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ORIGINAL PAPER

Activity of ivermectin long-acting injectable (IVOMEC® GOLD) in first-season grazing cattle exposed to natural challenge conditions in Germany

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Abstract The persistent activity of ivermectin long-acting injection (IVM LAI; IVOMEC® GOLD, Merial; 3.15 % ivermectin *w*/*v*) against nematode infections of cattle was evaluated under natural challenge conditions. Seventy nematode-free Brown Swiss calves were blocked by pre-treatment bodyweight and allocated randomly to seven groups of 10 animals each: saline (control) at 1 mL/50 kg bodyweight once on day 0 or IVM LAI at 1 mL/50 kg bodyweight (630 mcg IVM/kg) on either days 0, 7, 14, 21, 28, or 35. After housing until day 35, calves were grazed as one herd on a naturally contaminated pasture for 42 days. Calves were then weighed and housed for 4 weeks before being necropsied for parasite counting. Treatment with IVM LAI prevented the establishment (>90 %, $p<0.05$) of *Dictyocaulus viviparus* (100 %), *Bunostomum phlebotomum* (100 %), *Haemonchus contortus* (98.6 %), *Ostertagia ostertagi/lyrata* (94.9 %), and *Oesophagostomum radiatum* (93.3 %) for at least 77 days; *Ostertagia leptospicularis* (99.1 %) for 63 days; *Cooperia punctata* (97.7 %), *Trichostrongylus axei* (96.5 %), and *Ostertagia* spp. inhibited larvae 4 (93.3 %) for 56 days; *Cooperia oncophora/surnabada* (96.9 %), *Trichuris discolor* (93.6 %), and *Cooperia* spp. inhibited larvae 4 (98.8 %); and *Nematodirus* spp. inhibited larvae 4 (97.1 %) for 42 days. Calves of groups treated with IVM LAI had significantly ($p<0.001$) higher days 0 to 77 weight gains than the saline-treated controls (28.40–39.25 vs 2.60 kg); the weight gains of the IVM LAI-treated groups, however, were not different from one another ($p>0.3$). This study demonstrated a very high efficacy of IVOMEC® GOLD in preventing the establishment of a wide range of bovine nematodes for extended periods of time which was associated with a significant benefit to productivity in terms of weight gain.

Keywords Ivermectin · Long-acting injection · Efficacy · Chemoprophylaxis · Nematodes · Cattle

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Introduction

Although parasitic infections in the majority of the cattle are subclinical under current production conditions, gastrointestinal nematodes and lungworms are still considered to be of the highest economic importance for grazing cattle in temperate regions worldwide. Because they are lacking acquired and/or age-related functional immunity, young stock in their first grazing season are the most susceptible becoming impacted, as measured in terms of reduced performance (e.g., insufficient growth rate or feed conversion) and also clinical disease incidence. Thus, these animals benefit most significantly from well-implemented parasite management. However, the effects of parasitism may not only be evident during the first grazing season but may also affect the productivity of the animals in their later life as expressed in terms of body condition, reproductive performance including rebreeding efficiency, milk production, or carcass traits. In order to minimize the risk resulting from parasitic infections acquired through grazing, several measures including prophylactic strategic programs were developed based on the local parasite epidemiology and with respect to production thresholds. These control measures collectively aim to reduce or at least limit the contamination with infective stages on pasture or to avoid clinical disease and thus to enable economically viable cattle production in intensive pastoral systems. Both repeated use of short-acting anthelmintics and products providing prolonged control of parasites were demonstrated to be suitable measures in

the management of parasites of grazing cattle (e.g., Shaw et al. 1998; Stromberg and Gasbarre 2006; Sutherland and Scott 2010). In the cattle industry, long-acting products are widely accepted not only because they treat existing parasitic nematode infections and prevent new ones but also because of the substantial lower resources needed to treat the animals due to reduced frequency of administrations and associated livestock handling and restraint (labor and equipment) (Forbes 2013).

Macrocyclic lactones exhibit excellent efficacy against both endoparasites and ectoparasites including having inherent residual activity. More recently, long-acting injection formulations were developed and authorized for use in several countries (Cady et al. 2013). The 3.15 %*w/v* ivermectin long-acting injectable (IVM LAI; IVOMEC® GOLD, Merial) demonstrated effective parasite control associated with improved weight gains under field conditions in South America with challenge of gastrointestinal nematode infections, tropical grubs, and *Rhipicephalus* (*Boophilus*) *microplus* ticks (Carvalho et al. 1998, 1999a, b, 2000; Alva et al. 1999; Cruz et al. 1999; Serra-Freire et al. 1999; Bridi et al. 2000; Bordin et al. 2001; Malacco et al. 2001), and in laboratory studies with induced nematode infections (Gogolewski et al. 2006) and psoroptic mange (Bridi et al. 2001; Rehbein et al. 2002).

The study reported here assessed the persistent efficacy of 3.15 %*w/v* IVM LAI over 11 weeks under natural challenge conditions in central Europe.

Material and methods

The design of the study was in accordance with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL7, "Efficacy of Anthelmintics: General Requirements," and GL12, "Efficacy of Anthelmintics: Specific Recommendations for Bovine" (Vercruysse et al. 2001). The study was conducted in compliance with VICH GL9, entitled *Good Clinical Practice* and in compliance with the local animal welfare legislation.

The study was performed as a blinded study; i.e., all personnel involved in collecting efficacy data were masked as to the treatment assignment of the animals.

Study animals

Seventy healthy, ruminating Braunvieh (Brown Swiss) male calves, weighing 135.5 to 199.5 kg prior to first treatment (day 0), and aged approximately 4 to 8 months, were included in the study which was conducted in Upper Bavaria, Germany. The animals were not previously treated with an avermectin or milbemycin product within 60 days prior to day 0. They were raised indoors from birth and were free of strongylid nematode, *Strongyloides*, *Trichuris*, and lungworm as determined by fecal examination prior to study start, i.e., day −15/−14, using a modified McMaster method and the Baermann technique (MAFF 1986).

All animals were handled with due regard to their welfare and in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals, any applicable local regulations, and requirements of any local IACUC.

Experimental design

The study was a negative control (saline), clinical efficacy study using a randomized block design based on decreasing pre-treatment (day 0) bodyweight such that 10 replicates of seven calves each were formed sequentially. Within replicates, animals were allocated randomly to one of seven groups: saline (control) at 1 mL/50 kg bodyweight once on day 0 or IVM LAI (3.15 %*w/v* ivermectin in a LAI formulation; IVOMEC® GOLD, Merial) at 1 mL/50 kg bodyweight (630 mcg IVM/kg) on either days 0, 7, 14, 21, 28, or 35, for a total of 10 animals in each treatment group. Treatments and saline as well as IVM LAI were administered by subcutaneous injection in front of the right shoulder using commercial syringes and needles. Dose volumes were calculated based on bodyweights obtained on the day of dosing rounded to the next 0.1 mL above the calculated dose volume if the bodyweight was between increments.

All calves were observed hourly for 4 h post-treatment and thereafter once daily throughout the study for health problems or adverse drug events.

All study animals were weighed on day 0 for allocation to treatment groups and dose calculation of day 0-treated calves, and on day 77; calves treated on days 7, 14, 21, 28, or 35 were weighed prior to dosing for dose calculation.

From day 35 on, all saline-treated (control) and IVM LAI-treated calves grazed the same permanent, naturally contaminated pasture (total 8.6 ha) for 42 days (October to mid-November) to ensure continuous uniform nematode exposure. After conclusion of the grazing on day 77, all calves were housed for 4 weeks before being humanely euthanized for nematode recovery and count. Until turnout onto pasture on day 35 and after removal from pasture on day 77, animals were housed as one group in an open-front shed under conditions designed to preclude nematode exposure.

During housing, animals were offered 1–2-kg maize cobs per head per day and hay for ad libitum consumption; while on pasture, animals were maintained on grass exclusively with mineral salts presented as licking blocks. Potable water was available ad libitum throughout the study.

For proof of pasture infectivity (pasture contamination) with nematode larvae, herbage samples were collected on days 21 and 28, washed, and poured over different sized sieves, and the collected debris was subjected to the Baermann technique for the recovery of the larvae (MAFF

1986). Examination of the samples revealed the presence of third-stage larvae of the nematode genera *Cooperia* (dominating), *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, and *Trichostrongylus*.

Parasite counts

All study animals were humanely euthanized for nematode recovery and counting 27 to 30 days after removal from pasture. At necropsy, the lungs, abomasum, small intestine, and large intestine were removed. Lungs were examined completely for lungworms by lengthwise opening of all accessible air passages and soaking of the dissected lungs overnight. The contents of the abomasum and small and large intestines were collected separately and diluted with water. The abomasum and small intestine were incubated (saline soak) to recover mucosal stages of the parasites for identification and counting. To facilitate isolation and counting of nematodes, organ contents and soaks were screened over sieves of appropriate mesh sizes to remove the debris. While cestodes were collected directly from the small intestines during processing and counted totally, gastrointestinal nematode counts were made on 5 % (abomasum and small intestine contents and soaks) or 25 % aliquots (large intestine content). Counts of each nematode species for each animal were calculated by multiplying the number of worms actually counted from each organ by the aliquot factor and summing over all organs.

At necropsy, helminths were identified to species or genus level and stage of development according to recognized standard methods and procedures. For the relevant *Cooperia* and *Ostertagia* species, the concept of polymorphism was accepted; i.e., as both morphotypes, *Cooperia oncophora* and *Cooperia surnabada*, or *Ostertagia ostertagi* and *Ostertagia lyrata*, respectively, were identified, counts of the morphotypes were combined for analysis as *C. oncophora/surnabada* and *Os. ostertagi/lyrata*. Polymorphism was accepted because, based on molecular genetic studies, they belong to a single species each (Newton et al. 1998; Zarlenga et al. 1998).

Data analysis

Nematode counts for each species and stage were transformed to the natural logarithm of (count+1) for calculation of geometric means for each treatment group. Efficacy was determined for each parasite by calculating the percent efficacy as $100[(C-T)/C]$, where C is the geometric mean among the saline-treated controls and T is the geometric mean of the IVM LAI-treated animals. Each IVM LAI-treated group was compared to the saline (control) group using Wilcoxon rank sum tests (two-sided at $\alpha=0.05$), and the raw p values from Wilcoxon rank sum tests were adjusted using the Holm method for multiplicity adjustment.

Day 0 and day 77 bodyweights were analyzed using analysis of variance comparing each IVM LAI-treated group to the saline-treated (control) group, and the Dunnet-Hsu multiple comparison adjustment was used to adjust for multiple comparisons. The day 0 to day 77 weight gain was analyzed using analysis of variance, and pairwise comparisons among all treatment groups were done with the Tukey multiple comparison adjustment to adjust for multiplicity.

Results

All animals were reported as normal during hourly observations for 4 h post-treatment. There were no drug-related health problems or adverse drug events and no other health problems observed at any time during the study.

As demonstrated through the parasites recovered from the control animals, study animals were exposed to challenge of *Dictyocaulus viviparus* lungworms and gastrointestinal nematodes of the species *Haemonchus contortus*, *Ostertagia leptospicularis*, *Os. ostertagi/lyrata*, *Spiculopteragia boehmi*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Bunostomum phlebotomum*, *C. oncophora/surnabada*, *C. punctata*, *Nematodirus battus*, *Nematodirus helvetianus*, *Strongyloides papillosus*, *Oesophagostomum radiatum*, and *Trichuris discolor* while grazing. In addition, *Moniezia* spp. cestodes were recovered from 3 of 10 saline-treated animals (1–3 specimens), and from 6/10 (1–4 specimens), 5/10 (1–5 specimens), 8/10 (1–5 specimens), 8/10 (1–6 specimens), 8/10 (1–6 specimens), and 7/10 (1–5 specimens) animals treated with IVM LAI on days 0, 7, 14, 21, 28, or 35, respectively, and four *Trichuris ovis* whipworms were isolated from one calf treated with IVM LAI on Day 0.

The results of the study with respect to the assessment of the persistent efficacy of IVM LAI against gastrointestinal and pulmonary nematode infections under natural challenge conditions in cattle in Germany are summarized in Table 1.

With the exception of adult *N. helvetianus* and *Trichostrongylus colubriformis*, nematode counts were generally reduced in magnitude in all IVM LAI-treated groups compared to the saline-treated controls. Treatment with IVM LAI prevented the establishment of >90 % ($p<0.05$) of *D. viviparus* (100 %), *B. phlebotomum* (100 %), *H. contortus* (98.6 %), *Os. ostertagi/lyrata* (94.9 %) and *Oe. radiatum* (93.3 %) for at least 77 days; *Os. leptospicularis* (99.1 %) for 63 days; *C. punctata* (97.7 %), *Trichostrongylus axei* (96.5 %), and *Ostertagia* spp. inhibited larvae 4 (93.3 %) for 56 days; *C. oncophora/surnabada* (96.9 %) and *Cooperia* spp. inhibited larvae 4 (98.8 %); *Nematodirus* spp. inhibited larvae 4 (97.1 %); and *Trichuris discolor* (93.6 %) for 42 days. Adult

Table 1 Nematode counts of cattle administered saline (control) or IVM LAI at weekly intervals prior to turnout for grazing and resulting persistent efficacy (persistency interval [>90 % efficacy, $p<0.05$] bold)

Parasite	Nematode counts and efficacy							
	Saline (control)		IVM LAI Persistency, 42 days		IVM LAI Persistency, 49 days		IVM LAI Persistency, 56 days	
	NI/NG[a]	Geo mean[b] (range)	NI/NG	Geo mean (range) %efficacy[c]	NI/NG	Geo mean (range) %efficacy	NI/NG	Geo mean (range) %efficacy
Dictyocaulus viviparus	10/10	204.5 (2–989)	0/10	0 100**	0/10	0 100**	0/10	0 100**
Haemonchus contortus	8/10	28.4 (0–200)	0/10	0 100**	0/10	0 100**	1/10	0.4 (0–20) 98.7**
Ostertagia ostertagi/ lyrata	10/10	6,153.4 (1,716–11,024)	2/10	2.3 (0–1,241) >99.9**	2/10	1.2 (0–342) >99.9**	5/10	7.4 (0–180) 99.9**
Ostertagia leptospicularis	10/10	407.7 (146–1,355)	1/10	0.7 (0–219) 99.8**	4/10	3.7 (0–538) 99.1**	4/10	4.0 (0–140) 99.0**
Ostertagia spp., inhibited larvae 4	10/10	7,201.1 (1,120–11,420)	7/10	75.0 (0–14,360) 99.0*	10/10	205.7 (20–4,720) 97.1**	9/10	**482.5 (0–5,620) 93.3****
Trichostrongylus axei	10/10	2,751.3 (1,680–4,160)	4/10	7.7 (0–640) 99.7**	9/10	91.4 (0–960) 96.7**	9/10	**95.6 (0–680) 96.5****
Bunostomum phlebotomum	6/10	10.5 (0–80)	0/10	0 100**	0/10	0 100**	0/10	0 100**
Cooperia oncophora/ surnabada	10/10	14,893.7 (9,720–20,300)	9/10	**456.9 (0–22,740) 96.9***	10/10	3,156.7 (980–10,700) 78.8**	9/10	972.3 (0–18,920) 93.5*
Cooperia punctata	10/10	5,557.1 (4,440–7,180)	6/10	23.7 (0–1,640) 99.6**	10/10	149.4 (40–2,020) 97.3**	8/10	**127.1 (0–3,600) 97.7****
Cooperia spp., inhibited larvae 4	10/10	4,554.6 (1,200–15,020)	7/10	**53.1 (0–4,420) 98.8****	10/10	730.4 (180–2,600) 84.0**	10/10	630.6 (40–4,500) 86.2**
Nematodirus helvetianus	10/10	719.7 (130–2,680)	10/10	771.6 (40–2,980) <0, ns	10/10	976.2 (360–3,100) <0, ns	10/10	397.4 (20–2,640) 44.8, ns
Nematodirus spp., inhibited larvae 4	10/10	409.0 (80–1,380)	5/10	**11.7 (0–340) 97.1***	8/10	39.4 (0–640) 90.4, ns	9/10	98.9 (0–620) 75.8, ns
Trichostrongylus colubriformis	6/10	35.5 (0–760)	5/10	10.4 (0–640) 70.8, ns	7/10	15.6 (0–240) 56.2, ns	5/10	14.6 (0–760) 58.9, ns
Oesophagostomum radiatum	10/10	98.0 (40–176)	1/10	0.2 (0–4) 99.8	0/10	0 100**	0/10	0 100**
Trichuris discolor	9/10	25.0 (0–156)	5/10	**1.6 (0–12) 93.6***	6/10	3.0 (0–24) 88.2*	3/10	1.7 (0–32) 93.4*

Parasite	Nematode counts and efficacy							
	Saline (control)		IVM LAI Persistency, 63 days		IVM LAI Persistency, 70 days		IVM LAI Persistency, 77 days	
	NI/NG[a]	Geo mean[b] (range)	NI/NG	Geo mean (range) %efficacy[c]	NI/NG	Geo mean (range) %efficacy	NI/NG	Geo mean (range) %efficacy
Dictyocaulus viviparus	10/10	204.5 (2–989)	0/10	0 100**	0/10	0 100**	0/10	**0 100****

Table 1 (continued)

Haemonchus contortus	8/10	28.4 (0–200)	0/10	0 100**	0/10	0 100**	3/10	**0.4 (0–40) 98.6****
Ostertagia ostertagi/lyrata	10/10	6,153.4 (1,716–11,024)	6/10	8.6 (0–408) 99.9**	8/10	45.2 (0–1,144) 99.3**	9/10	**311.9 (0–3,175) 94.9***
Ostertagia leptospicularis	10/10	407.7 (146–1,355)	4/10	**3.6 (0–612) 99.1****	9/10	57.3 (0–587) 85.9*	10/10	299.2 (7–960) 26.6, ns
Ostertagia spp., inhibited larvae 4	10/10	7,201.1 (1,120–11,420)	10/10	1,806.2 (240–21,940) 74.9**	10/10	4,447.4 (220–16,860) 38.2, ns	10/10	6,661.0 (80–22,680) 7.5, ns
Trichostrongylus axei	10/10	2,751.3 (1,680–4,160)	10/10	587.9 (100–2,800) 78.6**	10/10	1,476.6 (460–4,660) 46.3*	10/10	2,157.5 (640–7,280) 21.6, ns
Bunostomum phlebotomum	6/10	10.5 (0–80)	0/10	0 100**	0/10	0 100**	0/10	**0 100****
Cooperia oncophora/surnabada	10/10	14,893.7 (9,720–20,300)	10/10	3,832.7 (1,220–16,720) 74.3**	10/10	11,673.3 (3,580–27,020) 21.6, ns	10/10	8,076.0 (820–21,400) 45.8, ns
Cooperia punctata	10/10	5,557.1 (4,440–7,180)	7/10	1,097.5 (500–3,300) 80.3**	10/10	1,836.4 (200–5,100) 67.0**	10/10	2,377.2 (220–6,300) 57.2**
Cooperia spp., inhibited larvae 4	10/10	4,554.6 (1,200–15,020)	10/10	1,347.1 (40–7,460) 70.4*	10/10	1,602.8 (960–3,160) 64.8**	9/10	1,865.7 (0–14,760) 59.0, ns
Nematodirus helvetianus	10/10	719.7 (130–2,680)	10/10	448.4 (20–1,620) 37.7, ns	10/10	818.6 (20–4,100) <0, ns	9/10	252.1 (0–2,240) 65.0, ns
Nematodirus spp., inhibited larvae 4	10/10	409.0 (80–1,380)	8/10	93.3 (0–760) 77.2, ns	10/10	142.4 (40–660) 65.2, ns	9/10	134.6 (0–620) 67.1, ns
Trichostrongylus colubriformis	6/10	35.5 (0–760)	8/10	58.6 (0–620) <0, ns	6/10	22.1 (0–680) 37.9, ns	8/10	32.2 (0–820) 9.5, ns
Oesophagostomum radiatum	10/10	98.0 (40–176)	0/10	0 100**	3/10	0.8 (0–8) 99.2**	7/10	**6.5 (0–60) 93.3****
Trichuris discolor	9/10	25.0 (0–156)	9/10	20.2 (0–80) 19.4, ns	8/10	10.4 (0–104) 58.6, ns	9/10	12.7 (0–108) 49.2, ns

ns not significant at $\alpha=0.05$

*$p<0.05$; **$p<0.01$; probability from Wilcoxon rank sum test adjusted using Holm method for multiplicity adjustment

[a] Number of cattle infected/number of cattle in group

[b] Geometric mean count (based on transformation to ln[count+1])

[c] %Efficacy=100x (Geo mean control−geo mean IVM LAI/Geo Mean Control)

Sp. boehmi, *N. battus*, and *St. papillosus* were recovered each from two saline-treated (control) animals, and *Trichuris ovis* was isolated from one IVM LAI-treated animal only; thus, no meaningful analysis could be performed for those parasites.

Pre-treatment (day 0) bodyweights were similar for the calves treated with IVM LAI and saline. Calves of groups treated with IVM LAI had significantly ($p<0.001$) higher day 77 bodyweights and gained on average between 25.80 and 36.65 kg more during days 0 to 77 than the saline-treated controls; there was, however, no evidence that the weight gains of the IVM LAI-treated groups were different from one another ($p>0.3$) (Table 2).

Discussion

Nematode recoveries from the saline-treated controls indicated a substantial challenge of a spectrum of parasites typical for cattle in central and northern Europe including a considerable number of inhibited stages because the animals were grazed at the end of the annual pasture season (e.g., Agneessens et al. 2000; Borgsteede et al. 2000; Rehbein et al. 2003; Murphy et al. 2006; Höglund 2010; Chartier et al. 2013). The spectrum of nematodes, comprising *Os. ostertagi/lyrata* and *Cooperia* species as predominant components of the total gastrointestinal parasite burden, and the lungworm *D. viviparus*, was

Table 2 Summary of bodyweight and weight gain data

Group	Mean day 0 bodyweight (kg)	Mean day 77 bodyweight (kg)	Mean weight gain to day 77 (kg)
Saline (control)	160.00[a]	162.60[b]	2.60[d] [1.6 %]
IVM LAI, persistency 77 days	160.25[a]	189.70[c]	29.45[e] [18.4 %]
IVM LAI, persistency 70 days	161.00[a]	195.10[c]	34.10[e] [21.2 %]
IVM LAI, persistency 63 days	161.80[a]	190.20[c]	28.40[e] [17.6 %]
IVM LAI, persistency 56 days	161.85[a]	191.80[c]	30.95[e] [19.1 %]
IVM LAI, persistency 49 days	161.85[a]	201.10[c]	39.25[e] [24.3 %]
IVM LAI, persistency 42 days	160.05[a]	193.70[c]	33.65[e] [21.0 %]
	p=0.4427[f]	p<0.0001[f]	p<0.0001[f]

[a] p>0.3 from analysis of variance adjusted using Dunnet-Hsu's method for multiple comparison adjustment comparing each IVM LAI-treated group to the saline (control) group

[b,c] p<0.0001 from analysis of variance adjusted using Dunnet-Hsu's method for multiple comparison adjustment comparing each IVM LAI-treated group to the saline (control) group

[d,e] Probability from analysis of variance adjusted using Tukey's method for multiple comparison adjustment pairwise comparisons among all treatment groups: p<0.0001 for comparing each IVM LAI-treated group to the saline (control) group; p>0.3 for the pairwise comparisons among the IVM LAI-treated groups

[f] Probability from analysis of variance

representative of those species known to cause subclinical parasitism and poor productivity but also clinical disease in grazing cattle in temperate regions (e.g., Ploeger 2002; Höglund 2010; Sutherland and Scott 2010). However, some species, i.e., *H. contortus*, *Trichostrongylus colubriformis*, *N. battus*, and *Trichuris ovis* which are characteristic parasites of sheep in Germany (Rehbein et al. 1996), were also recorded in the nematode-naïve calves because the pasture used for the study had been grazed by both cattle and sheep before the study animals were turned out for 6 weeks. In addition, the finding of *Os. leptospicularis* and particularly the incidental recording of *Sp. boehmi* reflect the access of roe deer to the pastures. Both nematodes are ubiquitous parasites of roe deer in Germany (Rehbein et al. 2000) which is the common species of deer in the area.

The observed relationship of the different nematode species and the period of minimum 90 % prevention of establishment in cattle following single IVM LAI treatment—increasing from 42 days for *C. oncophora/surnabada* and *Trichuris discolor*, through 56 days for *C. punctata* and *Trichostrongylus axei*, and up to at least 77 days for *D. viviparus*, *H. contortus*, *Os. ostertagi/lyrata*, *B. phlebotomum*, and *Oe. radiatum*—reveals in its sequence the findings of several studies during the development and practical use of macrocyclic lactones administered in conventional injection and pour-on formulations and is apparently correlated to the inherent different susceptibilities of the individual nematode species to this group of compounds (Benz et al. 1989; Vercruysse and Rew 2002) in relation to the plasma profile of IVM LAI (Lifschitz et al. 2007). Laboratory challenge studies with weekly repeated inoculations found partly different minimum intervals of persistent activity at the 90 % efficacy threshold level for some species (Gogolewski et al. 2006; Zanetti Lopes et al. 2013). However, the results of this controlled study are confirmed through two studies conducted to evaluate the therapeutic and prophylactic efficacy of IVM LAI under field conditions in Germany as measured in terms fecal nematode egg and lungworm larval count reductions over 12- and 14-week grazing periods following a mid-year treatment (Knaus et al. 2013). Importantly, this controlled study under natural challenge conditions demonstrated that IVM LAI treatment prevented effectively the establishment of inhibited larvae of several nematode genera for extended periods, including the accumulation of arrested early fourth-stage *Ostertagia* larvae. Before necropsy, all study animals were kept for about 4 weeks under conditions designed to prevent further nematode infections, thus allowing normally developing and inhibited nematode larvae to be distinguished (Williams et al. 1997).

The design of this natural nematode challenge study with the use of one and the same pasture for grazing saline-treated control and IVM LAI-treated animals as one herd excluded the potential effect of the factor "pasture" and ensured a uniform infection pressure to all animals during grazing (Bransby 1993). The high prophylactic anthelmintic efficacy of IVM LAI demonstrated in the present study resulted in significantly improved rates of weight gain of IVM LAI-treated versus saline-treated cattle with the IVM LAI-treated calves gaining an average of 20 % and the saline-treated controls gaining only 1.6 % of their initial weight. This difference impressively reflects the negative impact of the substantial parasite challenge the study animals were exposed to during the 6-week grazing period and thus underlines the high prophylactic efficacy of IVM LAI. This benefit of

treatment is confirmed through the results of field studies using IVM LAI in Germany (Knaus et al. 2013) and is in line with numerous studies in which strategic anthelmintic treatments in first-season grazing cattle (Shaw et al. 1998) or an ivermectin intraruminal slow-release bolus have been used to control nematode infection in young stock (e.g., Jacobsen et al. 1995; Pitt et al. 1996; Forbes et al. 2002; Mertz et al. 2005). The weight gains of the IVM LAI-treated groups were not different from one another, such that the weight gains observed in this study illustrate the different pathogenicity of the various bovine parasites. The significantly improved weight gains resulted mainly from the extended period of prevention of infection with *Os. ostertagi*/*lyrata* and *D. viviparus* while the impact of *Cooperia* species, with some counts up to the numbers seen in the saline-treated controls, appeared to be less important, presumably due to the lower pathogenicity associated with these species (Armour et al. 1987; Parkins et al. 1990; Fox 1997; Stromberg et al. 2012).

As with any other sustained anthelmintic use, the use of long-acting products raises concerns in terms of selection of resistant parasite populations. However, monitoring of effectiveness of treatments, appropriate grazing management, and creation of refugia may be ways to reduce the selective advantage for resistant parasites.

In conclusion, the results of this study confirm the importance of effective measures to prevent economically important consequences of nematode infections in grazing cattle. The controlled study reported here demonstrated a very high efficacy and acceptability of IVM LAI when administered subcutaneously at 630 mcg/kg bodyweight to cattle against a wide range of important nematode infections under natural challenge conditions for up to 77 days. With its extended activity, IVM LAI proved to be a very effective and safe product which can conveniently be administered to control the most important parasitic infections of grazing cattle.

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Conflict of interest All authors are current employees of Merial and assisted with the study design, conduct, data analysis, and manuscript preparation.

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