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First report of cattle farms with gastrointestinal nematodes resistant to levamisole in Mexico

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ABSTRACT

The objectives of the present study were: (1) to report the percentage of cattle farms with gastrointestinal nematodes (GINs) resistant to levamisole in Veracruz, Mexico, (2) to identify the genera of GINs involved in resistance, and (3) to identify factors associated with these resistances. The faecal egg count reduction test (McMaster technique) was used to detect the presence of resistant GINs. A questionnaire was given to owners to understand the history of anthelmintic use. The percentage of cattle farms with GINs resistant to levamisole was 36.4% (4/11). The percentage of faecal egg count reduction on resistant farms was 91%, 82%, 42% and 88%. A similar number of cattle farms (4/11) were identified as potentially having levamisole resistance. Only three farms had GIN populations susceptible to levamisole. *Cooperia* spp. was the genus most commonly found to be resistant, followed by *Haemonchus* spp., *Ostertagia* spp. and *Oesophagostomum* spp. No factors were identified that influenced the presence of GIN resistance. However, there were identified inappropriate anthelmintic practices in cattle farms that should be improved. None of the farmers weighed their animals in order to dose them correctly with anthelmintics. Six cattle farms (54.5%) applied anthelmintics to new arriving animals. This is the first report of levamisole resistant GINs in Mexico. Improving the use of anthelmintics and measures of quarantine for infected cattle will help control the spread of resistance.

1. Introduction

Gastrointestinal nematode (GIN) infections reduce the productivity and health of grazing cattle. Therefore, acceptable performance of animals depends on the availability of effective broad-spectrum anthelmintics (AHs) to control GIN populations (Sutherland and Leathwick, 2011). The most common AHs used in cattle belong to the benzimidazoles (BZs), macrocyclic lactones (MLs) and imidazothiazoles (IMZs) (Kaplan, 2004; Wolstenholme et al., 2004). The successful use and toxic effect of AHs on GINs depends on parasite susceptibility as well as environmental factors and medication protocols directed at controlling parasite-induced illnesses (Coles, 2002). The increasing development of GIN populations resistant to broad-spectrum AHs is a persistent and additional threat that affects the efficacy/sustainability of medications as control mechanisms (Leathwick et al., 2012).

Anthelmintic resistance to all available broad-spectrum drugs has mainly been reported in GIN populations from small ruminant farms (e.g., sheep and goats) worldwide

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(Coles, 2002; Kaplan, 2004; Kaplan and Vidyashankar, 2012; Alonso-Díaz et al., 2014). Resistance has reached high levels of prevalence in some regions, threatening the control of GINs and the productivity and welfare of small ruminant systems (Wolstenholme et al., 2004). Historically, the resistance to AHs in cattle nematodes is less common in comparison to small ruminants and in some regions it is present at very low levels (Coles, 2002; Kaplan, 2004). However, during the last 10 years an increasing number of reports on AH resistance in cattle nematodes have been published internationally (Sutherland and Leathwick, 2011; Alonso-Díaz et al., 2014). Most studies have discussed resistance to MLs and BZs (Canul-Ku et al., 2012; Encalada-Mena et al., 2008; Vermunt et al., 1995; Fiel et al., 2001; Márquez et al., 2008; Soto et al., 2007; Arnaud and Alonso-Díaz, 2012), and there are few reports worldwide concerning the resistance of cattle nematodes to IMZs (Arnaud and Alonso-Díaz, 2012; Kaplan and Vidyashankar, 2012).

In Mexico, field reports discussing the presence of GIN populations resistant to AHs in cattle farms are limited to a small number of studies that only focus on ivermectin (Encalada-Mena et al., 2008; Canul-Ku et al., 2012) or BZs (Arnaud and Alonso-Díaz, 2012). Data on resistance to broad-spectrum levamisole still remains limited. This information can help to improve GIN control programmes in cattle and to augment production indices for cattle farms. Early monitoring for resistance/susceptibility of cattle nematodes to AHs can help when designing measures to improve the use of efficacious drugs and avoid the dissemination of resistant nematodes among farms. The objectives of this study were: (i) to report the percentage of cattle farms with GINs resistant to levamisole in Veracruz, Mexico, (ii) to identify the genera of GINs showing resistance, and (iii) to identify possible factors associated with GIN resistance to levamisole.

2. Materials and methods

2.1. Study area and cattle farms selected

This study was carried out from January to October 2012 on 11 cattle farms in five municipalities (Martinez de la Torre, Misantla, Nautla, Tlapacoyan and Vega de Alatorre) in the state of Veracruz, Mexico (24°4′ N, 97°03′ W). The climate is humid tropical with an average annual temperature of 23.4 ± 0.5 °C, annual rainfall of 1991 ± 392 mm and relative humidity of 85% (INEGI, 2008). Twenty-five cattle farms where cattle grazing was practiced were tested, but only on 11 farms was it possible to evaluate AH resistance. Selection and sampling of cattle farms were made by convenience.

At each selected farm, grazing calves 3–8 months old and 50–150 kg live weight were used. The inclusion criteria for farm selection were: (i) herds with a population of calves (3–12 months of age), (ii) calves not dewormed during the 60 days prior to the study and (iii) herds with calves excreting more than 150 eggs per gram (EPG) of faeces. From a total of 958 calves sampled from 25 herds, only 278 calves from 11 herds complied with the selection criteria. The major factor that limited the number of herds evaluated was the lack of animals excreting more than 150 EPG.

2.2. Experimental design to evaluate resistance

To diagnose the presence of GINs resistant to levamisole, the Faecal Egg Count Reduction Test (FECRT), recommended by the World Association for the Advancement of Veterinary Parasitology was used (Coles et al., 1992). On day 0, within each cattle farm, a sample of faeces was collected directly from the rectum of each calf (using a labelled plastic bag) to calculate the number of EPG. Faeces were transported in a plastic cooler (4–5 °C) to the Animal Health Laboratory for analysis. Faecal egg counts (FECs) were determined using the McMaster technique with a sensitivity of 50 EPG (Rodríguez-Vivas and Cob-Galera, 2005). Coprocultures were made to identify the genera of GINs present in faeces. Then, on day one, calves were distributed according to their parasite loads (balanced) into two experimental groups: a control group having 10–15 calves maintained without treatment, and a treated group having 10–15 calves treated with levamisole (HELMISOL 12%®, TTOKKYO, México; REG. SAGARPA Q-6990-007) through intramuscular injection using a dose of 7.5 mg kg^{-1} live weight (Plumb, 2010). Prior to treatment, animals were weighed individually using the same mobile weighing scale (TRU-TEST MP600®) so as to administrate the correct dose of levamisole; thus avoiding variability among doses used for treatments.

Fourteen days after treatment, another faecal sample was obtained from each calf to calculate the FEC as mentioned previously. A new coproculture per group was performed to identify the genera of GINs (third larval stage) involved with resistance.

2.3. Questionnaire

A questionnaire was given to farm owners and managers to obtain information about herd size, cattle breeds, zootechnical purpose (dual purpose, milk or breeding stock), production system (extensive, intensive or semi-intensive), the family of AH currently used (ML, BZ, or IMZ), frequency of AH treatments per year, selection criteria for AH medications, correct dosage or not (e.g. weight measured or not), deworming new animals, other GIN control systems, rental of paddocks, contact with other cattle farms and whether or not rotations of AH families were used.

2.4. Statistical analysis

For each cattle farm, AH resistance was calculated following Coles et al. (1992) and RESO© software (CSIRO, 1990, Animal Health Division) by means of the formula:

$$\text{Percentage reduction}\,(\%) = \left(1 - \frac{T}{C}\right) \times 100$$

where T is the EPG arithmetic mean of the treated group, and C is the EPG arithmetic mean of the control group after treatment (at day 14).