Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 145 (2007) 94–99
veterinary parasitology
www.elsevier.com/locate/vetpar

Field evaluation of the efficacy and the safety of a combination of oxantel/pyrantel/praziquantel in the treatment of naturally acquired gastrointestinal nematode and/or cestode infestations in dogs in Europe

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Received 22 August 2006; received in revised form 13 November 2006; accepted 22 November 2006

Abstract

In five multicentre field trials, the efficacy and safety of a combination of oxantel/pyrantel/praziquantel (Dolpac®, Vetoquinol SA) in the treatment of naturally acquired gastrointestinal nematode and/or cestode infestation in dogs was evaluated in northern and southern Europe.

Forty-eight investigators from France, Belgium, Germany, Italy and Spain enrolled 329 dogs to be treated with the tested combination; 235 of these dogs complied with the inclusion criteria of the protocol and had a tested helminth identified on Day 0. A pooled analysis was performed on each of the following helminth species: *Toxocara canis*, *Ancylostoma caninum*, *Toxascaris leonina*, *Trichuris vulpis*, *Uncinaria stenocephala*, *Taenia* spp. and *Dipylidium caninum*, which were isolated on Day 0.

The main efficacy criterion was the egg per gram (epg) percent reduction of the nematodes and the absence of proglottids and or eggs for the cestodes. After treatment, dogs were examined on Day 7, Day 14 and Day 21. The efficacy of the combination against *Toxocara canis* was 99.1%, 98.8% and 98.9% on Day 7, Day 14 and Day 21, respectively. At the same occasions the efficacy was, respectively, 99.2%, 99.2% and 99.3% against *Ancylostoma caninum,* 97.3%, 97.2% and 98.4% against *Trichuris vulpis,* 98.4%, 98.8% and 98.8% against *Uncinaria stenocephala*, 98.9%, 99.5% and 99.9% against *Toxascaris leonina*, 97.1%, 100% and 100% against *Dipylidium caninum* and 100% against *Taenia* spp.

Keywords: Oxantel/pyrantel/praziquantel combination; Clinical trial; Dogs; Efficacy; Intestinal helminths

1. Introduction

Infections with intestinal helminths are common in dogs in Europe. *Toxocara canis* is the most frequently encountered, mainly in puppies, with prevalences ranging 3–30%. *Toxascaris leonina* is found less frequently, with 0.5–10% positive dogs. The prevalence of the whipworm, *Trichuris vulpis*, is highly variable

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0304-4017/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2006.11.013

(0–47.6%), with higher values in kennel dogs and adult dogs compared to household dogs and young dogs. The hookworm *Uncinaria stenocephala* is more commonly found in stray dogs and shelter dogs (2.2–34.7%) than in household dogs (0–13.1%). *Ancylostoma caninum* is only present in southern Europe. Only low prevalences are found for the tapeworms *Dipylidium caninum* and *Taenia* spp. (0–8%) (Vanparijs and Thienpont, 1973; Haralabidis et al., 1988; Vanparijs et al., 1991; Deplazes et al., 1995; Causapé et al., 1996; Franc et al., 1997; Overgaauw, 1997; Beugnet et al., 2000; Fok et al., 2001; Barutzki and Schaper, 2003; Habluetzel et al., 2003; Le Nobel et al., 2004; Epe et al., 2004).

Anthelmintics or combinations of anthelmintics with a broad spectrum of activity are relevant to treat the polyparasitism currently encountered in dogs in the field. The anthelmintic product (Dolpac®) tested in this study is a combination of pyrantel pamoate, oxantel pamoate, and praziquantel. It is a broad spectrum anthelmintic for oral use in dogs. Pyrantel is an L-type cholinergic agonist with good anthelmintic activity against ascarids and hookworms (Cadiergues and Franc, 1994), but no activity against whipworms. Oxantel is a *meta*-oxphenyl derivative of pyrantel (N-type cholinergic agonist, Martin et al., 2004) that was first introduced for the treatment of whipworms in children. It is also highly potent for the treatment of *Trichuris muris* in mice (Rajasekariah et al., 1991). Praziquantel is a pyrazinoisoquinoline that is currently the drug of choice for the treatment of a wide range of cestode infections (Day et al., 1992). Anthelmintic products combining pyrantel, oxantel and praziquantel are available in several countries for use in dogs (e.g. New-Zealand, Australia, Chile), but are not commonly used in Europe (except for Italy). Thus, in order to assess the efficacy of the combination in field conditions in Europe, a study with the same experimental design was carried out in five European countries. The primary objective was to evaluate in the field the efficacy and safety of such a combination in the treatment of nematode (*A. caninum*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *U. stenocephala*) and/or cestode (*D. caninum*, *Taenia* sp.,) infections in dogs.

2. Materials and methods

2.1. Experimental design

The experimental design was a multicentre field study. Overall five studies, one for each country, were carried out in Belgium, France, Germany, Italy and Spain. In each country, one reference laboratory was elected to carry out the faecal examinations for the inclusion of dogs into the study and for its follow up after treatment. All studies were conducted in compliance with the Committee for Veterinary Medical Products European Union Note for Guidance "Good Clinical Practice for Conduct of Clinical Trials For Veterinary Medical Products" (July 2001). Informed consent was obtained from the animals' owners before enrolment.

2.2. Selection of animals

Owned dogs of any breed and sex, aged more than 2 months and suspected to have an intestinal helmintic infection were selected for the trials. Pregnant bitches (in the first 4 weeks of pregnancy), dogs weighing less than 1 kg, dogs with a concurrent disease or pre-existing surgical conditions, dogs treated with anthelmintic compounds within the 60 previous days (except preventive products effective against heartworm larval stages only) and dogs with a history of idiosyncrasy to one or more of the compounds used in the study were not included. The presence of intestinal parasites was first confirmed by macroscopic/microscopic faecal examinations carried out by the veterinarians participating in the study or throughout a pre-inclusion analysis carried out by the reference laboratory. Dogs were included into the studies only after a definitive diagnosis of intestinal helminth infection throughout quali-quantitative faecal examinations carried out by the reference laboratory in charge for the faecal analyses in each country.

2.3. Treatment

Three formulations of the test drug were available (divisible in half tablets corresponding to 2 kg b.w., 10 kg b.w., or 25 kg b.w.) to treat the dogs according to their body weight allowing a mean dosage of 20 mg/kg of oxantel, 5 mg/kg of pyrantel and 5 mg/kg of praziquantel). The same formulations (Dolpac®, Vetoquinol SA, batch numbers: 010604A, 01064B, 010604C) were used for all the studies. The tested products were under evaluation and were not launched at the time of the study.

2.4. Efficacy and safety assessment and faecal analyses

The main efficacy criterion was the percent reduction of the faecal egg counts per gram (epg) for the nematodes and the presence/absence of proglottids/eggs for the cestodes after treatment. When the efficacy was

lower than 90% (nematodes) or when cestode proglottids/eggs were present in a given dog at a given time, it was considered as a treatment failure.

Dogs were clinically evaluated and their faeces sampled on Day 0 (inclusion visit), Day 7 (first follow-up visit), Day 14 (second follow-up visit) and Day 21 (final visit).

The epg counts were carried out by the reference laboratories using the same MacMaster method. Briefly, 4 g of faeces were suspended in 60 ml of saturated NaCl water solution (density: 1.2). The suspension was used to fill the MacMaster cells (sensitivity = 25 epg for four counted compartments and 50 for two counted compartments). When no eggs were found using the MacMaster slide, a direct faecal flotation using the prepared suspension was carried out to confirm the negative results. A test-tube of 10 ml was filled with the faecal suspension and centrifuged at low speed ($\leq 1500 \times g$). The surface of the suspension was gently collected and dropped on a glass slide and covered with a 20 mm × 20 mm cover glass. When parasite eggs were found as a consequence of the higher sensitivity of the direct flotation, the eggs under the cover glass were counted and these counts were used for the statistical analysis. Differentiation of hookworm eggs was done by morphological features (Thienpont et al., 1979).

The adverse events observed throughout the study were reported and analyzed.

2.5. Statistical analysis

The egg counts from all the studies were pooled and analyzed together. The percentage of epg reduction at each study time with respect to the counts on Day 0 was calculated after a natural log transformation of epg [ln(x + 1)]. Geometric mean (GM) egg counts for each species and for each study time were estimated from least squares means of the ln(epg count + 1). GM were used to estimate percent reduction in epg counts after treatment: % reduction = [epg GM on Day 0 − epg GM on day n (where n is the different times of the study)/epg GM on Day 0] × 100. The results were analyzed by a non-parametric paired test (Wilcoxon-signed rank test) to evaluate the statistical value of the epg count reduction after treatment (the significance level was set at 5%). When for a given dog a faecal sample was missed on a given day after treatment, the egg reduction was estimated on this day using the GM of Day 0 calculated without the egg count of this given dog in order to have the same sample size.

As the efficacy against the cestodes was assessed as presence/absence, no statistical analysis was performed.

Table 1
Number and percentage of dogs infected with intestinal helminths at inclusion in the trial (Day 0)

Parasite species	Number of dogs	Percentage of positive dogs
Ancylostoma caninum	14	6
Toxocara canis	61	26
Trichuris vulpis	56	24
Uncinaria stenocephala	17	7
Dipylidium caninum	27	11.5
Taenia spp.	7	3
Ancylostoma caninum, *Trichuris vulpis*	4	1.7
Ancylostoma caninum, *Uncinaria stenocephala*	1	0.4
Ancylostoma caninum, *Dipylidium caninum*	3	1.3
Toxocara canis, *Ancylostoma caninum*	3	1.3
Toxocara canis, *Toxascaris leonina*	2	0.9
Toxocara canis, *Trichuris vulpis*	14	6
Toxocara canis, *Uncinaria stenocephala*	1	0.4
Toxocara canis, *Dipylidium caninum*	2	0.9
Trichuris vulpis, *Taenia* spp.	1	0.4
Trichuris vulpis, *Uncinaria stenocephala*	13	5.5
Dipylidium caninum, *Taenia* spp.	1	0.4
Ancylostoma caninum, *Trichuris vulpis*, *Dipylidium caninum*	1	0.4
Toxocara canis, *Trichuris vulpis*, *Ancylostoma caninum*	2	0.9
Toxocara canis, *Trichuris vulpis*, *Toxascaris leonina*	1	0.4
Toxocara canis, *Toxascaris leonina*, *Uncinaria stenocephala*	1	0.4
Toxocara canis, *Trichuris vulpis*, *Uncinaria stenocephala*	1	0.4
Trichuris vulpis, *Uncinaria stenocephala*, *Taenia* spp.	2	0.9

Table 2
Geometric mean (GM), range and percentage reduction of faecal egg counts (epg) of nematode parasites in 235 dogs treated with a combination of pyrantel pamoate, oxantel pamoate and praziquantel

Parasite (infected dogs)		Day 0	Day 7	Day 14	Day 21
Ancylostoma caninum (28)	epg GM	173.1	1.4	1.3	1.2
	Range	1–25,000	0–50	0–50	0–150
	% reduction		99.2	99.2	99.3
Toxocara canis (88)	epg GM	422.9	4	5.3	4.65
	Range	1–85,150	0–7,150	0–5,100	0–4,400
	% reduction		99.1	98.8	98.9
Toxascaris leonina (4)	epg GM	882.2	10.1	4.1	0
	Range	175–3,350	0–200	0–275	0–0
	% reduction		98.9	99.5	99.9
Trichuris vulpis (95)	epg GM	207.9	5.6	6	3.3
	Range	1–105,000	0–700	0–2,500	0–600
	% reduction		97.3	97.2	98.4
Uncinaria stenocephala (36)	epg GM	151.1	2.5	1.8	1.8
	Range	1–3,600	0–250	0–300	0–350
	% reduction		98.4	98.8	98.8

3. Results

Overall, 20 veterinary investigators in France, 4 in Belgium, 8 in Germany, 11 in Italy and 5 in Spain enrolled 329 dogs. Two hundred and thirty-five dogs complied with the inclusion criteria and intestinal helminth infections were identified on D0.

Animals of different sexes (male, neutered male, female, spayed female) and breeds with a large range of weight (1.4–68.7 kg b.w., arithmetic mean of 20 kg, median 18 kg) and age (aged 2 months to 15 years, arithmetic mean of 3.5 years, median 2 years) were included in the studies. Four dogs had clinical signs of infection at inclusion (diarrhoea) and 22 were sharing kennels with untreated dogs throughout the study.

Out of the 235 positive dogs, 182 dogs (77%) had a single species infection, 45 were infected (19%) by 2 worm species and 8 by 3 worm species (4%) (Table 1). Thus, on Day 0, of 235 dogs 95 were positive to *Trichuris vulpis* (40.4%) and 88 to *Toxocara canis* (37.4%) as a single infection or in association with other parasites. *Toxocara canis* was found as a single infection in 61 dogs (26%) and *Trichuris vulpis* in 56 dogs (24%), respectively. *U. stenocephala* and *A. caninum* were found in 36 (15%) and 28 (12%) dogs, respectively. The prevalence of hookworm single infections accounted 7% for *U. stenocephala* and 6% for *A. caninum* (Table 1). *Toxascaris leonina* was found only in four dogs (2%) and always associated with other parasites. Proglottids of *D. caninum* were found in 34 dogs (15%); in 27 dogs the parasite was found as a single infection (11.5%). Proglottids of *Taenia* spp. were found in 11 dogs (5%); in 7 dogs the parasite was found as a single infection (3%).

Overall, the epg counts decreased >97% compared with Day 0 on each post-treatment sampling for the nematodes *Toxocara canis*, *A. caninum*, *Toxascaris leonina*, *Trichuris vulpis*, and *U. stenocephala* ($p < 0.0001$, Table 2). Similarly, the number of dogs positive for proglottids/eggs of *D. caninum* and/or *Taenia* spp. was reduced more than 97% on each faecal examination after treatment (Table 3).

Individual treatment failures (i.e. egg count reduction <90%) are summarized in Table 4. Most treatment failures were found for *Toxocara canis* and *Trichuris vulpis*. Twelve failures were observed for *Toxocara canis* on D7 and occurred in very young dogs or dogs presenting diarrhoea at inclusion. Four and five of these dogs, showed an efficacy >90% on day 14 and 21, respectively, while six and seven new failure cases were

Table 3
Number of dogs positive at faecal examinations for proglottids of cestodes before (day 0) and after (days 7, 14 and 21) treatment with a combination of pyrantel pamoate, oxantel pamoate and praziquantel and percentage reduction of infected dogs after treatment

Parasite	Day 0	Day 7	Day 14	Day 21
Dipylidium caninum				
Infected dogs	34	1	0	0
% reduction		97.1	100	100
Taenia spp.				
Infected dogs	11	0	0	0
% reduction		100	100	100

Table 4
Number of individual failures (epg counts reduction <90%) including number of new failures at the different times of the study in bold

Parasite	Day 7	Day 14	Day 21
Ancylostoma caninum	1	2 (**1**)	1 (**1**)
Toxocara canis	12	14 (**6**)	14 (**7**)
Toxascaris leonina	1	0	0
Trichuris vulpis	22	25 (**13**)	18 (**7**)
Uncinaria stenocephala	5	4 (**1**)	2 (**1**)

found at the same times. Twenty-two failures were observed on Day 7 for *Trichuris vulpis*. Twelve and 11 of these dogs showed an efficacy >90% on Day 14 and 21, respectively, while 13 and 7 new failure cases were found at the same times. Only a few cases were found for *A. caninum* and *U. stenocephala*.

Throughout the studies, veterinarians notified eight adverse reactions. Overall, the frequency of suspected adverse reaction was 2.4%. The reactions were diarrhoea, vomiting and one case of transient anorexia and reduced general health condition. All the observed reactions were transient and mild and dogs recovered spontaneously.

4. Discussion and conclusion

The results clearly show that the parasitism found in dogs included in the different trials was representative of worm infection in dogs in Europe. All the most frequent helminth species were found with prevalences and epg counts consistent with previous findings including frequent isolation of potential zoonotic worms, especially, *Toxocara canis*. *Trichuris vulpis* and *Toxocara canis* alone or in association with other helminth species were the most frequent parasites found at inclusion into the studies. Furthermore, in some case the number of eggs shed with faeces was very high (see *Toxocara canis* and *Trichuris vulpis* epg ranges on Day 0, Table 2), confirming the high risk of environmental contamination by faeces of infected dogs, untreated against intestinal parasites. It is also interesting to note that about 23% of dogs were infected by two or three worm species, showing that polyparasitism is quite common in field conditions. The anthelmintic efficacy of the combination of pyrantel pamoate, oxantel pamoate and praziquantel was close to 99% against all the tested parasites at each study day, except for *Trichuris vulpis*, where the efficacy was 97–98%.

Although faecal egg counts from different methods were pooled to calculate the anthelmintic efficacy, differences in sensitivity mainly affect low egg counts and probably had a minor influence on the results.

Although the overall epg reduction was very high, some treatment failures were observed throughout the study. Most *Toxocara canis* failures on Day 7 were found in very young dogs (2-month-old) and in dogs with diarrhoea. In these dogs, migrating larvae can be present, which are not affected by the pyrantel pamoate. Furthermore, diarrhoea can shorten the intestinal transit time, affecting the efficacy of the anthelmintic compounds. Most *Trichuris* failures were observed in dogs recovered in kennels together with untreated dogs. In some dogs, the failures were observed on days 14 and 21 and not on day 7 post-treatment. Pyrantel and oxantel have rather a limited efficacy against immature stages, therefore, after treatment some larvae may develop to adult female worms and excrete eggs. Recently, Sager et al. (2006) have shown that coprophagia should be an important factor interpreting parasitological analyses. In our studies, it is possible that immature, ingested eggs may have contributed to the egg counts. Moreover, residual eggs may have induced a bias. For both *A. caninum* and *U. stenocephala*, the treatment failures could be a consequence of the short prepatent period (2–3 weeks) and the faecal eggs present on Day 21 might be due to re-infections after treatment. No obvious association was observed between multiple infections or infection level and treatment failure. Furthermore, regurgitation of tablets by the dog could occur without owners and investigators' knowledge.

The pyrantel pamoate, oxantel pamoate and praziquantel combination tablets were always well accepted by dogs. The frequency of side effects was low and they were already described in the literature for pyrantel and praziquantel (Shmidl et al., 1981; Plumb, 1995).

Based on these results, it may be concluded that the tested anthelmintic combination for dogs had shown a satisfactory safety and efficacy, and is convenient for the treatment and prevention of helminth infestation in dogs.

Acknowledgements

The authors would like to thank the different participants of the study (veterinary surgeons and their staff, laboratories, dog's owners) and Professor Vercruysse for his advices.

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