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MULTIPLE AND MULTIGENERIC ANTHELMINTIC RESISTANCE ON A SHEEP FARM IN KENYA

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ABSTRACT

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The anthelmintic efficacy of benzimidazoles (albendazole, fenbendazole and oxfendazole), levamisole, oral ivermectin and closantel was evaluated on a farm in Kenya using faecal egg count reduction test, larval cultures and a controlled slaughter trial. The results of this study indicated simultaneous resistance of *Haemonchus contortus* against benzimidazoles, levamisole and ivermectin, and of *Trichostrongylus colubriformis* and *Oesophagostomum* spp. against levamisole on the same farm. Ivermectin resistance developed to 47% within 15 months of first use. Closantel was effective against the benzimidazoles, levamisole and ivermectin resistant *H. contortus.*

INTRODUCTION

In recent times there have been increasing reports from all parts of the world of anthelmintic resistance to trichostrongyle nematodes in small ruminants. Resistance to all groups of anthelmintics has been described, including ivermectin (Van Wyk and Malan, 1988; Sivaraj *et al.*, 1994). Recently, a survey on 42 sheep and goat farms in Kenya indicated that levamisole and benzimidazole resistance was present in 38% and 33% of the premises (Wanyangu *et al.*, 1996). The main species involved in drug resistance appeared to be *Haemonchus contortus.* An isolate of this species resistant to ivermectin and closantel has also been described in Kenya (Mwamachi *et al.*, 1995).

In this country institutional farms, that supply breeding stock to other farms, rely heavily on anthelmintics to control nematode infections because of the intensive grazing management. Under such conditions there is a high risk of developing resistant strains and of spreading these strains to other farms by the movement of stock. Following widespread mortality due to strongylosis on a commercial farm in spite of continued routine drenching during 1995, a study was undertaken to determine the anthelmintic resistance status on that farm.

MATERIALS AND METHODS

Study site and background information

The study was carried out on a commercial farm about 40 km to the north-west of Nairobi. The sheep ($n = 300$), consisting mainly of Dorper sheep, were allowed to graze on natural pastures for about 7 h per day, and were housed at night in pens with concrete floors. The animals had free access to water and minerals.

In order to control gastrointestinal nematode infections, which were the main cause of mortality on the farm in all age classes, anthelmintic treatments were carried out routinely. Thiabendazole, fenbendazole, albendazole, levamisole and rafoxanide had been used over the past five years. Deworming had been done using anthelmintics singly (benzimidazoles or levamisole) or in combination, i.e. benzimidazole plus levamisole or benzimidazole plus rafoxanide. Benzimidazole–levamisole combinations were replaced by oral ivermectin from January 1995.

Faecal egg count reduction test

Faecal egg count reduction test (FECRT) was done in April 1996 on sheep that had not been drenched during the 4 weeks prior to the trial. Seventy animals of mixed sexes and aged between 9 and 18 months were randomly allocated into 7 treatment groups, and on day zero (0) all the animals were weighed and sampled for faecal egg counts (FEC). Animals in groups 1–6 were then treated respectively with oral ivermectin (Oramec, MSD Agvet, Rahway, NJ, USA, 0.2 mg/kg body weight), levamisole (Wormicid, Cosmos, Kenya, 7.5 mg/kg body weight), albendazole (Valbazen, Smith Kline, 5 mg/kg body weight), oxfendazole (Systamex, MSD Agvet, 5.0 mg/kg body weight), fenbendazole (Panacur, Hoechst, Munich, Germany) and closantel (Flukiver, Janssen Pharmaceutica, Belgium, 5 mg/kg body weight), according to the manufacturer's recommendations. All animals in a given group received a constant dose based on the heaviest animal in the group; average weight of sheep was 18.5 kg (range 17.3–20.1 kg). Animals in group 7 were the untreated controls.

Faeces were collected from the rectum of each animal immediately prior to treatment (day 0) and again 14 days later. Faecal egg counts were performed using a modified McMaster technique (Anonymous, 1986).

Post-treatment faecal samples were pooled by treatment and cultured for 10–14 days, and the larvae recovered were compared with those recovered from a culture of the untreated control group and changes in species composition noted.

Slaughter trials

A controlled slaughter trial was conducted in July 1996. Forty-nine lambs of mixed sexes and aged between 7 and 9 months were reared in pens with concrete floors and fed a ration of lucerne hay and concentrates. Water and minerals were provided *ad*

libitum. The animals were parasitologically negative on faecal examination prior to experimental infection. Each lamb was infected orally with 5000 freshly harvested third stage infective larvae (L_3) of mixed gastrointestinal nematodes obtained from faecal cultures of faeces from untreated farm animals. Twenty-one days later eggs per gram of faeces (epg) of each animal were counted using a modified McMaster technique (Anonymous, 1986). Twenty-four days post-infection the sheep were randomly placed into 7 groups of 7 animals according to their body weight, identified by ear tags and dosed with anthelmintics (Table II). All animals were killed 10 days post-treatment (day 34 post-infection) and the worms present in the abomasum, small and large intestines were recovered, identified and counted (Anonymous, 1986).

Analysis

Percentage reduction (PR) was determined using arithmetic group means for FECs following treatment according to the recommendations given by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al*., 1992). It is calculated by the equation:

$$PR\% = 100\ (1–XT/XC)$$

where XT and XC are arithmetic means (day 14) for the treated and control groups respectively, following treatment. Resistance is present if the PR in egg count is less than 95% and the lower 95% confidence limit is less than 90%. If either of the two criteria is met, resistance is suspected.

Worm count reduction (WR)% was calculated by the equation:

$$WR\% = (C–T)/C \times 100$$

where C and T are geometric mean worm counts of control and treated groups at slaughter. WR% is defined as the difference between the geometric mean worm counts in the control and treated groups expressed as a percentage of the geometric mean worm counts in the control group (Presidente, 1985).

RESULTS

The results of the FECRT are shown in Table I. Closantel had a high efficacy (over 95%), but benzimidazoles, levamisole and ivermectin had low efficacies.

All pre-treatment faecal cultures had strongylid larvae and were composed of *H. contortus* (74%), *Trichostrongylus* spp. (17%) and *Oesophagostomum* spp. (9%). In post-treatment faecal cultures, *H. contortus* L_3 were recovered only from sheep treated with benzimidazoles and ivermectin, while *Trichostrongylus* spp. and *Oesophagostomum* spp. L_3 were recovered from animals treated with levamisole and closantel.

TABLE I
Efficacy of anthelmintics (faecal egg count reduction test) against *Haemonchus contortus*, *Trichostrongylus* and *Oesophagostomum* species in sheep[a]

	Faecal egg count reduction test										
	Pre-treatment (day 0)				Post-treatment (day 14)				95% confidence limits		
	Epg[c]	Species in cultures[d] (%)			Epg	Species in cultures (%)					
Treatment[b]		H	T	O		H	T	O	PR (%)	Upper (%)	Lower (%)
Control	1500	69	22	9	1900	74	17	9	–	–	–
Ivermectin	1500	82	12	6	900	100	0	0	52.6	79	–12
Levamisole	1280	70	21	9	750	68	20	12	60.5	82	31
Albendazole	1660	70	18	12	1116	100	0	0	41.3	72	–11
Fenbendazole	2320	66	24	10	1396	100	0	0	26.5	64	–109
Oxfendazole	1060	74	15	11	820	100	0	0	56.8	87	4
Closantel	1720	64	23	13	12	0	63	37	99.4	100	98

[a]10 animals per group; [b]all drugs given orally; [c]eggs per gram of faeces; [d]H, *Haemonchus contortus*; T, *Trichostrongylus* spp.; O, *Oesophagostomum* spp.

The results of the controlled slaughter trial are shown in Table II. Four species were recovered in the trial: *H. contortus*, *T. colubriformis*, *Oesophagostomum columbianum* and *Oesophagostomum venulosum*. A low efficacy of levamisole against all worm species was found, as well as low efficacies of benzimidazoles and ivermectin against *H. contortus*. Only closantel was active against *H. contortus*.

DISCUSSION

The results of this study indicate simultaneous resistance of *H. contortus* against benzimidazoles, levamisole and ivermectin and of *T. colubriformis* and *Oesophagostomum* spp. against levamisole on the same farm. In Kenya, this appears to be the first report of resistance of *Oesophagostomum* spp. to anthelmintics. Resistance to benzimidazoles and levamisole has previously been detected and confirmed in *Haemonchus* and *Trichostrongylus* species in sheep in Kenya (Maingi, 1991), and using generic (L_3 in faecal cultures) and specific richness (adult worms), a benzimidazole resistant strain of *O. venulosum* has recently been reported on a goat farm (Cabaret *et al.*, 1995). Resistance of *H. contortus* against oral ivermectin was not expected as the drug had only been introduced into the farm in 1995. The repeated intensive use of ivermectin coupled with failure to detect early development of resistance are characteristics of ivermectin resistance in the field (Shoop, 1993). In this study, resistance had developed in the flock following routine ivermectin treatment every 3 to 4 weeks during a 15 month period.

Indiscriminate use of benzimidazoles, levamisole and ivermectin on the farm had probably led to the selection of resistant *H. contortus*. Even after introduction of ivermectin, once resistance is present, counter selection with an alternative drug may not re-establish susceptibility to the original drug (Martin *et al.*, 1988). If reversion to susceptibility occurs, it may take several years to do so (Miller and Baker, 1980). The ineffectiveness of ivermectin against benzimidazole and levamisole resistant *H. contortus* population was consistent with earlier reports (McKellar and Marriner, 1987; Craig and Miller, 1990). Recently, similar results were obtained in Kenya by Mwamachi *et al.* (1995).

Closantel was the only drug used in this study that was effective against *H. contortus* as was reported earlier (Hall *et al.*, 1981; Waruiru *et al.*, 1996); thus, the problem of multiple resistant *H. contortus* may be temporarily overcome by its use. Moreover, the prolonged activity of closantel (Hall *et al.*, 1981), which could prevent the establishment of incoming larvae, could additionally reduce the rate of reinfection and pasture contamination and increase the intervals between treatments (Dash, 1986). In order to maintain the potential of the drug for as long as possible as an effective weapon against *H. contortus*, it is recommended that closantel be used, where relevant, three times per year. At other times, and against nematode species other than *H. contortus*, sheep should be treated with a broad spectrum anthelmintic (Vassilev, 1995).

In view of the high level of resistance to benzimidazoles, levamisole and the danger of development of resistance to other groups of anthelmintics, rational use of these drugs is necessary to preserve the life of currently available anthelmintics. Several

TABLE II
Efficacy of anthelmintics (controlled slaughter trials) against *H. contortus*, *T. colubriformis* and *Oesophagostomum* spp. in sheep[a]

Treatment[b] (mg/kg^{-1})	*H. contortus*		*T. colubriformis*		*Oesophagostomum* sp.[c]	
	Mean worm count (range)	WR (%)	Mean worm count (range)	WR (%)	Mean worm count (range)	WR (%)
Control	1379 (290–3050)	–	452 (80–1120)	–	263 (4–414)	–
Ivermectin (0.2)	1044 (813–1265)	24.9	0	100	3 (0–16)	98.9
Levamisole (7.5)	648 (40–1200)	53.0	291 (0–850)	35.6	131 (60–270)	50.2
Albendazole (5.0)	741 (210–2180)	46.3	0	100	0	100
Fenbendazole (5.0)	831 (381–2046)	39.7	5 (0–87)	98.9	0	100
Oxfendazole (5.0)	776 (150–1450)	43.7	8 (0–110)	98.2	1 (0–10)	98.9
Closantel (5.0)	0	100	320 (40–840)	–[d]	156 (31–272)	–[d]

[a]7 animals per group; [b]all drugs given orally; [c]*Oesophagostomum columbianum* and *O. venulosum*; [d]Closantel is ineffective against non-blood sucking nematodes

strategies for reducing the development and spread of anthelmintic resistant nematodes (Coles and Rouch, 1992) and for the control of resistance (Jackson, 1993) have been advocated, which can be used in different ecological situations. Other strategies of worm control, such as breeding of sheep for resistance to nematodes (Baker, 1995) and/or biological control of free-living stages of trichostrongylid nematodes of ruminants by using nematode-trapping microfungi (Githigia *et al.*, 1997), merit serious consideration.

The finding of resistance against three anthelmintic classes on the same farm is a matter of serious concern. It should serve as a timely warning of what is to be expected on other farms if no responsible and effective rotational programmes are formulated to conserve the efficacy of the limited number of anthelmintics with different modes of action. This report also illustrates the danger that exists in the sale and/or movement of stock from large commercial or institutional farms to smaller farms without evaluation of the efficacy of anthelmintic treatments. Improved awareness amongst managers of these large farms of the problem of anthelmintic resistance in their animals might be beneficial in minimizing the spread of resistance into the smallholder sector (Wanyangu *et al.*, 1996).

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Résistance aux antihelminthes chez des moutons au Kenya

Résumé – L'efficacité des benzimidazoles (albendazole, fenbendazole, et oxfendazole) du levamisole, de l'ivermectine orale et du closantel fut effectué dans une ferme au Kenya, après comptage des oeufs dans les faèces, la culture des larves et une étude après abattage. Les résultats de cette étude montrèrent une résistance de la part d' *Haemonchus contortus* contre les benzimidazoles, le levamisole et l'ivermectine et de *Trichostrongylus colubriformis* et *Oesophagostomum* spp. contre le levamisole. La résistance à l'ivermectine atteignit 47% après les 15 premiers mois d'utilisation. Le closantel fut même effectif contre les *H. contortus* résistants aux autres drogues.

Resistencia múltiple y multigenérica a los antihelmínticos en una granja ovina en Kenya

Resumen – Se evaluó la eficacia antihelmíntica de los benzimidazoles (albendazol, fenbendazol y oxfenbendazol), levamisol, ivermectina oral y closantel en una granja en Kenya utilizando análisis coprológicos, cultivo de larvas y sacrificios controlados. Los resultados del estudio indicaron resistencia simultánea de *Haemonchus contortus* frente a los benzimidazoles, levamisol e ivermectina, y de *Trychostrongylus colubriformis* y *Oesophagostomum* spp. frente a levamisol en la misma granja. La resistencia a la ivermectina alcanzó un 47% transcurridos 15 meses desde la primera administración. El closantel fue efectivo frente a *H. contortus* resistente a benzimidazoles, levamisol e ivermectina.