The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Brazil

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Received 8 June 1995; accepted 29 September 1995

Abstract

This survey was conducted in the southern Brazilian state of Rio Grande do Sul and involved 182 farms located in 26 counties. In addition to the three major broad-spectrum anthelmintic groups (viz. benzimidazole, levamisole and ivermectin) the combination benzimidazole and levamisole and the H. contortus specific anthelmintic, closantel, were tested by the faecal egg count reduction method for the prevalence of anthelmintic resistance. Resistance was found to be 90%, 84%, 13%, 73% and 20%, respectively. This is a crisis situation. Immediate, drastic action needs to be implemented, otherwise the sheep industry in this region (approx. 10 million head) will soon face a lack of any effective anthelmintics with the inevitable consequences of major restructuring or abandonment.

Keywords: Sheep-Nematoda; Haemonchus contortus; Trichostrongylus spp; Osteragia circumcincta; Resistance; Brazil

1. Introduction

The sheep population of Brazil is demarcated into two distinct, geographically separated regions which have different breeds, raised under different husbandry and
environmental conditions. In the North-East, hairy breeds predominate and the sheep population in this region constitutes approximately 40% of the national total. Rainfall tends to be low in this region, particularly in the inland areas. In the southern state of Rio Grande do Sul, there is a total shift to wool breeds which account for 55% of the total Brazilian flock of 17 million head (see Fig. 1). Approximately 12 million cattle are also raised in this state, which represents nearly 10% of the total number of cattle in Brazil. Rainfall in this region exceeds 1000 mm year\(^{-1}\) with many areas receiving well above this total. An example of seasonal rainfall and temperature variations for Bagé, an important sheep raising county in Rio Grande do Sul, is given in Fig. 2. Rainfall at this locality is relatively uniform throughout the year with a long-term average of 1200–1400 mm annually. Evaporation rates are only higher than rainfall during summer and mild to hot temperatures throughout the year ensure ideal conditions for development and survival of free-living stages of nematode parasites on pasture.

*Haemonchus contortus* is the major problem during the summer months, but *Trichostrongylus* and *Ostertagia circumcincta* assume dominance during winter and spring causing widespread clinical disease and productivity losses. Traditional anthelmintic treatments are empirical and range from 6–12 treatments year\(^{-1}\) (average 9.4 year\(^{-1}\)). Despite this intensive dosing regimen, growth rate of young sheep is still greatly compromised by parasitism. A strategic dosing programme which combined two broad spectrum anthelmintic treatments with an anthelmintic having prolonged efficacy against
*H. contortus* (e.g. disophenol or closantel) during the summer months resulted in young sheep reaching a target weight of 42 kg at 18 months of age. This was in stark contrast to traditionally treated sheep receiving 9 treatments year⁻¹. Such sheep do not reach this weight until 30 months of age and untreated flocks may suffer up to 40% mortality during this period (F. Echevarria, unpublished data, 1991).

Therefore, it is not surprising that resistance has been reported in this region as a consequence of the heavy use of anthelmintics. In fact, it was in Rio Grande do Sul that one of the first cases of anthelmintic resistance was recorded (Santos and Franco, 1967). Amaral (1985) reviewed the work by Santiago and colleagues on anthelmintic resistance and concluded that benzimidazole-resistant *H. contortus* populations are widespread in Brazil, with strains resistant to levamisole/morantel and rafoxanide also present. Resistance to both benzimidazoles and levamisole/morantel have also been reported in *Trichostrongylus* spp. (Santiago et al., 1978; Santiago and da Costa, 1979), *Ostertagia* spp. (Santiago and da Costa, 1979) and *Nematodirus* spp. (da Costa et al., 1985).

More recently Echevarria and Pinheiro (1989) conducted a limited survey involving 31 farms in the county of Bagé in Rio Grande do Sul. Of these farms, approximately 38% showed benzimidazole resistance, 26% levamisole resistance and 19% multiple resistance. Benzimidazole resistance was mainly associated with *H. contortus* and levamisole resistance mainly occurred in *Trichostrongylus* and *Ostertagia* populations. Of great concern also is a report by Echevarria and Trindade (1989) of ivermectin resistance in *H. contortus*, making it the first report of resistance to this class of compounds on the South American continent.

This survey was designed to comprehensively evaluate the prevalence of anthelmintic resistance in the wool production sheep flocks of southern Brazil.
2. Materials and methods

This survey was conducted on 182 sheep farms located in 26 counties in the state of Rio Grande do Sul from the beginning of June until the end of October 1994. From each county, three farms were selected at random from three flock size categories, namely
- less than 500 sheep;
- 500–1000 sheep;
- greater than 1000 sheep.

Field veterinary (Secretaria de Agriculture) and extension (EMATER) staff, who had been specifically trained in appropriate techniques, were responsible for the field aspects of the trial. This was conducted in accordance with the guidelines described in Anonymous (1989). For each farm, young sheep 3–6 months of age were used for the faecal egg count reduction test (FECRT). Animals were gathered and outliers were rejected to achieve a homogenous mob. They were then randomly allocated into the following six treatment groups of ten lambs:

- Group 1: Benzimidazole (oral) (Albendazole 3.8 mg kg\(^{-1}\), "Valbazen", SmithKline).
- Group 2: Levamisole (oral) (Levamisole 5.8 mg kg\(^{-1}\), "Ripercol", Cyanamid).
- Group 3: Combination (oral) (Albendazole 3.8 mg kg\(^{-1}\) + Levamisole 5.8 mg kg\(^{-1}\)).
- Group 4: Ivermectin (oral) (Ivermectin 0.2 mg kg\(^{-1}\), "Ivomec", Merck, Sharp & Dohme).
- Group 5: Closantel (oral) (Closantel 10 mg kg\(^{-1}\), Fatec).
- Group 6: Control.

Pre-treatment faecal samples were also collected for nematode faecal egg count estimates. Only on few occasions were the mean pre-treatment egg counts below 200 epg, which required a reserve farm to be substituted for the test. At this time a farmer questionnaire was also completed. The animals were each treated with an anthelmintic dose calculated for the heaviest lamb in each group. Ten days post-treatment each farm was re-visited to collect faecal samples which were consigned to the Parasitology Laboratory at the EMBRAPA regional field station at Bagé R.S. Laboratory procedures for the FECRT were carried out by staff at the Parasitology Laboratory according to the standardised procedures (Anonymous, 1989). Individual sheep egg counts were performed with a minimum detection level of 100 epg and for each treatment group 2 g of faeces from each sheep was bulked to provide a faecal culture. Faecal cultures were incubated for 7 days at 25°C to allow development of eggs to infective larvae and then these were harvested for larval differentiation into species. Faecal egg counts were expressed as a percentage for each parasite species and the results were analysed according to the RESO statistical package (Anonymous, 1989). For closantel treatment groups, results were considered for \(H.\ contortus\) only.

3. Results

Overall, anthelmintic resistance was recorded on 97% of the 182 sheep farms investigated in this survey. The distribution of resistance to the various anthelmintic groups is shown in Table 1.
Table 1
Number of farms (% of total) found to be susceptible (S) or resistant (R) to the different anthelmintics used in a survey in southern Brazil

<table>
<thead>
<tr>
<th>Resistance status</th>
<th>ABZ</th>
<th>LEV</th>
<th>COMB</th>
<th>IVM</th>
<th>CLOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>19 (10.4)</td>
<td>30 (16.5)</td>
<td>50 (27.5)</td>
<td>159 (87.4)</td>
<td>136 (80.5)</td>
</tr>
<tr>
<td>Resistant</td>
<td>63 (89.6)</td>
<td>152 (83.5)</td>
<td>132 (72.5)</td>
<td>23 (12.6)</td>
<td>33 (19.5)</td>
</tr>
</tbody>
</table>

There were 182 sampled farms for all groups with the exception of closantel where 169 farms were surveyed. BZ, benzimidazole; LEV, levamisole; COMB, combination BZ + LEV; IVM, ivermectin; CLOS, closantel.

Table 2
Anthelmintic resistance in nematode parasites of sheep flocks in southern Brazil

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Percentage resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BZ</td>
</tr>
<tr>
<td>Ostertagia</td>
<td>87</td>
</tr>
<tr>
<td>Haemonchus</td>
<td>68</td>
</tr>
<tr>
<td>Trichostrongylus</td>
<td>7</td>
</tr>
<tr>
<td>Others</td>
<td>35</td>
</tr>
</tbody>
</table>

BZ, benzimidazole; LEV, levamisole; COMB, combination BZ + LEV; IVM, ivermectin; CLOS, closantel.

Resistance existed to all drug groups and to the benzimidazole/levamisole combination. Approximately 90% of farms recorded resistance to albendazole, 84% to levamisole and 73% to the combination product. In addition, approximately 13% of farms recorded resistance to ivermectin. The parasite species composition on the farms which recorded anthelmintic resistance is shown in Table 2. The three major species (Oster-

Table 3
Distribution of anthelmintic resistance according to flock size in southern Brazil

<table>
<thead>
<tr>
<th>Flock size</th>
<th>Susceptible</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 500</td>
<td>3</td>
<td>76</td>
</tr>
<tr>
<td>501–1000</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>1</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 4
Frequency of anthelmintic treatment (percentage of farms) for the different classes of sheep and resistance status of flocks in southern Brazil

<table>
<thead>
<tr>
<th>Sheep class</th>
<th>Treatment year(^{-1}) (percentage)</th>
<th>Low (&lt; 3)</th>
<th>Medium (4–6)</th>
<th>High (&gt; 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs (% R)</td>
<td></td>
<td>12 (6.7)</td>
<td>80 (44.9)</td>
<td>86 (48.3)</td>
</tr>
<tr>
<td>Ewes (% R)</td>
<td></td>
<td>10 (5.75)</td>
<td>87 (50)</td>
<td>77 (44.3)</td>
</tr>
<tr>
<td>Wethers (% R)</td>
<td></td>
<td>13 (8.3)</td>
<td>90 (57.7)</td>
<td>53 (34)</td>
</tr>
</tbody>
</table>

\(^{a}\) Percentage of farms with resistant parasites according to anthelmintic treatment practices for each class of sheep.
tagia, H. contortus and Trichostrongylus spp.) all recorded resistance to not only the benzimidazole, levamisole and combination products but also to ivermectin. In addition, H. contortus populations resistant to closantel were recorded on 20% of surveyed farms.

There was no evidence that the size of the flock influenced the level of anthelmintic use (see Table 3). The frequency of drenching ranged from 2 to 12 treatments year\(^{-1}\) with an average of 7.1 treatments year\(^{-1}\). Although high frequency of treatment was recorded for the three classes of sheep (viz. lambs, ewes and wethers) there was a tendency for lambs to be treated most often (Table 4).

4. Discussion

Parasite control of sheep in southern Brazil is rapidly reaching a state of crisis. The benzimidazole, levamisole and combination products have virtually reached their limit of chemotherapeutic usefulness, with resistance to these groups higher than in any other major sheep producing country, or region, in the world (for comparisons see Coles et al., 1993; Waller et al., 1995). Undoubtedly, the main reason for this is the high frequency of anthelmintic treatments, with more than 90% of farmers drenching lambs more than 4 times year\(^{-1}\) (44.6% giving 4–6 treatments year\(^{-1}\); 48.3% giving more than 7 treatments year\(^{-1}\)). The great concern is that sheep farmers in Rio Grande do Sul are also setting the pace for selection of resistance to closantel and, except for Paraguay (Maciel et al., 1996), to ivermectin. Closantel has been shown to be an exceedingly valuable drug in the control of H. contortus. In the high summer rainfall regions of Australia where this parasite is a major problem to sheep producers, closantel is the critical component of the strategic parasite control schemes, “Wormkill” and “Wormbuster” (Waller et al., 1993). The former programme, “Wormkill”, was the vanguard of all the parasite control programmes, now being actively promoted in an attempt to arrest the further development of anthelmintic resistance in sheep flocks in Australia (Waller et al., 1995). This programme was based on the work of Dash (1986), which showed that closantel treatment provided effective control against H. contortus for up to 60 days.

“Wormkill” was launched in July 1984 on the Northern Tablelands of NSW (Dash et al., 1985). The primary aim of the programme was to reduce the frequency of treatment with broad spectrum anthelmintics by exploiting the persistent anthelmintic efficacy of closantel against H. contortus. The original “Wormkill” programme recommended 3 closantel treatments year\(^{-1}\), but with time, modifications to further reduce the frequency of treatment have been made (Holdsworth, 1993), because of the concerns that the pharmacokinetic profile of closantel may predispose to selection for resistance (Hennessy, 1993). However, despite virtually a decade of reliance on closantel to control H. contortus in the “Wormkill” and “Wormbuster” targeted regions, resistance to this drug was slow to emerge (Rolfe, 1990). Most recent estimates indicate that less than 2% of farms have resistance to closantel, based on faecal egg count reduction (Rolfe, 1993). This provides good albeit indirect, evidence that drug efficacy can be preserved over a large region, involving many hundreds of farmers with many thousands of sheep, when its use is carefully promoted and monitored. Unfortunately, closantel resistance in H. contortus infections
of sheep flocks in Rio Grande do Sul appears to be approximately ten-fold greater than in Australia. Furthermore, the fact that approximately 13% of farmers were found to have ivermectin resistant parasites in their flocks, means that immediate drastic action needs to be taken to overhaul nematode parasite control practices of sheep in southern Brazil.

Epidemiological studies conducted in this region where the survey was carried out, have shown that strategic parasite control in sheep flocks could be achieved by using few, but effective, anthelmintic treatments (F. Echevarria, unpublished data, 1991). However, these parasite control studies were carried out at a time when the worm populations were largely susceptible to anthelmintics. Unfortunately, the situation has now progressed to one of widespread, high level, multiple resistance and farmers will need to seek assistance from trained professionals in order to solve their parasite control problems on a case-by-case basis. It is also of utmost importance that resources be invested on evaluating the potential of a range of alternative parasite control methods, otherwise the sheep industry in southern Brazil may collapse for want of an effective, sustainable parasite control scheme in the near future.

Acknowledgements

This survey was financially supported by the Technical Co-operation Programme of the Food & Agriculture Organisation (FAO) of the United Nations. The senior author was supported by a grant from CNPq (Conselho Nacional de Pesquisa).

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