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The efficacy of eprinomectin extended-release injection against naturally acquired nematode parasites of cattle, with special regard to inhibited fourth-stage *Ostertagia* larvae

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ABSTRACT

The efficacy of eprinomectin in an extended-release injection (ERI) formulation in the treatment of cattle harboring naturally acquired nematode populations (including inhibited nematodes) was evaluated. Five studies were conducted under a similar protocol in the USA, the UK, and in Germany. All study animals were infected by grazing naturally contaminated pastures. The adequacy of pasture infectivity was confirmed by examining tracer calves prior to allocation and treatment of the study animals. The cattle were of various breeds or crosses, weighing 79–491 kg, and aged approximately 6–15 months. In each study, 20 animals were infected by grazing, and then removed from pasture and housed in a manner to preclude further nematode infections for 8–16 days until treatment. Animals were blocked based on descending pre-treatment body weight and randomly allocated to one of two treatments: ERI vehicle (control) at 1 mL/50 kg body weight or eprinomectin 5% (w/v) ERI at 1 mL/50 kg body weight (1.0 mg eprinomectin/kg). Treatments were administered once on Day 0 by subcutaneous injection in front of the shoulder. For parasite recovery and count, all study animals were humanely euthanized 14/15 days after treatment. Cattle treated with eprinomectin ERI had significantly ($p < 0.05$) fewer of the following nematodes than the controls with overall reduction of parasite counts of ≥94%: adult *Dictyocaulus viviparus, Capillaria* spp., *Cooperia oncophora*, *Cooperia pectinata*, *Cooperia punctata*, *Cooperia surnabada*, *Haemonchus placei*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Ostertagia lyrata*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Trichuris discolor*, *Trichuris skrjabini*, and *Trichuris* spp.; developing fourth-stage larvae of *Ostertagia* spp. and *Trichostrongylus* spp.; and inhibited fourth-stage larvae of *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia* spp., and *Trichostrongylus* spp.

Animal treatments were well accepted, with no adverse reactions to treatment observed in any study animals. The results of this series of controlled studies demonstrated high therapeutic efficacy and acceptability of eprinomectin ERI against pulmonary nematodes and a wide range of gastrointestinal parasitic infections, including inhibited gastrointestinal nematodes, in cattle.

1. Introduction

Eprinomectin, a recently commercialized member of the macrocyclic lactone class of parasiticides has been proven highly effective as a pour-on formulation for

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cattle in the prevention and control of a wide range of endo- and ectoparasites in cattle (e.g., Barth et al., 1997; Shoop et al., 1996; Gogolewski et al., 1997a,b; Holste et al., 1997, 1998; Pitt et al., 1997; Williams et al., 1997; Yazwinski et al., 1997; Cramer et al., 2000; Campbell et al., 2001; Davey and George, 2002; Shoop and Soll, 2002; Rehbein et al., 2005). While highly effective as a pour-on formulation in providing persistent therapeutic control, prophylactic success also has been demonstrated against important nematode species (e.g., Batty et al., 1999; Epe et al., 1999; Dorny et al., 2000).

In a concerted effort to further lengthen the persistent activity of eprinomectin an extended-release injection (ERI) formulation has been developed for cattle which releases the active material in an efficacious manner for up to 150 days (Soll et al., 2013). This new formulation provides flexibility for strategic protection and control of endo- and ectoparasites.

The studies reported here were designed to confirm the efficacy and acceptability of eprinomectin ERI when administered to cattle harboring naturally acquired gastrointestinal and pulmonary nematode infections, including inhibited nematodes. Inhibition of development in the immature stage is a characteristic feature in the life-cycle of ruminant trichostrongyloid nematodes, occurring in *Cooperia, Haemonchus*, *Trichostrongylus*, *Nematodirus*, and *Ostertagia species*. However, inhibition is of special epidemiological and pathogenic relevance in *Ostertagia ostertagi*, the most economically important gastrointestinal nematode in cattle. The larval inhibition appears to be induced by environmental stimuli – climatic conditions less favorable for development and/or survival of free-living larval stages (fall of temperatures in autumn in northern temperate regions or dry and hot conditions in southern temperate climates) – but might also be affected by the development of both acquired immunity and age resistance. The duration of arrested development has been documented to last from a few weeks up to several months, so potentially large numbers of larvae can accumulate (Bürger, 1992; Taylor et al., 2007).

2. Materials and methods

Five controlled studies were conducted according to a similar protocol, three in the USA, one in the UK, and one in Germany. Cattle harboring naturally acquired nematode infections were utilized. The studies were designed and conducted to comply with the regulatory requirements of both the FDA/CVM and the European Medicines Agency/Committee for Medicinal Products for Veterinary Use, and according to relevant guidelines for Good Clinical Practices (GCPs) and for establishing the efficacy of cattle anthelmintics.

All five studies were performed in a blinded fashion, and personnel involved with data collection were unaware of the treatment group assignments.

2.1. Experimental animals

A total of 100 (42 male, 35 male castrate, 23 female) healthy, ruminating Angus cross, other beef crossbreds, Holstein, Holstein Friesian, and Friesian cattle, weighing 79–491 kg prior to treatment (Days −1 or 0), and aged approximately 6–15 months at the time of treatment were used. The five studies were conducted in the USA (Study #2 in Arkansas during June, #4 in Louisiana during May, and #5 in Missouri during September to December), in Germany (Study #3 in Upper Bavaria during December), and in the UK (Study #1 in Scotland during November to December). The animal descriptions and details are presented in Table 1. None of the animals had been previously treated with an avermectin or milbemycin product.

Cattle for all five studies were grazed on naturally contaminated pastures prior to treatment and were expected to be harboring naturally acquired nematode infections. To confirm pasture infectivity and to demonstrate the presence of adequate nematode infections (including inhibited fourth-stage larvae), a total of 16 tracer animals (at least two animals per study site) were commingled with the study animals. They were removed from pasture, housed to preclude further nematode infection, and were necropsied 8–16 days later for parasite identification and enumeration. Examination of tracer calves was completed prior to Day 0 for animals enrolled in the efficacy studies.

2.2. Study design

Each of the studies was conducted utilizing a randomized block design. Ten replicates of two animals each were formed based on descending Day −1 or Day 0 body weights. Within replicates, one animal was randomly allocated to each treatment group. Group 1 animals received the ERI vehicle (control) at 1 mL/50 kg bodyweight subcutaneously once on Day 0. Group 2 animals received eprinomectin 5% (w/v) ERI solution at a targeted dosage of 1.0 mg eprinomectin/kg bodyweight (1 mL/50 kg) subcutaneously once on Day 0. The appropriate dose volume for each animal was administered in front of the right or left shoulder using commercial syringes and needles. Dose volumes were based on pre-treatment body weights and measured at 0.1 mL increments. Calculated doses that fell between increments were rounded up to the next greater 0.1 mL.

2.3. Parasite counts

All study animals were humanely euthanized and necropsied for nematode counting and identification 14/15 days after treatment (22–31 days after removal from pasture). The following organs were removed for content recovery and examination: heart–lung complex (Studies 4 and 5), lungs with trachea (Studies 2 and 3), abomasa, small intestines and large intestines (including the cecum, all studies). Total lungworms were collected and counted. The contents of the abomasa, small intestines and large intestines were collected separately and diluted with water. The abomasum and small intestine of each calf were incubated separately (saline soak) to recover mucosal stages of the parasites for counting and identification. For all animals within each study, the entire contents or a known percentage of the contents of each organ (abomasum and small intestine contents: 2.5%, 5%, 10% or 20%

Table 1
Animal description and details.

Study[a]	Treatment[b]/animals per treatment		Breed	Sex	~Age (months)	Pre-treatment body weight (kg)
1	Control	$n=10$	Holstein-Friesian, Friesian, Friesian cross	Male	11–15	195–315
	EpERI	$n=10$				
2	Control	$n=10$	Holstein	Male castrate	6	79–182
	EpERI	$n=10$				
3	Control	$n=10$	Holstein-Friesian	Male	12	353–491
	EpERI	$n=10$				
4	Control	$n=10$	Crossbred beef	2 male, 3 male castrate, 15 female	9–14	137–224
	EpERI	$n=10$				
5	Control	$n=10$	Angus cross	12 male castrate, 8 female	9–15	142–239
	EpERI	$n=10$				

[a] Study sites: Study 1 = Scotland, UK; Study 2 = Arkansas, USA; Study 3 = Upper Bavaria, Germany; Study 4 = Louisiana, USA; Study 5 = Missouri, USA.
[b] Control = ERI vehicle; EpERI = eprinomectin ERI.

aliquots; abomasum and small intestine soaks 2%, 2.5%, 5%, or 10% aliquots; large intestine content: 5%, 10%, 20% aliquots or total count) were examined. Preliminary counts were multiplied by the inverse of the aliquot percentage to calculate final worm counts.

Counts of individual parasite species were calculated by multiplying the numbers of worms recovered from each organ by the inverse of the aliquot percentage. Animal and treatment group worm counts were calculated, and summed over all locations for each parasite species. Nematodes were identified to genus or species and stage of development (where possible) according to recognized methods and procedures. Adult female nematodes and/or fourth-stage larvae (L4s) were not always identified to species level. For several nematode genera (i.e., *Cooperia*, *Ostertagia*, and *Trichostrongylus*), the total species counts were estimated by proportionally assigning adult females to each species based on the adult male counts. When adult females within genera were counted in the presence of no males, the counts were either proportioned equally or assigned based on the species present in other study animals. For *Trichostrongylus* spp., in some studies, non-speciated nematodes were assigned to species based on the location in which the nematodes were found.

2.4. Statistical methods

Nematode counts were transformed to the natural logarithm (count + 1) for analysis and calculation of geometric means. Treatment groups were compared using a Wilcoxon rank sum test. A two-sided test was used at $\alpha = 0.05$. Efficacy was calculated as $100[(C-T)/C]$, where C is the geometric mean for the ERI vehicle-treated (control) group and T is the geometric mean for the eprinomectin ERI-treated group.

3. Results

Nematode counts in the tracer animals at all study sites supported the presence of a sufficient number (>1000) of inhibited L4s of *Ostertagia* spp. and thus resulted in the enrollment of the cattle of all sites into studies. In addition, identification of adult gastrointestinal nematodes recovered from the tracer animals at Study sites 2 (Arkansas/USA), 3 (Upper Bavaria/Germany) 4 (Louisiana/USA), and 5 (Missouri/USA) revealed the presence of nematodes of the following genera: *Capillaria* (Study 2), *Cooperia* (Studies 2–5), *Dictyocaulus* (Study 3), *Haemonchus* (Study 5), *Nematodirus* (Studies 2 and 3), *Oesophagostomum* (Studies 2–5), *Ostertagia* (Studies 2–5), *Trichostrongylus* (Studies 4 and 5), and/or *Trichuris* (Studies 2–4).

In all five studies, treatment was well accepted and cattle were reported as normal during hourly observations through the first four hours post-treatment. There were no drug related health problems or adverse events related to the drug at any time during the studies. Prior to treatment, some animals exhibited clinical signs of parasitic disease but none required salvage treatment.

In total, 20 species of nematodes – 15 species in the cattle from the two study sites in Europe and 17 species in the cattle from the three study sites in the USA – were recorded in the gastrointestinal tracts and lungs of control animals in this set of studies. These species comprise all of the economically relevant nematode parasites of grazing cattle. The majority of these species occurred in high enough numbers and in a sufficient proportion of control animals for meaningful analyses to be performed.

At necropsy, 22–31 days after removal from pasture and 14/15 days after treatment, the groups that received eprinomectin ERI had significantly ($p < 0.05$) fewer of the following nematodes than the ERI vehicle-treated (control) cattle with overall reduction of nematode counts by ≥94% (Table 2): *Dictyocaulus viviparus*, *Capillaria* spp., *Cooperia oncophora*, *Cooperia pectinata*, *Cooperia punctata*, *Cooperia surnabada*, *Haemonchus placei*, *Nematodirus helvetianus*, *Oesophagostomum radiatum*, *Ostertagia lyrata*, *O. ostertagi*, *Ostertagia* spp. developing fourth-stage larvae, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Trichostrongylus* spp. developing fourth-stage larvae, *Trichuris discolor*, *Trichuris skrjabini*, and immature and adult *Trichuris* spp.

Additionally, cattle treated with eprinomectin ERI had significantly ($p < 0.05$) fewer inhibited fourth-stage larvae of the following nematode genera than the ERI vehicle-treated (control) cattle with overall reduction of counts by ≥94% (Table 3): *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia* spp., and *Trichostrongylus* spp. While the efficacy of eprinomectin ERI

Table 2
Therapeutic efficacy of eprinomectin ERI against adult and developing pulmonary and gastrointestinal nematodes.

Study #[a]	Control (vehicle)		Eprinomectin ERI		Efficacy[d]	Probability[e]
	Prevalence[b]	GM[c]	Prevalence	GM		
Dictyocaulus viviparus						
3	9/10	30	0/10	0	100%	<0.01
4	10/10	24	0/10	0	100%	<0.05
Cooperia oncophora						
1	10/10	1476	7/10	68	95%	<0.05
2	10/10	7686	9/10	208	97%	<0.05
3	10/10	809	5/10	4	>99%	<0.01
Cooperia pectinata						
4	9/10	800	0/10	0	100%	<0.05
Cooperia punctata						
2	10/10	558	3/10	2	>99%	<0.05
3	8/10	18	1/10	<1	98%	<0.01
4	10/10	4222	0/10	0	100%	<0.05
5	9/10	337	1/10	<1	>99%	<0.05
Cooperia surnabada						
1	7/10	79	3/10	4	95%	<0.05
2	10/10	534	8/10	28	95%	<0.05
3	4/10	14	1/10	<1	95%	ns[f]
Haemonchus placei						
2	10/10	653	1/10	<1	>99%	<0.05
4	4/10	13	0/10	0	100%	<0.05
5	5/10	12	1/10	<1	96%	ns[f]
Nematodirus helvetianus						
2	10/10	44	0/10	0	100%	<0.05
Oesophagostomum radiatum						
1	4/10	1	0/10	0	100%	<0.05
2	6/10	8	0/10	0	100%	<0.05
3	10/10	7	0/10	0	100%	<0.01
4	10/10	185	0/10	0	100%	<0.05
5	10/10	101	0/10	0	100%	<0.05
Ostertagia lyrata						
2	10/10	139	2/10	<1	>99%	<0.05
3	6/10	7	0/10	0	100%	<0.01
4	4/10	8	0/10	0	100%	<0.05
5	9/10	702	0/10	0	100%	<0.05
Ostertagia ostertagi						
1	10/10	7030	2/10	1	>99%	<0.01
2	10/10	4747	5/10	2	>99%	<0.05
3	10/10	1651	0/10	0	100%	<0.01
4	10/10	9236	1/10	<1	>99%	<0.05
5	10/10	30158	2/10	<1	>99%	<0.05
Ostertagia spp., developing (late) fourth-stage larvae						
1	10/10	327	1/10	<1	>99%	<0.01
2	10/10	113	0/10	0	100%	<0.05
4	8/10	211	5/10	13	94%	<0.05
5	10/10	1751	3/10	2	>99%	<0.05
Trichostrongylus axei						
1	8/10	141	0/10	0	100%	<0.01
2	5/10	3	0/10	0	100%	<0.05
3	10/10	224	0/10	0	100%	<0.01
4	10/10	3931	0/10	0	100%	<0.05
5	10/10	53640	2/10	1	>99%	<0.05
Trichostrongylus colubriformis						
1	8/10	163	0/10	0	100%	<0.01
2	5/10	3	0/10	0	100%	<0.05
Trichostrongylus spp., developing (late) fourth-stage larvae						
1	10/10	247	0/10	0	100%	<0.01
Trichuris discolor						
3	9/10	11	0/10	0	100%	<0.01
Trichuris skrjabini						
3	5/10	1	0/10	0	100%	<0.05
Trichuris spp.						
1	8/10	8	0/10	0	100%	<0.01
2	9/10	21	1/10	<1	98%	<0.05

Table 2 (*Continued*)

Study #[a]	Control (vehicle)		Eprinomectin ERI		Efficacy[d]	Probability[e]
	Prevalence[b]	GM[c]	Prevalence	GM		
Trichuris spp., immature						
2	9/10	8	2/10	<1	94%	<0.05

[a] Study sites: Study 1 = Scotland, UK; Study 2 = Arkansas, USA; Study 3 = Upper Bavaria, Germany; Study 4 = Louisiana, USA; Study 5 = Missouri, USA.
[b] Prevalence: number of cattle infected/Number of cattle in group.
[c] Geometric mean counts (based on transformation to the natural logarithm of [count + 1]).
[d] Efficacy = 100 [(geometric mean control – geometric mean eprinomectin ERI)/geometric mean control].
[e] Probability from Wilcoxon rank sum test.
[f] Not significant at $\alpha = 0.05$.

Table 3
Therapeutic efficacy of eprinomectin ERI against inhibited gastrointestinal nematodes.

Study[a]	ERI vehicle (control)		Eprinomectin ERI		Efficacy[d]	Probability[e]
	Prevalence[b]	GM[c]	Prevalence	GM		
Cooperia spp.						
1	9/10	1002	2/10	2	>99%	<0.01
2	10/10	34	0/10	0	100%	<0.05
4	8/10	155	1/10	<1	>99%	<0.05
5	3/10	2	0/10	0	–[f]	na[g]
Haemonchus spp.						
2	6/10	6	0/10	0	100%	<0.05
4	3/10	4	0/10	0	–	na
5	6/10	10	0/10	0	100%	<0.05
Nematodirus spp.						
1	7/10	160	0/10	0	100%	<0.01
2	8/10	13	0/10	0	100%	<0.05
Oesophagostomum spp.						
1	2/10	<1	0/10	0	–	na
2	1/10	<1	0/10	0	–	na
5	4/10	1	0/10	0	100%	<0.05
Ostertagia spp.						
1	10/10	201124	10/10	1422	99%	<0.01
2	10/10	2182	5/10	<1	>99%	<0.05
3	10/10	10803	10/10	908	92%	<0.01
4	10/10	30773	10/10	5726	81%	<0.05
5	10/10	5666	5/10	10	>99%	<0.05
Trichostrongylus spp.						
1	1/10	<1[h]	0/10	0	–	na
1	10/10	4842[i]	1/10	<1	>99%	<0.01
5	4/10	5	1/10	<1	93%	ns[j]

[a] Study sites: Study 1 = Scotland, UK; Study 2 = Arkansas, USA; Study 3 = Upper Bavaria, Germany Study 4 = Louisiana, USA; Study 5 = Missouri, USA.
[b] Prevalence: number of cattle infected/Number of cattle in group.
[c] Geometric mean counts (based on transformation to the natural logarithm [count + 1]).
[d] Efficacy = 100 [(geometric mean control – geometric mean eprinomectin ERI)/geometric mean control].
[e] Probability from Wilcoxon rank sum test.
[f] Not calculated due to a prevalence of <40% in the control group.
[g] Not analyzed.
[h] Abomasum.
[i] Small intestine.
[j] Not significant at $\alpha = 0.05$.

against inhibited L4s of *Ostertagia* spp. was 92% to >99% in four studies, reduction of inhibited *Ostertagia* spp. larvae was 81% in the study conducted in Louisiana. In the analysis of pooled data across the five studies for inhibited L4s of *Ostertagia* spp. overall efficacy was calculated to be 94.4% (overall efficacy was calculated as the arithmetic mean of individual study efficacies; $p < 0.05$, Friedman's test).

4. Discussion

Prior to removal from pasture, cattle in each of the five studies were subjected to prolonged nematode exposure on naturally contaminated pastures. Nematode recoveries from tracer cattle as well as controls supported the presence of a sufficient number of inhibited *Ostertagia* spp. larvae in addition to a range of adult nematodes of several genera. The species identified were well representative of those known to cause parasitic gastroenteritis and bronchitis in North America and Europe.

Based on parasite recoveries of the cattle carrying naturally acquired nematode infections, the current series of studies conducted at different geographical locations in the USA and in Europe consistently demonstrated a high level of therapeutic effectiveness of eprinomectin ERI (≥94%) against pulmonary nematodes and a wide range of bovine gastrointestinal parasites. This confirms results of controlled studies, conducted to assess the therapeutic efficacy of eprinomectin ERI against experimentally induced

adult and developing nematode infections (Rehbein et al., 2013a,b) and field studies where efficacy was demonstrated by ≥96% reduction of fecal egg or larval counts of eprinomectin ERI-treated cattle compared to vehicle-treated controls (Kunkle et al., 2013; Rehbein et al., 2013a,b).

Approximately four weeks before necropsy, all study animals were removed from infective pastures and held under conditions to prevent further nematode infection. This provided adequate time for nematodes with three- to four-week prepatent periods to complete development to the adult stage. Immature nematodes recovered at necropsy in the L4 stage were therefore considered to be inhibited (Williams et al., 1997; Taylor et al., 2007). The overall therapeutic efficacy of eprinomectin ERI against inhibited *Ostertagia* spp. L4s was high, and thus consistent with that of eprinomectin as a topical formulation (Williams et al., 1997). However, compared to studies conducted with topical eprinomectin (Williams et al., 1997), some variability was seen between the results in the current set of studies. This variability may be related to the length of the interval between treatment and slaughter employed in the two sets of studies. The three-week interval practiced by Williams et al. (1997) allowed extended time for clearance of larvae from the abomasal tissue (passage of and/or disintegration and resolution) compared to the two weeks used in the present studies.

Results of the current series of studies as well as other similarly controlled studies in the USA and Europe (Rehbein et al., 2013a,b) demonstrated the high effectiveness of eprinomectin LAI in cattle against naturally acquired and induced infections of adult, developing and inhibited immature nematode stages. Additionally, prophylactic efficacy under natural challenge conditions was demonstrated for at least 120 days using eprinomectin ERI, which prevented the accumulation of inhibited larvae of several nematode genera and stages, including the important early fourth-stage larvae of *Ostertagia* spp. (Rehbein et al., 2013a,b).

In conclusion, the results of these five controlled studies in cattle demonstrated excellent therapeutic efficacy and acceptability of eprinomectin ERI against a wide range of naturally acquired adult and inhibited gastrointestinal and pulmonary nematodes, including *O. ostertagi*, after a single treatment administered subcutaneously at 1 mL/50 kg.

Conflict of interest statement

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All authors are current employees (JH, SY, SR) or were contractors (TY, JW) of Merial and assisted with the study design, conduct, data analysis and review of the manuscript.

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