against mixed prepatent infections with *I. felis* and *I. rivolta*, and it was conducted as a dose determination study. The study design is summarised in Tab. 2.

Study animals

The kittens used in this study were purpose-bred animals that had not been treated previously with anthelmintic or anticoocidial drugs. Eighteen kittens were acclimatised to the study facility for at least 7 days prior to the start of the study. They were identified by ear tattoo number. At study start kittens were 12 weeks of age, and the body weight ranged between 1.0 and 1.3 kg. Until the 3rd day post infection (dpi), the kittens were housed in groups of two kittens each, and thereafter kittens were housed separately for the subsequent study period. The kittens were fed with commercially available dry kitten food once daily and water was available *ad libitum*.

General requirements for study inclusion were good health as determined by a veterinary examination

before study start and repeated negative faecal oocyst counts (FOC) for *Isospora felis* and *Isospora rivolta* before experimental infection.

Clinical observations

The health status of all kittens was assessed once before treatment and 2, 4 and 24 hours after treatment, and thereafter once daily. The kittens were evaluated for general attitude and behaviour, and faecal consistency was assessed.

Experimental infection

All kittens were infected orally with sporulated oocysts from *Isospora felis* and *Isospora rivolta* at an 1:5 ratio. The oocysts had been derived from an isolate originally obtained from naturally infected cats from an animal shelter in North Rhine-Westphalia. The isolate was purified and then stored in 2 % potassium dichromate until the day of infection. Oocysts were seven weeks old. At the day of infection, the oocysts were concentrated and washed by repeated centrifugation and controlled for sporulation. On day 0, individual inocula of 1 x 10^5 oocysts were prepared and administered to each of the eighteen kittens as a single oral dose.

Treatment

At day 0, the kittens were ranked according to their body weights and they were randomly allocated to the groups. At 3rd dpi, kittens of group 1 and 2 were treated with Procox® oral suspension for dogs at a dosage of 0.5 ml/kg body weight (group 1) and 1.0 ml/kg body weight (group 2) corresponding to 0.45 mg emodepside + 9.0 mg toltrazuril per kg body weight and 0.9 mg emodepside + 18.0 mg toltrazuril per kg body weight, respectively. Kittens of the control group were left untreated. The individual doses were based on the body weights taken on the day of treatment. The single treatment was performed orally. The kittens were observed after dosing to determine whether any suspension was regurgitated.

Parasitological techniques

Before the start of the experimental infection, FOC were performed on grouped faecal samples from two kittens each to determine whether natural *Isospora* infections were present (day –11 to day –6). On day 0, a faecal sample was collected from each individual kitten before experimental infection. From 5th dpi onwards, individual FOC were performed daily to detect the possible beginning of patency. FOC were conducted using a modified McMaster method (Schmidt 1971; Wetzel 1951). Adequacy of *Isospora* infection in the control group was assessed per study day. The control group was considered to be adequately infected on days with positive oocysts counts in all 6 control animals. Results are presented as number of oocysts per gram faeces (OPG).

Efficacy calculation

The criterion for effectiveness of the Procox® oral suspension for dogs was the post treatment OPG of *I. felis*/*I. rivolta* of each treatment group vs. the

Tab. 2 Study design of the negative controlled study on the efficacy of emodepside (E) plus toltrazuril (T) oral suspension against prepatent *Isospora felis*/*Isospora rivolta* infection (dpi: day post infection)

Breed	Age of cats	Body weight (kg) (one day before treatment)	Number of cats per group	Treatment dose (mg/kg b.w.)	Infection	Origin of isolate (age of isolate)	Treatment day	Faecal oocyst counts post treatment
Domestic shorthair	12 weeks	1.0–1.3	6	E: 0.45 / T: 9.0	Experimental (1 x 10^5 sporulated oocysts)	Animal shelter North Rhine-Westphalia, Germany (7 weeks)	3 dpi	5–14 dpi
			6	E: 0.9 / T: 18.0				
			6	Untreated control				

untreated control group. The efficacy of the treatment as seen by a reduction of OPG was evaluated from 5th dpi onwards for each day in accordance to the following formula:

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

N1: geometric mean OPG of *I. felis*/*I. rivolta* for the treated group (group 1 and 2)

N2: geometric mean OPG of *I. felis*/*I. rivolta* for the untreated control group (group 3)

Statistical significant differences between arithmetic mean number of excreted oocysts of the treatment groups and of the control were examined by One-way ANOVA with Bonferroni's Multiple Comparison post test using the programme GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

Repeated faecal examinations showing negative OPG before experimental infection confirmed the absence of natural *Isospora* infections in all kittens. The success of the experimental infection with *Isospora* oocysts was demonstrated by considerable numbers of both *I. felis* and *I. rivolta* oocysts in faecal samples from all six kittens of the untreated control, starting 6 dpi until 14 dpi with two peak oocyst excretions on days 7/8 and day 12 and declining OPG on 14 dpi.

Treatment with the single dose 9.0 mg toltrazuril/kg body weight in group 1 significantly reduced oocyst counts from day 6–8 dpi and day 12–13 dpi ($p < 0.001$) (> 99 % efficacy). Oocyst counts were markedly but not significantly reduced in group 1 on day 9 and 10 dpi (Fig. 1). Oocyst count reduction from the single 18.0 mg toltrazuril/kg body weight dose in group 2 showed significant differences (from $p < 0.01$ to $p < 0.001$) from day 7 dpi until day 13 dpi (> 99 % efficacy) (Tab. 3 and Fig. 1). As the OPG declined on day 14 dpi in the control cats, no significant differences in the oocyst excretion between treated and untreated cats could be detected on this day.

Although a successful infection could be established, no clinical signs of coccidiosis like diarrhoea or emesis were observed in any of the kittens during the observation period. The Procox® oral suspension was highly palatable and well accepted by the kittens. No regurgitation was observed. In both dose groups, no adverse reactions due to Procox® treatment were observed during the post treatment interval.

Discussion

The present study demonstrated that a single oral administration of 1 x 10^5 *I. felis*/*I. rivolta* oocysts in 12-weeks-old kittens resulted in a successful infection with distinct oocyst shedding in all kittens of the untreated control group starting on 6th dpi. This is consistent with data from the literature

Tab. 3 Efficacy of Procox® oral suspension for dogs against experimental prepatent *I. felis*/*I. rivolta* infection in kittens

Study group	% efficacy									
	5 dpi	6 dpi	7 dpi	8 dpi	9 dpi	10 dpi	11 dpi	12 dpi	13 dpi	14 dpi
1 0.45 mg emodepside + 9.0 mg toltrazuril/kg b.w.	100	99.8	99.8	99.8	82.4	64.0	69.2	98.8	98.1	57.2
2 0.9 mg emodepside + 18.0 mg toltrazuril/kg b.w.	100	100	100	100	100	99.9	100	99.3	99.8	96.7

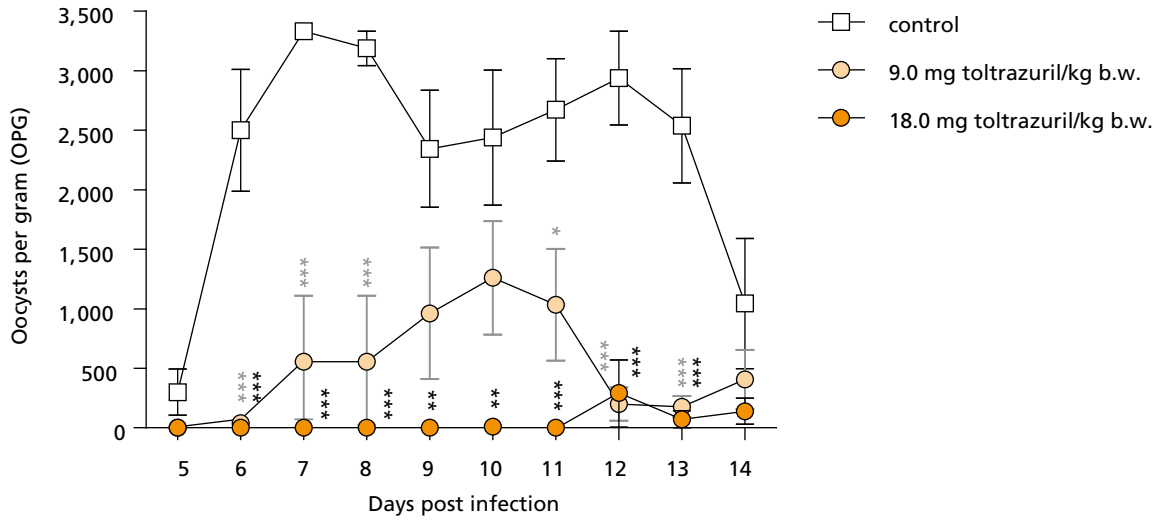

Fig. 1 *I. felis*/*I. rivolta* oocyst counts of experimentally infected kittens treated with two different doses of Procox® oral suspension for dogs. Data are mean (± SEM) OPG at the indicated dpi. Statistical significant differences between the treatment groups and the control are demonstrated by One-way ANOVA with Bonferroni's Multiple Comparison post test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

stating a prepatent period of 4–7 days for *I. rivolta* and 7–11 days for *I. felis*.

This study also proved that kittens with *Isospora felis*/*Isospora rivolta* infections usually manifest no clinical signs of coccidiosis, such as diarrhoea, vomiting, lethargy or weight loss as in none of the kittens of the control group any abnormal clinical signs were seen. As described in studies with *Isospora suis* infections in pigs where oocyst shedding was the most reliable objective variable of infection (Mundt et al. 2006), faecal oocyst counts were therefore chosen to be the primary criterion for efficacy in this study.

Re-infections and development of immunity have a great influence on the course of oocyst shedding. In order to evaluate efficacy of a product, the choice of length of the study period between the treatment day and the end of the examination period is of decisive importance, as with increasing duration of the examination period the distinction between treatment response and development of immunity or re-infections becomes increasingly difficult. Based on this experience a study design with a 14 days observation period was chosen to minimise the effects of these interference factors.

Starting at 6 dpi, both toltrazuril doses induced a rapid reduction in oocyst excretion. However, a more pronounced suppression of oocyst excretion was observed in the higher toltrazuril dose group. The Procox® application was well tolerated and no adverse events were seen with any of the applied dosages.

In cases with suspected or confirmed coccidiosis in cats and especially in very young kittens, Procox® will be a suitable treatment during the prepatent period by reducing the contamination of litters with *Isospora* oocysts. Especially in animal shelters or large catteries with a high *Isospora* infection pressure, this will be an effective measure to reduce or even to prevent problems due to intestinal coccidiosis, as it has already been demonstrated for puppies (Daugschies et al. 2000).

It has been clearly shown that Procox® oral suspension for dogs is effective against *Isospora felis*/*Isosopora rivolta* infections in kittens under experimental conditions.

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

Compliance statement

All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations. The study was conducted in accordance with the standards of Good Scientific Practice studies and followed the requirements given in the VICH guideline 7 "Efficacy requirements for anthelmintics: General requirements" (December 2000). 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).

Disclosure statement

The study was sponsored by Bayer Animal Health GmbH, all authors were employed by Bayer Animal Health GmbH, Germany, during the conduct of this study.

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