Stability of ivermectin resistance in a field strain of *Ostertagia* spp

T.G. WATSON, B.C. HOSKING AND P.F. McKEE

AgResearch, Ruakura, Private Bag 3123 Hamilton, New Zealand.

ABSTRACT

Multiple anthelmintic resistance (ivermectin (IVM), morantel (MOR), oxfendazole (OXF) was first demonstrated in 1988 from a goat property in New Zealand. Total spelling of all anthelmintics on the site has not been a management option. Since detection, a strict drench rotation has been adopted - levamisole (LEV) 1988-89, IVM 1990 and combination product 1991-92. IVM has been used very strategically as animal health demanded, 2 occasions in 1988, 6 in 1990 and twice in each of 1991 and 1992.

In 1992 surveillance monitoring of anthelmintic status using lambs indicated that LEV remained highly effective (99.3%) against the resistant *Ostertagia* spp. but there was a subtle shift downward for IVM (87.5%). The alternate milbemycin, moxidectin (MOX), removed 99.9% of the *Ostertagia* spp. population.

Stability of resistance is an important issue to address when designing and evaluating management options to delay or restrict resistance and minimise the impact on farming profitability. These results suggest that anthelmintic rotation and strategic use of IVM have not had a significant effect on the field isolate over the past 5 years. Currently in the field, there appears to be a significant difference in efficacy between IVM and MOX despite their close structural similarities. It is proposed that MOX will not be used on the site but efficacies will be monitored at regular intervals.

Keywords: Ivermectin, *Ostertagia*, resistance, persistence, goats, sheep, stability, nematode.

INTRODUCTION

Anthelmintic resistance has emerged as a major threat to animal production not only in New Zealand, but also in most developed countries where modern broad-spectrum anthelmintics are used to control nematode parasites in ruminants. In this country, resistance to benzimidazoles (BZ) was first detected in the late 1970's (Vlassoff and Kettle 1979). Recently, resistance to the milbemycin family member, ivermectin (IVM), was demonstrated in *Ostertagia* spp. from goats (Watson and Hosking 1990, Badger and McKenna 1990). Revelations of multiple resistance in both cases were of grave concern. In the latter case, to BZ and in the former to members of both BZ and levamisole (LEV) families of anthelmintics.

Levels of resistance intensify as selection pressure continues, removing both homozygous and heterozygous susceptible individuals from the population. There is a single important factor that has made BZ resistance so significant, once resistance has been diagnosed in roundworms on a property it appears to remain established in the population for prolonged periods. To date there is no published evidence that withdrawl of the anthelmintic from use will render the worms appreciably more susceptible (McKenna 1990).

There is little information concerning the stability of IVM resistance in a population where parasite control and drench management necessitates its strategic and rotational use. The present study was to determine if rotational and periodic strategic use of IVM on IVM-resistant *Ostertagia* spp. had intensified resistance and investigate claims that another milbemycin, moxidectin (MOX), would be effective against IVM-resistant strains.

MATERIALS AND METHODS

Site history

Multiple anthelmintic resistance involving BZ, morantel (MOR) and IVM was demonstrated in *Ostertagia* spp. from lambs grazing the study site during 1988 (Watson and Hosking 1990). The location had been used solely for Saanen milking goat research studies for the previous 6-8 years. Since then, a Cashmere goat flock consisting of approximately 120 does grazed the site between 1988 and 1991 when it was replaced with Angora goats. Parasite management on the property has required use of IVM under strategic circumstances for animal health and anthelmintic rotation considerations. These have been identified in Table 1. In general, rotation between anthelmintic families has been adopted. LEV was used during 1988 and 1989. IVM was used on 6 occasions during 1990 and the combination anthelmintic, Leviben (ricobendazole/ levamisole, Youngs Animal Health) was used during 1991-92. All drugs were given at twice the manufacturers' dose rate.

Experimental Procedures

During December 1991 and January 1992, 40 lambs free of nematodes and uniquely identified by ear tags were grazed across the contaminated site. When monitor mean faecal egg counts (FEC) reached approximately 1500 epg all stock were transferred indoors and penned on wire mesh to preclude re-infection.

After a 5 week conditioning period indoors, lambs were randomised into 4 similar anthelmintic treatment groups on the basis of liveweight and FEC. One group was given a water placebo as the untreated control; the second was given IVM

TABLE 1: Ivermectin use 1988-1992

Date	Stock Class
1988	
Feb 25	Does
Mar 11	Kids
1990	
Jan 01	Does
May 02	Kids
Jun 22	Does, Kids, Bucks
Jul 05	Does
Aug 03	Does, Kids
Dec 02	Does, Kids
1991	
Mar 04	Does, Kids, Bucks
Sep 18	Bucks
1992	
Jan 01	Kids
Apr 02	Does, Kids

(Ivomec oral formulation, 200mcg kg^{-1}, MSD; the third was given MOX (Cydectin oral formulation, 200mcg kg^{-1}, Cyanamid) and the final was given LEV (Nilverm oral formulation, 8.0mg kg^{-1}, Coopers). All products were given to individual animal liveweight by disposable syringe.

All stock were faecal sampled 2 days before and 7, 10, 15 and 18 days after treatments were administered. Lambs were killed humanely at the Ruakura abattoir 24 days after anthelmintics were given. Abomasa and small intestines were recovered to assess total worm burdens. Large intestines were not processed as pre-drench faecal larval cultures suggested extremely low worm numbers.

Drug Efficacy Assessments

Faecal egg count reduction testing (FECRT) was used to evaluate drug efficacies 10 days after administration of anthelmintics. FEC estimations were made by the modified McMaster technique to an accuracy of 34 epg. Bulked faecal larval cultures were prepared prior to drenching and for each treatment group 10 days after anthelmintics were administered. Worm burdens were assessed according to procedures outlined by Robertson and Elliott (1966) to an accuracy of 1/50 worms. Efficacies were determined based on untreated control burdens.

The data were transformed [$\log_{10}(X+1)$] to normalise distributions before statistical analysis. Least squares means analysis of variance were used to detect differences between treatment groups (SAS 1985). Drug efficacies were calculated from geometric means using the formula:

Efficacy (%) = ((Control - Treatment Group)/Control) x 100

RESULTS

FEC were reduced significantly after IVM or LEV treatments were given (Table 2). No FEC was demonstrated in lambs given MOX. Egg count reductions at Day10 were 99.4% and 95.0% for IVM and LEV groups, respectively.

Faecal cultures before treatment indicated that the predominant species were abomasal and small intestinal species, *Ostertagia* spp. (36%) and *Trichostrongylus* spp. (62%). The remaining larvae (2%) were large intestinal species made up of the *Chabertia/Oesophagostomum* complex. Post-treatment cultures demonstrated 100% *Ostertagia* spp. after IVM was given. No larvae were recovered from post-LEV or post-MOX cultures.

TABLE 2: Faecal egg count reduction tests

Treatment Group	Mean Faecal Egg Count Day-2	Day+10	Reduction(%)
Control	1125	2558	-
Ivermectin	1549	9	99.4
Levamisole	3027	151	95.0
Moxidectin	738	0	100.0

Slaughter worm burden data are reported in Table 3. Untreated lambs were heavily infected with *Ostertagia* spp. and *Trichostrongylus* spp. (predominantly *O. circumcincta* and *T. colubriformis*). Low numbers of *T. axei* and *Haemonchus contortus* were also present.

Although IVM removed 99.3% of *T. axei* it was only 87.5% effective against *Ostertagia* spp. (Table 3). LEV eliminated 99.3% of *Ostertagia* spp. but considerable numbers of *Trichostrongylus* spp. were recovered from lambs given this anthelmintic. Efficacies ranged between 91.6% and 99.3% for the intestinal and abomasal species, respectively. Small numbers of *Ostertagia* spp. and *Trichostrongylus* spp. remained following treatment with MOX with the lowest efficacy recorded for *T. axei* (99.3%). All 3 drugs were completely effective against *H. contortus*.

TABLE 3: Back-transformed geometric mean worm burdens and anthelmintic efficacies following anthelmintic application

Parasite Species	Treatment Group	Total Worms	Efficacy (%)
Ostertagia spp.			
	Control	4395	-
	Ivermectin	548	87.5
	Levamisole	31	99.3
	Moxidectin	2	99.9
Trichostrongylus spp.			
(Small Intestinal)	Control	11169	-
	Ivermectin	2	100.0
	Levamisole	938	91.6
	Moxidectin	5	100.0
T. axei			
	Control	281	-
	Ivermectin	2	99.3
	Levamisole	2	99.3
	Moxidectin	2	99.3
H. contortus			
	Control	59	-
	Ivermectin	0	100
	Levamisole	0	100
	Moxidectin	0	100

DISCUSSION

The present study has re-confirmed IVM resistance in stock grazing the study site. However, the level of IVM efficacy in the field population has not declined significantly.

IVM removed approximately 87.5% of *Ostertagia* spp. from lambs during experimental testing conducted in 1992.

Despite recovery of very low numbers of *H. contortus* there is good evidence that all 3 drugs used in the present study will continue to afford effective control. It is highly likely that this particular strain is BZ-resistant (Watson, unpublished).

Because of low worm numbers the results for *T. axei* should be regarded with caution. It is noteworthy that efficacies for IVM, LEV and MOX are identical and this in itself may indicate the possibility of an emerging problem. Alternatively, the findings may simply reflect natural reduced drug effectiveness. The data do suggest a need to isolate the strain for further intensive controlled investigations, particularly, in light of the possibility of emerging multiple resistance that has been demonstrated with *T. colubriformis* on the site.

IVM resistance by *Ostertagia* spp. was demonstrated on the trial site in 1988. At that time, the drug only removed 93.1% (arithmetic mean) of worms from lambs grazed across the property (Watson and Hosking 1990). This isolate exhibited resistance to oxfendazole (43.8%) and morantel (79.6%) in the same study. At that time, FECRT did not reveal the IVM resistance. Reduction testing conducted 8 days after the drug was given suggested all lambs had ceased shedding nematode eggs. This was not the case with OXF and MOR.

Since IVM resistance was confirmed on the site in 1988 a LEV or LEV/BZ combination has been used as the drug of choice during 1988-89 and 1991-92. IVM has been used strategically on welfare grounds twice annually during that time. The combination product was spelled in 1990 when IVM was used on 6 occasions. Despite application of LEV in the combination it remains highly effective against *Ostertagia* spp. in sheep even though resistance to its close associate, MOR was observed in 1988 (Watson and Hosking 1990). Evidence of MOR-resistance in *Trichostrongylus* spp. was demonstrated in 1988 when only 89.7% efficacy was recorded (Watson and Hosking 1990). Side resistance to LEV has now been shown. Elsewhere, studies have shown that side resistance between LEV and MOR may be related to dose-dependent selection pressure (Sangster *et al.* 1988; Martin and McKenzie 1990a). Resistance to MOR may be independent of development of resistance to LEV but LEV resistance always shows MOR resistance.

The parasite population including *Ostertagia* spp. currently under investigation appears to be largely controlled by IVM. The current data suggest that selection for resistance has been minimised and/or that susceptible individuals continue to dominate in the population. Most researchers accept that if reversion toward susceptibility were to occur it would do so extremely slowly (Martin and McKenzie 1990b; McKenna 1990). Reversion is unlikely where resistance is controlled by a single gene. Generally, polygenicity has been demonstrated for BZ resistant strains involving various parasitic nematode genera (Martin *et al.*1988). It remains to be shown what genetic factors govern IVM resistance.

ACKNOWLEDGEMENTS

The authors wish to note with appeciation the technical assistance of D Brears, C Cooke, B Mather and G Mather.

REFERENCES

Badger, S.B.; McKenna, P.B. 1990. Resistance to ivermectin in a field strain of *Ostertagia* spp. in goats. *New Zealand Veterinary Journal* **38**:72-74.

Martin, P.J.; McKenzie, J.A. 1990a. Levamisole resistance in *Trichostrongylus colubriformis*: a sex-linked recessive character. *International Journal for Parasitology* **20**:867-872.

Martin, P.J.; McKenzie, J.A. 1990b. Genetics and population biology of resistance. Implications for resistance management. Ed. J.A. McKenzie, P.J. Martin and J.H. Arundel. *In: Resistance Management in Parasites of Sheep*. Australian Wool Corporation, Parkville,VIC 3052. pp 3-15.

Martin, P.J.; Anderson, N.; Brown, T.H.; Miller, D.W. 1988. Changes in resistance of *Ostertagia* spp. to thiabendazole following natural selection of treatment with levamisole. *International Journal for Parasitology* **18**:333-340.

McKenna, P.B. 1990. The use of benzimidazole-levamisole mixtures for the control and prevention of anthelmintic resistance in sheep nematodes: an assessment of their likely effects. *New Zealand Veterinary Journal* **38**:45-49.

Robertson, T.G.; Elliott, D.C. 1966. The laboratory assessment of worm parasite populations in sheep. *New Zealand Journal of Agricultural Research* **9**:350-358.

Sangster, N.C.; Riley, F.L.; Collins, G.H. 1988. Investigation of the mechanism of levamisole resistance in trichostrongylid nematodes of sheep. *International Journal for Parasitology* **18**:813-818.

SAS 1985. SAS language guide for personal computers. Version 6 Edition. SAS Institute Inc., Cary, NC.

Vlassoff, A.; Kettle, P.R. 1979. Benzimidazole resistance in *Haemonchus contortus*. *New Zealand Veterinary Journal* **27**:23-24.

Watson, T.G.; Hosking, B.C. 1990. Evidence for multiple anthelmintic resistance in two nematode genera on a Saanen goat dairy. *New Zealand Veterinary Journal* **38**:50-53.