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Efficacy of ivermectin against gastrointestinal nematodes of cattle in Denmark evaluated by different methods for analysis of faecal egg count reduction

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ABSTRACT

The efficacy of ivermectin (IVM) against gastrointestinal nematodes in Danish cattle was assessed by faecal egg count reduction test (FECRT). Six cattle farms with history of clinical parasitism and avermectin use were included. On the day of treatment (Day 0), 20 naturally infected calves per farm (total n = 120) were stratified by initial faecal egg counts (FEC) and randomly allocated to a treatment group dosed with 0.2 mg IVM kg^{-1} body weight s.c. (IVM; n = 10) or an untreated control group (CTL; n = 10). Individual FEC were obtained at Day 0 and Day 14 post-treatment and pooled faeces by group were cultured to isolate L3 for detection of *Ostertagia ostertagi* and *Cooperia oncophora* by qPCR. Treatment efficacies were analysed using the recommended WAAVP method and two open-source statistical procedures based on Bayesian modelling: 'eggCounts' and 'Bayescount'. A simulation study evaluated the performance of the different procedures to correctly identify FEC reduction percentages of simulated bovine FEC data representing the observed real data. In the FECRT, reduced IVM efficacy was detected in three farms by all procedures using data from treated animals only, and in one farm according to the procedures including data from treated and untreated cattle. Post-treatment, *O. ostertagi* and *C. oncophora* L3 were detected by qPCR in faeces of treated animals from one and three herds with declared reduced IVM efficacy, respectively. Based on the simulation study, all methods showed a reduced performance when FEC aggregation increased post-treatment and suggested that a treatment group of 10 animals is insufficient for the FECRT in cattle. This is the first report of reduced anthelmintic efficacy in Danish cattle and warrants the implementation of larger surveys. Advantages and caveats regarding the use of Bayesian modelling and the relevance of including untreated cattle in the FECRT are discussed.

1. Introduction

Grazing cattle are continuously exposed to infection with gastrointestinal nematodes (GIN) that can severely impair the health and productivity of pasture-based livestock systems (Corwin, 1997; Shaw et al., 1998; Charlier et al., 2014). In practice, the control of GIN in cattle largely relies on the routine use of anthelmintic drugs, mainly from the macrocyclic lactone (ML) family (Vercruysse and Rew, 2002; Geurden et al., 2015). As a consequence, worm populations resistant to MLs have been selected, and anthelmintic resistance (AR) is now becoming a serious threat to the control of bovine nematodes in several countries (Sutherland and Leathwick, 2011; Gasbarre, 2014;

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Sutherland and Bullen, 2015). Coinciding with the development of AR, concerns regarding the prophylactic use of veterinary drugs and chemical residues in both food and environment have led to stricter regulations on the use of anthelmintics in some nations (Thamsborg et al., 1999). In 1999, Denmark became the first country to introduce prescription-only use of anthelmintics in livestock, requiring a mandatory veterinary diagnosis before treatment in both organic and conventional farms (Anonymous, 1998, 2013). Since 2000, there has been an additional requirement for all prescriptions in production animals to be registered in 'VetStat' – the Danish system for surveillance of the veterinary use of drugs (Stege et al., 2003). Preliminary analyses in VetStat indicate that MLs accounted for ~85% of all anthelmintics prescribed for Danish cattle between 2010 and 2012, with ivermectin (alone or in combination) representing 72% of all ML prescribed (Peña-Espinoza et al., unpublished data). However, and despite the significance of ivermectin for current parasite control strategies in cattle, its field efficacy against GIN has not been investigated in Denmark.

In the absence of quantitative molecular techniques for the detection of ML-resistance, and the high cost of the controlled efficacy test (the current gold standard method for verification of anthelmintic activity; Wood et al., 1995), the only readily available technique for investigating field drug efficacy is the faecal egg count reduction test (FECRT). This technique estimates the efficacy of an anthelmintic to reduce the faecal egg counts (FEC) of infected animals based on measurements pre- and post-treatment, or between treated and untreated individuals. The major advantages of the FECRT are that all drugs can be tested regardless of active compounds or formulation and that it relies on FEC detection methods readily available in most diagnostic laboratories. The current recommendations to conduct and analyse FECRT in cattle derive from guidelines by the World Association for Advancement of Veterinary Parasitology (WAAVP), which were originally developed for detection of AR in sheep nematodes (Coles et al., 1992). However, potential limitations have been highlighted concerning the use of FECRT with bovine nematodes, mainly due to the lower faecal egg excretion of cattle, compared to sheep, and the highly aggregated distribution of FEC in cattle groups (Coles, 2002; Coles et al., 2006; Demeler et al., 2010; El-Abdellati et al., 2010; Sutherland and Leathwick, 2011). These factors may limit the correct analysis of FECRT data and inference of drug efficacy in cattle using the WAAVP guidelines. More recently, Bayesian modelling using Markov chain Monte Carlo (MCMC) methods have been advocated as robust statistical analyses to cope with low and aggregated FEC data (Denwood et al., 2010; Torgerson et al., 2014). These MCMC-based procedures, available as open-source R packages or web-interface software, are being increasingly used to infer drug efficacy and to monitor AR in horse nematodes (Denwood et al., 2010; Fischer et al., 2015) and cattle helminths (Neves et al., 2014; O'Shaughnessy et al., 2014; Geurden et al., 2015; Novobilský and Höglund, 2015; Ramos et al., 2016). However, the performance of these MCMC procedures with the low mean FEC and parasite aggregation levels commonly found in cattle has not yet been evaluated. In addition, sensitive and species-specific tests to detect which GIN species survive treatment are critical for the surveillance of AR and are urgently required for cattle (Coles, 2002; Sutherland and Leathwick, 2011).

The objectives of the present study were: 1) to assess the efficacy of ivermectin (IVM) against GIN in naturally infected Danish cattle by FECRT, and 2) to evaluate the performance of different statistical approaches for estimating drug efficacy using simulated bovine FEC data of similar characteristics to those observed in Danish cattle. In addition, we investigated the prescription patterns of anthelmintics in the study farms in order to examine a possible relationship between previous use of avermectins and IVM efficacy in the FECRT.

2. Materials and methods

2.1. Selection of farms

Cattle farms (~50) with a history of clinical parasitism were contacted through local veterinarians across Denmark during spring 2013 and 2014. The farmers were offered free FEC analyses and evaluation of anthelmintic efficacy by FECRT. Farms were selected based on the following criteria: herd size ≥20 first-season grazing (FSG) calves with ≥4 weeks of grazing (before the initial screening) and not treated with anthelmintics within 8 weeks prior to sampling. In addition, the availability of a cattle crush or barn was required for the handling of animals. A total of 19 farms (8 in 2013 and 11 in 2014) that fulfilled these criteria accepted the invitation. Individual faecal samples were collected from 20 FSG calves in each farm between mid-June and early September of 2013 and 2014 for analysis of FEC (initial screening). Due to a low number of farms with mean FEC >150 strongyle eggs per g (epg) of faeces (as recommended by Coles et al., 1992), farms with a mean FEC ≥ 75 epg were selected for the FECRT. Of the six farms finally included in the study, one herd was a conventional beef farm (farm #1), three were organic dairy farms (#2, #4 and #6), one was an organic beef farm (#5) and one was a conventional dairy farm (#3). In Denmark, organic cattle farms should by law provide access to pasture from 15 April until 1 November (Anonymous, 2016), while conventional farms do not have to comply with this rule. The cattle breeds in the investigated farms were Danish Holstein crossbreeds (#1 and #5), Danish Holstein (#2, #3, and #6) and Danish Jersey (#4). All the selected farms were located in the Jutland Peninsula and the FECRT was conducted within one to four weeks after the initial screening.

2.2. Faecal egg count reduction test (FECRT)

The FECRT was performed to test the efficacy of IVM based on WAAVP recommendations (Coles et al., 1992). Pre- and post-treatment faecal samples from treated and untreated animals were included, and a total of 120 FSG calves were enrolled in the FECRT studies. On the day of treatment (Day 0), 20 FSG animals from each farm were stratified by FEC (based on the initial screening) and randomly allocated to a treatment group (IVM; n = 10) or an untreated control group (CTL; n = 10) of similar (initial) mean FEC. Due to a limited number of animals available in farms #4 and #6 at the start of the FECRT, the CTL groups at these properties consisted of nine calves. Oral formulations of IVM are not registered for use in cattle in Denmark, thus injectable IVM was used. At Day 0, individual body weights (BW) were estimated in the IVM group using a girth tape for cattle (Rondo combi®, Kruuse, Denmark), and the calves in the treatment group were injected with the recommended dose of IVM (0.2 mg kg^{-1} BW s.c., Ivomec® 10 mg/mL, Merial Norden A/S). A comparison of BW estimations between girth tape and electronic scale in a group of 30 FSG calves (BW range = 84–172 kg) was performed prior to the study and demonstrated a very high correlation between the methods (Pearson's correlation = 0.98). Faecal samples were collected rectally from all animals on Day 0 and 14 days post-treatment (Day 14). Immediately after collection, the faecal samples were vacuum packed (Freshield Touch, CSE Co, Gyeonggi-do, Korea) to create anaerobic conditions and transported to the laboratory in a cooling box. On all farms, animals in the IVM and CTL groups grazed together on the same pastures until Day 14, when all control calves