DEVELOPMENT OF ANTHELMINTIC RESISTANCE IN A CLOSED SHEEP FLOCK

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SUMMARY

Changes in anthelmintic resistance over the past 16 years is documented following the isolation of a field strain of *Haemonchus contortus* in sheep showing multiple anthelmintic resistance at Lawes in south eastern Queensland in 1979. Resistance to thiabendazole was first suspected in 1969 and continued to persist until the present time. In addition resistance to fenbendazole and oxfendazole also occurred and persisted for the entire period of the study. Resistance to levamisole ceased to exist after 3 years of withdrawal and remained highly effective even with regular challenge. Rafoxanide was moderately effective against the pre 1980 Lawes strain, but strong resistance developed after 3 years of exposure. Resistance to closantel developed after 25 exposures over a 9 year period. Ivermectin remained highly effective even after 10 applications over the past 6 years.

Keywords : Haemonchus contortus, anthelmintic resistance, sheep, parasites

INTRODUCTION

Anthelmintic resistance in Australia is widespread in nematode parasites of sheep, goats and horses (Prichard 1994). The resistance status of gastro-intestinal nematodes in Australian sheep flocks surveyed by Overend *et al.* (1994) revealed that 85% of farms had sheep infected with benzimidazole resistant nematodes, but there was no evidence of ivermectin resistance. The "Lawes" strain of *H. contortus* isolated in 1979 (Green *et al.* 1981) was resistant to all broad spectrum anthelmintics available for use in Australia at that time and was probably due to the selection pressure imposed by regular use of these agents. To minimise the effect of haemonchosis in this flock, a strategy of rotational grazing and pasture spelling combined with selective anthelmintic usage was implemented. All records of anthelmintic treatments along with a regular assessment of drug efficacy was conducted to quantify the changes in the resistance spectrum for a range of anthelmintics.

MATERIAL AND METHODS

The experimental flock consisted of approximately 600 breeding sheep of which about one half were Merinos and the remainder was Merino crossbreds. This flock was maintained in a relatively closed state with all newly introduced animals being subjected to a gastro-intestinal nematode elimination programme prior to being released on pasture. Parasite control in this flock changed from total anthelmintic reliance, where drenching occurred at approximately 3 weekly intervals during summer, to a combination of selected anthelmintic usage combined with pasture spelling using cattle and horses in rotation. The animals were drenched when mean egg counts from a randomly selected sample of 50 sheep exceeded 1000 per gram of faeces and the response was measured by post treatment reductions in egg counts. Larval cultures were regularly performed to ascertain species prevalence. To measure the change in susceptibility to anthelmintics of *H. contortus* nematodes in this flock of sheep, total worm count reduction experiments were performed from 1983 to 1995 inclusive, except for the years 1986, 1987, and 1992. Anthelmintic usage over this period is shown in Table 1. The resistance spectrum of the pre 1980 or Lawes strain was used as the basis for comparison.

Parasites

Random faecal samples were collected from 20 sheep in January - February of each year. This was to ensure a sample at that time of the year when *H. contortus* burdens were high. Faecal samples were pooled and larval cultures using standard techniques were performed. Infective larvae harvested from the cultures were maintained at 4^0 C in distilled water until used.

Sheep

For each test, weaner Merino crossbred sheep were obtained from a commercial flock on the western Darling Downs, drenched with double the manufacturer's dose of oxfendazole and held on slatted floor pens when a minimum of 2 consecutive zero egg counts were obtained 30 to 50 days post-treatment. Sheep were then regarded as worm free or carrying a negligible parasitic burden.

Table 1. Frequency of anthelmintic usage per year from 1980 to 1994

Year	80-82	83	84	85	86-88	89-90	91	92	93-94
Chemical									
Rafoxanide	6	6							
Closantel		1	3	1	3	3	3	3	2
Oxfendazole	1			2	1			3	
Levamisole		1	1	1	1		2		
Ivermectin						2	1	1	2

Parasitological techniques

Faecal egg counts were determined using a modified McMaster technique. Nematode infections were determined by washing and sieving abomasal contents, the volume of which was brought up to 1000 ml before parasites were counted in twenty, 10 ml aliquots, with zero or low counts being confirmed by examination of the whole sample.

Anthelmintics

Over the experimental period, commercial anthelmintic formulations of thiabendazole, fenbendazole, oxfendazole, levamisole, rafoxanide, closantel, ivermectin and napthalophos were administered at the manufacturer's recommended dose rates.

Experimental procedure

Sheep were each infected with 10000 infective *H. contortus* larvae which were administered by intraruminal inoculation. On day 28 post-infection, sheep were blocked on the basis of faecal egg counts and four animals each randomly allocated to each of the treatments.. Animals were slaughtered on day 35 and abomasal contents taken for total worm counts.

Statistics

Worm count data were analysed in a manner similar to that described by Campbell *et al.* (1978) by general linear model procedure using SAS (1985).

RESULTS

Anthelmintic efficacy over the 16 year experimental period is given in Table 2. To control the multiple anthelmintic resistant pre 1980 Lawes strain, rafoxanide was used extensively for 3 years after which its efficacy declined to less than 60% in 1983. The effectiveness of levamisole in the pre 1980 strain was only 45% and, after 3 years without exposure to this anthelmintic, the efficacy increased significantly to 99.8% and remained at that level even with the reintroduction of this drug in limited applications. Resistance to thiabendazole and fenbendazole persisted throughout the experimental period and although these anthelmintics were not used, cross resistance through the use of oxfendazole occurred. Closantel was first used for *H. contortus* control in 1983, and after continuous use showed the first signs of resistance in 1993 with an efficacy of 94% and with a subsequent dramatic reduction to 66% in 1994. Ivermectin was incorporated into the drenching programme in 1989 and over the past 6 years has remained an effective anthelmintic with an efficacy of greater than 99%.

DISCUSSION

Long term persistence of benzimidazole resistance demonstrated in this study has also been observed by Kelly and Hall (1979a,1979b), Webb and McCully (1979), Guinan and Kiernan (1980), Waller *et al.* (1989) and Echevarria *et al.* (1991). This would suggest that once resistance to benzimidazole anthelmintics occurs, it renders this group ineffective and unlikely to be of any value as a future control option. Although fenbendazole has never been used in this flock, cross resistance to thibendazole occurred and although efficacy improved in 1991 and continued to rise slowly over the next 3 years, this drug is still unsatisfactory for *H. contortus* control.

Table 2. The efficacy of anthelminthics aginst ***H. contortus*** in sheep. Post treatment geometric mean worm count (% reduction)

Year	Pre 1980	83	84	85	88	89	90	91	93	94	95
Chemical (Dose)											
Untreated Control	4738^{bA}	2142^{b}	1509^{c}	5270^{b}	3827^{b}	1379^{b}	3196^{b}	3462^{c}	705^{b}	1937^{c}	1030^{c}
Thibendazole	4424^{b}	2540^{b}	1847^{c}	4063^{b}	2120^{b}	1407^{b}	2723^{b}	1789^{c}	636^{b}	1480^{c}	258^{bc}
(44mg/kg)	(6.6)	(+18.6)	(+22.4)	(22.9)	(44.6)	(+2)	(14.8)	(48.3)	(9.8)	(23.6)	(75.0)
Fenbendazole	4188^{b}					749^{b}	1958^{b}	549^{b}	134^{b}	172^{b}	75^{b}
(5mg/kg)	(11.6)					(45.7)	(38.7)	(84.1)	(81)	(91.1)	(92.7)
Oxfendazole	1847^{b}	962^{b}			1703^{b}	527^{b}					451^{c}
(5mg/kg)	(61.0)	(55.1)			(55.5)	(61.8)					(56.2)
Levamisole	2540^{b}	5^{a}	25^{a}	27^{a}	30^{a}	0^{a}	1.2^{a}	10.2^{a}	0^{a}	8^{a}	2^{a}
(7.5mg/kg)	(46.4)	(99.8)	(98.3)	(99.5)	(99.2)	(100)	(99.9)	(99.7)	(100)	(99.6)	(99.8)
Rafoxanide	57^{a}	1001^{b}	239^{b}		1074^{b}	449^{b}	1163^{b}	595^{b}	300^{b}		
(7.5mg/kg)	(98.8)	(53.3)	(84.2)		(71.9)	(67.4)	(63.6)	(82.8)	(57.4)		
Closantel	59^{a}		6^{a}		2.3^{a}	0^{a}	4^{a}	3.5^{a}	42^{b}	659^{bc}	1372^{c}
(7.5mg/kg)	(98.9)		(99.6)		(99.9)	(100)	(99.9	(99.9)	(94.0)	(66.0)	(+33.2)
Ivermectin	0^{a}				0.8^{a}	0^{a}	1.2^{a}	0^{a}	0^{a}	7^{a}	2^{a}
(0.2mg/kg)	(100)				(99.9)	(100)	(99.9)	(100)	(100)	(99.6)	(99.8)
Napthalophos	72^{a}					0^{a}	0^{a}	12^{a}	0^{a}	2^{a}	2^{a}
(30mg/kg)	(98.5)					(100)	(100)	(99.7)	(100)	(99.9)	(99.8)

[A] Means within the same column with different superscript differ significantly (P<0.05).

The increased efficacy of levamisole from a low of 45% in the pre 1980 strain, to greater than 99% after only 3 years of withdrawal, is at variance with the opinion of Kelly and Hall (1979 b), and Green *et al.* (198 1), where the considered opinion was that once resistance had been established in a population, withdrawal of the defective product and its subsequent reintroduction offers no useful method of control.

In this study levamisole was reintroduced as a broad spectrum treatment and even with regular use, its efficacy did not decline in 12 years of challenge. A similar effect was observed by Waller *et al.* (1988) in Ostertagia where the conclusion was that resistant parasites have a lower fitness than non-resistant strains and reversion occurs when the particular anthelmintic is withdrawn.

Slight resistance to closantel developed after 26 applications over 10 years, but efficacy declined markedly in 1 year with only 3 subsequent applications. The speed at which resistance intensifies once established was also noted by van Wyk *et al.* (1982), where it took only 1 exposure to cause a dramatic increase in resistance.

Ivermectin when used on 10 occasions over 6 years in rotation with chemically unrelated anthelmintic compounds has remained highly effective. Its anticipated useful life is unknown as studies by van Wyk and Malan (1988), and Echevarria and Trindade (1989) indicate great variability in the rapidity with which ivermectin resistance develops.

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