Widespread resistance to macrocyclic lactones in cattle nematodes in Ecuador

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ABSTRACT

The aim of the present study was to assess the resistance status of bovine gastrointestinal nematodes (GINs) against ivermectin (IVM) and fenbendazole (FBZ) in Ecuador. The study involved five cattle farms located in different topographic zones of the country. Anthelmintic efficacy was assessed by calculating the percentage of fecal egg counts reduction (FECR) after treatment. Additionally, DNA from pooled larval cultures was screened to ascertain benzimidazole resistance alleles. For animals treated with IVM, FECR percentages ranged from 0 to 68%, indicating the presence of highly resistant worms. The opposite was found for animals treated with FBZ, where FECR percentages were above 90% on all the farms tested. Pooled coprocultures revealed that Cooperia spp. were the predominant species pre and post-treatment although minor proportions of Haemonchus spp. and Ostertagia spp. were also identified. No mutations conferring resistance to benzimidazoles were identified in the beta-tubulin isotype 1 gene of the isolated Cooperia spp. worms, which is in line with the results of the FECR performed with FBZ. Overall, the present study highlights widespread resistance of bovine GINs to IVM but no to FBZ in Ecuador.

1. Introduction

Gastrointestinal nematode infections are associated with serious detrimental effects on general performance and welfare of animals, therefore representing a major threat to the cattle industry. Broad spectrum anthelmintics, including macrocyclic lactones (MLs) and benzimidazoles (BZs), have been frequently used to treat GIN infections. However, the inherent biological plasticity of GINs has enabled them to counteract anthelmintic chemicals, which has given rise to worldwide development of resistance (Soutello et al., 2007; Suarez and Cristel, 2007; Waghorn et al., 2006).

Resistance in cattle nematodes appears to be spreading, but special concern surrounds the selection of worm populations resistant to MLs in tropical regions. In these environments, long lasting-MLs based pesticides are broadly used to control parasitic infestations (FAO, 2004; Henrioud, 2011). In Ecuador, dairy and beef cattle production is mainly based on extensive grazing all year round. With about 75% of livestock farms infested or at risk to be infested by ticks, IVM have been extensively employed to control such infestations. As a consequence, considerable selection pressure has been put on populations of cattle tick Rhipicephalus microplus, ultimately resulting in the development of resistance to IVM (Rodriguez-Hidalgo et al., 2017). Yet, the effect of this high drug pressure on the GIN populations in cattle and their susceptibility to MLs and other anthelmintics is so far unknown. Therefore, the present study was aimed to evaluate the resistance status of bovine gastrointestinal nematodes in different locations in Ecuador against two of the most frequently used anthelmintic compounds, i.e. ivermectin and fenbendazole.

2. Methods

2.1. Farm selection, treatment and parasitological analysis

The study took place from April to September 2018 on five cattle farms in the provinces of Pichincha (two farms) Napo (two farms) and Guayas (one farm) (Fig. 1). Farm inclusion was based upon the
fulfillment of the following criteria: (i) willingness to participate in the study; (ii) ten or more grazing calves per treatment group (both sexes, between 3 and 10 months of age); (iii) absence of any anthelmintic treatment for at least four weeks prior to testing and (iv) proximity to laboratory facilities to allow microscopical examination of the fecal samples. Between nine and 12 calves per treatment group were individually weighed with an electronic scale on the day of treatment and drug doses were calculated accordingly. IVM (Ivomec® Merial, IVM 1%, subcutaneous injection, 0.2 mg kg\(^{-1}\)) and FBZ (Panacur® Merck, FBZ 10%, oral administration, 5 mg kg\(^{-1}\)) were administered following manufacturer’s instructions. Individual fecal egg counts (FEC) were performed one week before the start of the study to ensure a minimum of 150 nematode eggs per gram (EPG) feces per animal. From the animals from farms 1 to 4, fecal samples were individually collected from the rectum on day 0 and 14- or 8-days following treatment with either IVM or FBZ, respectively. On farm 5, fecal samples were collected on day 0 and 14 from both the IVM and FBZ treated animals. The Mini-FLOTAC technique (Cringoli et al., 2013) with a diagnostic sensitivity of 5 EPG based on 45 ml saturated sucrose salt solution (specific density 1.21) was used to determine individual FEC. Samples were processed the same day or stored at 4 °C until the next day. In addition, larval cultures were set-up at each sampling time-point to determine the nematode species present. Briefly, 20 g of feces of each animal was thoroughly homogenized with vermiculite and mixed daily to keep a well aerated environment. After two weeks of incubation at 28 °C, 3rd stage larvae were harvested by the Baermann technique (Hendrix, 1998). The first 100 3rd stage larvae were microscopically identified according to (Van Wyk and Mayhew, 2013).

### 2.2. Statistical analysis

Assessment of treatment efficacies was performed using a Bayesian hierarchical model of FEC based on Markov chain Monte Carlo simulations (Torgerson et al., 2014). The selected approach intends to calculate the efficacy of the treatment (FECR percentage – FECR%) and to provide 95% confidence intervals (upper -U and lower -L) while addressing aggregation of egg counts and Poisson errors derived from sampling procedures. The FECR test was interpreted according to (Lyndal-Murphy et al., 2014), in which resistance is inferred when the estimated FECR percentage and U95 is below 95% and L95 is less than 90%. Hence, each treated group was classified as being susceptible to the treatment (FECR % and U95 ≥ 95% and L95 ≥ 90%), having confirmed resistance (FECR % and U95 < 95% and L95 < 90%) or being suspected for resistance (none of the aforementioned criteria fulfilled). Statistical analysis was carried out using R (version 3.6.3) (R Core Team, 2016).

### 2.3. Beta-tubulin Isotype 1 mutation analysis

DNA was extracted from pooled 3rd stage larvae using a commercial kit following manufacturer’s instructions (PowerSoil® DNA Isolation Kit, Mobio Laboratories Inc., USA). The isotype 1 β-tubulin gene sequence of the predominant Cooperia species was amplified using the primers described by Demeler et al. (2013): the forward primer (CoPCR167fw) 5′-TATGGGCACTTTGCTTATTTCA-3′ together with the reverse primer (PCR198 + 200rev) 5′-CCGGACATYGTGACAGACACTAGG-3. The PCR reaction was set up in 25 μl with 0.25 mM dNTP, 4 mM MgCl2, 1 U of Taq polymerase (GoTaq) and 0.25 μM of each primer. PCR conditions were as follow: 98 °C for 30 s, 40 cycles of 98 °C for 10 s, 52 °C for 30 s and 72 °C for 20 s followed by a single extension of 72 °C for 10 min. Desired PCR fragments were excised and purified from agarose gels using a commercial kit (GENECLEAN® II Kit, mpbio Laboratories Inc., USA) and cloned into the pGEM®-T Easy Vector (Promega) according to supplier’s instructions. Between 7 and 10 clones per PCR product were sequenced in both
3. Results

3.1. Fecal egg count reduction test

Results of the FECR test for treatments with IVM and FBZ are summarized in Table 1. Following the criteria to determine AR, nematode resistance to IVM was detected on all farms, with FEC reductions ranging between 53% to 99% (L95 ≥ 98, U95 = 99). In contrast, treatment with FBZ resulted in average reductions of FEC of ≥99% (L95 ≥ 98, U95 ≥ 99) in all but one farm, i.e. farm 1 with 92% reduction in FEC (L95 = 90, U95 = 94).

3.2. Species identification

Results of species identification performed on the larval cultures before and after treatment are summarized in Table 2. Before treatment, Cooperia was the predominant genus on all cattle farms, representing between 53% to 99% of all the larvae present in pooled cultures. Minor proportions of other strongyle genera were also identified, in particular Haemonchus and Ostertagia. All larvae recovered following the treatment with IVM belonged to Cooperia sp. on three farms (2, 4 and 5). On the two remaining farms (1 and 3), some larvae belonging to the other genera were also recovered, in particular Ostertagia sp. and Haemonchus sp. Following treatment with FBZ, viable 3rd stage larvae were isolated from the coprocultures from two farms (1 and 4). The majority of the larvae identified belonged to Cooperia sp. although a small proportion of Haemonchus sp. and Ostertagia sp. was also observed.

3.3. Mutation analysis of the beta-tubulin gene

In order to identify mutations associated with BZ resistance, DNA was extracted from the Cooperia sp. larvae collected following treatment with FBZ from farms 1 and 4. Extracted DNA was used to amplify, clone and sequence the isotype 1 β-tubulin gene. Ten and seven clones for farms 1 and 4 respectively were finally analyzed. Each clone was identified as belonging to C. pectinata by the Local Alignment Search Tool (Madden, 2013). None of the sequenced clones contained any of the known resistance amino-acid substitutions. Although Cooperia sp. are acknowledged to be less pathogenic than other worms (Coop et al., 1979), failing to control this parasite can result in significant economic losses due to their detrimental effect on appetite and nutrient uptake (Stromberg et al., 2012). In Ecuador, there is little information about the epidemiology of GIN nematodes, but for the data reported here, Cooperia sp. were by far the most prevalent parasite species present both before and after treatment.

4. Discussion

Our findings show widespread resistance to IVM in cattle GIN parasites in Ecuador, as the efficacy of treatment on all the farms included in this study were far below the threshold of 95% reduction in worm burden. Reports of resistance to this drug has become commonplace in recent years (Kaplan and Vidyashankar, 2012). Prevalence data from the North Island of New Zealand (Waghorn et al., 2006) and Brazil (Soutello et al., 2007) for example indicated evident resistance to IVM on 92% of the farms tested. Moreover, data from the same surveys identified Cooperia sp. as the most prevalent and largest contributor to the resistant nematode populations. Although Cooperia sp. are acknowledged to be less pathogenic than other worms (Coop et al., 1979), failing to control this parasite can result in significant economic losses due to their detrimental effect on appetite and nutrient uptake (Stromberg et al., 2012). In Ecuador, there is little information about the epidemiology of GIN nematodes, but for the data reported here, Cooperia sp. were by far the most prevalent parasite species present both before and after treatment. Increasing numbers of Cooperia sp. in cattle have been reported in several studies (Edmonds et al., 2010; El-Abbadi et al., 2010; Gasbarre, 2014), and is likely a result of the repeated use of MLs, providing a competitive advantage to Cooperia sp. and allowing them to predominate (El-Abbadi et al., 2010; Stromberg et al., 2012).
frequently been found in other countries (Mejía et al., 2003; Rendell, 2010; Soutello et al., 2007; Vermunt et al., 1995; Wagorn et al., 2006), increased usage of BZs should be accompanied by continuously monitoring the efficacy of the treatments.

5. Conclusions

This study provides the first evidence of AR in Ecuador. Extensive use of MLs to control ectoparasites parasites might account for the severity of IVM resistance reported here. Although further research to assess the real extend of the problem throughout the whole country may be necessary, our findings clearly highlight the threat posed to the sustainability of the cattle industry in Ecuador. Closely monitoring drug efficacy as well as the development of alternative control and management strategies, which need to be based on the local epidemiological situation, will be crucial to manage the further spread of resistance.

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Conflict of interests

None.

Ethical statement

This study was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare. Owners provided their informed consent before each procedure, which involved oral and subcutaneous administration of anthelmintics following manufacturer’s instructions and fecal samples collection.

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References


