Field Evaluations of the Efficacy and Safety of Emodepside plus Toltrazuril (Procox® Oral Suspension for Dogs) against Naturally Acquired Nematode and *Isospora* spp. Infections in Dogs

Gertraut Altreuther[1] (✉), Nadine Gasda[1,2], Kerstin Adler[2], Klaus Hellmann[2], Heloise Thurieau[3], Annette Schimmel[1], Douglas Hutchens[1], Klemens J. Krieger[1]

[1] Bayer Animal Health GmbH, Leverkusen, Germany
[2] Klifovet AG, Munich, Germany (Gasda: present address)
[3] Bayer Santé Division Santé Animale, Puteaux, France

✉ E-mail: gertraut.altreuther@bayer.com

Abstract

Three controlled, blinded and randomised multicentre field studies evaluated the efficacy and safety of a new formulation containing emodepside plus toltrazuril (Procox® suspension for dogs) against naturally acquired parasite infections in dogs. In two studies dogs positive for gastrointestinal nematodes and/or *Isospora* spp. were treated with emodepside/toltrazuril suspension (at least 0.45 mg emodepside plus 9 mg toltrazuril per kg body weight) or a reference product containing either milbemycin oxime plus praziquantel (Milbemax®) or sulfadimethoxine (Kokzidiol SD®) at recommended dose rates. The third study investigated efficacy against prepatent natural *Isospora* spp. infections in comparison to an untreated control group by enrolling *Isospora*-negative dogs that were at risk to develop a patent infection during the study.

No suspected adverse drug reactions were observed in any of the 403 dogs enrolled in the three studies including 234 dogs treated with emodepside/toltrazuril suspension. In dogs treated with emodepside/toltrazuril suspension against nematode infection faecal egg counts were reduced by 100 % (reference product: 99.7 %). Similarly, in the dogs that had been treated against patent *Isospora* spp. infection, faecal oocyst counts were reduced by 100 % (reference product: 99.0 %). In both studies, statistical analysis demonstrated non-inferiority and even superiority to the reference products ($p \leq 0.009$). Dogs treated with emodepside/toltrazuril suspension during suspected prepatent *Isospora* spp. infection had 98.7 % lower faecal oocyst counts after treatment compared to untreated dogs ($p < 0.0001$).

The studies demonstrated that emodepside/toltrazuril suspension is safe and highly efficacious against nematodes and *Isospora* spp. under field conditions.

Introduction

Emodepside plus toltrazuril suspension (Procox® suspension for dogs) has been developed as a combined nematocide and coccidiocide for dogs. Especially young dogs and puppies are at risk of a mixed infection of nematodes, in particular *Toxocara canis*, and coccidia of the genus *Isospora*. Even though serious complications are not common, both parasites can be clinically relevant, predominantly as causes of enteritis and thus of diarrhoea. Milder infections may still cause subclinical mucosal damage which can prepare the way for other enteropathogenic agents, e.g. bacterial infections, and may have a significant impact on the further development of the dog (Daugschies et al. 2000; Eckert et al. 2008). Therefore, concurrent treatment against nematodes and coccidia may be indicated where a mixed infection is suspected or demonstrated.

Emodepside plus toltrazuril suspension contains emodepside for its nematocidal effect while toltrazuril acts as a coccidiocide. The efficacy of the suspension against nematodes (*Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*) and *Isospora* spp. (*I. canis*, *I. ohioensis*-complex: i.e. *Isospora ohioensis*, *Isospora burrowsi*, *Isospora neorivolta*) has been demonstrated in a series of laboratory dose confirmation studies (Altreuther et al. 2011; Schimmel et al. 2011).

This paper reports the results of three controlled, blinded and randomised multicentre clinical field studies that evaluated the efficacy and safety of emodepside plus toltrazuril suspension at the recommended dose range in the treatment of naturally acquired gastrointestinal nematode and *Isospora* spp. infections in dogs compared to currently licensed reference products as well as the efficacy against prepatent natural *Isospora* spp. infections in comparison to an untreated control group.

Materials and methods

Study design

The three studies were conducted according to the VICH guidelines 9 ("Good Clinical Practice", July 2000), 7 ("Efficacy requirements for anthelmintics: overall guidelines", December 2000) and 19 ("Efficacy of anthelmintics: specific recommendations for canines", July 2001) and the WAAVP guideline for evaluating the efficacy of anthelmintics in dogs and cats (Jacobs et al. 1994) as applicable.

The first two studies were conducted in parallel to evaluate the efficacy against patent nematode (study no. 1) or *Isospora* spp. infection (study no. 2) while the third study evaluated the efficacy against prepatent *Isospora* spp. infection. The different study designs are summarised in Tab. 1.

In studies no. 1 and 2 dogs that had been positive for nematode and/or *Isospora* spp. infection at an initial screen at the veterinary practice were enrolled and randomly allocated to the treatment or the control group in a ratio of ~ 2:1. Prior to treatment, a faecal sample was taken for quantitative analysis at a diagnostic laboratory. Post treatment, faecal samples were taken to monitor the course of the faecal nematode egg or *Isospora* spp. oocyst counts. In study no. 1 two faecal samples, of different defaecations, were collected 7 to 13 days post treatment, and in study no. 2 three faecal samples were taken 3, 7 and 8 to 10 days post treatment.

In study no. 3 dogs to be enrolled were required to be negative for *Isospora* spp. but considered to be at risk to develop a patent infection during the study period. For this purpose *Isospora*-negative litters or housing groups were enrolled at breeders with a known history of *Isospora* spp. infection. Within the litters/housing groups the puppies were randomly allocated to the treatment or untreated control group in a ratio of ~ 1:1. Three faecal samples were taken during the post treatment period 3, 5 and 6 to 8 days after treatment of the dogs in the treatment group. Only the data of litters/housing groups where at least one of the control dogs had turned positive during the study were included in the evaluation of efficacy.

Tab. 1 Study design of three studies evaluating efficacy and safety of emodepside plus toltrazuril suspension in dogs under field conditions

Study no.	Parasite infection	Treatment	Minimum dose (per kg body weight)	Dose route and frequency	Day of treatment	Faecal samples taken		Veterinary examination
						pre treatment	post treatment	
1	Patent nematode infection	Emodepside plus toltrazuril suspension	0.45 mg emodepside + 9.0 mg toltrazuril	oral, once	day 0	1 sample: day –1 or 0	2 samples: day 10 ± 3	day 0, 10 ± 3
		Milbemycin oxime plus praziquantel tablets	0.5 mg milbemycin oxime + 5.0 mg praziquantel					
2	Patent *Isospora* spp. infection	Emodepside plus toltrazuril suspension	0.45 mg emodepside + 9.0 mg toltrazuril	oral, once	day 0	1 sample: day –1 or 0	3 samples: day 3, 7 and 9 ± 1	day 0, 9 ± 1
		Sulfadimethoxine powder	day 0: 40 mg day 1–6: 25 mg	oral, once daily for 7 days	day 0–6			
3	Prepatent *Isospora* spp. infection	Emodepside plus toltrazuril suspension	0.45 mg emodepside + 9.0 mg toltrazuril	oral, once	day 0	1 sample: day –1 or 0	3 samples: day 3, 5, and 7 ± 1	day 0, 7 ± 1
		No treatment	–	–	–			

Study animals and health evaluation

A total of 403 purebred (more than 30 breeds) or crossbred dogs were included in the studies. Dogs in studies no. 1 and 2 were enrolled from 17 veterinary practices in Germany (7 practices, 3 regions), France (6 practices, 3 regions), Portugal (3 practices, 3 regions) and Albania (1 practice, 1 region). Up to 5 dogs per owner were included in the efficacy evaluation in these studies. Dogs in study no. 3 were enrolled from 13 commercial or private dog breeders in Germany (7 sites), France (4 sites), Hungary (1 site) and Ireland (1 site). The numbers of dogs that were included in the evaluation of efficacy and safety are shown in Tab. 2.

The 234 dogs treated with emodepside plus toltrazuril suspension were between 3 weeks and 11 years of age (82.5 % were ≤ 3 months of age). Their body weight ranged between 0.4 and 33.4 kg. Informed consent was obtained from the animal owners before enrolment of the dogs.

Tab. 2 Numbers of dogs included in the evaluation of efficacy and safety

Group	Efficacy			Safety			
	Study 1	Study 2	Study 3	Study 1	Study 2	Study 3	Total
Emodepside plus toltrazuril suspension	66[a]	37[b]	40	80	50	104	**234**
Control (milbemycin oxime plus praziquantel tablets, sulfadimethoxine powder or untreated)	31	14	40	41	26	102	**169**
Total	97	51	80[c]	121	76	206	**403**

[a] 16 animals with nematode infection originally enrolled in study no. 2 were also included in the efficacy evaluation of study no. 1

[b] 2 animals with *Isospora* infection originally enrolled in study no. 1 were also included in the efficacy evaluation of study no. 2

[c] i.e. data from 19 litters/housing groups

Two veterinary examinations were performed in each of the studies: the first was done at study enrolment and the second 6 to 13 days post treatment (see Tab. 1). After treatment, the health of the dogs was observed by their owners.

Faecal examination

As an initial screening qualitative faecal flotation using Fasol® (magnesium-sulphate solution, Jørgen Kruuse A/S, DK-5290 Marslev) or other commercially available flotation solutions was done in all three studies. Quantitative faecal egg counts (FEC) and faecal oocyst counts (FOC) were then performed in the diagnostic laboratory with a modified McMaster method using glucose sodium chloride solution. Results of the FEC/FOC were expressed as eggs or oocysts per gram of faeces (EPG or OPG). The diagnostic laboratory was blinded to the group allocation of the dogs.

Treatment

In the three studies a total of 234 dogs were treated with emodepside plus toltrazuril suspension. Dogs were treated once orally at a dosage of at least 0.45 mg emodepside and 9 mg toltrazuril per kg body weight according to the label instructions. The veterinarian rated the acceptance of the suspension for each dog as good, medium or poor.

In study no. 1 the dogs in the control group were treated with a tablet containing milbemycin oxime plus praziquantel (Milbemax®, Novartis Tiergesundheit GmbH, Eschborn, Germany and Novartis Santé Animale S.A.S., Rueil Malmaison, France) at a minimum dose of 0.5 mg milbemycin oxime and 5 mg praziquantel per kg body weight according to the manufacturer's instructions.

In study no. 2 the dogs in the control group received powder containing sulfadimethoxine for oral application with food or water (Kokzidiol SD®, Pharmawerk Weinboehla GmbH, Weinboehla, Germany) over 7 days starting with a dose of 40 mg sulfadimethoxine per kg body weight on the first day and continuing with a dose of 25 mg/kg body weight on the following six days according to the manufacturer's instructions.

In study no. 3 the dogs in the control group were left untreated.

Data analysis

Efficacy (% reduction in FEC/FOC) was calculated according to the following formulae:

Studies no. 1 and 2:

% Reduction = (N1 – N2)/N1 x 100

N1: geometric mean pre treatment FEC/FOC
N2: geometric mean post treatment FEC/FOC

Study no. 3:

% Reduction = (N1 – N2)/N1 x 100

N1: geometric mean FOC of untreated control group during the post treatment period
N2: geometric mean post treatment FOC of treated group

In study no. 1 post treatment geometric mean FEC were calculated from the mean post treatment FEC of each dog. In study no. 2 where the control product had to be applied for 7 days, only the mean FOC of day 7 and 9 ± 1 were used for the overall efficacy calculation. Additionally, efficacy per day was calculated for day 3, 7 and 9 ± 1. In study no. 3 geometric mean FOC were calculated using the highest FOC of each dog during the post treatment period.

In studies no. 1 and 2 the relative efficacy of the treatment was assessed using a non-inferiority test on mean post treatment FEC/FOC. Calculations were carried out on the log-transformed scale (ln [count+1]) with a non-inferiority margin of 0.20. The upper 95 % two-sided confidence limit of the least square mean of the investigational veterinary product was required to be lower than 1.2 times the post baseline FEC/FOC of the control product to achieve non-inferiority. Superiority was assessed according to EMEA/CPMP/EWP/482/99, and a two-sided t-test was performed.

Tab. 3 Occurrence of mixed parasite infection in dogs enrolled in studies no. 1–3

Studies no. 1 + 2	Parasite species found	n (of 197)	%
Nematodes 9.6 %	*T. canis*, *A. caninum*	1	0.5
	T. canis, *U. stenocephala*	3	1.5
	T. canis, *T. vulpis*	3	1.5
	T. canis, *U. stenocephala*, *T. vulpis*	1	0.5
	T. leonina, *T. vulpis*	1	0.5
	U. stenocephala, *T. vulpis*	10	5.1
Nematodes and *Isospora* spp. 12.7 %	*T. canis*, *I. ohioensis*-complex	22	11.2
	U. stenocephala, *I. ohioensis*-complex	1	0.5
	T. canis, *I. canis*, *I. ohioensis*-complex	1	0.5
	I. canis, *T. vulpis*	1	0.5
Isospora spp. 2.5 %	*I. canis*, *I. ohioensis*-complex	5	2.5
Study no. 3	**Parasite species found**	**n (of 206)**	**%**
Nematodes and *Isospora* spp. 8.3 %	*I. ohioensis*-complex, *T. canis*	10	4.9
	I. ohioensis-complex, *I. canis*, *T. canis*	7	3.4
Isospora spp. 2.9 %	*I. canis*, *I. ohioensis*-complex	6	2.9

T. vulpis: *Trichuris vulpis*, *T. leonina*: *Toxascaris leonina*

In study no. 3 a test for superiority on mean FOC during the post treatment period was carried out to compare the effect of the treatment to the untreated control. Calculations were also carried out on the log-transformed scale (ln [count+1]) using a superiority margin of 0.20. Superiority was shown if the upper 95 % two-sided confidence limit of the least square mean of the investigational veterinary product was lower than 0.8 times of the post baseline counts of the control group.

Results

T. canis and *U. stenocephala* were the most frequently observed nematode species while the predominant *Isospora* species was *I. ohioensis*-complex. For 12.7 % of the dogs enrolled in studies no. 1 and 2 a mixed nematode and *Isospora* spp. infection was demonstrated (Tab. 3). For dogs up to 3 months of age the percentage of mixed nematode and *Isospora* spp. infection was 17.2 %. In study no. 3 mixed nematode and *Isospora* spp. infection was observed in 8.3 % of the dogs enrolled.

The efficacy results of the three studies are shown in Tabs. 4 to 6.

Studies no. 1 and 2 demonstrated a 100 % reduction in faecal nematode egg and *Isospora* spp. oocyst counts after treatment with emodepside plus toltrazuril suspension (Tabs. 4 and 5). Non-inferiority as well as superiority to the control products was shown in both studies ($p \leq 0.009$). In an additional evaluation on nematode subpopulations, 100 % and 99.9 % FEC reduction was observed after emodepside plus toltrazuril treatment of 49 dogs infected with *T. canis* (geometric mean FEC before treatment: 1,369 EPG) and 21 dogs infected with hookworms (geometric mean FEC before treatment: 500 EPG), respectively.

Study no. 3 demonstrated 98.7 % lower faecal oocyst counts in dogs treated with emodepside plus toltrazuril suspension during suspected prepatent *Isospora* spp. infection compared to untreated dogs also confirming superiority of the treatment ($p < 0.0001$; Tab. 6).

Tab. 4 Results of study no. 1: faecal nematode egg count (FEC) reduction

Group	Geometric mean FEC (EPG)		Reduction
	Pre tr.	Post tr. (95 % CI)	
Emodepside plus toltrazuril suspension	1,129	0.4 (0–1.1)	100.0 %
Milbemycin oxime plus praziquantel tablets	974	3 (1.1–6.4)	99.7 %

CI: confidence interval, EPG: eggs per gram faeces, tr.: treatment

No adverse drug reactions were observed in any of the studies.

The veterinarians rated the acceptance of emodepside plus toltrazuril suspension as good in over 90 % and medium in 9 % of the treatments with just one of 234 dogs showing poor acceptance.

Discussion

The three multicentre studies demonstrated that emodepside plus toltrazuril suspension is highly efficacious in the treatment of naturally acquired nematode and *Isospora* spp. infections in dogs under field conditions. In addition to the treatment of patent infections, also the efficacy of a treatment during suspected prepatent *Isospora* spp. infection was demonstrated. The dogs originated from a wide range of breeds, age and body weight and no adverse reactions to the treatment were observed, thus also confirming the safety of this new formulation. Acceptance was rated as good in the majority of cases so that this oral suspension offers a convenient treatment option especially in puppies, which were the predominant age group in the studies.

The efficacy results on patent *Isospora* spp. infection fit to previous work of Daugschies et al. (2000), who investigated the therapeutic efficacy of toltrazuril against natural infection with *Isospora* spp. at single doses of 10 or 20 mg/kg body weight applied orally to pups under field conditions. Both doses were shown to be highly effective, and 99 % of the litters treated with 10 mg/kg body weight had turned negative on the following day. Efficacy > 99 % against canine nematodes under field conditions had also been previously demonstrated for emodepside using a tablet formulation (Profender®) at a minimum dose of 1 mg emodepside per kg body weight combined with praziquantel (Altreuther et al. 2009).

Tab. 5 Results of study no. 2: *Isospora* spp. faecal oocyst count (FOC) reduction

Group	Geometric mean FOC (OPG)					FOC reduction			
	Pre tr.	Day 3	Day 7	Day 9 ± 1	Post tr. day 7/9 ± 1 (95 % CI)	Day 3	Day 7	Day 9 ± 1	Post tr. (day 7/9 ± 1)
Emodepside plus toltrazuril suspension	2,852	3	0.5	0.6	1 (0.01–3.4)	99.9 %	100.0 %	100.0 %	100.0 %
Sulfadimethoxine powder	1,404	157	13	7	14 (3.3–47.7)	(88.8 %)[a]	99.1 %	99.5 %	99.0 %

CI: confidence interval, OPG: oocysts per gram faeces, tr.: treatment
[a] treatment was to be applied until day 6

Tab. 6 Results of study no. 3: *Isospora* spp. faecal oocyst count (FOC) reduction – "metaphylactic" treatment

Group	Geometric mean FOC (OPG)		Range of FOC during post treatment period	Reduction versus control
	Pre treatment	Post treatment period = max of day 3, 5, 7 ± 1 (95 % CI)		
Emodepside plus toltrazuril suspension	0	1.7 (0.06–5.9)	0–3,750	98.7 %
Untreated control	0	128.8 (49.8–330.9)	0–109,150	n.a.

CI: confidence interval, OPG: oocysts per gram faeces

Of the dogs enrolled in the studies 8.3 % (study no. 3) resp. 12.7 % (studies no. 1 and 2) harboured a mixed nematode and *Isospora* spp. infection, with *T. canis* plus *I. ohioensis*-complex being the most frequent combination. A certain bias regarding the prevalence of mixed nematode and *Isospora* spp. infection can be assumed for all three studies: In studies no. 1 and 2 the veterinarians may have tended to select dogs which they expected to be at a higher risk of infection. However, dogs presented in veterinary practice may be generally less likely to harbour infection, and the prevalence evaluation was based on just one faecal sample per dog. Study no. 3 was conducted at breeders with a known history of *Isospora* spp. infection. However, the patent mixed infections were identified at a time when the investigators had expected the dogs to still be negative for *Isospora* spp. and, similarly to the other two field studies, the calculation of prevalence was based on just one sample per dog. For a comparison with other published data several factors need to be considered with the number of faecal samples taken and the age of the population probably being the most important ones. Shedding of nematode eggs as well as oocysts can be highly variable so that a single faecal sample in a mere cross-sectional survey may lead to significant underestimation of the actual prevalence, especially for mixed infections. Also the time period over which a survey is conducted can have a considerable influence on the results. Sager et al. (2006a,b) found a significantly higher prevalence of helminths as well as protozoa when dogs were tested in monthly intervals for approximately 1 year instead of just once. Only 3.7 % of dogs were positive for *Isospora* spp. while the annual incidence/prevalence was above 40 %. Similarly, 7.1 % of the dogs were positive for *Toxocara canis* while 32 % tested positive at least once during the year despite quarterly anthelmintic treatment. Only few publications evaluate gastrointestinal parasite prevalence in unweaned pups for the obvious difficulty in obtaining faecal samples in the first few weeks of life. Seeliger (1999) monitored parasite infection of unweaned pups at a breeding facility between their third and tenth week of life. During the course of the study 100 % of the pups were found positive for *Isospora* spp. and 79.2 % were also positive for *T. canis*. Bode (1999) identified 88.5 % of 104 litters from four dog breeding units to be positive for *Isospora* spp. with 19.2 % of positive pups being also positive for helminths. These data may represent extreme cases in problematic environments, however, they illustrate that high co-infection rates are possible. Even though the prevalence of mixed infections observed in studies no. 1 and 2 was lower, the data demonstrate relevant exposure to nematodes as well as *Isospora* spp. in a less extreme environment. The significance of gastrointestinal parasite infection especially in the first weeks of life should therefore not be underestimated, and potential epidemiological as well as clinical implications need to be considered taking account of the individual situation.

Acknowledgements

The authors thank the diagnostic laboratory of the Institute of Parasitology, University of Veterinary Medicine Vienna, Austria, headed by A. Joachim for the diagnostic part of the studies, the dog owners, breeders and the participating veterinary practices.

Compliance statement

All of the studies reported herein were performed in compliance with current, applicable, local laws and regulations.

Disclosure statement

G. Altreuther, N. Gasda, A. Schimmel and K.J. Krieger were employed by Bayer Animal Health GmbH, Germany; D. Hutchens was employed by Bayer HealthCare LLC, USA, and H. Thurieau was employed by Bayer Santé Division Santé Animale, France, during the conduct of the studies. K. Hellmann ist the managing director and K. Adler an employee of Klifovet AG, a CRO contracted to conduct the studies. All studies were sponsored by Bayer Animal Health GmbH.

References

Altreuther G, Radeloff I, LeSueur C, Schimmel A, Krieger KJ (2009) Field evaluation of the efficacy and safety of emodepside plus praziquantel tablets (Profender® tablets for dogs) against naturally acquired nematode and cestode infections in dogs. Parasitol Res 105:23–29.

Altreuther G, Gasda N, Schroeder I, Joachim A, Settje T, Schimmel A, Hutchens D, Krieger KJ (2011) Efficacy of emodepside plus toltrazuril suspension (Procox® oral suspension for dogs) against prepatent and patent infection with *Isospora canis* and *Isospora ohioensis*-complex in dogs. Parasitol Res 109:9–20.

Bode K (1999) Endoparasitenbefall in kommerziellen Hundezuchten unter besonderer Berücksichtigung der Isosporose. Dissertation, Tierärztliche Hochschule Hannover.

Daugschies A, Mundt H-C, Letkova V (2000) Toltrazuril treatment of cystoisosporosis in dogs under experimental and field conditions. Parasitol Res 86:797–799.

Eckert J, Friedhoff KT, Zahner H, Deplazes P (2008) *Toxocara canis*: Erreger der Toxocarose des Hundes und anderer Caniden. In: Lehrbuch der Parasitologie für die Tiermedizin, Enke Verlag, Stuttgart, pp 317–322.

EMEA guideline EMEA/CPMP/EWP/482/99 (2000) Points to consider on switching between superiority and non-inferiority.

Jacobs DE, Arakawa A, Courtney CH, Gemmell MA, McCall JW, Myers GH, Vanparijs O (1994) World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics in dogs and cats. Vet Parasitol 52:179–202.

Sager H, Steiner Moret C, Grimm F, Deplazes P, Doherr MG, Gottstein B (2006a) Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelmintic treatment. Parasitol Res 98:333–338.

Sager H, Steiner Moret C, Mueller N, Staubli D, Esposito M, Schares G, Haessig M, Staerk K, Gottstein B (2006b) Incidence of *Neospora caninum* and other intestinal protozoan parasites in populations of Swiss dogs. Vet Parasitol 139:84–92.

Schimmel A, Schroeder I, Altreuther G, Settje T, Charles S, Wolken S, Kok DJ, Ketzis J, Young D, Hutchens D, Krieger KJ (2011) Efficacy of emodepside plus toltrazuril (Procox® oral suspension for dogs) against *Toxocara canis*, *Uncinaria stenocephala* and *Ancylostoma caninum* in dogs. Parasitol Res 109:1–8.

Seeliger U (1999) Feldstudie zur Epidemiologie und Bekämpfung der Isosporose des Hundes. Dissertation, Tierärztliche Hochschule Hannover.

VICH guideline 7: Efficacy requirements for anthelmintics: overall guidelines. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, December 2000.

VICH guideline 9: Good clinical practice. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, July 2000.

VICH guideline 19: Efficacy of anthelmintics: specific recommendations for canine. Veterinary International Cooperation on Harmonization, European Agency for the Evaluation of Medicinal Products, London, July 2001.