Main results obtained at Embrapa Southeastern Region Animal Husbandry: multidrug resistance in sheep flocks from São Paulo state, Brazil

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Short title: Multidrug resistance in sheep.

Abstract

Economic importance of sheep production has increased worldwide simultaneously to the emergence of parasitic resistance. *Haemonchus contortus* is the most prevalent and pathogenic nematode of sheep in tropical areas and it is responsible for economical losses and health problems. This study aimed to survey the current situation of parasite resistance in sheep flocks from São Paulo state, Brazil, and to verify the association between management practices and parasitic resistance. A questionnaire survey was applied in 35 sheep flocks to obtain information related to management practices. Feacal egg reduction count test (FERCT) was performed with five anthelmintics: albendazole, closantel, ivermectin, levamisole and moxidectin, and compared to untreated control. The frequency of polymorphism F200Y on isotype 1 of β-tubulin gene was evaluated in field isolates of *Haemonchus contortus*. Multidrug resistance to anthelmintics by FERCT was widespread in sheep flocks from São Paulo state, Brazil. The frequency of allele of resistance of F200Y was very variable among flocks. Resistance genotype frequencies higher than 40% were associated to several management risk factors. This information may increase the control and slow the emergence of resistance to anthelmintics in sheep.

Introduction

The economic importance of sheep production has grown worldwide. In Brazil, the number of ovine remained constant over the past 40 years, whereas in São Paulo state the growth of the sheep flocks was higher than 300% (PRODUÇÃO, 2010). However, the main limiting factor for sheep farming is the high prevalence of gastrointestinal nematodes, of which *Haemonchus contortus* is the most prevalent and pathogenic. Besides high prevalence the control of gastrointestinal nematodes is aggravated by parasitic resistance to anthelmintics, which use is the main strategy employed for helminth control (CHARLES, 1989). Unfortunately, easy access and lack of appropriated guidelines for anthelmintics usage contributed to the large scale application of these drugs and led to reduction in treatment effectiveness and increase in parasitic resistance.

Resistance is an evolutionary process, in which individuals that survive to treatments contribute with their genes to the next generation. So after a few generations, the frequency of surviving individuals will increase in the population (BLACKHALL et al., 2008). However, besides genetic factors, other events such as drug dosage, treatment frequency, turnover speed of chemical compounds (CRAIG, 1993), and amount of helminth population in refugia (PRICHARD, 1990) can influence the spread of resistance. Thus, understanding the relationship between risk factors of management practices in sheep flocks and anthelmintic resistance can guide preventive measures to control or to reduce the emergence and establishment of helminth resistance.

Among the tests to detect anthelmintic resistance, faecal egg count reduction test (FECRT) is the most widely employed. FECRT is a practical and inexpensive test appropriate for all anthelmintic drugs, which only requires the technician ability to perform egg per gram (EPG) counts (COLES, 2005). Molecular diagnosis of resistance may overcome some limitations observed in traditional parasitological methods (GASSER et al., 2008). The molecular mechanism of resistance to benzimidazole was the first to be elucidated. The first mutation described on isotype 1 of β-tubulin gene was a T > A transversion (codon TTC is modified to TAC), which leads to the substitution of phenylalanine for tyrosine at position 200 (F200Y) and confers resistance to benzimidazole (KWA et al., 1994).

For that purpose, the objective of this study was to assess helminth frequency and resistance status to five anthelmintics (albendazole, closantel, ivermectin, levamisole, and moxidectin) by FECRT, to determine the frequency of F200Y polymorphism on isotype 1 of β-tubulin gene in field isolates of *Haemonchus contortus* and to investigate the association between anthelmintic resistance and management practices employed in sheep flocks in São Paulo state, Brazil.

Material and Methods

This study was performed in 35 sheep flocks in São Paulo state from November 2008 to July 2010. A questionnaire survey was applied to obtain information related to management practices in sheep flocks.

Faecal egg count reduction test (FECRT) was adapted from COLES et al. (2006) (NICIURA et al., 2009). For FECRT, 60 animals were randomly distributed into six groups and treated with one of the following treatments: 1) albendazole (3.4 mg/kg); 2) closantel (10 mg/kg); 3) ivermectin (0.2 mg/kg); 4) levamisole (7.5 mg/kg); 5) moxidectin (0.2 mg/kg); 6) control.

From each of the 33 farms studied, at least 48 L3 larvae were subjected to DNA extraction and genotyping of the F200Y polymorphism on isotype 1 of β-tubulin gene by nested- and ARMS-PCR according to Silvestre and Humbert (2000) and Coles et al. (2006) with modifications (GROMBONI et al., 2009).

Anthelmintic efficiency by FECRT was calculated in RESO 2.0 (WURSTHORN and MARTIN, 1990). Helminths were considered susceptible when efficiency value was superior to 91%; suspect from 81 to 90%; and resistant when inferior to 80%. Regarding the polymorphism F200Y, genotypic frequencies were tested for Hardy-Weinberg equilibrium using GENEPOP (ROUSSET, 2008). According to resistance genotypic frequencies (rr), *Haemonchus contortus* larvae were separated into two groups: high resistance when rr was superior to 40% and low resistance when rr was inferior to 40%. The association between resistance (low or high) and descriptive statistics of management data were analyzed by Chi-square test in Minitab (version 13).

Results

The results of management practices in ovine flocks were previously published (NICIURA et al., 2010). In all treatments, there was a predominance of *Haemonchus* sp. (75.8%), followed by *Trichostrongylus* sp. (19.1%). The mean efficiency results of five anthelmintics in FECRT were -17.3% for

albendazole, 53.0% for closantel, -4.7% for ivermectin, 57.1% for levamisole, and 11.8% for moxidectin. Resistance (anthelmintic efficiency lower than 80%) was observed in 93.3% of the sheep flocks to albendazole, 77.8% to closantel, 96.4% to ivermectin, 46.4% to levamisole, and 86.3% to moxidectin.

The frequency of the genotype of resistance (rr) of F200Y ranged from 0 to 66.7%, and the frequency of the allele of resistance (r) varied from 9 to 74%. Most isolates (81.8%) were under Hardy-Weinberg equilibrium ($P>0.05$).

The risk factors in management practices associated ($P<0.05$) to high resistance were: sheep farming for shorter period of time; absence of zootechnical control of herds; use of crossbred, Dorper and Suffolk sheep breeds, compared to Ile de France, Santa Ines and Texel; use of rotational grazing; absence of wetlands in farm; sharing ovine pasture with bovine and equine; frequent incorporation of new animals to the herd; use of semi-intensive farming system compared to extensive or intensive; use of whole-flock treatment compared to selective treatment of some animals or lots; no use of Famacha method for treatment; no change of animals from pasture after treatment; rotation of the anthelmintic group after each application compared to the change based on FECRT or effectiveness; use of visual estimation of weight for treatment compared to weighing; no use of drug combinations for treatment.

Conclusions

It is possible to conclude that multidrug resistance to anthelmintics is widespread in sheep flocks from São Paulo state, Brazil. Also, due to the high prevalence of multidrug resistance, polymorphisms other than F200Y may be associated with resistance in the studied field isolates of *Haemonchus contortus*. Information about the management practices associated with resistance may increase the control and slow the development of resistance to anthelmintics in sheep.

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