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Field efficacy and safety of an oral formulation of the novel combination anthelmintic, derquantel-abamectin, in sheep in New Zealand

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Abstract

AIM: To evaluate the efficacy and safety of the novel anthelmintic combination, derquantel-abamectin, against gastrointestinal nematode populations in sheep, under field-use conditions.

METHODS: Controlled faecal egg count reduction tests (FECRT) were conducted in New Zealand in 14 trials, covering a range of geographic locations, farming enterprises, breeds, nematode populations, and anthelmintic-resistance profiles. Enrolled animals were naturally infected with mixed populations of gastrointestinal nematodes. All trials included a group treated with derquantel-abamectin, and a negative control group. Nine trials included additional groups each treated with a single- or dual-active oral reference anthelmintic, selected from albendazole, levamisole, albendazole-levamisole, ivermectin, abamectin and moxidectin. A total of 838 animals were enrolled across all trials, and were randomly allocated to treatment groups within blocks defined by faecal nematode egg counts (FEC) pre-treatment. On Day 0 derquantel-abamectin was administered orally at 1 ml/5 kg bodyweight (2 mg/kg derquantel, 0.2 mg/kg abamectin), and each reference anthelmintic was given at the recommended label dose. Faecal samples were collected on Day 14 (± 1 day), to determine the percentage reduction in mean FEC for each anthelmintic tested. Larval differentiation was also performed post-treatment, to estimate efficacy at the genus level. Animals were weighed on or before Day 0, and on Day 14 (± 1 day) in 13 trials.

RESULTS: The efficacy of derquantel-abamectin against mixed strongyle populations was ≥99.2%, based on the percentage reduction in geometric mean FEC. *Nematodirus* sp. was present in six trials at a level sufficient for efficacy calculations to be conducted; in all cases, the efficacy of derquantel-abamectin was 100%. In those trials where the efficacy of at least one reference anthelmintic was <95% against strongyles and/or *Nematodirus* sp., derquantel-abamectin was 100% effective. In five trials, the mean gain in bodyweight was significantly greater in the derquantel-abamectin group than the negative controls.

CONCLUSIONS AND CLINICAL RELEVANCE: When administered orally at 1 ml/5 kg bodyweight, derquantel-abamectin is highly effective for the treatment of gastrointestinal nematodes in sheep, including populations of strongyles and *Nematodirus* sp. with resistance to one or more single- or dual-active anthelmintics. Derquantel-abamectin presents sheep producers with a unique opportunity to introduce a new class of anthelmintic to their nematode control programmes, with the added benefits offered by a combination anthelmintic.

KEY WORDS: *Spiroindole (SI), derquantel, abamectin, anthelmintic efficacy, sheep, gastrointestinal, nematode*

Introduction

Gastrointestinal parasitism, and emerging resistance to single-, dual- and triple-active anthelmintic products, continues to represent a major production cost to sheep farmers throughout the world. Treatment with effective anthelmintics continues to be the cornerstone of internal parasite control, when used strategically in conjunction with other nematode-management practices.

Several reviews of the status of anthelmintic resistance in small ruminants have been published (Besier and Love 2003; Kaplan 2004; Jabbar *et al*. 2006). In New Zealand, resistance in gastrointestinal nematodes to the macrocyclic lactones, as well as emerging resistance to dual- and triple-combination anthelmintics, has been reported (Wrigley *et al*. 2006; Hughes *et al*. 2007; Sutherland *et al*. 2008). A nationwide survey of sheep farms conducted in 2005 showed widespread resistance to commercially available anthelmintics (Waghorn *et al*. 2006), while Leathwick (2004) conducted an analysis that estimated the total discounted cost of anthelmintic resistance accumulated over 30 years (from 2002) would exceed NZ$1.3 billion. Similar reports of anthelmintic resistance have been published in Australia (Wooster *et al*. 2001; Love *et al*. 2003), South Africa (Van Wyk *et al*. 1989, 1999), and the United Kingdom (UK) (Yue *et al*. 2003; Bartley *et al*. 2005; Sargison *et al*. 2007). A trial conducted in the southern sheep-producing zone of Western Australia, in weaned Merinos, estimated that production losses associated with using an ineffective anthelmintic compared with a fully effective one would be >A$2/

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FEC	Faecal nematode egg count(s)
FECRT	Faecal egg count reduction test(s)
L4	Fourth-stage larvae
WAAVP	World Association for the Advancement of Veterinary Parasitology

animal in wool returns and >A$4/animal in animal value over a 12-month period (Besier *et al*. 1996). In the UK, resistance to all available broad-spectrum anthelmintics has resulted in the culling of flocks on individual farms (Sargison *et al*. 2005; Blake and Coles 2007).

It is clear there is an urgent need for new anthelmintics to be introduced for sheep, particularly in those countries where products currently available are beginning to fail (Besier 2007). From the introduction of ivermectin in the early 1980s, until the recent discovery of the amino-acetonitrile derivatives and identification of monepantel as a drug-development candidate (Ducray *et al*. 2008; Kaminsky *et al*. 2008; Mason *et al*. 2009), over a quarter of a century had elapsed before a chemical class with a new mode of action was developed for use in livestock.

The discovery of paraherquamide and its semi-synthetic derivative, derquantel (2-desoxoparaherquamide), both members of the spiroindole class of anthelmintics, has been reported previously (Shoop *et al*. 1990; Lee *et al*. 2001, 2002; Johnson *et al*. 2004). The efficacy of derquantel against gastrointestinal nematodes of sheep was assessed early in its development, and in subsequent dose-determination studies; in the dose range tested (0.5–8.0 mg/kg), derquantel was found to be a mid-spectrum anthelmintic.

Derquantel has been developed as an oral anthelmintic for sheep in combination with abamectin, to provide broad-spectrum utility, efficacy against strains of nematodes resistant to existing anthelmintics, and a means of protecting the new class from the rapid emergence of anthelmintic resistance. Combination anthelmintics have been shown to be effective against populations of gastrointestinal nematodes that have developed resistance to single-active anthelmintics, thus extending the useful life of existing classes (McKenna 1990; Anderson *et al*. 1991ab). The potential for combinations to delay or slow the development of resistance to their individual components has also been proposed (Anderson *et al*. 1988; Smith 1990; Barnes *et al*. 1995; Dobson *et al*. 2001; Leathwick *et al*. 2009).

At the dose rate selected for the combination product, derquantel alone was found to have excellent anthelmintic activity (>95% reduction in mean worm count) against adults and fourth-stage larvae (L4) of *Trichostrongylus* and *Nematodirus* spp., and the adult stage of *Haemonchus contortus*. It was less than 95% effective against *Teladorsagia* (=*Ostertagia*) *circumcincta* (adults and L4), L4 of *H. contortus*, and some large intestinal nematodes (PR Little and SJ Maeder, unpubl. data). Here, we report the field efficacy and safety of the proposed commercial formulation of derquantel-abamectin (10 mg/ml derquantel, 1 mg/ml abamectin), in a series of trials conducted on 14 farms throughout New Zealand.

Materials and methods

Experimental design

Controlled FECRT were conducted during 2006–2009 in 14 trials, to evaluate the anthelmintic efficacy of the proposed commercial formulation of derquantel-abamectin (Startect; Pfizer New Zealand Ltd, Auckland, NZ) when administered orally to sheep. They were conducted against naturally acquired, mixed infestations of gastrointestinal nematodes, on farms that were considered representative of sheep enterprises in the region, and managed as a commercial operation. Trial sites were selected to capture a range of geographic locations, climatic conditions, farming operations, breeds, sexes and ages, and to ensure representation of the economically important species of gastrointestinal nematodes. All trials were approved by either the AgResearch Grasslands Animal Ethics Committee, Palmerston North, New Zealand, or the Kaiawhina Animal Ethics Committee, Palmerston North, New Zealand.

The design of the study and technical procedures were similar across all farms, and consistent with the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for evaluating the efficacy of anthelmintics in ruminants (Wood *et al*. 1995). For each type of nematode egg (strongyle and/or *Nematodirus* sp.), efficacy of the product under investigation and each reference anthelmintic was given by the percentage reduction in geometric mean FEC compared with a negative control group. Percentage reductions in arithmetic mean FEC were also determined for completeness. In all trials, the interval between treatment (Day 0) and collection of faecal samples post-treatment was 14 (± 1) days. Pooled larval cultures were as-

Table 1. Details of individual trials conducted to evaluate the field efficacy of the novel combination anthelmintic derquantel-abamectin in sheep, with range of pre-treatment strongyle faecal nematode egg counts (FEC), target group size (n), and reference anthelmintics used.

Trial	Region	Breed	Age (months)	Sex	Weight range (kg)	FEC range (epg)	n	Reference anthelmintic
1	Manawatu	Romney	3–6	F	21.8–34.4	350–1,750	20	Nil
2	Otago	Poll Dorset x Romney	6–7	M/F	32.0–48.0	400–2,000	20	Nil
3	Southland	Coop x East Friesian	6–7	F	30.4–50.0	250–3,700	20	Nil
4	Pahiatua	Romney x Coop	7	F	27.0–38.5	300–2,000	20	Nil
5	Canterbury	Merino x Romney	9	M/F	18.5–35.5	100–2,600	20	Nil
6	Waipukurau	Romney	9–10	F	26.5–45.5	500–2,900	15	LEV, BZ-LEV, IVM
7	South Otago	Coop x Texel	2–3	M/F	19.0–44.0	300–1,400	15	BZ, BZ-LEV, IVM
8	Southland	Coop x Dorset Down	4–5	M/F	25.1–39.7	200–1,800	15	BZ, BZ-LEV, IVM
9	Rangitikei	Composite	5	F	21.0–33.2	600–2,000	15	IVM, ABA, MOX
10	Ruapehu	Finn/Romney/Suffolk	5–6	M/F	20.2–32.4	200–4,800	15	LEV, BZ-LEV, IVM
11	Ruapehu	Romney	6–7	F	41.4–53.0	100–2,700	12	BZ-LEV, IVM
12	Manawatu	Romney	8	F	30.2–43.0	750–2,500	15	BZ, LEV, BZ-LEV
13	Canterbury	Romney	10	F	31.0–45.0	200–900	13	LEV, BZ-LEV, IVM
14	Wellington	Composite	12	F	27.0–43.0	100–2,600	15	LEV, BZ-LEV, IVM

Coop = Coopworth; Finn = Finnish Landrace; F = female; M = male; LEV = levamisole; BZ = benzimidazole (albendazole); IVM = ivermectin; ABA = abamectin; MOX = moxidectin

sessed pre- and post-treatment, to identify the strongyle genera present.

A summary of the location, animal details, pre-treatment strongyle FEC, group size and reference anthelmintics used in each trial is presented in Table 1. Each was a single-site, negatively controlled efficacy trial, using a randomised block design, with the individual animal as the experimental unit. Negative control animals either received tap water as a placebo or remained untreated.

Trials 1–5 were conducted to support registration of the derquantel-abamectin combination product, and did not include reference anthelmintics; a prior history of anthelmintic resistance was not a requirement for selection of these farms. Trials 6–14 were conducted to generate additional efficacy data on sheep farms with a history of resistance to single-active and/or combination anthelmintics available commercially. The selection of reference anthelmintics for these trials was guided by the results of previous anthelmintic-resistance tests on each farm, and were selected from albendazole (Albendazole Sheep; Ancare New Zealand Ltd, Auckland, NZ), levamisole (Levicare; Ancare New Zealand Ltd), combined albendazole-levamisole (Arrest; Ancare New Zealand Ltd), ivermectin (Ivomec Liquid for Sheep and Goats; Merial New Zealand Ltd, Manukau City, NZ), abamectin (Genesis Oral Drench; Ancare New Zealand Ltd), and moxidectin (Vetdectin Oral Drench for Sheep; Fort Dodge Animal Health Ltd, Auckland, NZ).

Experimental animals

The sheep used were aged 2–12 months, weighed 18.5–53.0 kg at the time of treatment, and represented a range of breeds. A single sex (female) was used in nine trials, and mixed sex (female/castrated male) in the other five (see Table 1). A total of 838 animals were enrolled across the 14 individual trials.

On each trial site, individual mobs were screened in the weeks leading up to commencement of the trial, to determine the parasite burden and range of species present. Source flocks with a mean strongyle FEC of ≥400 epg, and with at least two nematode genera present, were required. On some farms the study animals had significantly higher nematode burdens than this, with several genera represented. Potential source flocks were only considered on the basis that they had not been treated in the previous 60 days with a persistent macrocyclic lactone, or the previous 150 days with a sustained-release anthelmintic.

Faecal samples were collected 2–5 days prior to treatment, for the determination of individual FEC, as well as for differentiation of larvae from pooled coproculture. In each trial, up to 50% more animals than the target number were sampled, to ensure that all enrolled animals had an established nematode burden. Individual animals were included on the basis of good general health and a strongyle FEC of ≥100 epg; the animals with the highest egg counts were selected for each trial. In certain trials, animals with a very high FEC were excluded on welfare grounds, due to the potential for clinical parasitism in the event that those animals were allocated to the negative control group.

Allocation to experimental groups

The selected animals were sorted and blocked by pre-treatment strongyle FEC and, when possible, *Nematodirus* sp. FEC, and randomly allocated to experimental groups within each block. As a result of this procedure, each group had a similar mean and range of FEC prior to treatment.

Administration of test and reference anthelmintics

Animals were weighed for calculation of the dose on Day 0 (the day of treatment), or up to 5 days prior. In Trials 1–5, animals were treated with either derquantel-abamectin or tap water placebo; the dose for each animal was calculated on individual bodyweight at the rate of 1 ml/5 kg (nominal dose rates of 2 mg/kg derquantel and 0.2 mg/kg abamectin). In Trials 6–14, the doses of derquantel-abamectin and each reference anthelmintic were based on the heaviest animal enrolled in the trial; in Trial 7, the animals were split into two lines in order to avoid excessive overdosing, and as such the doses in this trial were based on the heaviest animal in each line. Based on the stated concentration(s) and recommended label dose for each reference anthelmintic, the nominal (minimum) dose rate of each active drug was 4.75 mg/kg albendazole, 7.5 mg/kg levamisole, 0.2 mg/kg ivermectin, 0.2 mg/kg abamectin, and 0.2 mg/kg moxidectin. The negative control animals remained untreated in Trials 6–14. In all trials, individual doses were administered using a 10-ml or 20-ml disposable plastic syringe. Except in Trial 1, the presence or absence of coughing immediately following treatment was assessed and recorded.

Clinical observations and bodyweight

Clinical observations were performed by a veterinarian, who was blinded to allocation to treatment groups, from the commencement of treatment until at least 30 minutes after the final animal was treated. In Trials 1–5, additional clinical observations were made at 2 and 6 hours following treatment. After completion of clinical observations on Day 0, the study animals were returned to pasture and run as a single group until Day 14 (± 1 day). During this period, the animals were observed in the paddock by the farm manager on the day following treatment, then at least three times a week. The animals were weighed on the final day of the trial, except in Trial 7.

Parasitological techniques

Faecal samples were collected per rectum pre- and post-treatment, and transported to a commercial veterinary laboratory (Gribbles Veterinary Pathology, Palmerston North or Dunedin), for individual FEC and pooled larval culture. In several trials it was not possible to obtain a faecal sample on the final day of the trial from every enrolled animal; this resulted in no more than one animal from any experimental group being excluded from the efficacy calculations. FEC were performed according to standard laboratory procedures, using a modified McMaster technique, and reported as epg; pre-treatment samples were counted at a sensitivity of 1:100 epg, while post-treatment samples were counted at either 1:50 epg (eight trials) or 1:100 epg (six trials). To reduce observational bias, post-treatment faecal samples were not sorted into their respective treatment groups prior to counting, and laboratory personnel were blinded to the allocation to treatment groups. In Trial 5, a repeat FEC was performed due to a suspected mix-up or identification error of samples; this repeat count was performed 6 days after collection of the samples, using new subsamples from stored faeces, and laboratory personnel remained blinded to the allocation to treatment groups.

Once FEC were completed, faecal samples collected post-treatment were pooled by treatment group for larval culture and identification, according to standard laboratory procedures. Differentiation of larvae for strongyle genera is reported as the percentage of each genus identified. In line with standard practice, *Chabertia* and *Oesophagostomum* spp. larvae were not differentiated, thus

Table 2. Range and geometric mean (GM) strongyle faecal nematode egg counts, and percentage reductions compared with negative controls (Control), for sheep treated with derquantel-abamectin (DQL-ABA) and reference anthelmintics, 14 (± 1) days post-treatment in 14 trials.

Trial	Treatment group	Strongyle range (epg)	Strongyle GM (epg)	% Reduction GM (AM)	P-value[a]
1	Control	200–3,350	707.0	–	–
	DQL-ABA	0–50	0.5	99.9 (99.5)	<0.001
2	Control	250–2,550	1,179.8	–	–
	DQL-ABA	0–0	0	100	<0.001
3	Control	50–3,350	733.0	–	–
	DQL-ABA	0–0	0	100	<0.001
4	Control	300–7,250	1,553.4	–	–
	DQL-ABA	0–150	0.3	>99.9 (99.7)	<0.001
5	Control	0–2,250	358.2	–	–
	DQL-ABA	0–500	2.9	99.2 (93.3)[b]	<0.001
6	Control	450–4,100	1,142.0	–	–
	DQL-ABA	0–0	0	100	<0.001
	LEV	0–250	5.4	99.5 (96.7)	<0.001
	BZ-LEV	0–0	0	100	<0.001
	IVM	0–0	0	100	<0.001
7	Control	0–800	66.1	–	–
	DQL-ABA	0–0	0	100	<0.001
	BZ	0–800	4.4	93.4 (65.7)	0.004
	BZ-LEV	0–100	0.4	99.5 (97.9)	<0.001
	IVM	0–300	10.2	84.5 (70.3)	0.031
8	Control	100–3,000	1,262.5	–	–
	DQL-ABA	0–200	0.4	>99.9 (99.2)	<0.001
	BZ	0–200	0.4	>99.9 (99.2)	<0.001
	BZ-LEV	0–0	0	100	<0.001
	IVM	0–0	0	100	<0.001
9	Control	400–2,900	1,131.2	–	–
	DQL-ABA	0–0	0	100	<0.001
	IVM	0–700	14.6	98.7 (85.9)	<0.001
	ABA	0–200	8.0	99.3 (96.0)	<0.001
	MOX	0–400	7.6	99.3 (92.5)	<0.001
10	Control	50–3,600	931.7	–	–
	DQL-ABA	0–0	0	100	<0.001
	LEV	0–100	0.8	99.9 (99.3)	<0.001
	BZ-LEV	0–50	0.3	>99.9 (99.8)	<0.001
	IVM	0–400	4.8	99.5 (95.3)	<0.001
11	Control	200–950	441.2	–	–
	DQL-ABA	0–0	0	100	<0.001
	BZ-LEV	0–400	13.1	97.0 (84.2)	<0.001
	IVM	0–150	5.5	98.8 (91.2)	<0.001
12	Control	500–4,000	2,130.4	–	–
	DQL-ABA	0–0	0	100	<0.001
	BZ	100–1,100	422.3	80.2 (77.3)	<0.001
	LEV	100–1,000	394.4	81.5 (80.3)	<0.001
	BZ-LEV	0–600	53.8	97.5 (91.4)	<0.001
13	Control	0–1,000	72.0	–	–
	DQL-ABA	0–0	0	100	<0.001
	LEV	0–100	1.0	98.6 (94.3)	<0.001
	BZ-LEV	0–0	0	100	<0.001
	IVM	0–100	0.4	99.4 (97.1)	<0.001
14	Control	0–700	35.6	–	–
	DQL-ABA	0–0	0	100	<0.001
	LEV	0–100	0.5	98.6 (94.3)	<0.001
	BZ-LEV	0–500	4.7	86.9 (60.7)	0.003
	IVM	0–0	0	100	<0.001

[a] Significance of difference in GM compared with Control

[b] Following a repeat count of Day 14 samples, the percentage reductions were 99.9% (GM) and 99.2% (AM)

AM = arithmetic mean; LEV = levamisole; BZ = benzimidazole (albendazole); IVM = ivermectin; MOX = moxidectin

percentages of larvae of those genera are reported as a single combined figure.

Statistical analysis and efficacy calculations

As per WAAVP guidelines (Wood *et al*. 1995), the primary outcome measure was the percentage reduction in the geometric mean of individual FEC, compared with a negative control group, for each type of nematode egg identified, i.e. strongyle or *Nematodirus* sp. A log-transformation [ln(x+1)] was applied to the FEC data prior to analysis, and the transformed values were analysed using a GLM including the fixed effect of treatment group and the random effect of block. Geometric means were obtained using back transformation, and treatment differences were assessed at the 5% level of significance (two-tailed). Arithmetic means for each treatment group were also determined using a corresponding analysis of untransformed data.

Provided there was overall evidence of a treatment effect ($p<0.05$), each treated group was compared with the negative control group, to determine the statistical significance of treatment differences, with no further adjustments for multiple comparisons, and to estimate treatment efficacy. Statistical comparisons between the derquantel-abamectin group and the reference groups have not been presented, as the reference anthelmintics were used in these trials solely to establish or confirm the resistance profile of the nematode population present on each farm.

Geometric and arithmetic means were used to estimate efficacy for each of the treated groups (derquantel-abamectin, and each reference anthelmintic where relevant), using the following formula:

$$\% \text{ Reduction} = 100 \times \frac{\text{mean count (T01)} - \text{mean count (T0X)}}{\text{mean count (T01)}}$$

where T01 represents the negative control group, and T0X the treated group of interest

In the case of *Nematodirus* sp., efficacy calculations were not performed where the number of egg-positive animals in the negative control group at Day 14 (± 1 day) was fewer than six.

In Trials 6–14, larval differentiation figures post-treatment were used to estimate the percentage efficacy of derquantel-abamectin, as well as the reference anthelmintics, against each strongyle genus identified. The arithmetic mean count for each genus was determined by multiplying the arithmetic mean strongyle FEC by the larval culture percentage, for each experimental group. Efficacy was then calculated using the arithmetic mean genus counts using the formula above; geometric means were not calculated due to larval differentiation being based on a pooled culture rather than culture of individual faecal samples. Where the derived arithmetic mean in the negative control group was <50 epg, efficacy calculations were not considered a valid estimate of the true efficacy against that genus (McKenna 1996; Miller *et al*. 2006), and are therefore not reported.

The presence of resistance to any one of the broad-spectrum reference anthelmintics used was defined as <95% reduction in mean FEC (Presidente 1985; Coles *et al*. 2006).

For those trials where the animals were weighed twice (all except Trial 7), the change in bodyweight was analysed using a GLM, with the fixed effect of treatment group, the random effect of block, and the bodyweight pre-treatment fitted as a covariate. LSM changes in bodyweight are reported for each experimental group. Where there was overall evidence of a treatment effect ($p<0.05$), pair-wise differences significant at the 5% level are reported. For trials with more than two experimental groups, Tukey's method was used to adjust for multiple comparisons.

Statistical analyses were performed using SAS for Windows v9.1 (SAS Institute Inc, Cary NC, USA).

Results

Summaries of the FEC data post-treatment with treatment efficacies (based on geometric and arithmetic means) for strongyles are presented in Table 2. *Nematodirus* sp. was present at an adequate level to enable efficacy calculations to be conducted in six trials; these results are presented in Table 3.

Based on percentage reductions in both geometric and arithmetic mean FEC, the efficacy of derquantel-abamectin against strongyles was ≥99.2%, except in Trial 5, in which the geometric and arithmetic mean efficacies were 99.2% and 93.3%, respectively. In that trial, results from a repeat egg count indicated a geometric mean efficacy of 99.9% and an arithmetic mean efficacy 99.2%. Treatment efficacies of <95%, for at least one reference anthelmintic, were found in three trials (based on geometric means) and six trials (based on arithmetic means). In the six trials in which *Nematodirus* sp. was present at an adequate level, the efficacy of derquantel-abamectin was 100%; in three of those trials, efficacy of <95% was found for at least one reference anthelmintic.

Table 3. Range and geometric mean (GM) *Nematodirus* sp. faecal egg counts, and percentage reductions compared with negative controls (Control), for sheep treated with derquantel-abamectin (DQL-ABA) and reference anthelmintics, 14 (± 1) days post-treatment in six trials.

Trial	Treatment group	*Nematodirus* range (epg)	*Nematodirus* GM (epg)	% Reduction GM (AM)	P-value[a]
1	Control	0–300	32.4	–	–
	DQL-ABA	0–0	0	100	<0.001
2	Control	0–400	78.7	–	–
	DQL-ABA	0–0	0	100	<0.001
5	Control	0–200	3.4	–	–
	DQL-ABA	0–0	0	100	0.010
7	Control	0–700	14.6	–	–
	DQL-ABA	0–0	0	100	<0.001
	BZ	0–300	6.8	53.8 (40.8)	0.296
	BZ-LEV	0–0	0	100	<0.001
	IVM	0–0	0	100	<0.001
10	Control	0–200	5.7	–	–
	DQL-ABA	0–0	0	100	<0.001
	LEV	0–50	0.3	94.8 (92.2)	<0.001
	BZ-LEV	0–0	0	100	<0.001
	IVM	0–0	0	100	<0.001
11	Control	0–150	7.8	–	–
	DQL-ABA	0–0	0	100	<0.001
	BZ-LEV	0–150	0.5	93.3 (70.0)	0.003
	IVM	0–0	0	100	<0.001

[a] Significance of difference in GM compared with Control

AM = arithmetic mean; BZ = benzimidazole (albendazole); LEV = levamisole; IVM = ivermectin

Table 4. Percentage larvae at the genus level, based on culture of pooled faeces, arithmetic mean (AM) genus faecal nematode egg count (FEC) in the negative control group, and percentage reduction in FEC for sheep treated with derquantel-abamectin (DQL-ABA) and reference anthelmintics, 14 (± 1) days post-treatment, in eight trials.

		Negative control group		% Reduction in AM count for each anthelmintic				
Trial	Genus	Larvae (%)	AM (epg)	DQL-ABA	BZ	LEV	BZ-LEV	IVM
6	*Haemonchus*	0	0	–	nt	–	–	–
	Teladorsagia	5	70.5	100		57.0	100	100
	Trichostrongylus	82	1,156.2	100		98.9	100	100
	Cooperia	5	70.5	100		100	100	100
	Oesoph/Chab	8	112.8	100		97.1	100	100
7	*Haemonchus*	0	0	–	–	nt	–	–
	Teladorsagia	29	91.1	100	11.4		93.6	3.7
	Trichostrongylus	14	44.0	–	–		–	–
	Cooperia	0	0	–	–		–	–
	Oesoph/Chab	57	179.1	100	100		100	99.5
8	*Haemonchus*	11	173.1	100	100	nt	100	100
	Teladorsagia	4	62.9	100	97.9		100	100
	Trichostrongylus	40	629.3	98.9	98.9		100	100
	Cooperia	24	377.6	98.6	98.7		100	100
	Oesoph/Chab	21	330.4	99.6	99.9		100	100
10	*Haemonchus*	58	828.6	100	nt	100	100	100
	Teladorsagia	1	14.3	–		–	–	–
	Trichostrongylus	27	385.7	100		98.7	99.8	99.3
	Cooperia	14	200.0	100		100	100	77.7
	Oesoph/Chab	0	0	–		–	–	–
11	*Haemonchus*	0	0	–	nt	nt	–	–
	Teladorsagia	6	28.5	–			–	–
	Trichostrongylus	49	232.8	100			72.9	100
	Cooperia	23	109.3	100			100	96.6
	Oesoph/Chab	22	104.5	100			98.6	100
12	*Haemonchus*	6	139.3	100	100	100	100	nt
	Teladorsagia	2	46.4	–	–	–	–	
	Trichostrongylus	72	1,671.4	100	73.5	74.0	88.3	
	Cooperia	15	348.2	100	98.5	100	100	
	Oesoph/Chab	5	116.1	100	31.9	80.3	96.6	
13	*Haemonchus*	0	0	–	nt	–	–	–
	Teladorsagia	1	2.7	–		–	–	–
	Trichostrongylus	39	105.0	100		90.3	100	92.8
	Cooperia	2	5.4	–		–	–	–
	Oesoph/Chab	58	156.2	100		97.1	100	100
14	*Haemonchus*	17	31.7	–	nt	–	–	–
	Teladorsagia	0	0	–		–	–	–
	Trichostrongylus	67	125.1	100		92.0	41.4	100
	Cooperia	17	31.7	–		–	–	–
	Oesoph/Chab	0	0	–		–	–	–

BZ = benzimidazole (albendazole); LEV = levamisole; IVM = ivermectin; *Oesoph/Chab* = *Oesophagostomum* and/or *Chabertia* spp.; nt = not tested

The percentage larval differentiation post-treatment, arithmetic mean FEC (by genus) for the control group, and treatment efficacies for Trials 6–8 and 10–14 are summarised in Table 4, in which the reference anthelmintics were selected from albendazole, levamisole, albendazole-levamisole and ivermectin. Table 5 presents the data from Trial 9, in which ivermectin, abamectin and moxidectin were the reference anthelmintics used.

The change in mean bodyweight for each experimental group for 13 trials is presented in Table 6. In five trials, the change in bodyweight was significantly greater in the derquantel-abamectin group than for the negative control group. In three trials, one or more groups treated with a reference anthelmintic also had significantly greater increases in mean bodyweight compared with the negative control group.

Mild, transient coughing occurred immediately following treatment in 123/209 animals treated with derquantel-abamectin, 13/113 treated with ivermectin, 2/15 treated with abamectin, and 1/15 treated with moxidectin. No coughing occurred in animals treated with albendazole (n=43), levamisole (n=73), or albendazole-levamisole (n=113). No other adverse events occurred that could be attributed to treatment with the test product.

Table 5. Percentage larvae at the genus level, based on culture of pooled faeces, arithmetic mean (AM) genus faecal nematode egg count (FEC) in the negative control group, and percentage reduction in FEC for sheep treated with derquantel-abamectin (DQL-ABA) and reference anthelmintics, 14 (± 1) days post-treatment, in one trial.

		Negative control group		% Reduction in AM count for each anthelmintic			
Trial	Genus	Larvae (%)	AM (epg)	DQL-ABA	IVM	ABA	MOX
9	*Haemonchus*	29	384.7	100	99.0	100	100
	Teladorsagia	10	132.7	100	0	65.0	32.2
	Trichostrongylus	49	650.1	100	98.3	98.9	98.5
	Cooperia	8	106.1	100	94.7	100	100
	Oesoph/Chab	4	53.1	100	100	100	100

IVM = ivermectin; MOX = moxidectin; *Oesoph/Chab* = *Oesophagostomum* and/or *Chabertia* spp.

Table 6. Mean change, and percentage change, in bodyweight from pre-treatment (Day –5 to Day 0) to Day 14 (± 1 day) for sheep treated with derquantel-abamectin (DQL-ABA) or reference anthelmintics, or negative controls (Control) in 13 trials evaluating the field efficacy and safety of DQL-ABA. In Trial 7, the sheep were not weighed on Day 14.

	Mean change (95% CI) in bodyweight (kg)[a]							
Trial	Control	DQL-ABA	BZ	LEV	BZ-LEV	IVM	ABA	MOX
1	1.5 (0.8, 2.2) [5.7%]	2.2 (1.9, 2.6) [8.9%]	nt	nt	nt	nt	nt	nt
2	2.8^{x} (2.2, 3.4) [7.2%]	3.8^{y} (3.2, 4.3) [9.8%]	nt	nt	nt	nt	nt	nt
3	0.9 (0.4, 1.5) [2.4%]	1.6 (1.1, 2.1) [4.1%]	nt	nt	nt	nt	nt	nt
4	–1.1^{x} (–1.8, –0.3) [–3.2%]	1.5^{y} (0.7, 2.2) [4.5%]	nt	nt	nt	nt	nt	nt
5	1.1 (0.6, 1.6) [4.0%]	1.6 (1.1, 2.2) [5.5%]	nt	nt	nt	nt	nt	nt
6	–2.4 (–4.1, –0.7) [–6.8%]	–1.1 (–2.3, 0.0) [–3.3%]	nt	–1.8 (–3.3, –0.3) [–5.0%]	–1.8 (–2.5, –1.1) [–5.1%]	–2.1 (–3.9, –0.2) [–5.9%]	nt	nt
8	2.5^{x} (1.5, 3.4) [7.0%]	4.4^{y} (3.6, 5.2) [12.9%]	4.0^{y} (3.5, 4.6) [11.6%]	nt	4.0^{x,y} (2.5, 5.5) [11.4%]	3.9^{x,y} (3.0, 4.8) [10.8%]	nt	nt
9	4.0 (3.3, 4.8) [14.7%]	5.3 (4.6, 6.1) [20.9%]	nt	nt	nt	4.7 (4.0, 5.5) [17.1%]	4.4 (3.6, 5.1) [15.9%]	4.8 (4.1, 5.5) [18.3%]
10	0.3^{x} (–0.8, 1.4) [1.1%]	1.9^{x,y} (–0.1, 3.8) [7.0%]	nt	3.6^{y} (2.1, 5.1) [13.2%]	2.5^{y} (1.4, 3.6) [9.0%]	1.6^{x,y} (0.6, 2.5) [5.6%]	nt	nt
11	2.1 (1.2, 2.9) [4.4%]	2.0 (1.1, 2.8) [4.3%]	nt	nt	1.6 (0.8, 2.4) [3.5%]	2.1 (1.3, 3.0) [4.6%]	nt	nt
12	–0.3^{x} (–0.8, 0.1) [–0.9%]	0.8^{y} (0.2, 1.4) [2.0%]	0.6^{y} (0.4, 0.8) [1.6%]	0.5^{x,y} (–0.1, 1.0) [1.2%]	0.6^{x,y} (0.1, 1.1) [1.7%]	nt	nt	nt
13	1.8^{x} (0.9, 2.7) [4.8%]	4.3^{y} (3.5, 5.2) [11.2%]	nt	2.6^{x,y} (1.8, 3.5) [6.8%]	2.9^{x,y} (2.1, 3.8) [7.6%]	3.2^{x,y} (2.3, 4.0) [8.2%]	nt	nt
14	4.8 (4.1, 5.5) [13.9%]	6.1 (5.4, 6.8) [17.4%]	nt	5.0 (4.3, 5.7) [14.3%]	5.9 (5.2, 6.6) [17.2%]	6.0 (5.2, 6.7) [17.4%]	nt	nt

[a] Figures in square brackets represent the mean change in bodyweight expressed as a percentage of the mean bodyweight pre-treatment

^{x,y} Within rows, means sharing the same superscripts are not statistically different at the 5% level of significance. Superscripts are not presented where there was no significant difference overall between the treatment groups, or no significant pair-wise comparisons

BZ = benzimidazole (albendazole); LEV = levamisole; IVM = ivermectin; MOX = moxidectin; nt = not tested

Discussion

In 13/14 trials, the efficacy of derquantel-abamectin against populations of strongyles was ≥99.9% based on geometric means and ≥99.2% based on arithmetic means. In the other trial, efficacy was 99.2% and 93.3%, respectively; this difference was largely due to an outlier in the FEC data, with one animal in the treated group having a post-treatment strongyle FEC of 500 epg. Based on a repeat count of all post-treatment faecal samples in this trial, the geometric mean efficacy was 99.9% and the arithmetic mean efficacy 99.2%.

Defining anthelmintic resistance as percentage reduction in mean FEC of <95%, resistance in the strongyle population to at least one reference anthelmintic was confirmed in three trials (based on geometric means) and six trials (based on arithmetic means). In each of these trials, the efficacy of derquantel-abamectin was 100%. In the population of *Nematodirus* sp., resistance to at least one reference anthelmintic was confirmed in three trials, with derquantel-abamectin again being 100% effective in each instance.

There is debate as to whether the percentage reduction in geometric or arithmetic mean FEC provides the more appropriate measure of efficacy in the FECRT (Dash *et al*. 1988; McKenna 1997a; Smothers *et al*. 1999; Dobson *et al*. 2009). In the trials reported here, percentage reductions based on arithmetic means were less than those based on geometric means (except when 100%), consistent with the view that the use of arithmetic means may provide a more stringent test of anthelmintic efficacy (Vercruysse *et al*. 2001). In accordance with the anthelmintic efficacy guidelines published by the WAAVP and the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (Wood *et al*. 1995; Vercruysse *et al*. 2001), we used the geometric mean FEC as the primary measure of anthelmintic efficacy against strongyles and *Nematodirus* sp.; arithmetic mean efficacies are also reported for completeness.

In Trials 6–14, where efficacy calculations at the genus level were considered valid, i.e. a mean genus FEC of ≥50 epg in the negative control group, the efficacy of derquantel-abamectin was 100% for *Haemonchus* (four trials), 100% for *Teladorsagia* (four trials), ≥98.9% for *Trichostrongylus* (eight trials), ≥98.6% for *Cooperia* (six trials), and ≥99.6% for *Oesophagostomum/Chabertia* (seven trials) spp. Of particular interest are the data generated that indicate established or emerging anthelmintic resistance in one or more genera, where that resistance was not always detectible in the strongyle population as a whole. Although the calculation of means at the genus level contains some inherent inaccuracies, due to a margin of error in both the mean strongyle FEC and percentage larval culture post-treatment, valuable information on emerging anthelmintic resistance in individual strongyle genera may be obtained using larval culture post-treatment (Presidente 1985; McKenna 1997b). In Trial 9, for example, the efficacy of ivermectin, abamectin and moxidectin against *Teladorsagia* sp. was 0%, 65% and 32%, respectively, compared with efficacy against the undifferentiated strongyle population of 86%, 96% and 93% (based on arithmetic means). The efficacy of derquantel-abamectin against *Teladorsagia* sp. in that trial was 100%, underscoring the role of derquantel in the combination anthelmintic.

While just over half of the animals treated with derquantel-abamectin coughed immediately following treatment, this was of a mild and transient nature, with no recurrence or adverse sequelae. There were no reports of loss of the anthelmintic associated with the episodes of coughing. No other adverse events occurred that could be attributed to the test product.

In conclusion, these results demonstrate a high therapeutic efficacy of the combined oral formulation of derquantel-abamectin against naturally acquired gastrointestinal nematode populations in sheep, under a range of farming conditions in New Zealand. The proposed commercialisation of derquantel-abamectin offers sheep producers a unique opportunity to use a novel anthelmintic from a new chemical class as part of a highly effective combination anthelmintic, while resistance alleles are still rare, this being identified as one of the conditions required for combinations to be effective in delaying the emergence of resistance (Dobson *et al*. 2001; Leathwick *et al*. 2009). In association with sustainable anthelmintic treatment practices and maintaining a proportion of susceptible nematodes in refugia (either as free-living stages or adult nematodes in untreated sheep), the prudent use of highly effective combination anthelmintics may be a key element of internal parasite control in the future.

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