A survey on the occurrence of resistance to anthelmintics of gastrointestinal nematodes of goats in Mozambique

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ABSTRACT

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A survey to study the extent of anthelmintic resistance was conducted in Maputo and Gaza, two of the ten provinces of Mozambique, during February and March, 1999. A total of 12 flocks, six in Maputo and six in Gaza, was surveyed. The faecal egg count reduction test was used to assess the efficacy of three anthelmintics most often used in Mozambique, namely albendazole, fenbendazole and levamisole.

The degree of resistance was calculated using two different methods, and varied according to the method used. Using the formula of Coles, Bauer, Borgsteede, Geerts, Klei, Taylor & Waller (1992), resistance to the benzimidazoles was detected in one flock in Maputo and one in Gaza, and to levamisole in three flocks in Maputo and one in Gaza. When the formula of Dash, Hall & Barger (1988) was used, however, resistance to the benzimidazoles was detected in only one flock in Maputo, and no resistance to levamisole was detected.

The 12 farms surveyed were too few for conclusions to be made on the prevalence of anthelmintic resistance in goats in Mozambique as a whole. Therefore, an extensive survey at national level is needed. This study gives evidence, however, that anthelmintic resistance in nematode parasites of goats is an emerging problem, to which special attention should be paid.

Keywords: Albendazole, anthelmintic resistance, fenbendazole, gastrointestinal nematodes, goats, levamisole, Mozambique

INTRODUCTION

The control of helminth parasites is often essential for the economic production of animals, especially small ruminants, and anthelmintics are used as the primary means of control (Prichard 1990). Resistance of nematodes to the generally used groups of anthelmintics is an increasing problem in sheep and goat production worldwide (Waller 1997; Gopal, Pomroy & West 1999). The significance of the problem varies between and within countries and farming systems (Waller 1987). Recently, a strain of *Haemonchus contortus* resistant to all classes of anthelmintics has developed in South Africa, probably because of frequent use of anthelmintics and inappropriate dosage rates (Van Wyk, Malan & Randles 1997).

The objective of this survey was to determine whether anthelmintic resistance occurs in goats in

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the provinces of Maputo and Gaza, two of the provinces where anthelmintics are most often used.

MATERIALS AND METHODS

Study animals and location

The study was carried out during February and March 1999. Twelve goat flocks, six in Maputo Province and six in Gaza Province, comprising 568 goats, were selected. The number of goats in each of the flocks varied from 24 to 60 animals and all were kept extensively on communal grazing for the duration of the survey.

Faecal examination

Reinecke's (1961) modification of the McMaster technique was used for quantitative determination of nematode eggs in faecal samples. The number of nematode eggs per gram of faeces (epg) was calculated using the formula: epg = Te/Tc x 200 where Te is the number of eggs and Tc the number of chambers of the McMaster slide examined (Reinecke 1983).

Larval cultures were made (Anonymous 1986) and 100 larvae per culture were identified unless there were fewer than 100, in which case all were identified. The larvae were identified using the descriptions of Reinecke (1983), Georgi, Theodorides & Georgi (1985) and Anonymous (1986).

Determining anthelmintic resistance

The efficacy of oral suspensions of the three anthelmintics mostly used in Mozambique, namely albendazole (Valbazen®, Pfizer, South Africa), fenbendazole (Ecomintic®, Eco, South Africa), and levamisole (Levicon®, Milborrow, South Africa) was assessed using the faecal egg count reduction test (FECRT). The latter was performed according to the methods recommended by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) (Coles, Bauer, Borgsteede, Geerts, Klei, Taylor & Waller 1992) and also those of Dash, Hall & Barger (1988). The results were interpreted according to Pomroy (1996) and McKenna (1997).

Once a flock was selected, the animals were randomly allocated to four groups, tagged with coded ear tags, and weighed. Each group consisted of at least six goats that had not been dewormed during the preceding 12 weeks in order to reflect the naturally acquired helminth population. An untreated control group was also included to monitor changes in the nematode egg counts during the test period.

A dose of 5 mg kg^{-1} albendazole was administered to one group, 5 mg kg^{-1} fenbendazole to another and 7.5 mg kg^{-1} levamisole to the third group. All anthelmintics were given with a separate 10 mℓ plastic syringe. Faecal samples were collected on the day of treatment and again 10 days later.

Goats with less than 100 nematode epg in the pre–treatment sample and those with missing values for either the pre– or post–treatment epg were excluded from the analyses.

The percentage reduction was calculated, firstly, as 100 (1 – Xt/Xc), where Xt was the mean egg count of the treated group and Xc was the mean egg count of the untreated control group 10 days after treatment (Coles *et al.* 1992), and secondly, as 100 x $\{1 - (T_2/T_1 \times C_1/C_2)\}$, where T and C are the means for the treated and control groups and subscripts 1 and 2 designate the counts before and after treatment, respectively (Dash *et al.* 1988). Resistance to an anthelmintic was considered to be present if the percentage reduction in egg counts was less than 95 % and 80 %, respectively (Coles *et al.* 1992; Dash *et al.* 1988).

RESULTS

The arithmetic means of the faecal egg counts for each of the four groups used in Maputo and Gaza Provinces are presented in Tables 1 and 2, respectively, while the efficacy of the treatments, as measured by the FRCRT and calculated according to the two methods for each anthelmintic tested, are presented in Tables 3 and 4.

Using the formula recommended by Coles *et al.* (1992), resistance to two of the benzimidazoles was detected on two farms, one in Maputo and one in Gaza, and to levamisole on four farms, three in Maputo and one in Gaza. Using the formula of Dash *et al.* (1988), however, resistance to the benzimidazoles was detected only in one farm in Maputo, and no resistance against levamisole was detected.

In pre-treatment larval cultures, *Haemonchus* spp., *Oesophagostomum* spp. and *Strongyloides papillosus* were the predominant nematode species, while *Trichostrongylus* spp., *Bunostomum* spp. and *Gaigeria pachyscelis* were present in small numbers. Post-treatment faecal cultures indicated that *Haemonchus* spp., and to a lesser extent *Oesophagostomum* spp. and *Trichostrongylus* spp., were

TABLE 1 The arithmetic means of faecal nematode egg counts before and after treatment on six farms in Maputo

Farm no.	Control			Albendazole			Fenbendazole			Levamisole		
	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment
1	8	988	1 000	9	1 144	11	11	1 027	0	9	500	67
2	7	1 586	2 829	7	7 300	0	8	13 675	0	7	4 014	114
3	14	4 521	4 679	14	1 893	50	13	1 969	0	15	3 553	0
4	11	6 818	8 436	12	5 117	242	12	4 367	75	12	5 558	550
5	14	1 300	1 521	15	1 440	0	15	1 427	0	14	1 079	200
6	6	1 750	2 317	6	4 717	3 317	6	2 117	1 183	6	1 517	0

TABLE 2 The arithmetic means of faecal nematode egg counts before and after treatment on six farms in Gaza

Farm no.	Control			Albendazole			Fenbendazole			Levamisole		
	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment
7	9	1 156	1 311	10	940	0	10	610	0	10	1 740	0
8	14	1 279	1 650	13	2 692	0	13	2 000	0	13	3 015	208
9	10	3 570	5 620	11	3 255	0	11	4 336	0	10	2 200	30
10	12	1 622	2 692	13	608	0	11	581	0	12	792	8
11	11	3 209	6 145	12	3 275	0	13	3 054	0	12	2 975	200
12	21	1 176	2 081	22	1 118	355	22	959	259	22	1 691	14

TABLE 3 The percentage faecal egg count reduction calculated according to two methods for the anthelmintics tested on six goat farms in Maputo

Farm no.	Albendazole		Fenbendazole		Levamisole	
	Dash *et al.* (1988)	Coles *et al.* (1992)	Dash *et al.* (1988)	Coles *et al.* (1992)	Dash *et al.* (1988)	Coles *et al.* (1992)
1	99.1	98.9	100	100	86.8	93.3R
2	100	100	100	100	98.4	96
3	97.5	98.9	100	100	100	100
4	96.2	97.1	98.6	99.1	92.0	93.5R
5	100	100	100	100	84.2	87.0R
6	46.9R	–43R	57.8R	49R	100	100

R – Resistant

TABLE 4 The percentage faecal egg count reduction calculated according to two methods for the anthelmintics tested on six goat farms in Gaza

Farm no.	Albendazole		Fenbendazole		Levamisole	
	Dash *et al.* (1988)	Coles *et al.* (1992)	Dash *et al.* (1988)	Coles *et al.* (1992)	Dash *et al.* (1988)	Coles *et al.* (1992)
7	100	100	100	100	100	100
8	100	100	100	100	94.7	87.4R
9	100	100	100	100	99.1	95
10	99.2	99.7	100	100	99.4	99.7
11	100	100	100	100	96.5	96.7
12	81.9	82.9R	84.6	87.6R	99.2	99.3

R – Resistant

resistant to both benzimidazoles and levamisole. *Strongyloides papillosus* appeared also in large numbers in post-treatment larval cultures.

DISCUSSION

This is the first report on the existence of anthelmintic resistant of nematodes of goats in Mozambique. The results of the FECRT are in agreement with studies done in the Netherlands (Borgsteede, Pekelder & Dercksen 1996), Denmark (Maingi, Bjørn, Thamsborg, Bogh & Nansen 1996a), Thailand (Pandey, Pralomkarn, Kochapakdee & Saithanoo 1996) and Spain (Requejo-Fernández, Martínez, Meana, Rojo-Vázquez, Osoro & Ortega-Mora 1997) where resistance of *H. contortus, Trichostrongylus* spp. and *Oesophagostomum* spp. to the benzimidazoles, and to levamisole (Yadav & Uppal 1992; Hunt, Hong & Coles 1994; Sangster & Bjørn 1995; Maingi *et al.* 1996a) was present. Resistance of *H. contortus* to the benzimidazoles in goats was also reported by Hong, Hunt & Coles (1996) in England. Waruiru, Kogi, Weda & Ngotho (1998) reported strains of *H. contortus* resistant to albendazole and levamisole, and *T. colubriformis* and *Oesophagostomum* spp. that showed resistance against levamisole, from goats in Kenya.

In this study resistance to the benzimidazoles and levamisole was detected but no case of multiple resistance was observed on any of the farms surveyed.

The formula of Dash *et al.* (1988) failed to detect resistance to the benzimidazoles and to levamisole in the four farms where resistance was detected by using the formula of Coles *et al.* (1992). This discrepancy could be influenced by the inclusion or not of the pre-treatment egg counts in the formulas used. The formula of Dash *et al.* (1988) includes the pre-treatment egg counts but that of Coles *et al.*

(1992) only uses the post-treatment egg counts to calculate the percentage faecal egg reduction. Maingi *et al.* (1996a) stated that inclusion of pre-treatment egg counts in the calculation influenced the declaration of resistance. On the other hand, it is not so much the formula as the arbitrary level chosen below which resistance is regarded to be present, and it could be argued that a level of 80 % is very insensitive. Presidente (1985), using the same formula as Dash *et al.* (1988), refers to a number of workers who regard a arithmetic mean reduction of less that 90 % as indicative of resistance in sheep. However, a FECR of 80 % or less has been used to indicate resistance in goats because reduced efficacy may not be due to resistance but to the rapid breakdown of benzimidazole anthelmintics in these animals, as well as to inappropriate dose rates (McKenna 1984, cited by Presidente 1985).

The arithmetic mean of the faecal egg counts was used to calculate the percentage reduction in the two formulas. Dash *et al.* (1988) and Coles *et al.* (1992) stated that the arithmetic mean is preferable to the geometric mean as it is easier to calculate, it provides a better estimate of the worm egg output and it is a more conservative measure of anthelmintic efficacy. Based on these points, McKenna (1997) concurs, suggesting that the estimates of anthelmintic efficacy should be calculated from the arithmetic mean.

On two farms, one in each province, where evidence of benzimidazole resistance was found, both albendazole and fenbendazole had been used for more than five consecutive years. On the farm in Maputo Province, the owner had been complaining of the failure of these anthelmintics to control nematode infections since 1993 when fenbendazole was used. The farmer was advised to change to an anthelmintic from another group. He changed to oxfendazole, however, and for the last two years has been using albendazole; the efficacy of levamisole was still 100 % on this farm. According to Maingi, Bjørn, Thamsborg, Dangolla & Kyvsgaard (1996b) and Prichard (1999), frequent anthelmintic treatments, the use of anthelmintics with similar mode of action for several years and underdosing are some of the factors that contribute to the development of resistance. Martin, Anderson, Brown & Miller (1988) state that the use of levamisole following the emergence of thiabendazole resistance provided poor control of *Ostertagia* spp. Therefore, as a means of preserving susceptibility of the worms to anthelmintics, it would seem logical to consider alternate anthelmintics before any resistance develops to a specific group. Van Wyk (2001), however, suggests that refugia play a much more important role than is currently recognized.

On one of the farms in Maputo Province, where resistance to levamisole was detected, a batch of expired injectable levamisole solution had been used until 1997. From 1998, albendazole has been used to control nematode infections in this farm, with efficacy of 98.9 % and 99.1 %, respectively. The efficacy of fenbendazole on this farm was 100 % using both formulas.

On the only farm in Gaza Province, where resistance to levamisole was detected, most of the goats were imported from Zimbabwe during 1996 through a restocking program implemented by the Mozambican government. It should be noted that the animals were not dewormed for two years after they had been imported. Mukaratirwa, Charakupa & Hove (1997) reported the occurrence of nematodes resistant to levamisole in sheep in Zimbabwe, and it is conceivable that goats acquired the helminths from sheep, since goats and sheep often feed together on the same communal pastures. The absence of a parasite control policy for newly introduced animals could imply the possible introduction of resistant worms from within or outside the country (Maingi *et al.* 1996b). According to Waller (1997), to prevent importation of anthelmintic resistance, a quarantine anthelmintic treatment should be used, and the grazing of purchased stock should be restricted whilst faecal egg counts can be conducted to confirm worm removal.

Strongyloides papillosus appeared in post-treatment larval cultures from the different groups treated with each of the drugs tested. The occurrence or not of resistance of *S. papillosus* to the anthelmintics tested could, however, not be determined due to the low egg counts for this nematode in pre- and post-treatment epg. It is known, for example, that fenbendazole is a class A anthelmintic for the L3, L4 and adult stages (Reinecke 1983). Large numbers of infective larvae of *S. papillosus* in post-treatment larval cultures compared with the pre-treatment larval cultures in seven of the flocks were observed in this study. This could be the result of reinfection, since the lifespan of the benzimidazoles after administration is short, about 4 h, and the prepatent period of this nematode varies between 8 and 14 days, or is the result of the nematode completing its heterogonic life cycle in the larval culture media. Oosthuizen, Erasmus, Boelema & Grové (1993), in experimental infections of sheep, made no reference to the resistance of this nematode to either the benzimidazoles or levamisole, but found moxidectin to be ineffective. Therefore, the occurrence or not of resistance of *S. papillosus* to the drugs tested should be investigated further.

The 12 farms surveyed were too few and no conclusions can be made on the prevalence of anthelmintic resistance in goat farms in Mozambique as a whole. Therefore, an extensive survey on a national level is needed. However, this study gave evidence that anthelmintic resistance in nematode parasites of goats is an emerging problem, to which special attention should be paid.

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