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Anthelmintic resistance in nematode parasites from goats in Spain

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Abstract

In a flock of cashmere goats sited in Asturias (NW, Spain) diminished efficacy was observed following treatment with netobimin and consequently a study to demonstrate the existence of anthelmintic resistance and the species of nematode involved was conducted. Results from faecal egg count reduction tests before and after treatment with netobimin or ivermectin showed efficacies of 89.4 (81.8–94.8) and 99.7% (93.9–99.9%), respectively. *Teladorsagia circumcincta* was found to be the dominant species involved in netobimin resistance. Effective dose (ED₅₀) values in the egg hatch assay of 0.22 µg thiabendazole ml⁻¹ confirmed the existence of benzimidazole carbamate resistant nematodes. To the best of our knowledge, this is the first report on the existence of anthelmintic resistant nematodes from ruminants in Spain. © 1997 Elsevier Science B.V.

Keywords: Goat; Drug resistance; *Teladorsagia circumcincta*

1. Introduction

During the past 15 years, there have been increasing numbers of reports of anthelmintic resistance in goats from several countries including Australia (Barton et al., 1985), New Zealand (McKenna et al., 1990), Cameroon (Ndamukong and Sewell,

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1992), Kenya (Mwamachi et al., 1995), India (Yadav and Uppal, 1992) and Malaysia (Dorny et al., 1994). In Europe, nematode resistance has been described mainly in northern countries (Coles et al., 1993). By contrast, reports of anthelmintic resistance from the mediterranean basin have been scarce and mainly associated with the introduction of small ruminants from areas where anthelmintic resistance has already been detected (Himonas and Papadopoulos, 1994). Either in northern or southern countries of Europe, fibre producing goats (Cashmere and Angora goats) have been one of the principal breeds implicated in anthelmintic resistance (Jackson et al., 1992b; Varady et al., 1993).

In Spain, the dry climate in most of the country results in low levels of gastro-intestinal nematode infections and only necessitates a maximum of two anthelmintic treatments per year (Spring and Autumn). The first report of benzimidazole (mebendazole) resistance in cyathostomes of horses in Spain has recently been published (García-Pérez et al., 1994) and anthelmintic resistance has been suspected in some sheep flocks in northern Spain (Coles et al., 1993). However, until now there has been no real evidence of anthelmintic resistant nematodes in ruminants in Spain.

The three aims of the present study are first to use the faecal egg count reduction test (FECRT) to investigate the susceptibility of nematodes in goats in northern Spain to drugs within the benzimidazole and ivermectin families. The second aim of the study was to determine which species, if any, were implicated in resistance and the third was, to investigate the susceptibility of nematode ova to the benzimidazole group of anthelmintics, using the egg hatch assay (EHA).

2. Materials and methods

2.1. Farm history

During the Autumn of 1995 clinical signs of parasitic gastroenteritis were observed in a flock of cashmere goats (73 animals) located in Asturias (northern Spain). Following treatment of some goats with the recommended dosage of the nitrophenylguanidine compound netobimin (Hapasil[®], 5%, Schering-Plough) a diminished treatment efficacy was observed when pre- and post-treatment coprological examination results were compared, and a more exhaustive study to demonstrate the existence of anthelmintic resistance was designed. The cashmere flock was located in an institutional farm together with a flock of sheep (Lacha and Gallega breeds) and cattle (Asturiana de los Valles breed). Cashmere goats had been introduced in December 1993 and no sign of anthelmintic resistance had been observed on the farm before this date.

2.2. Faecal egg count reduction test

Three groups of randomly selected cashmere does, each containing 13 animals, were used in this test. Animals included in this study had not been treated with anthelmintics during the previous 5 months. All the does were individually identified, weighed and a faecal sample taken before those in the two treated groups were dosed with netobimin

(Hapasil®, Schering-Plough) at 20 mg kg^{-1} (fluke recommended dose) or ivermectin (Ivomec®, MSD Agvet) at $200 \text{ } \mu\text{g kg}^{-1}$. Ten days after the first treatment a faecal sample was taken from all the does.

Mean faecal egg counts, percentage reductions and 95% confidence intervals were calculated by using the formulae recommended by the World Association for the Advancement of Veterinary Parasitology guidelines for detecting anthelmintic resistance in nematodes of ruminants (Coles et al., 1992). An efficacy of less than 95%, and 95% confidence limits of less than 90% were taken as indicating the presence of anthelmintic resistant nematodes.

2.3. Faecal egg count and identification of species

Faecal samples collected from each animal were processed to determine the number of nematode eggs per gram of faeces using a modified McMaster method (MAFF, 1986). Diagnosis of nematode species present in each goat group before and after anthelmintic treatment was determined following the recommended guidelines (Coles et al., 1992).

2.4. Egg hatch assay

Faecal samples obtained from 12 cashmere does in the same flock were taken on day 0, anaerobically stored at room temperature (Hunt and Taylor, 1989) and processed within two days. The egg hatch assay (Le Jambre, 1976) was performed using pure thiabendazole in nine concentrations ranging from 0.05 to $1.5 \text{ } \mu\text{g ml}^{-1}$. Effective dose (ED_{50}) values were calculated using a regression analysis after correcting for natural mortality. Animals in which the ED_{50} value is in excess of $0.1 \text{ } \mu\text{g thiabendazole ml}^{-1}$ are considered to be carrying benzimidazole-resistant strains of nematodes.

3. Results

The arithmetic mean faecal egg counts ($\pm \text{SD}$) before and after treatment with netobimin or ivermectin are given in Table 1. Netobimin at 20 mg kg^{-1} body weight reduced the faecal egg count by 89.4% with a 95% confidence intervals of 81.8–94.8%. Nematode parasites were sensitive to ivermectin with an efficacy of 99.7% and confidence intervals of 93.9–99.9%.

Table 1

Mean faecal egg counts ($\pm \text{SD}$) and efficacies from cashmere goats prior to and following anthelmintic treatments with netobimin or ivermectin

Group	Mean faecal egg counts ($\pm \text{SD}$)		Efficacy (%)
	Pre-treatment	Post-treatment	
Control	1355 ± 793	2185 ± 1791	–
Netobimin treated	1080 ± 1130	231 ± 215	89.4
Ivermectin treated	1880 ± 1668	6 ± 17	99.7

Table 2

Percentages of nematode larvae present in each goat group before and after anthelmintic treatments with netobimin or ivermectin

Genera	Control group		Netobimin treated group		Ivermectin treated group	
	day 0	day 10	day 0	day 10	day 0	day 10
<i>Teladorsagia</i>	15	8	12	60	10	NL
<i>Oesophagostomum</i>	52	50	40	25	48	NL
<i>Trichostrongylus</i>	10	10	13	15	8	NL
<i>Haemonchus</i>	13	18	22	0	18	NL
<i>Chabertia</i>	10	14	13	0	16	NL
Total	100	100	100	100	100	NL

NL: no larvae

The results from the faecal culture and larval identification, representing the nematode genera present in the different doe groups on days 0 and 10, are shown in Table 2. In does in the netobimin treated group, the genera present before treatment were *Oesophagostomum* (40%) and *Haemonchus* (22%), with smaller numbers of *Chabertia* (13%), *Trichostrongylus* (13%) and *Teladorsagia* (12%). After treatment with netobimin the predominant genus was *Teladorsagia* (60%), followed by *Oesophagostomum* (25%) and *Trichostrongylus* (15%).

The ED₅₀ value obtained after probit analysis of data in the EHA was 0.22 µg thiabendazole ml⁻¹, demonstrating the presence of benzimidazole resistant nematodes based on the interpretations of Le Jambre (1976).

4. Discussion

The results from the FECRT and the EHA both provided evidence for the presence of benzimidazole resistant, but ivermectin susceptible populations of nematodes in goats from North of Spain (Asturias). This is the first report on the existence of anthelmintic resistant nematodes from ruminants in this country.

Studies of the efficacy of benzimidazoles in goats (Pomroy, 1996) suggest that it may be better to treat goats with drugs in the benzimidazole family at one-and-a-half or twice the recommended dosage. For instance, Bogan et al. (1987) found only a 42% bioavailability of oxfendazole in goats when compared with sheep after oral administration but no after intravenous administration. The dosage of netobimin used in the FECRT was the fluke recommended dose which is 2.6 times the recommended dose rate for gastro-intestinal nematodes.

In this study, *Teladorsagia* was the main genera involved in resistance. *T. circumcincta* is one of the most prevalent specie parasitising small ruminants in Spain (Compairé Fernández and Tarazona Vilas, 1985), while benzimidazoles are the most frequently used anthelmintics in this country (Coles et al., 1993). The results are in accordance with studies in Scotland in goats and sheep which have shown *Teladorsagia* species to have the greatest propensity for developing resistance to the benzimidazoles

(Jackson et al., 1992a), while in England, *T. circumcincta* seems to be the main species in sheep and *Haemonchus contortus* in goats (Hong et al., 1996). The presence in the post-treatment larval cultures of *Oesophagostomum* and *Trichostrongylus* suggests either the possibility of resistance in these species or a lower efficacy of netobimin against these species. Further studies are required in order to clarify the situation in goats.

There could be two main reasons for the existence of anthelmintic resistance in this cashmere flock. First, goats could have acted as carriers of anthelmintic resistant strains of nematodes since they were originally imported from the UK, where a high prevalence of anthelmintic resistance in fibre producing goats has been recorded (Jackson et al., 1992b; Hong et al., 1996). The other possibility is the acquisition of resistance once the animals arrived in Spain. Although the prevailing climatic conditions in this part of Spain favour a continuous cycle of infection between the host and the pasture, the low frequency of anthelmintic treatments (2–3 annual treatments, alternating benzimidazoles and ivermectin), the limited time the cashmere goats were on the farm and the absence of diminished anthelmintic efficacy in other sheep and goat flocks in the area support the idea of imported anthelmintic resistance.

The existence of resistant strains of nematodes in breeding animals from an institutional farm increases the risk of propagation of resistance (Coles and Roush, 1992) and livestock movement, in or between countries, has been suggested as one of the principal mechanisms of resistance propagation (Varady et al., 1993; Dorny et al., 1994; Himonas and Papadopoulos, 1994). Animal movement presents a particular risk for Spanish sheep and goat flocks since small ruminants harbour the same species of nematodes, anthelmintic resistance is retained after passage through sheep and a large number of Spanish sheep flocks also contain small numbers of goats.

In conclusion, this study demonstrates the need to investigate the prevalence of anthelmintic resistance in Spain. Results from this and similar studies (Varady et al., 1993; Himonas and Papadopoulos, 1994) show that investigation of resistant strains of nematodes may be required not only in animals imported from countries outside the EU, but also from EU countries with a previous record of anthelmintic resistance.

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