Parasitol Res (2016) 115:4639–4648
DOI 10.1007/s00436-016-5256-2

ORIGINAL PAPER

Control of parasitic infection with ivermectin long-acting injection (IVOMEC® GOLD) and production benefit in first-season grazing cattle facing a high-level larval challenge in Germany

Steffen Rehbein[1] · **M. Knaus**[1] · **M. Visser**[1] · **R. Rauh**[1] · **S. Yoon**[2]

Received: 4 August 2016 / Accepted: 7 September 2016 / Published online: 15 September 2016

Abstract Gastrointestinal and pulmonary nematode infections are affecting the health and productivity of grazing cattle worldwide. To evaluate the effects of a single treatment with ivermectin long-acting injection (IVM LAI; IVOMEC® GOLD, Merial; 3.15 % ivermectin *w*/*v*) in first-grazing season cattle, two studies were conducted under continued stocking conditions for 84 or 100 days in Bavaria, Germany. Each study involved 68 naturally infected, approximately 4- to 6-month-old Brown Swiss bull calves. Animals were blocked based on pretreatment body weights. Within each block of four animals, animals were randomly assigned to treatments: one to saline (control) and three to IVM LAI. Treatments were injected at 1 mL/50 kg body weight subcutaneously in front of the shoulder. Animals in both studies were managed as one herd each grazing together. Cattle were weighed and fecal samples were collected pretreatment and at intervals thereafter for determination of weight gain and treatment efficacy, respectively. Fecal examination including composite fecal culture indicated the presence of nematodes of the genera *Cooperia* (dominating), *Haemonchus*, *Nematodirus*, *Ostertagia*, *Strongyloides*, *Trichostrongylus*, *Trichuris*, and *Dictyocaulus*, and *Moniezia* cestodes in the cattle. Following treatment, IVM LAI-treated cattle did not shed any *Dictyocaulus* larvae for 84 days while controls continued to pass larvae. Compared to the controls, IVM LAI-treated cattle had significantly ($p < 0.01$) lower strongylid egg counts at each occasion. Percentage reductions were ≥94 % up to 70 days after treatment and were ≥83.9 and 58.9 % at 84 and 100 days. Over the 84- or 100-day study periods, IVM LAI-treated cattle gained significantly more weight than the controls: 22.7 and 12.4 kg, respectively. The two studies demonstrated a high efficacy of IVM LAI against gastrointestinal and pulmonary nematode infections under field conditions in Germany which was associated with significant benefit as to weight gain.

Keywords Ivermectin · Long-acting injection · Field efficacy · Therapy · Prophylaxis · Nematodes · Cattle

✉ Steffen Rehbein
steffen.rehbein@merial.com

[1] Merial GmbH, Kathrinenhof Research Center, Walchenseestr. 8-12, 83101 Rohrdorf, Germany

[2] Merial Inc., 3239 Satellite Blvd., Duluth, GA 30096, USA

Introduction

Gastrointestinal nematode parasitism is widely regarded as the primary reason for reduced or suboptimal productivity of grazing cattle, especially under intensively managed grass-based management systems in temperate climate regions. In addition, liver fluke and, in fewer situations, lungworm infections are to be considered as additional causes of losses and disorders. Production losses resulting from both parasite challenge and parasitism have been documented in cattle of all classes and ages, from the calf to the lactating dairy cow. However, impact, economically as well as with respect to impaired animal welfare, is most important in young cattle because of the lack of acquired and/or age-related functional immunity. The magnitude of losses due to helminth infections can vary considerably, despite that under current conditions in cattle production, the majority of infections are considered to remain subclinical in nature in developed countries (e.g., Gross et al. 1999; Vercruysse and Claerebout 2001; LeBlanc et al. 2006; Stromberg and Gasbarre 2006; Sutherland and Scott 2010; Forbes 2012; Morgan et al. 2013; Charlier et al. 2014a, b).

In principle, control of parasitic nematodes in grazing cattle can be achieved either by evasion or by suppression, mainly through anthelmintic treatments. Evasive management practices are, however, often not viable alternatives for an economic farming of cattle. Although numerous work investigating the use of anthelmintics in cattle and their impact on the gastrointestinal and pulmonary nematode infection on growing cattle under field use conditions has been published (e.g., Hawkins 1993; Shaw et al. 1998; Dimander et al. 2000; Forbes et al. 2002; Mertz et al. 2005; Kunkle et al. 2013), respective reports of work conducted in grazing cattle in Germany have been relatively sparse and studies were based mostly on relatively small groups of animals only (e.g., Schnieder et al. 1996; Bauer et al. 1997; Epe et al. 1999; Rehbein et al. 2013). The objectives of the work reported here were to study the therapeutic and prophylactic anthelmintic efficacy of a single administration of an ivermectin long-acting injectable product (IVM LAI; IVOMEC® GOLD, Merial) in first-season grazing cattle and to assess the effect of treatment on the performance of the animals with respect to weight gain in two single-pasture studies under natural challenge conditions in the south of Germany. Results of a recently reported necropsy study demonstrated IVM LAI to provide >90 % efficacy against natural challenge of *Dictyocaulus viviparus*, *Bunostomum phlebotomum*, *Haemonchus contortus*, *Ostertagia ostertagi/lyrata*, and *Oesophagostomum radiatum* for at least 77 days; *Ostertagia leptospicularis* for 63 days; *Cooperia punctata* and *Trichostrongylus axei* for 56 days; and *Cooperia oncophora/surnabada* and *Trichuris discolor* for 42 days (Rehbein et al. 2015).

Material and methods

The two studies were conducted in compliance with VICH GL9, entitled *Good Clinical Practice*, and in compliance with the local animal welfare legislation. Design and conduct of the studies 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 studies were blinded studies, i.e., all personnel involved in collecting efficacy data were masked as to the treatment assignment of the animals.

Study animals and animal management

A total of 136 single-sourced male Braunvieh (Brown Swiss) calves, weighing 100 to 188 kg prior to treatment and aged approximately 4 to 6 months, were included in two studies. Animal descriptions and details are presented in Table 1.

The studies were conducted at two locations in Upper Bavaria, Germany, in two consecutive years on permanent native pastures, naturally contaminated by cattle and sheep (study 1) or cattle (study 2) grazing the pastures previously. Cattle in study 1 grazed pastures of total 8.6 ha located in the Bavarian Alpine Forelands at 470 m above sea level. Study 2 cattle grazed pastures of total 7.6 ha in the Chiemgau Alps at approximately 850 m above sea level. Three or four smaller pastures, located closely together, were used per location, and the herd was moved from one pasture to another based on availability of grass. Cattle used had no previous experience of grazing but were allowed to acquire infection with parasitic nematodes naturally during grazing the pastures for 5 or 6 weeks prior to treatment. At commencement of the studies, all cattle harbored naturally acquired nematode infections as demonstrated through shedding strongylid (other than *Nematodirus*) eggs, *Nematodirus* eggs, and/or *Dictyocaulus* larvae prior to treatment. Following treatment, saline-treated (control) and IVM LAI-treated calves remained together as one herd and grazed the same pastures for further 84 days (study 1, early July to September end) or 100 days (study 2, mid of July to mid of October), respectively, to ensure continuous uniform exposure to infective stages of parasitic nematodes. While on pasture, animals were maintained on grass exclusively supplemented only with mineral salts presented as licking blocks. Potable water was available ad libitum throughout the studies.

Experimental design

The two studies were conducted under one protocol using a randomized block design based on pretreatment body weight. Seventeen blocks of four cattle each were formed

Table 1 Description of study animals

Study	Treatment/animals per treatment	Breed	Sex	~Age (months)	Pretreatment[a] body weight (kg)
1	Control (saline), $n = 17$ IVM LAI (IVOMEC® GOLD), $n = 51$	Brown Swiss	Male	4–5	100–159
2	Control (saline), $n = 17$ IVM LAI (IVOMEC® GOLD), $n = 51$	Brown Swiss	Male	5–6	150.5–188.0

[a] Body weights measured 5 (study 2) or 4 days (study 1) prior to treatment (=day 0)

sequentially based on pretreatment (day −5 or day −4) body weights. Within blocks, each animal was randomly allocated to treatment: one to the saline (control) group at 1 mL/50 kg body weight once on day 0 and three to the IVM LAI (3.15 % *w*/*v* ivermectin in a LAI formulation; IVOMEC® GOLD, Merial) group at 1 mL/50 kg body weight (630 mcg IVM/kg) once on day 0, resulting in a total of 17 saline- and 51 IVM LAI-treated animals per study. Treatments, 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 rounded to the next 0.1 mL above the calculated dose volume if the body weight was between increments.

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

Study animals were weighed on day −5 (study 2) or day −4 (study 1) for allocation to treatment groups and dose calculation and on days 28, 56, 70, and 84 (studies 1 and 2) and day 100 (study 2). Individual rectal fecal samples were collected from all study animals once five (study 2) or four (study 1) days relative to treatment (=day 0) and 28, 56, 84 (studies 1 and 2), and 100 days (study 2) after treatment for fecal egg and lungworm larval count determination.

Fecal examination

For fecal egg counting, a modified McMaster method with one egg counted representing 10 eggs per gram of feces (EPG) was used with saturated sodium chloride solution for floatation (MAFF 1986). For lungworm (*Dictyocaulus*) larval recovery, 20-g fecal samples were subjected to the Baermann/Wetzel technique (Wetzel 1930) to establish lungworm larval counts per gram of feces (LPG). When present, eggs were referred to as “strongylid” (nematode genera including *Bunostomum*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, *Ostertagia*, and *Trichostrongylus*), *Nematodirus*, and *Trichuris* (nematode) and/or *Moniezia* (cestode) eggs.

In addition, a coproculture procedure was employed for the identification of the larvae of strongylid nematodes. Composite fecal cultures on strongylid egg-positive fecal samples (20 g per animal) were performed (fecal samples of all animals combined prior to treatment; fecal samples of animals combined by treatment group on each sampling occasion following after treatment) to determine nematode composition by genera. For coproculture, samples of fecal composites were mixed with vermiculite and incubated for 7 days after which the third-stage larvae were collected. Per culture, 100 larvae were identified to genus using a standard identification key (MAFF 1986).

Postmortem parasite counts

Because six cattle from study 1 died or were euthanized on humane grounds, animals were necropsied by a qualified veterinarian in an attempt to determine the reason of death and/or disease. Lungs and gastrointestinal tract of the animals were processed for helminth recovery and count as described previously (Rehbein et al. 2015).

Data analysis

Data of all animals were included in the analyses for all time points the data were available. Fecal nematode EPG and *Dictyocaulus* LPG counts were compared by treatment group at each time point using Wilcoxon rank sum tests (two sided at $\alpha = 0.05$). Fecal nematode egg and *Dictyocaulus* larval counts were transformed to the natural logarithm of (count + 1) for calculation of geometric means for each treatment group. Efficacy was determined 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. Exclusion criterion for individual analysis for nematodes was based on a rate of <40 % animals shedding eggs or lungworm larvae in the saline-treated controls at the posttreatment time points.

Body weight and weight gain for each treatment group from pretreatment to each posttreatment time point were analyzed using analysis of variance for a randomized block design. Data analyses were performed using procedures in SAS® version 8.2.

Results

Fecal helminth egg and *Dictyocaulus* lungworm larval count data by treatment group for the two studies are summarized in Tables 2 and 3. Pretreatment fecal examination shows that, by grazing for approximately 4 to 5 weeks, cattle had acquired patent gastrointestinal nematode and lungworm infections. Pretreatment strongylid egg and *Dictyocaulus* larval counts were not significantly different between the IVM LAI-treated and saline-treated (control) cattle in the two studies. Animals in study 1 excreted higher numbers of eggs and larvae prior to treatment compared to the animals in study 2. Following treatment, cattle treated with IVM LAI produced significantly ($p < 0.01$) fewer strongylid eggs and lungworm larvae than the saline-treated (control) cattle at each posttreatment time point. Day 28 counts demonstrate a 100 % therapeutic effect in terms of the reduction of the output of strongylid eggs and lungworm larvae. IVM LAI treatment suppressed strongylid egg production consistently throughout both studies, with reductions of ≥94 % up to 70 days after treatment and ≥83.9 and 58.9 % at 84 and 100 days.

Table 2 Summary of fecal egg and larval count data and percentage efficacy

Study	Treatment[a]	Geometric mean[b] fecal egg or larval counts					
		% efficacy[c]					
		Before treatment	After treatment				
		Day −5/−4[d]	Day 28	Day 56	Day 70	Day 84	Day 100
Strongylid eggs per gram							
1	Control, $n = 17$[e]	213.9	276.0	329.8	253.5	289.2	NS[f]
	IVM LAI, $n = 51$[g]	230.8[h]	0[i]	2.7[i]	14.4[i]	46.6[i]	NS
		NA	100 %	99.2 %	94.3 %	83.9 %	NA
2	Control, $n = 17$	133.8	239.6	310.6	342.5	326.9	255.6
	IVM LAI, $n = 51$	131.1[h]	0[i]	0.2[i]	4.6[i]	33.0[i]	104.9[i]
		NA	100 %	99.9 %	98.7 %	89.9 %	58.9 %
Nematodirus eggs per gram							
1	Control, $n = 17$	–[j]	2.0	–	–	–	NS
	IVM LAI, $n = 51$[g]	–	0.2[i]	–	–	–	NS
		NA	92.1 %	NA	NA	NA	NA
Dictyocaulus larvae per gram							
1	Control, $n = 17$[e]	11.3	11.9	22.4	5.0	3.4	NS
	IVM LAI, $n = 51$[g]	8.9[h]	0[i]	0[i]	0[i]	0[i]	NS
		NA	100 %	100 %	100 %	100 %	NA
2	Control, $n = 17$	0.7	1.5	0.4	1.0	0.9	0.5
	IVM LAI, $n = 51$	1.0[h]	0[i]	0[i]	0[i]	0[i]	0.004[i]
		NA	100 %	100 %	100 %	100 %	99.3 %

NA not applicable

[a] Control = saline, IVM LAI = IVOMEC® GOLD

[b] Geometric mean egg and larval counts based on transformation to ln (count + 1)

[c] Efficacy = 100 [(geometric mean control − geometric mean IVM LAI/geometric mean control]

[d] Day −5: study 2, day −4: study 1

[e] Day 56, $n = 14$; days 70 and 84, $n = 12$

[f] Not sampled because study was terminated on day 84

[g] Days 28, 56, 70, and 84, $n = 50$

[h] (Two sided) probability from Wilcoxon rank sum test comparing the two treatments per time point: not significant at $\alpha = 0.05$

[i] (Two sided) probability from Wilcoxon rank sum test comparing the two treatments per time point: $p < 0.01$ at $\alpha = 0.05$

[j] Not analyzed due to a prevalence of <40 % in the control group

Nematodirus and *Trichuris* eggs were observed infrequently at fecal examinations with less than 40 % of the animals shedding eggs in the saline-treated (control) groups in most instances (Table 3); meaningful analysis was thus possible only for *Nematodirus* at one occasion indicating a therapeutic efficacy of 92 % (Table 2). No lungworm larvae were detected in the feces of any IVM LAI-treated animal up to 84 days following treatment, and only one of 51 cattle in study 2 had evidence of *Dictyocaulus* infection at examination at 100 days following treatment.

Coproculture and fecal flotation revealed that gastrointestinal nematodes of the genera *Cooperia*, *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, and *Trichostrongylus* were present in the animals in both studies and *Strongyloides* and *Trichuris* in study 1. In addition, *Moniezia* eggs were detected in variable frequency in animals of both treatment groups in both studies (Table 3). Identification of nematode larvae collected from the fecal cultures indicated that *Cooperia* species were the predominant contributors to strongylid fecal egg counts in both studies (Table 4).

Pretreatment body weight was not significantly different between the IVM LAI-treated and saline-treated (control) cattle in the two studies. Day 84 and day 100 body weights and weight gain to day 84 and day 100 were significantly ($p < 0.05$) higher for the animals treated with IVM LAI than

Table 3 Frequency of detection of fecal eggs of intestinal helminths which were not analyzed apart from day 28 *Nematodirus* egg counts in study 1 (see Table 2)

Study	Treatment[a]	Number of positive animals/number of animals in the group					
		Before treatment	After treatment				
		Day −5/−4[b]	Day 28	Day 56	Day 70	Day 84	Day 100
Nematodirus eggs							
1	Control	2/17	7/17	1/14	1/12	2/12	NS
	IVM LAI	4/51	3/50	5/50	6/50	9/50	NS
2	Control	0/17	2/17	4/17	0/17	6/17	3/17
	IVM LAI	0/51	5/51	5/51	1/51	4/51	8/51
Trichuris eggs							
1	Control	0/17	0/17	1/14	2/12	0/12	NS
	IVM LAI	0/51	0/50	0/50	0/50	2/50	NS
Moniezia eggs							
1	Control	0/17	0/17	1/14	2/12	3/12	NS
	IVM LAI	0/51	0/50	4/50	14/50	9/50	NS
2	Control	0/17	0/17	1/17	2/17	2/17	1/17
	IVM LAI	0/51	5/51	11/51	11/51	8/51	9/51

NS not sampled

[a] Control = saline, IVM LAI = IVOMEC® GOLD

[b] Day −5: study 2, day −4: study 1

they were for the saline-treated (control) animals in both studies (Table 5). Weight gain of cattle treated with IVM LAI was 83 % (study 1, 84 days grazing) and 31 % (study 2, 100 days grazing) higher than the weight gained by the respective saline-treated (control) cattle.

In both studies, animals were reported as normal during hourly observations for 4 h posttreatment, and there were no treatment-related health problems or adverse drug events observed at any time during the studies. All cattle were clinically normal throughout the course of study 2. However, following a sudden fall in temperature during study 1 (marked drop of temperature combined with heavy rainfall and wind resulting in very wet and cold midsummer weather), progressing respiratory signs (bouts of coughing and dyspnea) were observed in several cattle from about 8 weeks after turnout accompanied by impaired growth and loss of body condition. Despite treatment of 15 affected saline-treated animals with injectable antibiotics and anti-inflammatory and tonic drugs, five cattle (saline-treated) died or were euthanized on welfare grounds during the period from day 31 to day 64. In addition, one IVM LAI-treated animal was found dead on pasture on day 17.

At postmortem examination, the five saline-treated cattle were in poor bodily condition and presented lesions consistent with bacterial pneumonia associated with lungworm infection and parasitic gastroenteritis. Parasite counts revealed burdens of 10 to 817 *D. viviparus* in the lungs and 3746 to 26,859 total nematodes in the gastrointestinal tract. Individual animals harbored 40 to 2240 *H. contortus*, 55 to 8100 *Os. ostertagi/lyrata*, 0 to 190 *Os. leptospicularis/kolchida*, 0 to 360 *T. axei*, 2225 to 8635 *C. oncophora/surnabada*, 850 to 11,125 *C. punctata*, 0 to 25 *Cooperia curticei*, 5 to 40 *Nematodirus battus*, 70 to 375 *Nematodirus helvetianus*, 0 to 10 *B. phlebotomum*, 0 to 15 *Capillaria bovis*, 0 to 100 *Oe. radiatum*, 0 to 1 *Oesophagostomum venulosum*, 9 to 168 *Tr. discolor*, and 0 to 4 *Trichuris ovis*. Necropsy of the IVM LAI-treated animal did not reveal any specific finding, and no helminths were recovered from the gastrointestinal tract and lungs.

Discussion

The results of the two studies confirm the relevance of bovine gastrointestinal and pulmonary parasitic infections which may still not only constitute a considerable threat to the health and well-being but comprise the most important factor which limits the productivity of grazing cattle, i.e., mainly the growth in young, naive stock in temperate regions of the world. Sustainable cattle production with maintaining acceptable thresholds of animal performance within intensive grass-based production systems is dependent upon the effective control of internal parasite infections with gastrointestinal nematodes being considered as primary target because of having the greatest overall consequences worldwide (Sutherland and Scott 2010). However, and observed through the present work, grazing cattle may be confronted with clinical parasitic disease due to infection with *D. viviparus* which was shown previously to

Table 4 Summary of nematode genera identified from composite fecal cultures made prior to (all animals) and at each time point after treatment (per treatment)

Study	Coproculture of day	Nematode larval population composition (%)[a]					
		Strongyloides	*Cooperia*	*Haemonchus*	*Ostertagia*	*Trichostrongylus*	*Oesophagostomum*
1	All animals						
	Day −4	0	59	16	23	2	0
	Treatment: control (saline)						
	Day 28	2	38	27	13	9	11
	Day 56	2	53	2	36	5	2
	Day 70	4	53	20	14	3	6
	Day 84	0	71	7	14	3	5
	Treatment: IVOMEC® GOLD						
	Day 28	NC[b]	NC	NC	NC	NC	NC
	Day 56	0	98	0	2	0	0
	Day 70	0	100	0	0	0	0
	Day 84	0	92	0	8	0	0
2	All animals						
	Day −5	0	76	1	11	12	0
	Treatment: control (saline)						
	Day 28	0	78	1	9	4	10
	Day 56	0	78	0	3	2	17
	Day 70	0	73	0	6	5	16
	Day 84	0	87	0	4	3	6
	Day 100	0	83	0	8	4	5
	Treatment: IVOMEC® GOLD						
	Day 28	NC	NC	NC	NC	NC	NC
	Day 56	0	100	0	0	0	0
	Day 70	0	72	0	0	28	0
	Day 84	0	95	0	5	0	0
	Day 100	0	88	0	7	5	0

[a] Identification of 100 third-stage larvae per pool to genus level

[b] NC = no coproculture made as all animals had zero fecal strongylid egg counts

interact positively with gastrointestinal nematode co-infections and to cause greater effects (Kloosterman et al. 1990).

The first objective of the two studies was to assess the therapeutic and prophylactic efficacy of IVM LAI in young grazing cattle subjected to continuous challenge of infective larvae and/or eggs of parasitic nematodes under field conditions in Germany. Fecal examination prior to treatment demonstrated medium to high strongylid egg counts, generally in the realm of counts reported recently for young cattle in Germany (e.g., Demeler et al. 2009; Rehbein et al. 2013; Geurden et al. 2015). In both studies, control and IVM LAI-treated animals were grazed together to exclude the factor of "pasture" (Bransby 1993). This allowed for concluding that, per study, all animals were exposed to the same level of pasture contamination as shown by the fecal egg and larval counts of the control animals and/or the spectrum and counts of nematodes recovered from the five control cattle in study 1. Results of fecal examination including qualitative coprocultures (and control animal parasite counts in study 1) confirmed that the identified diverse spectrum of nematodes, including *Cooperia* spp. and *Ostertagia* spp. (mainly *Os. ostertagi*/*lyrata*) as main components of the total gastrointestinal nematode burdens, was representative of those species known to cause subclinical parasitism associated with impaired productivity and/or clinical disease in grazing cattle in temperate regions including Germany (Ploeger 2002; Rehbein et al. 2003; van Dijk 2004; Höglund 2010; Sutherland and Scott 2010).

Interestingly, similar to recently reported cases of death of three 4- to 6-month-old cattle associated with burdens of 1200, 1800, and 4100 *H. contortus* from the UK (Hogg et al. 2010), three of the five necropsied control cattle of study 1 harbored considerable burdens of 470, 900, or 2240 *H. contortus*, and coprocultures of the control animals revealed that *Haemonchus* was an important contributor to the strongylid fecal egg counts

Table 5 Summary of body weight and weight gain data

Study	Treatment[a]	Mean[b] day −5/−4[c] body weight (kg) probability[d]	Mean day 84 body weight (kg) probability	Mean weight gain (kg) to day 84 probability	Mean day 100 body weight (kg) probability	Mean weight gain (kg) to day 100 probability
1	Control, $n = 17$[e]	131.9	159.5	27.4	NA[f]	NA
	IVM LAI, $n = 51$[g]	131.1	181.2	50.1	NA	NA
		ns[h]	$p < 0.05$	$p < 0.05$	NA	NA
2	Control, $n = 17$	167.4	198.1	30.6	207.1	39.6
	IVM LAI, $n = 51$	167.5	208.1	40.6	219.5	52.0
		ns	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

[a] Control = saline, IVM LAI = IVOMEC® GOLD

[b] Least-squares mean

[c] Day −5: study 2, day −4: study 1

[d] Probability values from analysis of variance

[e] Day 84, $n = 12$

[f] Not applicable because study 1 was terminated on day 84

[g] Day 84, $n = 50$

[h] Probability from analysis of variance: not significant at $\alpha = 0.05$

throughout this study. In Germany, *H. contortus* is primarily a parasite of sheep but is abundant in goats, roe deer, and mouflon, too (Rehbein et al. 1996, 1998, 2000; Hille et al. 2003). In the relevant study 1, recovery of *H. contortus* in the cattle was likely related to the previous use of the pasture by sheep which is further indicated by the recovery of other typical ovine nematodes, e.g., *C. curticei*, *N. battus*, and *Oe. venulosum*. As sheep are considered to be central hosts of *H. contortus* (Hoberg et al. 2004), this species is likely not well adapted to cattle such that immune mechanisms may result in a relatively early expulsion of the majority of the parasite burden, if cattle are not compromised otherwise. However, experimental studies confirm that *H. contortus* can establish well in young calves under monospecies infection conditions, at similar levels as *Haemonchus placei*, which is primarily a parasite of cattle (Fávero et al. 2015). Importantly, this study demonstrated *H. contortus* to be very prolific maintaining egg excretion as high as *H. placei*. Because of being highly pathogenic, at least for small ruminants, *H. contortus* infections may be considered in young cattle presenting with a history of ill-thrift in systems of mixed or sequential grazing of cattle and sheep.

The prior-to-treatment fecal examinations including coprocultures substantiated that all animals were patent infected pretreatment. The repeated posttreatment fecal examinations confirmed that significant continuous pasture contamination and thus animal exposure occurred throughout the grazing period as fecal egg counts provide a quantitative measure of pasture contamination (Shaw et al. 1998; Höglund et al. 2009). The level of challenge/exposure varied between studies and over time. Based on fecal egg counts of the control cattle, challenge of the cattle appeared to be relatively similar in the two studies with respect to gastrointestinal nematodes; however, considering coproculture results, *Cooperia*/*Ostertagia* ratios indicate that challenge of study 2 cattle was probably less severe given that the bovine *Cooperia* species are considered to be less pathogenic than the *Ostertagia* species (Armour et al. 1987; Parkins et al. 1990; Fox 1997). With respect to the challenge with *D. viviparus*, fecal larval counts in the control animals indicated a substantial greater exposure of the cattle in study 1 compared to that of the cattle in study 2.

The anthelmintic efficacy of IVM LAI both therapeutically and prophylactically was evident in both studies by the significant ($p < 0.01$) reduction of fecal strongylid egg and *Dictyocaulus* larval counts in the IVM LAI-treated animals when compared to the saline-treated controls at day 28 and throughout the remaining course of the two studies, respectively. The reduction of the fecal output of strongylid eggs and lungworm larvae resulting from the protection from establishment of incoming larvae will reduce the contamination of the pasture as was observed elsewhere (e.g., Rickard et al. 1991; Claerebout et al. 1994; Stromberg and Averbeck 1999). The observed relationship of the lungworm larval and strongylid fecal egg count reductions (including the coproculture results) over the course of the two studies is consistent with the findings of a controlled study reported earlier (Rehbein et al. 2015). This study, based on parasite counts of cattle treated with IVM LAI in intervals prior to challenge with nematode parasites, has shown that the length of the period of protection from incoming infection was correlated with the inherent different susceptibility to macrocyclic lactone compounds of the individual nematode species (Benz et al. 1989; Vercruysse and Rew 2001). The dose-limiting nature of bovine *Cooperia* species for macrocyclic lactones is reflected in the predominance of *Cooperia* larvae in the posttreatment fecal cultures and likely explains, in a general perspective, that for macrocyclic lactone products, anthelmintic resistance in cattle has mainly been reported with respect to *Cooperia* species (Sutherland and Leathwick 2011; Geurden et al. 2015).

The high therapeutic and prophylactic anthelmintic efficacy of IVM LAI demonstrated in the present studies was reflected in an improved rate of weight gain of the IVM LAI-treated versus the saline-treated (control) cattle in both studies and also in the protection from clinical disease in the IVM LAI-treated cattle in study 1. The weight gain advantage is in line with the results of other studies which evaluated a recently authorized eprinomectin extended-release injection formulation (Kunkle et al. 2013; Rehbein et al. 2013) and numerous studies which documented the benefit of the control of nematode infection in growing cattle using an ivermectin intraruminal slow-release bolus (e.g., Rickard et al. 1991; Jacobsen et al. 1995; Pitt et al. 1996; Ryan et al. 1997; Forbes et al. 2002; Mertz et al. 2005).

The higher benefit of IVM LAI treatment on weight gain recorded in study 1 compared to study 2 is related to the overall substantial lower challenge in the latter study as reflected in the lungworm larval counts and the composition of the gastrointestinal nematode population indicated through the coprocultures. In addition, cattle in study 1 were slightly younger and weighed, on average, approximately 35 kg less than the cattle enrolled in study 2. Thus, study 1 animals were more vulnerable to the negative impact of nematode parasitism, and control animals experienced clinical disease over the course of the study while infection in study 2 control animals remained apparently at the subclinical level. The association seen in the magnitude of the weight gain advantage and the occurrence of clinical disease or subclinical infection is consistent with the growth response recorded in studies following repeated strategic anthelmintic treatments in first-season grazing cattle (Shaw et al. 1998).

Despite the clear benefits for animal health and production, the use of products providing extended activity raises concerns in terms of selection of resistant parasite populations as does any other sustained anthelmintic use. Monitoring the efficacy of treatments, appropriate grazing management, better understanding of the parasite epidemiology, and exclusion of a proportion of the nematode population from the exposure to the treatment (creation of refugia for dilution) may be ways to reduce the selective advantage for resistant survivors. Use of anthelmintics, in combination with pasture management, is assumed to remain the cornerstone of parasite control programs for the foreseeable future (Bennema et al. 2010; Höglund 2010). Sustainable nematode control strategies require the responsible use of anthelmintics aiming a balance between maintaining acceptable levels of animal productivity and welfare and the inevitable evolution of anthelmintic resistance because future intensification of pasture-based livestock production systems will likely increase the risk of losses from nematode infections.

In conclusion, the results of the two studies confirm the need of effective measures to prevent the consequences in grazing cattle resulting from subclinical nematode parasitism and clinical parasitic disease. The field studies reported here demonstrated a high therapeutic and extended efficacy against established and incoming nematode infections and acceptability of IVM LAI when administered subcutaneously to first-season grazing cattle against a wide range of important nematode infections under heavy natural challenge conditions in temperate regions.

Compliance with ethical standards

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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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