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Limited efficacy of pour-on anthelmintic treatment of cattle under Swedish field conditions ☆

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ABSTRACT

A study on the effect of topical macrocyclic lactones (ML) against gastrointestinal nematodes (GIN) in Swedish first season grazing cattle (FSG) was performed during the grazing seasons of 2009 and 2010. Herds were recruited through farming press and both dairy and beef cattle farms were invited. A questionnaire revealed that 64% of participating farmers dewormed their animals in previous years, and of these 76% used topical formulations with ML. Four to six weeks after turnout, 107 (2009) and 64 (2010) farmers sent in individual faecal samples from 6–10 FSG. Faecal egg counts (FEC) were determined by the FECPAK®-method in 2009 and the McMaster-method in 2010, when also larvae were cultured. Average FEC of ⩾100 eggs per gram faeces (EPG) was seen in 39% of the herds in 2009 and 42% in 2010 and with arithmetic means of 258 ± 110 and 252 ± 350 EPG, respectively. Interestingly, FSG in dairy and beef herds had similar mean FEC. In herds with mean FEC of ⩾100 EPG, farmers dewormed all FSG in the tested grazing group with ivermectin (IVM) or doramectin (DOR) pour-on. In 2009, 33 (31%), and in 2010, 26 (40%) of the herds were retested 7–16 days post treatment. Mean reduction was 89% and 88%, respectively, and in only 12 (36%) and 10 (38%) herds it was ⩾95%. Beef herds had mean reductions similar to those of the dairy herds. No significant difference ($P = 0.66$) in reduction was seen between the groups treated with three different pour-on formulations, nor was there any correlation between the previous year's usage of anthelmintics and the efficacy. Larvae from post-treatment cultures analysed in 2010 with a species-specific ITS2 qPCR showed that *Cooperia oncophora* was the predominant species after deworming. Four (15%) groups also harboured surviving *Ostertagia ostertagi* post treatment.
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1. Introduction

Parasitic gastrointestinal nematodes (GIN) are common worldwide among grazing cattle, and cause welfare problems and associated economic losses due to reduced performance of their hosts (Sutherland and Leathwick, 2011). In Sweden, the most important GIN include *Cooperia oncophora* and the more pathogenic *Ostertagia ostertagi*, which usually are present as mixed infections in grazing cattle (Höglund, 2010). According to Dimander et al. (2000), untreated first season grazing (FSG) cattle, even with subclinical infections, suffered from growth depression and weighed on average 30 kg less than treated animals at the end of the grazing season (October). Strategic treatments with anthelmintic drugs, remain the principal means of control of helminth infections in grazing livestock (Prichard et al., 2007). Between 50–85% of the conventional cattle farmers in Sweden rely on prophylactic strategic treatments with anthelmintics, and generally only the FSG are subjected to suppressive deworming early in the season (Höglund, 2010). However, there is also an increasing number of farmers who under certain circumstances also rely on tactical treatments at housing.

A new challenge for European livestock farmers is the increasing evidence of emerging anthelmintic resistance (AR), which today is widespread in sheep parasites, and seems to be an emerging problem also among GIN in cattle (Demeler et al., 2009). Recent reports have shown that the extensive use of anthelmintics in the cattle livestock industry, has led to a worldwide spread of AR (Demeler et al., 2009; Gasbarre et al., 2009; Sutherland and Leathwick, 2011). Under field conditions, the detection of AR is usually based on the faecal egg count reduction test (FECRT). According to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al., 1992), resistance is declared if the group based mean reduction

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in egg counts after macrocyclic lactone (ML) treatment is ⩽95% and when the lower 95% confidence interval is ⩽90%. If only one of these two criteria is met, resistance against anthelmintics is suspected. AR in trichostrongyloid cattle nematodes detected by FECRT appears against all major anthelmintic classes, both against ML and to a lesser extent also to benzimidazoles (BZ), particularly on the southern hemisphere (Mejia et al., 2003; Waghorn et al., 2006; Soutello et al., 2007; Suarez and Cristel, 2007; Almeida et al., 2013). However, ML-resistant *C. oncophora* has also been reported from the United States (US) (Edmonds et al., 2010), the United Kingdom (UK) (Stafford and Coles, 1999; Coles et al., 2001; Sargison et al., 2009, 2010; Orpin, 2010; Stafford et al., 2010; McArthur et al., 2011), and Belgium, Germany and Sweden (Demeler et al., 2009; El-Abdellati et al., 2010b). The reason for AR development has not been fully investigated, but the way in which anthelmintics are used in cattle is believed to be the main cause (*op. cit.*). Regular treatment when deworming is not required, the continued use of the same anthelmintic compound despite lack of efficacy, and the absence of FEC sampling procedures prior to and after deworming have all been identified as major risk factors (Stafford et al., 2010).

Due to increasing problems with AR, alternatives to strategic whole-herd based parasite control strategies are constantly being evaluated globally. An example of this is targeted treatments (TT), given to whole groups of animals but with consideration to prolonged susceptibility to anthelmintics by maintaining parasites in refugia. The same applies to targeted selected treatments (TST), where only the most heavily infected individuals are identified for treatment (Kenyon and Jackson, 2012).

To date, the 2 year study by Demeler et al. (2009) is the only presented field study investigating Swedish AR conditions in FSG cattle, but only five farms located in a restricted area of central Sweden were included. Another study by Charlier et al. (2010) investigated GIN burden (*O. ostertagi*) in dairy herds, in relation to herd management and anthelmintic usage, in Belgium, Germany and Sweden in 2006, as a baseline for future investigations but without focusing on AR. The primary aim of the current study was to investigate with FECRT the effect of the most commonly used pour-on anthelmintics – avermectins – the way they are used today under field conditions among Swedish cattle including both dairy and beef herds. An additional aim was to introduce and test a novel TT concept, where deworming decisions were based on the information from FEC in fresh faecal samples collected directly from the pasture.

2. Material and methods

2.1. Animals

To investigate the efficacy of ML under field conditions among Swedish FSG, a two-year field study was conducted during the grazing seasons of 2009 and 2010. Herds were recruited through advertisements in the farming press, and both ecologically certified and conventional dairy and beef cattle herds were invited to participate in the study. The inclusion criteria were that FSG were turned out no later than May, no anthelmintics were given before test results were communicated, and there were no less than 10 FSG in the investigated grazing enclosures. Farmers received sample material including detailed instructions and a questionnaire about their herd management.

2.2. Questionnaire

A questionnaire was designed to collect herd information on the age of the calves at turnout, herd size, pasture management and anthelmintic control measures in FSG calves. Most questions were closed, except the questions about herd size and date of turnout. To determine the anthelmintic treatment method, there was a closed question (calves were not dewormed/dewormed when showing clinical symptoms/dewormed preventively) and an open question that asked for a list of the anthelmintics (commercial products) used. The questionnaires were completed by the farmers and sent in together with the first faecal samples, pre treatment. The questionnaire results were validated by determining the response rate for all questions and evaluating the agreement between information that was asked for and the anthelmintic treatment the previous year.

2.3. Sampling, FECRT and larval cultures

The infection level of each farm was determined by faecal egg counts from 6-10 randomly chosen FSG calves, collected individually by the farmers from fresh dung pats directly after deposition, 4–6 weeks after cattle turnout in April and May. Farmers were instructed to exclude air from the sample bags and store them in a cool place until they were mailed the same day for individual FEC.

In 2009, the FECPAK® method was used to determine the number of GIN eggs in 10 g of faeces from each sample, giving a diagnostic sensitivity of ⩾10 EPG (www.techiongroup.co.nz, 2013). In 2010, samples were analysed by a commercial diagnostic laboratory (Vidilab) using a modified McMaster method (Anonymous, 1986) based on 5 g of faeces and 25 ml flotation fluid, with a diagnostic sensitivity of ⩾20 EPG. The anthelmintic efficacy of the drug was interpreted through the FECRT based on each group's arithmetic mean faecal egg counts: FECRT = $100 \times (1 - [T2/T1])$ where the arithmetic FEC means before (T1) and *X*–*Y* days after (T2) deworming are compared (Kochapakdee et al., 1995).

In herds with a mean EPG of ⩾100, advice was given to the farmers to apply anthelmintic treatment to all FSG in the tested grazing groups within one week with ML, either IVM (2009 and 2010) or DOR (2010) pour-on. This is in accordance with the instructions for FECPAC®. The anthelmintics applied were randomly selected and prescribed by us, but the animals were dewormed by the farmers in accordance with the manufacturer's dosage recommendations (2009: Ivomec pour-on® 0.5 mg IVM per kg bodyweight, or Noromectin pour-on® 0.5 mg IVM per kg, 2010; Ivomec pour-on® 0.5 mg IVM per kg, Noromectin pour-on® 0.5 mg IVM per kg, Dectomax pour-on® 0.5 mg DOR per kg). Farmers were also instructed to send in new samples within 7–16 days post treatment, for follow up parasite egg counts to determine the efficacy of the treatment.

In 2010, the concept for TT was further developed as the study was conducted in collaboration with Vidilab. In addition to FECs, 10 g of the individual samples from each grazing group were also pooled by farm both before and after deworming, mixed with vermiculite and incubated under moist conditions for 2 weeks at 25 °C. Infective third stage larvae (L3) were harvested by the inverted cover glass technique, and larval cultures were saved at −20 °C for species identification by a species-specific ITS2 qPCR.

2.4. Species-specific ITS2 qPCR

Species-specific ITS2 qPCR was performed as described by Höglund et al. (2013b). Briefly, DNA from fresh frozen mixtures of pooled L3 were isolated with QIAamp® DNA Micro Kit (Qiagen). Two sets of primers (Eurofins), targeting species-specific regions in the ITS2 of rDNA in *C. oncophora* and *O. ostertagi,* respectively, and TaqMan® minor groove binder (MGB)-probes labelled with FAM™ dye at the 5' end and non-fluorescent quencher at the 3' end, were then added to 25 µl reaction tubes with 0.65 U SureStart™ Taq DNA Polymerase (Agilent Technologies), 0.3 µM of

forward and reverse primers and 0.2 μM probe, and 200 μM dNTP in a final concentration of 5 mM $MgCl_2$. The relative abundance of both species was then determined against a standard curve created from a serially diluted plasmid DNA stock solution ranging between 10^9 and 2×10^3 ITS2 copies of both species μl^{-1}. Samples and standards were run in technical duplicates in a Rotor-Gene 3000 (Corbett), with the data analysed using Rotor-Gene 6.1.90 software. Cycling conditions were: 95 °C for 10 min followed by 50 cycles of 95 °C for 15 sec and 62 °C for 60 sec.

2.5. Statistical analysis

Data were summarised in Microsoft® Excel® (2007), and statistical analyses (ANOVA, GLM, Pearson's Chi-square test) were conducted and graphs were created in Minitab® (Version 15) or GraphPad Prism (Version 4.0c). The significance level was set to $p < 0.05$.

3. Results

3.1. Questionnaire

3.1.1. Management practices

All farms ($n = 59$) that met the inclusion criteria and then participated in the study both pre and post sampling in 2009 ($n = 33$) or 2010 ($n = 26$) answered a questionnaire about herd management. The majority of the farms were geographically located in south-central Sweden in the region of Götaland, with a few exceptions in the northern and southern part of the country (Fig. 1). Results were similar in both years, with a few exceptions described below. Among the participants, 44% (26/59) were FSG on dairy farms and 56% (33/59) suckling calves on beef farms. Altogether, 76% (45/59) of the herds on these farms had more than 60 animals, and 25% (15/59) used permanent grazing paddocks for their FSG, of which 67% (10/15) were dairy herds. FSG were turned out before 6 months of age (53%, 31/59) or between 6 and 12 months of age (47%, 28/59). In total, 41% (24/59) grazed their FSG in one enclosure, and 49% (29/59) of farmers answered that the enclosure grazed by FSG this year was also grazed by FSG the year before.

3.1.2. Anthelmintic treatments

Regarding anthelmintic treatment, more than one third, 36% (21/59) of farmers did not deworm their FSGs the year before and among these, 67% (14/21) were beef producers. On the farms that did deworm the year before, the proportion that did so due to parasite problems was 14% (3/21) in 2009 and 53% (9/17) in 2010. Among the dairy farmers, 73% (19/26) dewormed their FSG the previous year. The proportion among beef producers was 58% (19/33), but 31% (6/19) of these used an anthelmintic only at housing. Most farmers estimated animal weights by eye (65%), whereas 27% used girth tape and 4% weighed their FSG to calculate the dose. All farmers carried out the deworming in previous years by themselves, and 76% (29/38) used topical formulations such as ivermectin (Ivomec® and Noromectin®), doramectin (Dectomax®), and eprinomectin (Eprinex®). Only 16% (6/38) used an intraruminal intermittent release device with oxfendazol (Systamex®), which is the only bolus capsule available in Sweden today. As few as 3% (1/38) used injectable drugs as ivermectin (Ivomec®), whereas 5% (2/38) used oral granules of febantel (Rintal®). The most common treatment frequency was once per year (81% or 17/21 in 2009 and 53% or 9/17 in 2010) followed by twice per year (19% or 4/21 in 2009 and 47% or 8/17 in 2010), which was the maximum number of annual treatments reported. Farmers answered that deworming was conducted at turnout (37%, 14/38), 2–3 weeks after turnout (16%, 6/38), 6–8 weeks after turnout (42%, 16/38), during the last month on pasture (3%, 1/38), or at housing (26%, 10/38, mainly in beef herds). Eleven of the farmers (29%, 11/38) answered that they dewormed twice, and in general the second treatment was conducted during the grazing season 6–8 weeks after the first treatment. When treatment was performed at turnout, 57% (8/14) of farmers used a topical formulation, whereas 43% (6/14) used the oxfendazol bolus.

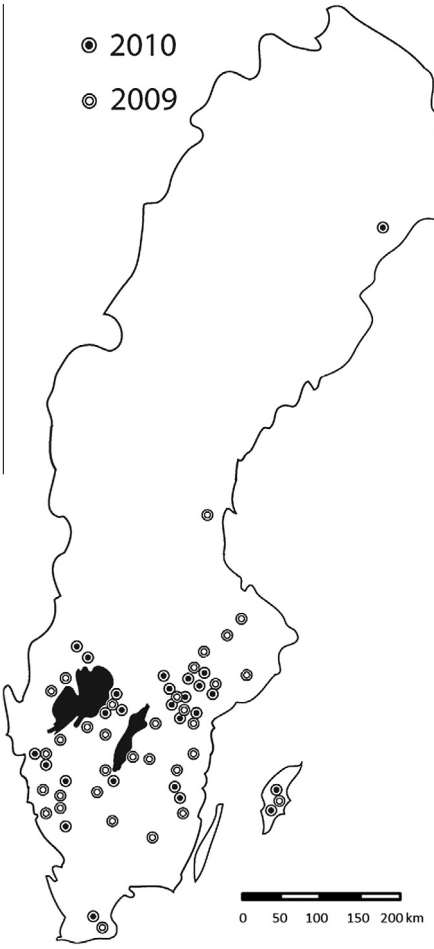

Fig. 1. Geographical distribution of farms participating with faecal samples both pre and post treatment during 2009 and 2010.

3.1.3. Preventive strategies and animal growth

Regarding preventive or prophylactic strategies, 6% (2/33) of farmers in 2009 and 31% (8/26) in 2010 had none. Of these, 70% (7/10) were beef producers. Of all farmers, 61% (20/33) in 2009 and 12% (3/26) in 2010 replied that they usually use prophylactic deworming, and 15% (5/33) in 2009 and 35% (9/26) in 2010 awaited signs of clinical disease. Altogether, 25% (15/59) of farmers use a pasture rotation strategy, and 33% (11/33) in 2009 and 8% (2/26) in 2010 fed extra forage at pasture. In 2009, 18% (6/33) of farmers shifted pastures between animal species as a preventive action, but in 2010 none of them did. Of all farmers, 78% (46/59) claimed that their animals did not suffer from reduced growth the previous season and 12% (7/59) said they did.

3.2. Parasitological data

In 2009, FEC conducted 4–6 weeks post turnout revealed a mean of 122 EPG (max 670) in 107 participating animal groups before treatment. Of the tested herds, 42 (39%) had an average egg

Table 1
Grazing groups composed of FSG from beef and dairy herds sampled 4–6 weeks post turnout, and then again 7–16 days post deworming. Groups were tested during grazing seasons 2009 ($n = 33$) and 2010 ($n = 26$). The table shows mean faecal egg counts, expressed in EPG, for each category divided into anthelmintic treatment and herd management. Values in brackets are the minimum and maximum mean EPG from the grazing groups in the category, both before (pre) and after (post) treatment. The table also shows reductions with 95% confidence intervals in brackets.

	Herds, *n*=	EPG_{pre}	EPG_{post}	Mean red%
2009				
Ivermectin	33	258 (100–600)	28 (0–170)	89 (83–93)
Doramectin	–	–	–	–
Beef herds	16	267 (110–600)	26 (0–80)	90 (84–94)
Dairy herds	17	250 (120–400)	29 (0–170)	88 (75–94)
2010				
Ivermectin	18	251 (100–1247)	34 (0–186)	86 (76–92)
Doramectin	8	253 (130–460)	19 (0–73)	92 (83–97)
Beef herds	16	232 (100–551)	32 (0–186)	86 (75–92)
Dairy herds	10	286 (107–1247)	25 (0–160)	91 (81–96)

count ⩾100. Of the 42 herds, 33 (arithmetic mean 258 EPG) completed the study with a second sampling 7–14 days post ML treatment (Table 1). Nine herds were excluded due to late or no second sampling. Among the 33, dairy herds ($n = 17$) and beef herds ($n = 16$), had similar initial mean egg counts of 250 EPG and 267 EPG, respectively. Mean FECR after treatment among the 33 re-tested herds was 89% (95% CI 83–93, max 100%, min 47%) and mean EPG was 28 (min ⩽10, max 170). Of these, 12 herds had mean reductions ⩾95%, and in only five herds did IVM eliminate the GIN (100% FECR). Of the retested grazing groups, 17 were from dairy herds with a mean FECR of 88 (75–94)%, and 16 were from beef herds with suckling calves and with a mean FECR of 90 (84–94)% (Table 1).

In 2010, FEC conducted 4–6 weeks post turnout revealed a mean of 119 EPG (max 1247) in all 64 grazing groups investigated before deworming. In total, 26 (41%) had egg counts ⩾100 EPG, and the arithmetic mean among these was 252 EPG. Dairy herds ($n = 10$) and beef herds ($n = 16$) had similar mean egg counts of 286 (±414) EPG and 232 (±305) EPG, respectively. Between 7 and 16 days post treatment, 26 herds were retested. The mean reduction was 88% (CI 81–93, min 50%, max 100%) and in 10 herds there was a mean reduction of ⩾95%. Maximum reduction (100%) was seen in only eight herds. Beef herds had a mean reduction of 86 (CI 75–92)%, which was slightly lower than the 91 (CI 81–96)% reduction observed in the dairy herds. No significant difference ($p = 0.66$) was seen between the groups treated with ivermectin (Ivomec pour-on®, $n = 8$, or Noromectin pour-on®, $n = 10$) or doramectin (Dectomax pour-on®, $n = 8$) (Table 1).

No significant differences were found when comparing FEC reductions and the previous year's reported anthelmintic treatment ($p = 0.42$) or choice of drug ($p = 0.36$). Neither was there any significant pattern when first sample EPG was compared to the previous year's treatments. No correlation ($R^2 = 0.0007$) was seen when comparing days between deworming and second sampling with reduction of EPG (Fig. 2).

3.3. qPCR, larval differentiation

In the 26 larval cultures collected pre treatment, 35% of the identified ITS2 copies were specific for *O. ostertagi* and 65% for *C. oncophora*. In the 26 cultures from post-treatment samples, *C. oncophora* was the predominant species (99% of the total copy number) with ITS2 copies ranging from 108 to 17.48×10^6 (mean 2.98×10^6). Only the pooled cultures from four grazing groups had ⩽2000 *C. oncophora* copies after deworming (Fig. 3). Four groups showed strong positive (⩾2000 ITS2 copies) results for *O. ostertagi*, (min: 4166, max: 5.83×10^5 ITS2 copies), while the other 22 groups were weakly positive (min: 8, max: 1374 ITS2 copies) after deworming. Three of the four strong positive samples were from suckling calves. Reaction efficiency was always ~0.9.

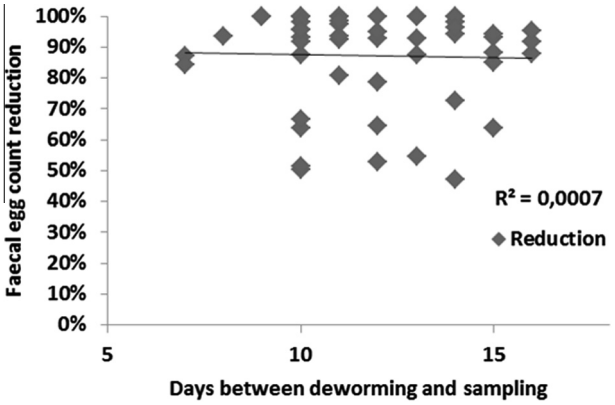

Fig. 2. Faecal egg count reductions in percentage, compared to days between deworming and second sampling during grazing seasons of 2009 and 2010.

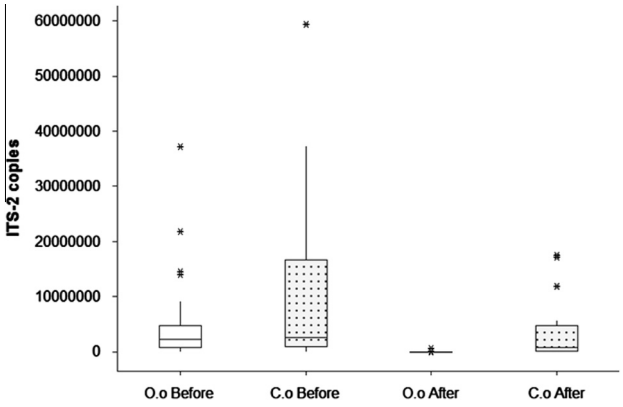

Fig. 3. Box plots of species-specific ITS2 qPCR on larval culture material from 26 Swedish farms in 2010. In pre-treatment samples, 35% of total ITS2 copies were specific for *O. ostertagi* and 65% for *C. oncophora*. In the 26 cultures from post-treatment samples, *C. oncophora* was the predominant species (99% of total copies). Four groups showed strong positive (>2000 copies) results for *O. ostertagi*, (4166-583050 ITS2 copies) while the other 22 groups were close to negative.

4. Discussion

Our study provides the first investigation about the efficacy of topical formulations of ML on trichostrongylid nematodes in FSG beef and dairy cattle in Sweden. According to the FECRT guidelines of WAAVP (Coles et al., 1992), our data indicate widespread avermectin-resistant GIN in Swedish cattle herds. Importantly, we investigated anthelmintic efficacy in commercial herds under practical farming conditions. Thus, this study reflects how ML pour-on products work when farmers estimate the dose and dispense the drug themselves, rather than under strict experimental conditions when animals are weighed. Even if the instructions were uniform to all farmers, there are several possible opportunities for mistakes when different persons administer the drug and collect samples. All of the prescribed anthelmintics were freshly delivered from the pharmacies. Still, examples of confounding factors to consider when AR is investigated by FECRT include drug storage conditions and failure of drug application that may result in underdosing. Another limitation of the FECRT is the lack of sensitivity due to a

possible overdispersed distribution of worms in the host population and non-uniform distribution of eggs in samples, which may lead to the false interpretation of reduced anthelmintic efficacy (Vidyashankar et al., 2007; Levecke et al., 2009; El-Abdellati et al., 2010a). The FECRT measures the effects on egg production by gravid female worms, and accordingly is not highly correlated with the actual worm burdens (Eysker and Ploeger, 2000). Thus, the interpretation of FEC of cattle nematodes is complicated by the presence of mixed populations of both adult and immature worms of different species, and with varying egg production over time in relation to the level of acquired immunity (Jackson et al., 2006). According to the WAAVP guidelines, follow-up samples should be collected within a certain time frame, depending on the anthelmintic compound tested (Coles et al., 1992). In this study it was, for practical reasons, impossible to collect the follow-up samples after an exact number of days, as we relied on the active participation of the farmers. To evaluate whether the number of days that elapsed between deworming and the second sample had an effect on the egg output reduction, we decided to test the correlation of percentage egg reduction with sampling time. Since they were uncorrelated, we conclude that the results, in this case collected from 7–16 days post treatment, were unaffected by the sampling day within this range. This is consistent with Demeler et al. (2009), where the samples were examined between 1 and 3 weeks after deworming.

In order to confirm AR, it is desirable to have a controlled efficacy test including naïve calves experimentally infected with field isolates and control isolates with a known susceptibility status (De Graef et al., 2012). However, such tests are costly, time consuming, and need an ethical approval for use and sacrifice of laboratory animals. Regardless of potential confounders, our results indicate that the way in which cattle farmers use pour-on ML today may cause an incomplete efficacy towards GIN among Swedish cattle herds.

The low efficiency of avermectins was, just as in the previous survey by Demeler et al. (2009), somewhat surprising, following the low frequency of anthelmintic use (64%) and usually only with a few treatments (e.g. once or twice) per year. Compared to the southern hemisphere (for a review, see Sutherland and Leathwick 2011), the level of treatment must be considered low, and thus raises the question of how AR has been selected for. Many farmers (25%) graze their FSG on the same confined pasture year after year, and it is well known that L3 of both species overwinter on pasture, which contributes to the formation of refugia (Dimander et al., 2000; Höglund et al., 2013c). It has been suggested that the common use of pour-on anthelmintics with persistent activity, in this case 76%, is likely to select for AR when drug profiles decline over time (Sutherland and Leathwick, 2011). A model of Barnes et al. (1995) suggested that once a certain level of resistant GIN has been established, the following treatments will result in an exponential increase of drug-resistant nematodes. However, this study is descriptive, and we have not experimentally investigated the potential mechanisms behind AR.

The dose-limiting species *C. oncophora* (Vercruysse and Rew, 2002) was clearly the main survivor following anthelmintic treatment, according to the species-specific PCR conducted in 2010. Although there are several possible explanations for why *C. oncophora* survives deworming, it is natural to expect that ML resistance would first emerge in this species among the cattle parasites (Coles, 2002). It has previously been shown that repeated sub-therapeutic treatments rapidly select for AR in trichostrongyloid nematodes of cattle (Molento et al., 1999; Van Zeveren et al., 2007). Thus, regardless of whether the poor efficacy on our studied farms reflects only underdosing, this is a risk factor, and is likely to select for AR in the future. Lifschitz et al. (2000) showed that the concentration of IVM is lower in the intestinal mucosa than in the abomasal mucosa, which are the predilection sites of *C. oncophora* and *O. ostertagi*, respectively. The pharmacokinetic properties of avermectins may to some extent explain why *C. oncophora* has a higher resilience to the drug than does *O. ostertagi*. Pharmacokinetic causes of reduced anthelmintic efficacy and how they are affected by body condition are, as yet, little explored in this context, but we have recently shown that simultaneous treatment with dexamethasone interferes with the pharmacokinetics of IVM (Areskog et al., 2012). Such causes of lack of efficacy and their potential involvement in the selection for AR need to be further studied.

In agreement with the AR survey made by Demeler et al. (2009), some farms harboured survivors of the more pathogenic species *O. ostertagi*. Whether this should be interpreted as a sign of early AR development is currently unknown, but no relationships were found when comparing FEC reductions and previous year's reported anthelmintic treatment or choice of drug. The overall use of anthelmintics the year before was generally low in the tested grazing groups. Although we noted large differences in drug use between years, 64% of farmers regularly dewormed their animals, and among these 76% mainly relied on topical ML formulations. This is in agreement with Charlier et al. (2010), who performed a similar survey in 2006 and reported that 69% of Swedish farmers dewormed their FSG. In Germany and Belgium, the corresponding proportions were 83% and 78%, respectively. In contrast, most (63%) Swedish farmers relied mainly on BZ, whereas German and Belgian farmers primarily used ML (78% and 62%, respectively). Thus, the present study indicates that the use of topical formulations of ML has recently increased in Sweden.

In our study, three out of the four farms with surviving *O. ostertagi* were from suckling calves in beef herds and only one was from FSG on a dairy farm. Interestingly, the mean FEC in initial faecal samples were similar in FSG both on dairy and beef farms. Still, 70% of the farmers with no preventive strategies were beef producers. These also constituted a higher proportion of those who did not deworm the previous year. However, of the beef farmers who did use anthelmintics, 31% used ML at housing, with the synergistic effect that the animals are also treated against common ectoparasites. Nevertheless, this study indicates that suckling calves in beef herds should be taken into consideration for GIN control to a greater extent than is done in Sweden today, where the common idea is that suckling calves are not exposed to parasites on pasture (Höglund et al., 2013c). It has recently been demonstrated that the effect of calving date was greater than the level of residual contamination, and early-born calves was found to be more heavily exposed to trichostrongyloid nematodes than late-born calves (Höglund et al., 2013c). As there are such natural causes generating variation in the exposure levels to pasture borne parasites, attention should be paid to opportunities for targeted parasite control also in suckling calves grazing with their dams.

With TST strategies, herds and/or animals that require treatment are identified, and therefore this is also a possible approach to postpone development and spread of AR. The use of anthelmintics can be reduced and selection pressure on susceptible parasite isolates thereby decreased (Kenyon and Jackson, 2012). Another advantage is that these treatment strategies are accepted by organic producers (Höglund et al. 2013a). There are several possibilities for TST, either based on performance factors such as live weight gain (Höglund et al., 2013a), or parasitological variables such as FEC and/or serum pepsinogen levels. In the present study we attempted a TT strategy based on FEC 4–6 weeks after turnout. Only 40% of the tested FSG herds in our study fulfilled the inclusion criterion and had mean egg counts of $\geqslant$100 EPG, which supports the idea of TT rather than preventive blanket treatment without prior diagnosis.

In conclusion, this study indicates that anthelmintic treatment efficacy of topical ML under Swedish field conditions is insufficient

according to the FECRT data, and highlights an incipient development of resistance or risk factors for a future spread of AR. Complementary controlled efficacy tests, as well as pharmacokinetic studies of drug administration and uptake, might further elucidate the phenomenon of poor efficacy under field conditions. In the future, more attention should also be paid to opportunities for parasitic nematodes in suckling calves grazing together with their dams, and to the development of TST approaches for parasite control.

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