

# Differences in efficacy of monepantel, derquantel and abamectin against multi-resistant nematodes of sheep

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**Abstract** Drug resistance has become a global phenomenon in gastrointestinal nematodes of sheep, particularly resistance to macrocyclic lactones. New anthelmintics are urgently needed for both the control of infections with multi-resistant nematodes in areas where classical anthelmintics are no longer effective, and the prevention of the spread of resistance in areas where the problem is not as severe. Recently, two new active ingredients became commercially available for the treatment of nematode infections in sheep, monepantel (Zolvix®) and derquantel, the latter used only in a formulated combination with the macrocyclic lactone, abamectin (Startect®). In order to assess the potential of the new actives for the control and prevention of spread of anthelmintic resistance, two characterized multi-resistant field isolates from Australia were used in a GLP (good laboratory practice) conducted efficacy study in sheep. Eight infected sheep in each group were treated orally according to the product labels with 2.5 mg/kg body weight monepantel, 0.2 mg/kg abamectin, or with the combination of 2.0 mg/kg derquantel and 0.2 mg/kg abamectin. The results demonstrate that mon-

epantel was fully effective against multi-resistant species, *Trichostrongylus colubriformis* and *Haemonchus contortus* (99.9%). In contrast, the combination of derquantel and abamectin was effective against *T. colubriformis* (99.9%), but was not effective against larval stages of the barber's pole worm *H. contortus* (18.3%).

## Introduction

Drug resistance in gastrointestinal nematodes is a severe global problem for sheep farmers, and in particular resistance of pathogenic nematodes to the macrocyclic lactones (MLs); Besier and Love 2003; Yue et al. 2003; Sargison et al. 2005; Wrigley et al. 2006; Blake and Coles 2007. The MLs were introduced into the market in the early 1980s as the third broad-spectrum anthelmintic class. However, resistance of nematodes to MLs is now reported frequently from all major sheep farming countries (Besier 2007). The advent of new compounds with anthelmintic activity was urgently needed for both the control of gastrointestinal nematodes resistant to the classical broad-spectrum drugs, and to help delay the development of drug resistance in regions where this phenomenon is not yet a major problem.

Monepantel is the first novel anthelmintic compound for sheep from a new chemical class (Kaminsky et al. 2008a, b) and was introduced to the market as Zolvix® (Novartis Animal Health Inc.) in 2009. The second anthelmintic compound from another class is derquantel, which was introduced into the market in 2010 as Startect® (Pfizer Animal Health), a combination of derquantel and abamectin, a long-known ML. While the spectrum and efficacy of monepantel has been well documented (Hosking et al. 2008, 2009a, b, 2010; Kaminsky et al. 2009; Mason et al.

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2009; Sager et al. 2009, 2010; Jones et al. 2010), there is little information published on the spectrum of efficacy of derquantel (Little et al. 2010). Of particular interest is the efficacy of these new compounds against ML-resistant nematodes. The objective of the present study was to determine the efficacy of monepantel and derquantel against ML-resistant nematodes in order to evaluate their potential in the control of such parasites and in delaying the development of drug resistance in sheep nematodes.

## Materials and methods

Thirty-two ruminating, gastrointestinal nematode-free sheep (as assessed by fecal egg counts) were used in the study. Two recent field isolates of sheep nematodes from Australia were used for experimental infection of the study animals: *Haemonchus contortus* (Haecon-51) was isolated in June 2008 and *Trichostrongylus colubriformis* (Tricol-56) in January 2009. Both isolates were shown in vivo to be resistant to benzimidazoles, levamisole, and MLs. All animals were infected intraruminally with 6,000 third-stage larvae of Tricol-56 and 3,000 third-stage larvae of Haecon-51 5 days prior to treatment. The nematode infections were, therefore, at the fourth-larval stage at treatment. Eight animals were treated orally with monepantel in a 2.5% (w/v) formulation (Novartis Animal Health Inc., CH-4002 Basel, Switzerland) at the minimum recommended dose of 2.5 mg/kg. A second group of eight sheep received abamectin (oral formulation 0.08% (w/v); Ancare Ireland Limited, Dublin, Ireland) at the recommended minimum dose of 0.2 mg/kg. A third group ( $n=8$ ) was treated with a commercially formulated oral combination of derquantel and abamectin (1% (w/v) derquantel and 0.1% (w/v) abamectin; Pfizer New Zealand Limited, Auckland, New Zealand) at the minimum recommended dose of 2 mg/kg and 0.2 mg/kg, respectively. Eight animals were left untreated and served as controls.

Twenty-four days after infection, fecal samples were collected from all animals and worm egg counts performed as described by Kaufmann (1996). On the two subsequent days, the animals were euthanized and worm counts were completed on the abomasum and small intestine contents as described in Hosking et al. (2008). In order to assess efficacy, the arithmetic and geometric mean counts from each group were used in Abbott's formula (Abbott 1925). Statistical significance was evaluated with a two-tailed Mann–Whitney  $U$  test (SAS® procedure *npar1way*, SAS 9.2 Help and Documentation, SAS Institute Inc. Cary, NC, SAS Institute Inc., 2002–2009) on fecal egg and worm counts.

The study was performed blinded according to the VICH guidelines 7 and 13 (anonymous, 1999, 2000; Vercruysse et

**Table 1** Fecal egg counts (FEC)

	FEC			FEC-Efficacy (%)	
	AMean	SEM ( $\pm$ )	GMean	AMean	GMean
Control	2944	528	2390		
MPL	0	0	0	100	100
ABA	1356	262	1067	53.9	55.3
DER+ABA	1225	322	430	58.4	82.0

AMean arithmetic mean; GMean geometric mean; SEM standard error of the mean; MPL monepantel; ABA abamectin; DER derquantel

al. 2001) under the principles of good laboratory practice (GLP) (anonymous, 2005).

## Results

The results of the fecal egg count reductions are summarized in Table 1. The average reduction of egg counts was 54% in the abamectin-treated group and 58% in the derquantel/abamectin combination group. Reduction in the monepantel-treated group was 100%, and this differed significantly from the control and other treated groups (Table 2).

The reduction of worm burdens after treatment of the fourth-stage larval nematodes is summarized in Table 3. Monepantel had an efficacy of 99.9% against both *H. contortus* and *T. colubriformis*. The combination treatment of derquantel and abamectin had an efficacy of 18.3% against *H. contortus* and 99.9% against *T. colubriformis*. Similarly, abamectin alone had an efficacy of 15.3% against *H. contortus* and 90.8% against *T. colubriformis*. While the worm count reduction for *H. contortus* in the monepantel-treated group was highly significant to all other groups (Table 4), there was no significant difference between the monepantel- and the derquantel/abamectin-treated groups for *T. colubriformis*. Both groups had highly significant worm count reductions compared to the control and abamectin-treated groups (Table 5).

**Table 2** Mann–Whitney  $U$  test ( $P$  values, FEC)

Vs.	Control	MPL	ABA	DER+ABA
Control	–	0.0004	0.0207	0.0156
MPL	–	–	0.0004	0.0015
ABA	–	–	–	0.7524
DER+ABA	–	–	–	–

MPL monepantel; ABA abamectin; DER derquantel

Significance is considered for  $P$  values  $\leq 0.05$ , high significance if  $P \leq 0.01$

**Table 3** Worm counts and calculated efficacy (%)

	<i>Haemonchus contortus</i>		<i>Trichostrongylus colubriformis</i>	
	GMean	AMean	GMean	AMean
Control (worm counts $\pm$ SEM)	2316 (–)	2349 (140)	3369 (–)	3401 (178)
MPL (efficacy)	99.98	99.8	99.9	99.8
ABA (efficacy)	15.3	15.0	90.8	89.6
DER+ABA (efficacy)	18.3	17.1	99.97	99.93

AMean arithmetic mean; GMean geometric mean; SEM standard error of the mean; MPL monepantel; ABA abamectin; DER derquantel

## Discussion

The objective of this study was to determine the efficacy of the recently developed anthelmintics, monepantel, and derquantel against ML-resistant nematodes. The results of this study on the efficacy of monepantel confirm those of previous studies (summarized in Hosking et al. 2010). Due to its unique mode of action (Kaminsky et al. 2008a; Rufener et al. 2009), monepantel is highly efficacious against gastrointestinal nematodes, including those resistant to MLs. The efficacy of 99.9% against the ML-resistant *H. contortus* isolate is in agreement with 100% efficacy observed against two well-characterized resistant *H. contortus* isolates (Kaminsky et al. 2008b), as well as with the >99% pooled efficacy data, including ML-resistant nematodes from controlled laboratory and field studies (summarized in Hosking et al. 2010). The efficacy of monepantel in these studies was independent of the life stage of the nematode, and was observed against the adult and larval stages, as was confirmed in the present study.

The results of the presented study on the efficacy of the combination treatment of derquantel and abamectin show that this combination has no efficacy against fourth-stage larvae of the ML-resistant *H. contortus* isolate used. The efficacy of abamectin alone was 15.3%, and 18.3% when used in combination with derquantel (Table 3). Underlying worm counts for both groups did not differ significantly ( $p=1.000$ ; Table 4). In the study published by Little et al. (2010), the combination was efficacious against four *Haemonchus* isolates (as determined by fecal egg count reduction);

however, all of these *Haemonchus* isolates were also sensitive to the ML ivermectin. It remains undetermined if the observed efficacy of the combination was due to derquantel or due to the ML abamectin. Unfortunately, the contribution of each active in the combination to efficacy was not determined in this study.

The efficacy of abamectin alone or in combination with derquantel against *T. colubriformis* differed. Abamectin had an efficacy of 90.8%, and the combination treatment resulted in 99.9% efficacy. The ML-resistance in the Tricol-56 isolate is border-line, just below the 95% definition level described by Coles et al. (1992). Thus, derquantel appears to have efficacy against fourth larval stages of *T. colubriformis* with a minor degree of ML-resistance, but clearly has no efficacy against fourth-stage ML-resistant *H. contortus*.

The results presented herein indicate that the combination of derquantel with abamectin has potential to control *T. colubriformis*, which cannot be fully eliminated with abamectin alone. However, the combination of derquantel with abamectin will not be able to control all infections with ML-resistant *H. contortus*.

Furthermore, this combination may also not be effective in delaying the development of drug-resistance if *H. contortus* is present. This is particularly indicated by the reduction of fecal egg counts (Table 1). There were a high number of eggs excreted from nematodes in sheep treated with the combination of derquantel and abamectin, which developed from larval stages to mature egg-laying adults. This poses a risk that resistant alleles from nematodes, which were not cured by the combination treatment, will be

**Table 4** Mann–Whitney *U* test (*P* values, *H. contortus*)

Vs.	Control	MPL	ABA	DER+ABA
Control	–	0.0006	0.1272	0.0831
MPL	–	–	0.0006	0.0006
ABA	–	–	–	1.0000
DER+ABA	–	–	–	–

MPL monepantel; ABA abamectin; DER derquantel

Significance is considered for *P* values  $\leq 0.05$ , high significance if  $P \leq 0.01$

**Table 5** Mann–Whitney *U* test (*P* values, *T. colubriformis*)

Vs.	Control	MPL	ABA	DER+ABA
Control	–	0.0008	0.0009	0.0007
MPL	–	–	0.0008	0.2016
ABA	–	–	–	0.0007
DER+ABA	–	–	–	–

MPL monepantel; ABA abamectin; DER derquantel

Significance is considered for *P* values  $\leq 0.05$ , high significance if  $P \leq 0.01$

selected and ultimately contaminate the pasture, and thus, increase the resistant worm population.

Emodepside, another anthelmintic with a new mode of action, has been shown to be effective against drug-resistant nematodes, including ML-resistant *H. contortus* (von Samson-Himmelstjerna et al. 2005) and would have the potential to be used in resistance control programs. Unfortunately, emodepside is not available for nematode control in sheep yet.

The results presented herein show that monepantel can be used to control ML-resistant *H. contortus* and *T. colubriformis* infections. Furthermore, the reduction of worm egg counts to zero (Table 1) demonstrates that monepantel is a suitable compound in resistance management programs to delay the onset of development of drug resistance. Monepantel has been reported to have efficacy not only against *H. contortus* and *T. colubriformis*, but to adult and larval stages of all the key gastrointestinal nematode species (summarized in Hosking et al. 2010), and can, therefore, be used as a broad-spectrum anthelmintic in sheep infected with any of these parasites.

It remains to be investigated if the efficacy of derquantel against ML-resistant nematodes is limited to *T. colubriformis* isolates or whether this could be extended to other species. Little et al. (2010) have reported on reduced efficacy (<95%) against larval and adult *Teladorsagia* (= *Ostertagia*) *circumcincta*. Here, we report on the lack of efficacy of the combination of derquantel and abamectin against ML-resistant *H. contortus* (<20%). Further studies are required to determine if the lack of efficacy of this combination is restricted to *T. circumcincta* and fourth-stage *H. contortus* or also other nematodes. It is important for veterinarians and other animal health advisors to know the spectrum of efficacy of the available compounds for the design of anthelmintic control programs.

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