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Diagnosis of anthelmintic resistance in cattle in Brazil: A comparison of different methodologies

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ABSTRACT

The occurrence of anthelmintic resistance to levamisole, albendazole, ivermectin and moxidectin was investigated in cattle from 10 farms located in São Paulo State, Brazil, using two techniques for counting eggs in faeces: McMaster with a sensitivity of 50 eggs per gram (EPG) and FLOTAC with a sensitivity of two EPG. We also evaluated the use of different mathematical and test design approaches to determine the efficacy of the anthelmintic treatments: one formula/design that compares post-treatment arithmetic mean EPG counts for the treated and control groups (FECRT1) and two methods to analyse data from pre- and post-treatment EPG counts in the same group (FECRT2 and FECRT3, respectively). Treatment groups received either ivermectin (0.2 mg/kg of body weight (BW); moxidectin (0.2 mg/kg BW); albendazole (2.5 mg/kg BW); levamisole (4.7 mg/kg BW); or no treatment (control group). The number of animals in each group ranged from 8 to 11. Faecal samples from each animal were collected 2 days before the treatment and again 10 and 28 days post-treatment. The FEC reduction (FECR) confidence intervals were usually wider when based on data obtained using the McMaster method than when data were obtained using the FLOTAC method. Efficacy estimated from pre- and post-treatment EPG counts in the same group presented smaller confidence intervals. Ivermectin proved to be totally ineffective in all herds evaluated. *Cooperia* spp. was the major parasite displaying resistance, followed by *Haemonchus* spp. The results also indicated the presence of *Oesophagostomum* spp. and *Trichostrongylus* spp., meaning they, too, were resistant to ivermectin. Resistance to moxidectin was found on nine of the 10 farms investigated; however, only three farms had previously used moxidectin. In contrast, albendazole and levamisole demonstrated high efficacy on the majority of farms. In surveys for anthelmintic resistance in cattle, the use of a diagnostic method with higher sensitivity to detect eggs is recommended, as is the case with the FLOTAC method. This study indicates that by using techniques with high sensitivity and by testing the same animals pre- and post-treatment, good precision can be achieved with group sizes from 8 to 11 animals.

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1. Introduction

Anthelmintic resistance has become a global problem in the cattle industry (reviewed by Sutherland and Leathwick, 2011). The primary method used for the diagnosis of anthelmintic resistance is the faecal egg count reduction test (FECRT), which can be used to detect the presence of nematodes with resistance to all groups of anthelmintics (Coles et al., 1992). However, there is a need for improved methods of detecting anthelmintic resistance as several factors complicate the diagnosis of resistance in cattle. First, it is recommended that each group consist of at least 15 animals and that the animals shed a minimum of 150 eggs per gram of faeces (EPG) (Coles et al., 1992). Often, most of the animals in a herd, even the young ones, have lower faecal egg counts (FEC). Furthermore, most of the farms do not have a sufficient number of animals to allow testing several drugs simultaneously. For example, to test four anthelmintics, 75 animals with an EPG count higher than 150 would be necessary, considering also an untreated control group. An innovation was introduced by Dobson et al. (2012) in a formula that uses the total number of eggs counted rather than eggs per gram of faeces to determine efficacy. This approach focused on how many eggs were observed, rather than on the number of animals in each group or the mean FEC. Dobson et al. (2012) suggest that rather than attempting to estimate the mean, it would be more effective to count a large number of eggs pre-treatment from high shedding animals (e.g., the four animals in the group with the highest counts) and then count the number of eggs from the same animals post-treatment. Torgerson et al. (2014) propose using the R package "eggCounts" that incorporates both sampling error and over-dispersion between animals to calculate the true egg counts in samples of faeces. Based on a hierarchical Bayesian framework, the software estimates the percentage reduction of FEC and the 95% uncertainty intervals of data generated by an FECRT.

We investigated the occurrence of anthelmintic resistance to levamisole, albendazole, ivermectin and moxidectin in cattle from 10 farms using two techniques for counting eggs in faeces: McMaster, with a sensitivity of 50 EPG (Ueno and Gonçalves, 1998), and FLOTAC, with a sensitivity of two EPG (Cringoli et al., 2010). We also evaluated the use of different mathematical approaches to determine the efficacy of the anthelmintic treatments.

2. Materials and methods

This work was developed in accordance with the ethical principles of animal experimentation and was approved by the local ethics committee on animal use (protocol 44/2012/CEUA-FMVZ).

2.1. Description of cattle herds and management

Ten cattle farms located in São Paulo State, Brazil (Fig. 1) were evaluated between May 2012 and June 2013. The presence of a balance for weighing animals was a prerequisite for the test to be conducted on the property to enable the accurate administration of anthelmintic. The number of cattle ranged from 210 to 800 head, and the age, sex and breed of animals on each farm are presented in Table 1. The bovines did not receive any anthelmintic treatment during the 8 weeks preceding the experiment.

On all farms, the administration of anthelmintics was the only approach used to control GIN infections. The frequency of anthelmintic treatments ranged from 2 to 12/year and included primarily macrocyclic lactones, especially ivermectin (Table 1), as it also controls certain ectoparasites. Albendazole and levamisole were also used on farm 1 and farm 5, respectively. The farmers did not weigh the animals before the administration of the anthelmintic, excepting farm 1. The dose administered to the animals was based on a visual estimation of body weight. Most owners drenched the cattle in combination with the vaccine against foot-and-mouth disease, following the official national calendar for prophylaxis of that disease. One farm reported that treatments were given when animals showed clinical signs of parasitism (farm 5). On six farms, treatments were administered to all animals in the herd, while on two farms, only animals up to 18 months of age were treated.

2.2. Experimental description

The bovines were assigned to experimental groups based on their previous stratification according to their FEC using the FLOTAC method, which was performed on individual faecal samples 2 days prior to treatment. The animals were classified in increasing order of EPG into FEC classes of five animals each. Randomly, one bovine from each class was allocated to the following groups: Group 1 – treated with injectable ivermectin (0.2 mg/kg of body weight (BW), Ivomec®, Merial, Brazil); Group 2 – treated with injectable moxidectin (0.2 mg/kg BW, Cydectin®, Fort Dodge, Brazil); Group 3 – treated with injectable albendazole sulphoxide (2.5 mg/kg; Albendathor®, Tortuga, Brazil); Group 4 – treated with injectable levamisole phosphate (4.7 mg/kg BW, Ripercol®, Fort Dodge, Brazil); and Group 5 – control (not treated). Animals were treated with each anthelmintic according to the manufacturer's directions, and the number of animals in each group ranged from 8 to 11, depending on the number of young animals available from each farm.

2.3. Faecal examination

Faecal samples from each animal were collected 2 days before the treatment and again 10 and 28 days post-treatment. Samples were individually processed using the modified McMaster technique with a sensitivity of 50 EPG (Ueno and Gonçalves, 1998) and the FLOTAC dual technique with a sensitivity of two EPG (Cringoli et al., 2010). Saturated sodium chloride (NaCl, specific gravity 1.2) was the flotation solution used in both techniques. On the same collection days, composite faecal cultures were prepared for each group to obtain and differentiate third stage larvae into parasitic genus (Ueno and Gonçalves, 1998; van Wyk and Mayhew, 2013). In the case of *Haemonchus* larvae, the distance between the tip of the larval tail and the end of the sheath tail was measured. Those larvae with measures of