ANTHELMINTIC RESISTANCE IN FIVE SHEEP FLOCKS IN NORTHWESTERN REGION OF SÃO PAULO STATE

RESISTÊNCIA ANTI-HELMÍNTICA EM CINCO REBANHOS OVINOS NO NOROESTE DO ESTADO DE SÃO PAULO

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ABSTRACT

Infection by endoparasites is among the main factors that affect the performance of sheep raised on pasture. The aim of this was to identify the resistance of helminths to four active ingredients used in the northwest of the state of São Paulo, Brazil. We carried out the Test Reduction in Fecal Egg Count, where it was detected resistance of gastrointestinal nematodes to all groups anthelmintics tested. Therefore, it is recommended to adopt a number of measures for the control of nematode parasites with monitoring via fecal examinations, which can allow minimization of the need to use anthelmintics, avoiding unnecessary costs and prolonging the efficacy of the principles assets available.

Key words: Endoparasites, Haemonchus, Helminths, Resistance.

INTRODUCTION
In Brazil, the total herd of sheep is bigger than 13.770 million head (IBGE, 2017), and one of the main problems that affect the health of sheep is the infection with gastrointestinal nematodes, which cause great losses in production and consequently a large impact on the production of such ruminants.

Several methods to control and/or minimize losses caused by worms in sheep are described in the literature, such as the use of alternating and rotational grazing with cattle (Fernandes et al., 2004), use of resistant breeds (Amarante, et al., 2004), crosses breeds between resistant and breeds specialized in meat production (Amarante et al., 2009), identification and exclusion of animals susceptible to infections with gastrointestinal nematodes, because they are major polluters of pasture (Bassetto et al., 2009), however, the most widely used remains the use of anthelmintic drugs.

The incorrect and exacerbated use of these drugs, was probably the main factor in the rapid spread of anthelmintic resistance in sheep, being described in various places of the world, like Scotland, Trinidad Tobago, Argentina, Costa Rica and Spain (Sargison et al., 2007; George et al., 2011; Fiel et al., 2011; Maroto et al., 2011; Martínez-Valladares et al., 2013).

In Brazil, anthelmintic resistance has been reported in 10 states, of which 431 farms, 367 (85%) showed resistance to one or more classes anthelmintic available in the country (Table 1).
Anthelmintics class, BZ (benzimidazoles), LEV (levamisole), ML (macrocyclic lactones), SAL (salicinalinidas), ORG (organophosphorates), MO (Monepantel).

The genera/species which exhibit anthelmintic resistance Brazilian described in the literature are shown in Table 2.

**TABLE 2. Anthelmintic classes and genera/species resistant nematodes found in Brazil.**

<table>
<thead>
<tr>
<th>Anthelmintics class</th>
<th>Nematodes (genera/species)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZ, LEV</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Ostertagia spp.</strong></td>
<td>Echevarria &amp; Trindade 1989</td>
</tr>
<tr>
<td>ML</td>
<td><strong>Haemonchus contortus</strong></td>
<td>Echevarria et al., 1996</td>
</tr>
<tr>
<td>BZ, LEV, BZ+LEV, ML, SAL</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Ostertagia spp.</strong></td>
<td>Cunha Filho et al., 1998; 2004; 2009</td>
</tr>
<tr>
<td>BZ, ML</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Ostertagia spp., Cooperia spp., Strongyloides spp., Oesophagostomum spp., Bunostomum spp.</strong></td>
<td>Melo et al., 1998; 2004; 2009; Ramos et al., 2002</td>
</tr>
<tr>
<td>BZ, SAL</td>
<td><strong>Haemonchus spp., Cooperia spp., Ostertagia spp., Bunostomum spp.</strong></td>
<td>Cunha Filho &amp; Yamamura 1999</td>
</tr>
<tr>
<td>ML, BZ</td>
<td><strong>Haemonchus spp., Cooperia spp., Ostertagia spp., Bunostomum spp.</strong></td>
<td>Thomaz-Soccol et al., 2004</td>
</tr>
<tr>
<td>ML, LEV, SAL, BZ</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Ostertagia spp., Cooperia spp., Nematodirus spp., Oesophagostomum spp.</strong></td>
<td>Rosalinski-Moraes et al., 2007</td>
</tr>
<tr>
<td>ML, LEV, SAL, BZ</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Nematodirus spp.</strong></td>
<td>Pereira et al., 2008</td>
</tr>
<tr>
<td>BZ, ML</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Oesophagostomum spp., Strongyloides spp.</strong></td>
<td>Falbo et al., 2009</td>
</tr>
<tr>
<td>SAL</td>
<td><strong>Haemonchus spp.</strong></td>
<td>Almeida et al., 2010</td>
</tr>
<tr>
<td>ML, LEV, SAL, BZ, ORG</td>
<td><strong>Haemonchus contortus, Trichostrongylus colubriformis</strong></td>
<td>Sczesny-Moraes et al., 2010</td>
</tr>
<tr>
<td>BZ, BZ+ML+LEV, SAL, ML, LEV, ORG</td>
<td><strong>Cooperia spp., Haemonchus spp., Trichostrongylus spp., Oesophagostomum spp., Strongyloides spp.</strong></td>
<td>Lima et al., 2010</td>
</tr>
<tr>
<td>BZ</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Oesophagostomum spp.</strong></td>
<td>Cezar et al., 2011</td>
</tr>
<tr>
<td>BZ, LEV</td>
<td><strong>Cooperia spp., Haemonchus spp., Trichostrongylus spp., Oesophagostomum spp., Strongyloides spp.</strong></td>
<td>Veríssimo et al., 2012</td>
</tr>
<tr>
<td>ML, LEV, SAL, BZ</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Cooperia spp., Strongyloides spp., Oesophagostomum spp.</strong></td>
<td>Ramos et al., 2018</td>
</tr>
<tr>
<td>MO</td>
<td><strong>Haemonchus spp., Trichostrongylus spp., Cooperia spp.</strong></td>
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</tbody>
</table>
This study aimed to detect the occurrence of resistance to gastrointestinal parasites to major anthelmintics used in sheep properties in the region of Andradina, in São Paulo state, Brazil.

MATERIALS AND METHODS

Approved by the Ethics Committee in Research of the FEA/MV protocol under FCAA 02/2009.

The experiment was conducted in five properties in the region of Andradina, northwest of the state of São Paulo, between the years 2009 and 2010, being used in each property 100 ewes purebred and crossbred, with different ages, naturally infected by gastrointestinal nematodes and fecal egg count (FEC) greater than 200.

The animals were weighed and divided according to the EPG count, in five of 20 treatments: T1 Ivermectin 1% (200 mg/kg, Ivomec®, Merial) injectable, T2 Abamectin 1% (200 mg/kg; Duotin®, Merial) injectable, T3 Closantel 10% (10 mg/kg; Diantel®, IRFA), orally, T4 moxidectin 2% (200 mg/kg, Cydectin®, Fort Dodge), orally and T5 untreated (control). The doses used were those recommended by the manufacturers of each product.

Fecal samples were collected on the day of treatment (day zero) and seven days after, being processed by the method described by Ueno and Gonçalves (1988).

The technique used was "Fecal Egg Count Reduction-Test" (Test for reduction of eggs per gram of feces - EPG) and the estimated effectiveness of the products, according to the equation R (%) = 100 (1-EPG group arithmetic mean treated/EPG arithmetic mean of the control group) as recommended by WAAVP (COLES et al, 1992).

Data were subjected to analysis of variance (Oneway analysis of variance) using the Minitab® (version 11). Means were compared by Tukey test with a significance level of 5%. The data relating to EPG counts were analyzed under logarithmic transformation (log 10 (x +1)). However, for ease of understanding, the results presented are the arithmetic averages (± standard error).
TABLE 3. Average count of EPG (± standard deviation) on the day of treatment (Day 0) and seven days after treatment (day 7) and percentage reduction in faecal egg (RPEF) in five properties in the northwest region of the state of São Paulo, Brazil.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Breed</th>
<th>Day</th>
<th>Ivermectin</th>
<th>Abamectin</th>
<th>Closantel</th>
<th>Moxidectin</th>
<th>Control</th>
<th>efficacy % (IC 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Texel</td>
<td>0</td>
<td>1555±1754a</td>
<td>1475±1653a</td>
<td>1373±1469a</td>
<td>1345±1444a</td>
<td>1310±1385a</td>
<td>29,83 (67; -50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>380±515bc</td>
<td>455±905c</td>
<td>50±83a</td>
<td>85±135ab</td>
<td>540±505c</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ile de France</td>
<td>0</td>
<td>2330±2207a</td>
<td>2240±2053a</td>
<td>2155±1996a</td>
<td>2100±1986a</td>
<td>2010±1894a</td>
<td>13,75 (52; -57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>1945±1483bc</td>
<td>1715±1926b</td>
<td>100±138a</td>
<td>5485±3920c</td>
<td>2255±2386bc</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mixed race</td>
<td>0</td>
<td>2140±1709a</td>
<td>2115±1590a</td>
<td>2125±1600a</td>
<td>2100±1545a</td>
<td>2105±1613a</td>
<td>86,60 (93; 75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>400±428ab</td>
<td>470±478ab</td>
<td>825±959bc</td>
<td>295±494a</td>
<td>2985±2514bc</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mixed race</td>
<td>0</td>
<td>935±645a</td>
<td>920±641a</td>
<td>895±621a</td>
<td>870±627a</td>
<td>855±615a</td>
<td>45,37 (74; -16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>295±369c</td>
<td>215±296bc</td>
<td>85±179ab</td>
<td>5±22a</td>
<td>540±583d</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Mixed race</td>
<td>0</td>
<td>1835±1517a</td>
<td>1740±1392a</td>
<td>1710±1383a</td>
<td>1665±1353a</td>
<td>1630±1333a</td>
<td>41,67 (67; -2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>385±322ab</td>
<td>510±492ab</td>
<td>295±314ab</td>
<td>260±333a</td>
<td>660±588b</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by different lowercase letters in the line differ by Tukey test P <0.05.
Upper and lower limit of the confidence interval (95%) are in parentheses.

RPEF (%) = 100 * (1 - arithmetic mean of the treated group EPG/EPG arithmetic mean of the control group).

RESULTS AND DISCUSSION

It was found resistance of the gastrointestinal nematodes to all the anthelmintics groups tested, 100% of the properties analyzed showed resistance to ivermectin and abamectin, 80% for Closantel and 80% moxidectin (Table 3).

According to Coles et al., (1992), for anthelmintic to be effective, treatment must reduce by at least 95% of the EPG count, with a lower limit of the confidence interval to 95% greater than 90%, and from 18 studies (Table 1), 39% didn’t report and/or didn’t found this confidence interval as a parameter.

In herd 2, instead of decreasing the number of eggs after treatment with moxidectin, an increase of EPG count compared to the control group was related, resulting in a negative balance displayed in the table above, probably due to several
concurrent factors such as limited availability and poor forage quality, being Ile de France breed, that is more susceptible to worms (ROCHA et al., 2005), and the fact of selection pressure (PRICHARD et al., 2012), due to the use of moxidectin monthly in all the flock, without any criteria for drug administration.

The results obtained are similar in most studies found in other states such as Santa Catarina (ROSALINSKI-MORAES et al, 2007), which found 100% of resistance to ivermectin, 66.7% to moxidectin and 55.6% to closantel, and another study in the same state (RAMOS, et al, 2002), covering 65 farms, in which the resistance was 77% for ivermectin, 65% to albendazole and 15% to levamisole. In Mato Grosso do Sul (SCZESNY-MORAES et al, 2010) where 15 of the properties studied were found 100% resistance to ivermectin, 93.3% for closantel and 86.7% moxidectin.

The animals in herd 3 didn’t receive ivermectin and abamectin for more than twelve years, however, there was cross-resistance to moxidectin, which was being used for years. Although the moxidectin doesn’t differ significantly from the avermectins (P>0.05), it has greater efficiency. However, moxidectin significantly differed (P<0.05) when compared with abamectin in herds 1 and 4, and ivermectin in herd 4, which can be explained because they are similar, but not identical (PRICHARD et al., 2012), which was also observed by Cunha Filho et al., (1998), Cunha Filho & Yamamura (1999) and Rosalinski-Moraes et al. (2007).

All owners reported that they used visual estimation rather than weighing the animals before administration of anthelmintics, which was applied to all the flock, when some animals began to show clinical manifestations of worms, which is the same criterion adopted by most of the farmers in the state of Minas Gerais, Brazil (DELGADO et al., 2009).

CONCLUSION

The results demonstrate that the anthelmintic resistance is present in all farms. In addition to production losses resulting from parasitism, the owners are taking unnecessary wastes with ineffective anthelmintics. Therefore, it is recommended to adopt a number of measures to control the nematodes with monitoring the effectiveness of the anthelmintic through fecal examinations, which can allow minimization of the
need to use anthelmintics, avoiding needless spends and prolonging the effectiveness of the active ingredients available.

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