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Short communication

Broad spectrum anthelmintic resistance of *Haemonchus contortus* in Northern NSW of Australia

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ABSTRACT

On a sheep farm in Northern New South Wales (NSW) of Australia a degree of anthelmintic resistance was suspected. With noticeable clinical signs of infection and sheep not responding to treatment, a faecal egg count reduction test was conducted to ascertain the broad spectrum of anthelmintic resistance at this farm. A number of classes of anthelmintics were assessed including organophosphate, macrocyclic lactone (ML) and in combination an ML, benzimidazole, levamisole and salicylanilide. In addition, the more recently registered classes of anthelmintics, monepantel (amino-acetonitrile derivative) and derquantel/abamectin combination (spiroindole + ML) were included.

Ninety merino sheep naturally infected with a field strain of *Haemonchus contortus* were randomly allocated to 6 treatment groups (15 animals/group). Sheep were subsequently treated based on label recommendations and individual bodyweight. Faecal samples were collected post-treatment on Days 7, 14 and 21 to conduct faecal egg counts and group bulk larval cultures.

Broad spectrum anthelmintic resistance was confirmed at this site with treatment efficacies ranging from 21.3% (monepantel) to 93.8% (derquantel/abamectin combination) against the *H. contortus* strain. Furthermore, resistance to the multi-combination anthelmintic containing 4 active ingredients was evident (52.5%). This broad spectrum of resistance highlights the need for integration of alternative sustainable methods in parasite control in order to slow development of resistance and increase the life time effectiveness of anthelmintics.

1. Introduction

Regular faecal egg count monitoring in sheep at Invetus (Armidale, New South Wales (NSW) Australia) has revealed an increasing prevalence of anthelmintic resistance of gastrointestinal worms in Northern NSW. Variable degrees of resistance to all major classes of anthelmintics exist (unpublished reports) including those anthelmintics with multiple active ingredients (actives).

The increasing prevalence of anthelmintic resistance is not only limited to the older classes of drenches but includes those drenches first registered less than 7 years ago. Resistance to the amino-acetonitrile derivative (monepantel) registered in Australia in 2010, has been reported in New Zealand (Scott et al., 2013) with *Teladorsagia circumcincta* and *Trichostronglyus colubriformis* and in the Netherlands (Van den Brom et al., 2015) with *Haemonchus contortus*. Furthermore, reduced efficacy of the spiroindole/ML combination (derquantel/abamectin) registered in Australia in 2014 was recently reported by Sales and Love (2016) in NSW, Australia.

A faecal egg count reduction test (FECRT) conducted over late July–August 2016 sought to investigate the nature of broad spectrum anthelmintic resistance on a sheep farm in Northern NSW. A degree of anthelmintic resistance was suspected as a mob of sheep failed to respond to treatment and a number of sheep had died. The FECRT subsequently conducted aimed to investigate the level of resistance, incorporating a number of anthelmintics including the more recently registered products (monepantel and derquantel/abamectin combination) in order to establish treatment efficacies and the broad spectrum of resistance at this test site.

2. Materials and methods

Ninety merino sheep (approx. 12 months old) with a natural field infection of *H. contortus* were selected from a larger flock based on faecal egg counts (FECs). Sheep were randomly allocated to 6 treatment groups on Day–7 based on FEC (15 animals/group). Each group had a similar group mean FEC and range of FECs at allocation with no significant differences ($p < 0.05$) between groups (Table 1). On Day 0, sheep were weighed then treated based on label recommendations and

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Table 1
Faecal egg count (FEC) reduction test – arithmetic and geometric mean treatment efficacies based on differentiated larval data (*H. contortus*).

Treatment[a]	Mean FEC Day–7 (epg[b])	Mean FEC Day 7 (epg)	Mean FEC Day 14 (epg)	Mean FEC Day 21 (epg)
Arithmetic Means (AM)				
Untreated Controls	579.4[1]	1000.0[1]	606.3[1]	370.2[1]
Startect®	522.5[1]	11.4[2]	37.3[2]	96.0[2]
Q Drench®	481.1[1]	277.3[1]	288.0[1]	296.0[1]
Zolvix®	499.2[1]	621.3[1]	477.3[1]	405.3[1]
Rametin®	563.9[1]	46.4[2]	138.5[1]	127.4[1]
Cydectin®	465.6[1]	338.7[1]	168.0[1]	184.0[1]
AM Treatment Efficacies (%)				
Untreated Controls		–	–	–
Startect®		98.9	93.8	74.1
Q Drench®		72.3	52.5	20.0
Zolvix®		37.9	21.3	−9.5
Rametin®		95.4	77.2	65.6
Cydectin®		66.1	72.3	50.3
Geometric Means (GM)				
Untreated Controls		687.4	388.6	347.7
Startect®		1.3	7.9	48.4
Q Drench®		191.7	234.4	120.5
Zolvix®		498.9	361.2	251.7
Rametin®		14.4	72.0	13.7
Cydectin®		132.2	101.6	107.7
GM Treatment Efficacies (%)				
Untreated Controls		–	–	–
Startect®		99.8	98.0	86.1
Q Drench®		72.1	39.7	65.3
Zolvix®		27.4	7.1	27.6
Rametin®		97.9	81.5	96.1
Cydectin®		80.8	73.9	69.0

[1,2] Means within the SAME column with the SAME superscript are NOT significantly different at $p < 0.05$.

[a] Startect® – derquantel and abamectin; Q Drench® – abamectin, albendazole, closantel and levamisole hydrochloride; Zolvix® – monepantel; Rametin® – naphthalophos; Cydectin® – moxidectin; Epg – eggs per gram.

individual bodyweight (Table 2). All treatments were administered orally, using graduated plastic Terumo syringes deep within the oral cavity; Group 1–Untreated controls; Group 2–Startect® Broad Spectrum Oral Drench for Sheep (derquantel/abamectin combination); Group 3–Q Drench® Multi-combination Drench for Sheep (abamectin, albendazole, closantel and levamisole hydrochloride); Group 4–Zolvix® (monepantel); Group 5–Rametin® Sheep Drench (naphthalophos); Group 6–Cydectin® (moxidectin). Following treatment, sheep co-mingled as one group in a single paddock to graze for the duration of the study.

Individual faecal samples were collected post-treatment on Days 7, 14 and 21 to conduct individual FECs and group bulk cultures. Faecal egg counts were conducted according to a modified McMaster method (Hutchinson, 2009) such that each egg counted represents 40 eggs per gram (epg) i.e. 2.5 g of faeces examined. Group faecal cultures were prepared by combining a sub-sample of equal weight (as available) from each animal within the group. Faecal cultures were mixed with vermiculite and moistened with water for incubation at 25–27 °C for 7 days. Third-stage larvae (L3) harvested from group cultures were examined microscopically and 100 larvae (as available) differentiated by genus (Van Wyk and Mayhew, 2013).

This study was conducted with the approval of the University of New England Animal Ethics Committee (AEC no. 16-054) and in accordance to Good Clinical Practice (VICH GL9) and VICH GL7, & GL13 guideline recommendations (VICH, 1999, 2000b).

2.1. Statistical analyses

FEC data was collated by group using Microsoft EXCEL and group arithmetic and geometric means were calculated. Treatment efficacies (Days 7, 14 and 21) were determined by comparison of treated and untreated group means using Abbott's formula (Abbott, 1925)-

$$\text{Treatment Efficacy (\%)} = 100 \times (1 - \text{Treated Mean/Control Mean})$$

FECs pre- and post-treatment were compared between groups at $p < 0.05$ using One Way Analysis of Variance and statistical analyses software (Statistix 10.0). In addition to point estimates of treatment efficacy, the 95% confidence intervals for treated efficacies (based on undifferentiated larval data) were calculated using an EXCEL FECRT template (Perkins, 2013). This interval indicates the possible upper and lower limits of treatment efficacies and assists in identifying emerging resistance (Playford et al., 2014).

3. Results

Based on Day 14 arithmetic means, treatment efficacies ranged from 21.3% (monepantel) to 93.8% (derquantel/abamectin combination) against the *H. contortus* at this test site. Mid-level resistance was observed to the organophosphate (naphthalophos–77.2%), ML (moxidectin–72.3%) and the multi-combination drench containing 4 actives (abamectin, albendazole, closantel and levamisole hydrochloride – 52.5%). Treatment efficacies calculated on arithmetic and geometric means and, based on differentiated larval data for each time point (Day 7, 14 and 21) are detailed in Table 1. Based on undifferentiated data, significant differences at $p < 0.05$ were seen between Group 2 (derquantel/abamectin combination) and the untreated control group (Group 1) on Day 14. Calculating the lower 95% confidence limit of the untreated control group geometric mean, as a percentage of the

Table 2
Treatment regime and dose level.

Group	Treatment	Dose Level	Mean Body Weight (kg) Day 0	Dose Volume	Route
1	Untreated Control	–	21.8	–	–
2	Startect®	0.2 mg/kg abamectin + 2.0 mg/kg derquantel	22.4	1 mL/5 kg	Oral
3	Q Drench®	0.2 mg/kg abamectin, 5.0 mg/kg albendazole, 8.0 mg/kg levamisole hydrochloride + 7.5 mg/kg closantel	23.0	1 mL/5 kg	Oral
4	Zolvix®	2.5 mg/kg monepantel	21.7	1 mL/10 kg	Oral
5	Rametin®	36.3 mg/kg naphthalophos (120 g/L naphthalophos)	21.5	6.5 mL/21.5 kg	Oral
6	Cydectin®	0.2 mg/kg moxidectin	25.0	1 mL/5 kg	Oral

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