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Original Article

Gastrointestinal nematode populations with multiple anthelmintic resistance in sheep farms from the hot humid tropics of Mexico

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ABSTRACT

This study evaluated the status of anthelmintic resistance against the three available classes of commercial drugs in seven sheep farms in the hot humid tropics of Mexico. Drug classes included benzimidazole (BZ), ivermectin (IVM) and levamisole (LV). Respective faecal egg count reduction tests (FECRT) were performed in each farm. Faecal samples were obtained from the rectum of > 100 sheep in each farm. Adult sheep shedding > 150 eggs per gram of faeces (EPG) were included. In each farm, animals were allotted to one of four groups with similar mean EPG: Control Group (untreated), BZ group (albendazole sulfoxide 5 mg/kg LW), IVM group (ivermectin, 0.2 mg/kg LW) and LEV group (levamisole 7.5 mg/kg LW). Drugs were administered subcutaneously. A second faecal sampling was performed on the same animals of each farm 14 days post-treatment. The GIN genera obtained from faecal cultures were identified for each group in different farms. Percentage faecal egg count reduction (%R) and 95% confidence intervals were estimated using the RESO© software. A questionnaire was applied to farm owners to describe anthelmintic management practices. All sheep farms had GIN populations with multiple resistance to the three anthelmintic classes tested. The %R ranged from 0 to 48% for BZ, 29 to 82% for IVM and 1 to 88% for LEV. *Haemonchus* spp. and *Trichostongylus* spp. were found in all treated groups of the study farms. Resistant *Oesophagostomum* spp. larvae (BZ or IVM) were found in respective farms. Treatment practices in study farms included frequent mass treatment every two months with extra treatments applied individually in the presence of clinical signs. Drug dosage used visual estimation of body weight rather than the exact weight of each animal. Quarantine anthelmintic treatment of incoming stock was used but efficacy was not confirmed.

1. Introduction

Commercial anthelmintic drugs have been the main tools for the control of gastrointestinal nematodes (GIN) in sheep farms for nearly four decades in hot humid tropical areas of Mexico. However, the irrational use of antiparasitic drugs led to the emergence of GIN populations able resist such drugs, endangering the sustainability of control strategies based on anthelmintics. In recent years, the situation seemed to worsen with the identification of GIN strains showing multiple resistance against different anthelmintic drug groups. Several surveys confirmed that anthelmintic resistant GIN are present in sheep flocks all over Latin America including Argentina, Brazil, Paraguay and Uruguay (Echavarria et al., 1996; Eddi et al., 1996; Maciel et al., 1996; Nari et al., 1996; Bonino and Mederos, 2003; Caracostantogolo et al., 2005). Some studies reported multi-resistant GIN strains against two or more anthelmintic drugs classes (Eddi et al., 1996; Nari et al., 1996; Caracostantogolo et al., 2005; Almeida et al., 2010; Skrebsky-Cezar et al., 2010; Martínez-Valladares et al., 2013). In Mexico, the diagnosis of anthelmintic resistance has been carried out in some sheep farms located mainly in hot humid tropical regions, but the diagnostic effort has been negligible (Torres-Acosta et al., 2012a, 2012b). The latter may imply that the situation of anthelmintic resistance may have been

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overlooked at field level for many years, even when the presence of multi-drug resistant GIN was reported > 10 years ago in 58% of sheep farms surveyed in a hot and humid area of Tabasco, México (n = 19; Nuncio-Ochoa et al., 2005). Although many sheep farmers in Mexico have been exposed to information highlighting the presence of anthelmintic resistant worm populations in sheep farms, the use of those drugs has not changed (Torres-Acosta et al., 2012c). Thus, it is likely that the situation of anthelmintic resistance may have worsened especially for sheep farms in the hot, humid tropical areas of Mexico such as Tabasco. The objective of the present study was to determine the frequency of sheep farms with GIN resistant to the three available anthelmintic drugs classes, Benzimidazole (BZ), Macrociclic lactones (IVM) and Levamizole (LV), as well as to identify the genera of gastrointestinal nematodes involved in anthelmintic resistance in seven sheep farms of the State of Tabasco, Mexico.

2. Materials and methods

2.1. Study area

The study was carried out in seven commercial sheep farms located in the central region of the State of Tabasco, Mexico. According to the Köppen classification, the climate of the study area is classified as tropical rain forest (Af) (Chen and Chen, 2013). It is characterized for its hot, humid climate with mean temperature of 27 °C, mean high ambient temperature of 36 °C, mean low ambient temperature of 18.5 °C, average annual rainfall of 2550 mm and average relative humidity of 80% (De Dios-Vallejo, 2001; INEGI, 2011).

2.2. Selection of sheep farms and experimental individuals

Sheep farms were selected according to the following criteria: (i) collaborating farmers were willing to participate in the study, (ii) farms had an adult sheep flock of at least 100 ewes, and (iii) the main source of food for the adult sheep was grazing in paddocks of introduced tropical grass.

Experimental animals in the different sheep flocks were selected according to the following criteria: (i) female adult animals, (ii) GIN egg faecal excretion > 150 EPG.

2.3. Faecal egg count reduction test protocol

The Faecal Egg Count Reduction Test (FECRT) was implemented to detect the presence of GIN populations resistant to the three anthelmintic drug classes as recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992). Faecal samples were collected directly from the rectum of adult ewes in each farm to determine their EPG. Faecal samples were kept in cool-boxes with refrigerants during transportation to the laboratory. Faecal egg counts (FEC) were determined using the McMaster technique with a sensitivity of 50 EPG (Rodríguez-Vivas and Cob-Galera, 2005). On day 0, animals selected for the FECRT were randomly assigned to one of four experimental groups as follows:

1. Control group: Without anthelmintic treatment.
2. BZ group: Treated with injectable albendazole sulfoxide (5 mg/kg live weight, subcutaneous route; Ricozol© 15% Bayer).
3. IVM group: Treated with injectable ivermectin (0.2 mg/kg live weight, subcutaneous route; Ivomec© 1% Merial).
4. LEV group: Treated with injectable levamisole (7.5 mg/kg live weight, subcutaneous route; Helmicin© 12% Sanfer).

Individual sheep were weighed to determine the correct dose of the respective anthelmintic drug. Fourteen days after treatment, a second faecal sampling was performed on the same individual sheep sampled the first time in each surveyed farms. Faecal samples were used to calculate the FEC and were also used to produce respective coprocultures produced for each treatment group. Larvae (L_3) produced in coprocultures were harvested using the Baerman technique. The keys of identification by Bowman and Lynn (1999) were used to identify the different genera of GIN L_3 harvested from respective coprocultures of each group. A total of fifty L_3 larvae were morphologically identified from each group coproculture to provide percentage values.

2.4. Data analysis

The anthelmintic resistance status of each drug was estimated using the RESO© software (Ver. 2.0; CSIRO, 1990) in the respective sheep farm, thought the following formula:

Percentage faecal egg count reduction (%R) = (1-T/C) × 100%.

Where: T is the EPG arithmetic mean of a treated group on the second sampling (day 14 post-treatment), and C is the EPG arithmetic mean of the control group also obtained on the second sampling.

The 95% confidence interval (95%CI) was calculated using the same RESO© software. A GIN population was considered "resistant" for a given anthelmintic class when the FEC reduction percentage was < 95% and the lower limit of the 95%CI was < 90%. A GIN population was considered "suspect" when one of the two criteria was met. The GIN population was considered susceptible to any given anthelmintic class when none of the criteria mentioned above was satisfied.

2.5. Questionnaire

A questionnaire was applied to farm owners and managers to gather information on the use of anthelmintic drugs in their farm. It included deworming frequency, drugs used, criteria for dosing, treatment criteria, treatment protocols applied to incoming animals arriving from other farms and the nutritional management of animals.

3. Results

3.1. Sheep farms with gastrointestinal nematodes resistant to the anthelmintic drugs

All the evaluated sheep farms (n = 7; 100%) were diagnosed to have GIN populations that are resistant to the three anthelmintic drug classes tested (Table 1). The resistance seemed to be severe as the %R ranged from 0 to 48% for BZ, 29 to 82% for IVM and 1 to 88% for LEV.

According to the post-treatment coprocultures (Table 2), genera of GIN involved in anthelmintic resistance against BZ, IVM and LEV were *Haemonchus* spp. and *Trichostrongylus* spp. The *Oesophagostmun spp.* L_3 were detected for the BZ group in one of the farms tested and the IVM group in another farm tested. In both cases the presence of *Oesophagostomum* spp. was low with percentage < 10%.

3.2. Questionnaire survey

All surveyed farms had several common answers related to the anthelmintic drug management. None of them had performed an anthelmintic resistance diagnostic test in their farms before the present survey. All farmers received non-specialized technical advice such as a systematic deworming schedule, the drugs to be used, application route and dosage (mg/Kg of live weight). However, at the moment of anthelmintic application, animals were not weighed in order to estimate the correct dosage, and the weight of animals was estimated visually.

All the seven farms dewormed their animals with a frequency of every two months. However, some animals were treated earlier if individuals showed signs of diarrhea or reduced body condition score, particularly around parturition. Five farmers used the three anthelmintic family drugs and two farmers used two drug families (Benzimidazole y Levamisole) in the same year. The combined use of drug families was