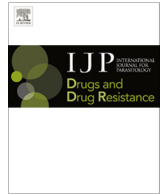


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Limited efficacy of pour-on anthelmintic treatment of cattle under Swedish field conditions

Marlene Areskog^{a,1}, Bitte Ljungström^{b,2}, Johan Höglund^{a,*}^a Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, Swedish University of Agricultural Sciences, SE-751 89 Uppsala, Sweden^b Vidilab, Enköping, Sweden

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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 $\geq 95\%$. 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 sub-clinical 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

* Corresponding author. Tel.: +46 18672371.

E-mail addresses: marlene.areskog@slu.se (M. Areskog), bitte@vidilab.se (B. Ljungström), johan.hoglund@slu.se (J. Höglund).¹ Tel.: +46 18672390.² Tel.: +46 171441260.

in egg counts after macrocyclic lactone (ML) treatment is $\leq 95\%$ and when the lower 95% confidence interval is $\leq 90\%$. If only one of these two criteria is met, resistance against anthelmintics is suspected. AR in trichostrongyloid cattle nematodes detected by FEC-RT 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 FECPAK[®]. 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 μ l reaction tubes with 0.65 U Sure-Start[™] Taq DNA Polymerase (Agilent Technologies), 0.3 μ M of

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