A survey of anthelmintic resistance on ten sheep farms in Mashonaland East Province, Zimbabwe

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ABSTRACT

A survey to detect anthelmintic resistance in nematode parasites of sheep was conducted on 10 randomly-distributed farms in the Chivhu District, Mashonaland East Province, Zimbabwe. Before the survey, a questionnaire was circulated to the farmers concerning nematode parasite control. Results showed that parasite control using anthelmintic treatment was the only method practised and that the benzimidazoles were the most frequently used anthelmintic drugs. The faecal egg count reduction test was used to detect resistance. The anthelmintic groups tested were benzimidazoles, levamisole and ivermectin. Resistance to benzimidazoles was detected on 6 of 10 farms and levamisole resistance on 2 of 3 farms. Ivermectin resistance was not observed on the farms surveyed. Post-treatment larval cultures indicated that *Haemonchus contortus* survived administration of fenbendazole, albendazole, oxfendazole and levamisole. A *Cooperia* sp. strain resistant to albendazole was detected and this is the first report in Zimbabwe of a resistant parasite in this genus.

Key words: anthelmintic resistance, *Cooperia* sp., *Haemonchus contortus*, sheep nematodes.

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INTRODUCTION

Gastrointestinal nematodes of importance in sheep in southern Africa belong to the genera *Haemonchus, Cooperia, Trichostrongylus* and *Oesophagostomum.* The nematodes cause marked loss in production due to poor weight gains, poor wool quality and most frequently losses due to high mortalities. In Zimbabwe, *Haemonchus contortus* is by far the most predominant nematode in sheep[6].

The intensification of sheep production has led to an increasing reliance on anthelmintics for control of gastrointestinal parasites[2] and anthelmintic resistance has become an increasingly widespread problem in many countries[14]. It is a cause of concern for all those interested in controlling helminth parasites of sheep. Zimbabwe is no exception owing to the indiscriminate use of anthelmintics by sheep farmers. Once resistance has developed, there are fewer options to control these parasites, and greater costs are incurred owing to reduced production efficiency or profit.

To date, no extensive studies have been carried out on anthelmintic resistance in sheep in Zimbabwe and, as in many other countries, marketing of anthelmintics to farmers has not taken into account the best options in formulation, duration of effect and dose rate to slow the development of resistance. The objective of this study was to investigate the extent of anthelmintic resistance in sheep in the Mashonaland East Province of Zimbabwe.

MATERIALS AND METHODS

The survey on anthelmintic resistance was conducted on commercial sheep farms in the Chivhu District of Mashonaland East Province situated in the highveld area of Zimbabwe. Chivhu District is 140 km south of Harare and lies in geographical regions II and III (between 31–32° E and 18–19° S). In geographical region II, rainfall is confined to summer and is moderately high (750–1000 mm). The region rarely experiences severe dry spells in summer and it is suitable for intensive systems of farming based on crops and/or livestock production. In region III rainfall is moderate (650–800 mm), much of it is accounted for by infrequent heavy falls and temperatures are generally high.

Farming systems are based on livestock production. In both regions the rainy season starts in late October and ends in May, with maximum rainfall between December and March. The monthly minimum and maximum temperatures vary between 9 and 14 °C and 19 and 25 °C respectively.

Ten commercial farms were selected with a flock size ranging from 35 to 350 sheep. All sheep were of the same breed (*i.e.* Dorper cross) and were raised solely for mutton. A questionnaire designed to seek information on census of sheep on the farm, breed of sheep, anthelmintics used on each farm for the past 5 years and the frequency of drenching, was completed by the farmers concerned. The questionnaire also gathered information on whether the farmers had experienced or suspected anthelmintic failure.

Sheep between the age of 3 and 6 months were randomly selected from each farm by the lottery method and their faecal samples were examined for eggs per gram of faeces (epg). All the lambs involved were vaccinated against pulpy kidney disease (*Clostridium perfringens* Type D enterotoxaemia) before commencement of the survey. The sheep are kept on permanent pastures with little or no supplementary feed throughout the year. Based on the results of faecal examination, sheep with a faecal worm egg count of 150 or more were selected and ear-tagged for identification. The selected number of sheep on each farm shown in Table 1 comprised approximately 10 % of the flock. No control groups were included in our study because the majority of the farmers were reluctant to allow more than 10 % of their lambs to be included in the study.

All the anthelmintics used in the study were commercial formulations (Table 1). They were administered orally using a

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Table 1: **Summary of anthelmintics used on the 10 commercial sheep farms between 1991 and 1995.**

Farm name	Census	Anthelmintic	Number of treatments per year	Drugs tested for resistance	Number of animals tested
1) Altrina	100	Ivermectin	4	Ivermectin	20
2) Constantia	350	Oxfendazole	4	Fenbendazole	20
		Fenbendazole	(during rain season only)		
3) Hezelden	75	Levamisole	12	Levamisole	9
		Albendazole		Albendazole	9
4) Morreson	175	Albendazole	6	Albendazole	26
5) Riversdale	170	Albendazole	6	Levamisole	20
		Levamisole			
6) Singlethorn	160	Rafoxanide	12	Oxfendazole	21
		Oxfendazole			
7) Slangfontein	200	Oxfendazole	12	Albendazole	25
		Albendazole			
8) Victor	35	Albendazole	3	Albendazole	14
9) Widgen	280	Albendazole	4	Albendazole	15
		Oxfendazole		Oxfendazole	15
10) Wilderbeeste	140	Levamisole	12	Oxfendazole	17
		Oxfendazole		Levamisole	15

drenching gun (except ivermectin, which was administered subcutaneously) at the manufacturers' recommended dose rates, based on the weight of the heaviest sheep in each group. When 2 drench tests were carried out on a farm, the drench gun and pack were rinsed and cleaned between each test. Sample collection was carried out from February to May 1996.

Faecal samples were collected from each sheep prior to drug administration, and worm egg counts determined by using the modified McMaster technique[1].

Seven days post-treatment, faecal samples from animals in each experimental group were examined again for worm eggs. Data on epg was transformed to logarithm (count + 1) to calculate the geometric mean (GM). Geometric means of pre- and post-treatment faecal egg counts (FEC) were compared for each treatment group on each farm and the percentage change in FEC calculated for each group[13]. To determine the genera or species of the nematodes that were resistant to the anthelmintic administered, pre-treatment and post-treatment faecal samples were pooled in each group for culture. Standard procedures for the preparation and identification of L_3 larvae were followed[1].

Table 2: **Recommended dose rates for anthelmintics tested for resistance.**

Farm name	Anthelmintic	Dose rate
1) Altrina	Ivomec[a] (ivermectin)	0.02 mg/kg
2) Constantia	Panacur[b] (fenbendazole)	5 mg/kg
3) Hezelden	Valbazen[c] (albendazole)	10 mg/kg
	Tramisol[d] (levamisole)	7.5 mg/kg
4) Morreson	Valbazen	10 mg/kg
5) Riversdale	Tramisol	7.5 mg/kg
6) Singlethorn	Systamex[e] (oxfendazole)	5 mg/kg
7) Slangfontein	Valbazen	10 mg/kg
8) Victor	Valbazen	10 mg/kg
9) Widgen	Valbazen	10 mg/kg
	Systamex	5 mg/kg
10) Wilderbeeste	Tramisol	7.5 mg/kg
	Systamex	5 mg/kg

[a]Logos Agvet; [b]Hoechst; [c]SmithKline Beecham; [d]Zimbabwe Pharmaceuticals; [e]Cooper Zimbabwe.

RESULTS

Information about the identities and the frequencies of use of the anthelmintics on the 10 farms in the last 5 years is shown in Table 2. The most commonly used anthelmintics in the areas of study were the benzimidazole compounds (*i.e.* albendazole, oxfendazole and fenbendazole), levamisole and to a lesser extent, rafoxanide. Only 1 farm reported the use of ivermectin. The number of treatments per year differed from one farm to another, ranging from 3 to 12 drenches per year (Table 1).

Table 3 shows the percentage faecal egg count reduction (FECR) after administration of the different anthelmintics on each farm. The ranges of FECR were 56–99 % for albendazole, 82.3–95.2 % for oxfendazole, 89.9–97.9 % for levamisole, 83.34 % for fenbendazole and 100 % for ivermectin. In this study, resistance to anthelmintics was presumed present when % FECR value is lower than 95 %. On the basis of this criterion, there was resistance to fenbendazole (FECR 83.4 %) on Constantia farm, to levamisole (FECR 89.9 %) on Hezelden farm, to oxfendazole on Widgen and Wilderbeeste farms (FECR 82.9 % and 82.3 %, respectively). Resistance to albendazole was detected on 4 of 5 farms tested for this anthelmintic (Table 3).

Data relating to the identities of the nematode genera involved in the study are presented in Table 4. Larval cultures before treatment showed that *H. contortus* was the predominant nematode on all farms, followed by *Cooperia* sp. and *Oesophagostomum* sp. respectively. The results of post-treatment larval cultures indicated that the species of parasite that survived after administration of anthelmintics, with the exception of

Table 3: **Geometric mean pre- and post-treatment faecal egg counts and percentage reduction** (% FECR) **following treatment with different anthelmintics on 10 commercial sheep farms.**

Farm name	Anthelmintic	Geometric mean		% FECR	Susceptible/resistant
		Pre-Rx[a]	Post-Rx[b]		
1) Altrina	Ivermectin	141 (50–9500)	0 (0)	100	Susceptible
2) Constantia	Fenbendazole	891 (300–5800)	148 (0–1900)	83.3	Resistant
3) Hezelden	Levamisole	525 (150–1100)	53 (0–400)	89.9	Resistant
	Albendazole	339 (150–3200)	148 (0–2300)	56	Resistant
4) Morreson	Albendazole	3548 (150–35000)	54 (0–12750)	79	Resistant
5) Riversdale	Levamisole	339 (150–17350)	7 (0–3500)	97.9	Susceptible
6) Singlethorn	Oxfendazole	1122 (150–5900)	54 (0–1250)	95.2	Susceptible
7) Slangfontein	Albendazole	4786 (600–1650)	603 (0–7500)	87	Resistant
8) Victor	Albendazole	1950 (200–9900)	11 (0–450)	99.4	Susceptible
9) Widgen	Albendazole	234 (150–1800)	30 (0–1800)	87	Resistant
	Oxfendazole	692 (150–7600)	48 (0–2900)	82.9	Resistant
10) Wilderbeeste	Oxfendazole	1950 (300–13600)	345 (0–8250)	82.3	Resistant
	Levamisole	1906 (350–12850)	59 (2800)	96.9	Susceptible

[a]Pre-Rx = pre-treatment.
[b]Post-Rx = post-treatment.

Table 4: **Pre-treatment and post-treatment composition of trichostrongylids on 10 commercial sheep farms.**

Farm name	Pre-treatment (%)				Post-treatment (%)				
	H[a]	C	T	O	H	C	T	O	
1) Altrina	98	0	1	1	0	0	0	0	
2) Constantia	92	4	3	1	100	0	0	0	
3) Hezelden	93	2	5	0	100	0	0	0	
4) Morreson	100	0	0	0	100	0	0	0	(lev)[b]
					100	0	0	0	(alb)
5) Riversdale	100	0	0	0	100	0	0	0	
6) Singlethorn	100	0	0	0	100	0	0	0	
7) Slangfontein	94	6	0	0	97	3	0	0	
8) Victor	82	4	4	10	100	0	0	0	
9) Widgen	76	3	4	12	100	0	0	0	(alb)
					100	0	0	0	(oxf)
10) Wilderbeeste	89	8	3	0	100	0	0	0	(oxf)
					100	0	0	0	(lev)

[a]H = *Haemonchus contortus;* C = *Cooperia* sp.; T = *Trichostrongylus* sp.; O = *Oesophagostomum* sp.
[b]lev = levamisole; alb = albendazole; oxf = oxfendazole;

ivermectin, was *H. contortus.* On Slangfontein farm, *Cooperia* sp. survived albendazole treatment (see Table 4).

DISCUSSION

The information obtained from the questionnaire survey indicated intensive use of anthelmintics as a method of helminth control on commercial farms. Benzimidazole compounds were the most commonly used anthelmintics, followed by levamisole. Ivermectin, an expensive anthelmintic, was only used on 1 farm (Table 1). Alternating unrelated anthelmintics periodically is not practised on most farms and the benzimidazoles had been used for 5 consecutive years.

The results of the present study are in agreement with results of other studies[8,17,20] that found *H. contortus* to have multiple resistance to 2 groups of anthelmintics with different modes of action (*i.e.* benzimidazoles and levamisole). The reason why *H. contortus* was the only resistant species on most farms might be due to its high biotic potential that allows small populations of resistant worms to swell into large populations in a short time[3]. Apart from *H. contortus,* a strain of *Cooperia* sp. resistant to albendazole was detected on 1 farm (Table 4) and this is the first report of a *Cooperia* sp. strain resistant to the benzimidazoles in Zimbabwe. Few cases of benzimidazole-resistant *Cooperia* spp. have been reported in the literature so far in cattle[4] and in sheep[7,9,11].

The fact that *H. contortus* resistance to benzimidazoles and levamisole was established on all farms tested suggests that the resistant strains could have been introduced from one farm to another through the purchase of breeding animals.

The current study did not investigate husbandry procedures concerning the introduction of new stock or the degree of sheep trading undertaken among the sheep farms. Both activities could have had an impact on the development of anthelmintic resistance. Quarantine and treatment of all replacement stock prior to movement is a critical management practice that is likely to prevent the introduction of resistant parasite strains onto farms without resistant strains[2].

The study failed to detect resistant strains of either *Trichostrongylus* sp. or *Oesophagostomum* sp. Elsewhere, resistant strains of these genera have been reported in sheep[12,19]. It is possible that resistant populations of the 2 genera were not detected in these flocks because they were either absent or were only present in low numbers during the time of sampling. Caution must be exercised in interpreting the results from larval cultures, because highly fecund nematode species (especially *H. contortus*) may mask species with low fecundity[18], and species that have low mortality in culture may be overestimated relative to those that suffer high mortality in culture[10]. Reinecke *et al.*[15] described a 1st-stage larval reduction test as an interesting alternative to the faecal egg count reduction assay. In this case 1st-stage larval populations (numbers and generic compositions) that developed from eggs recovered from treated animals are compared to those from non-treated animals. The main advantage of this assay is that it is more sensitive in detecting minor contributors to populations.

Anthelmintic resistance in the flocks tested suggests the need to redress the worm control programme for sheep in Zimbabwe. The recommended dosing programme of drenching every 3–4 weeks during the rainy season and every 6 weeks during the dry season might be contributing to the increase in the frequency of genes that confer resistance to benzimidazoles and levamisole.

There was no evidence of selection for resistance to ivermectin as it is rarely used (1 of 10 farms surveyed used ivermectin). However, there is a threat of ivermectin resistance emerging once it is used intensively. Recently, resistance to ivermectin has been reported in South Africa[16] and South America[5].

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