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Short communication

Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Tracer studies in sheep

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ABSTRACT

The aim of these studies was to determine the reduction in pasture infectivity likely to be achieved by the supplementation of grazing sheep with BioWorma®, a product containing the chlamydospores of the nematophagous fungus *Duddingtonia flagrans* strain IAH 1297. Four placebo-controlled trials were conducted between 2009 and 2013 in sheep in different climatic regions of New South Wales and Queensland, Australia and across several seasons. The effectiveness of BioWorma was assessed by total worm counts in tracer sheep placed in paddocks grazed by parasitised sheep which were fed a daily supplement with and without BioWorma under group-feeding conditions. Further proof of concept was obtained by assessing the worm burdens and weight gains of the parasitised sheep, as well as the number of anthelmintic ("salvage") treatments required when faecal egg counts exceeded a threshold level.

Significant reductions ranging from 57 to 84% ($P < 0.05$) in worm burdens of the tracer sheep placed in the paddock grazed by BioWorma treated sheep were obtained in all four trials, compared to the Control group. In two of the studies the treatment effect was greater at the end of the trial, indicating that pasture infectivity in the Control paddocks had risen considerably. The main nematodes encountered were *Haemonchus* spp., *Trichostrongylus* spp., and *Teladorsagia* spp. (including multi-resistant strains) and significant reductions were demonstrated for each of these species.

Given the results of the four trials it can be concluded that supplementation of pastured sheep with BioWorma was effective in reducing the numbers of parasitic nematode larvae ingested by tracer sheep. It is considered that these levels of reduced pasture larvae would result in productivity increases in grazing sheep and reduce the requirement for intervention with anthelmintic chemicals. Therefore, use of BioWorma will provide an alternative means for control of gastrointestinal nematode (GIN) parasites on pasture.

1. Introduction

Gastrointestinal nematode parasites (GIN) are of great concern for producers of sheep and other grazing livestock worldwide. Several species of nematodes affect sheep including *Haemonchus* spp., *Teladorsagia* (*Ostertagia*) spp., and *Trichostrongylus* spp. although differences in prevalence and abundance occur in different geographic locations due to local ecological and climatic zones. Infection with GIN results in significant losses in productivity and reproductive performance and impacts negatively on animal health, causing diarrhoea, anaemia and, in some cases, death. In higher rainfall areas of Australia where sheep contribute to on-farm profitability through wool and meat production, GIN parasites severely impact production if effective control measures are not undertaken. In Australia, internal parasites were identified as the highest cost disease of sheep (Sackett et al., 2006) and the annual cost associated with parasitic diseases in sheep and cattle has been estimated at A$1 billion (Roeber et al., 2013).

Since the 1960s, the regular appearance and availability of a number of effective anthelmintic chemicals has provided a ready solution to this problem. Over the past 25 years however, it has become apparent that the regular application of anthelmintic chemicals has led to the development of strains of the major pathogenic nematode species that are resistant to all of the currently available anthelmintics (Besier and Love, 2003; Kaplan and Vidyashankar, 2012; Playford et al., 2014, Lamb et al., 2017). It is also evident that the rate of development and registration of "new" anthelmintics is not keeping pace with the rate of emergence of strains of nematodes resistant to available anthelmintics (Hennessy, 2000). Concerns over the increasing prevalence of

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anthelmintic resistance and the increasing consumer demand for products with minimal chemical inputs has led researchers to place greater emphasis on finding non-anthelmintic means of combating this problem (Gill and Le Jambre, 1996; Knox et al., 2012).

Unlike nearly all other methods for parasite control in livestock, which are aimed at the parasitic stage within the host animal, biological control methods can be targeted at the free living parasitic stages on pasture (Knox, 2003; Waller, 2006). Nematode trapping fungi are found in both natural and agricultural soils (Duddington, 1951; Fernandez et al., 1999) where they live saprotrophically or predatorily (in the presence of nematodes) (Bogus et al., 2005). A large number of nematode-destroying fungi have been identified to date however only a few have been studied for use in controlling parasitic nematodes in animals (Waller and Larsen, 1996; Knox, 2003). *Duddingtonia flagrans* is currently the most studied fungus due to the ability of its thick walled chlamydospores to survive gut passage, germinate and grow rapidly in fresh faeces and its avid nematophagous capacity (Larsen, 1999; Knox, 2003).

A number of studies using *D. flagrans* have reported success in its use to control GIN (Waller et al., 2001; Fontenot et al., 2003; Chandrawathani et al., 2004; Paraud et al., 2005). The chlamydospores of *D. flagrans* can be added to animal feed (Mendoza de Gives et al., 2006) and pelleted feed (Hernandez et al., 2016) and pass through the animal's gastrointestinal tract, which ultimately leads to decreased numbers of pre-parasitic nematode larvae in faeces and on the surrounding pasture (Ojeda-Robertos et al., 2009; Paz-Silva et al., 2011). When used in combination with other control strategies, predacious fungi have the potential to decrease the reliance of farmers on anthelmintics.

Several studies have shown the passage of *D. flagrans* chlamydospores into the faeces of sheep after oral drenching (Larsen, 2000) and efficacy has been demonstrated in a number of field trials where *D. flagrans* spores were fed to sheep (Githigia et al., 1997; Knox and Faedo, 2001; Peart, 2002; Fontenot et al., 2003; Chandrawathani et al., 2004; Gomez-Rincon et al., 2006; Santurio et al., 2011). In this report, a series of field trials were carried out to investigate the effect of supplementation of BioWorma, a product containing the chlamydospores of an Australian isolate of the fungus *D. flagrans,* on GIN burdens in sheep in different regions and seasons in Australia.

2. Materials and methods

2.1. Experimental procedure

In these placebo-controlled trials the following products were used:

Livamol®: Placebo Product - a nutritious and highly palatable animal feed supplement containing molasses, protein and oilseed meals, fish oil, vitamins and minerals, made by International Animal Health Products Pty Ltd.

BioWorma®: Investigational Veterinary Product manufactured by International Animal Health Products Pty Ltd, providing 3×10^4 viable chlamydospores of *D. flagrans* strain IAH 1297/kg bodyweight (b.w.)/day. In these trials BioWorma was homogeneously dispersed in Livamol.

In each of the 4 trials, the groups of sheep used had one of two designated roles:

(1) "seeder" sheep, which harboured natural infections of a range of parasitic GIN representative of the region (including multi-resistant strains), were used to contaminate the pasture (Paddocks 1 or 2) with faeces infected with worm eggs. Seeder sheep were allocated to one of two equal groups (Control – Paddock 1; BioWorma – Paddock 2) based on pre-treatment FECs (except Trial 1, where group allocation was by bodyweight). Each group had a similar mean FEC and range of FECs within the group and with no significant differences between groups ($p < 0.05$). Independent faecal egg count reduction tests (FECRTs) were conducted with a variety of drenches at the study sites of Trials 2, 3 & 4 to determine the extent of anthelmintic resistance in the nematodes carried by the seeder sheep.

(2) "tracer" sheep, which were young, worm-susceptible animals and confirmed free of any worm burden, were used to assess the degree of worm contamination of the pasture on which they grazed. Being young, recently weaned and having low prior exposure to GINs, tracers were considered highly susceptible to infection and suitable for assessment of the level of worm-contamination on pasture. Tracer sheep were allocated to either trial Paddock 1 (Control group) or Paddock 2 (BioWorma) based on bodyweight. Each group had a similar mean bodyweight and range of bodyweights, with no significant difference between groups ($P < 0.05$).

In each trial a pair of matched paddocks (Paddock 1 and 2) was used to graze sheep. Paddock 1 was grazed with a group of seeder sheep which received a daily supplement of the placebo (Control Group) whilst Paddock 2 was grazed by a matching group of seeder sheep that received an equivalent amount of a daily supplement of BioWorma (BioWorma Group). Individual faecal egg counts (FECs) were conducted regularly (weekly or fortnightly) to monitor and confirm patent infections, with samples collected per rectum. FECs were conducted according to a modified McMaster method (Hutchinson, 2009) with sensitivity of 40 eggs per gram (2.5 g samples examined). Individual bodyweights were monitored monthly using electronic livestock scales (verified before and after weighing using calibrated test weights). The daily supplements were prepared using verified electronic scales (to 0.01 kg) and administered in a group setting in covered troughs at the same time each day. Any uneaten supplement was removed daily and weighed.

Trial paddocks chosen for the 4 independent studies were located in well-known sheep grazing regions of Australia and had not been grazed by sheep or goats for a minimum period of 2 months prior to introduction of seeder sheep/commencement of the study. Trials were conducted over a range of climatic conditions and the pastures were typical of those used to graze sheep in the region, with stocking density equivalent for each paddock. In Trial 1 the stocking density was higher than the regional average to increase the likelihood of larval ingestion (approx. 20 sheep per 0.5 ha) whilst in Trials 2–4, stocking densities conformed to regional averages. Throughout all trials there was sufficient pasture available to maintain liveweight gain in sheep at the stocking density used without need for supplementation, according to the established regional requirements. In Trial 1, paddocks were confirmed free of contamination by pre-trial grazing with worm-free tracer animals (group geometric mean FECs of pre-trial tracers were 5.4 and 0.1 epg for paddocks 1 and 2 respectively). In all subsequent trials paddocks had a similar history of grazing and period of rest (no grazing) to those in Trial 1 and hence pre-trial tracers were considered unnecessary.

After the seeder sheep had grazed the paddocks for two months, the degree of pasture contamination by infective nematode larvae was assessed by grazing paddocks with worm-susceptible tracers for a period of 3 weeks. Prior to grazing, the tracers were confirmed to be free of nematode infections by treatment with a broad-spectrum, short-acting non-residual anthelmintic combination drench and subsequent individual FEC. Anthelmintics were administered orally and based on label recommendations and individual bodyweight. Tracers were maintained in pasture-free pens for a period of 2 weeks to allow dissipation of anthelmintics prior to relocating tracers onto the trial paddocks. Tracers were subsequently allocated randomly to two groups (Group A and B). Group A grazed on the Control paddock (Paddock 1) whilst Group B grazed the BioWorma paddock (Paddock 2). The quantity of supplements provided (placebo and BioWorma) were proportionally increased while the tracers were grazing. The tracer animals were then removed from paddocks to raised pens to allow any worm

burdens to mature. The level of infection in tracer sheep was determined by conducting total worm counts (TWCs) after sacrifice, on aliquots collected from gut washings. In Trials 2–4 the seeder sheep continued to graze the trial paddocks for a further two months at which time another group of tracers were introduced as above and their TWC was determined. The additional groups of tracers were designated groups C and D (grazing Control and BioWorma paddocks respectively).

A salvage treatment was administered to any seeder sheep throughout the study if FECs (monitored weekly/fortnightly) exceeded a trigger threshold and/or veterinary indications based on ethical considerations, to prevent occurrence of clinical parasitosis between visits to trial sites. Salvage treatments consisted of a short-acting non-residual anthelmintic administered orally based on label recommendations and individual bodyweight. In Trials 3 and 4 the FEC trigger threshold was reduced because *Haemonchus* spp. was predominant amongst the species present and the more remote trial locations meant that veterinary oversight would be less frequent. The number of salvage treatments required throughout each trial formed an integral assessment of treatment efficacy.

The trials were conducted according to VICH Good Clinical Practice (Wood et al., 1995) and WAAVP guidelines (Duncan et al., 2002), with approval granted by the University of New England's Animal Ethics Committee. No adverse reactions to the BioWorma treatment were observed in any supplemented sheep throughout any of the trials.

An overview of the individual trials is presented in Table 1.

2.2. Statistics

Data were entered into Microsoft EXCEL (and verified following entry) and EXCEL used to calculate group summary data including arithmetic and geometric mean FECs, TWCs and bodyweights. Statistical analyses were performed using Statistix 8.0 and 9.0 (Analytical Software 2008 and 2010). Means were compared in the first instance using parametric Analysis of Variance (and Tukey's All Pairwise Comparison Test) or 2-sample *T*-Tests; homogeneity of variances was not assumed. If variances appeared to be unevenly distributed (based on Levene's Test) the equivalent non-parametric tests (primarily Kruskal–Wallis Analysis of Variance) were used. Means were compared in all cases at $P < 0.05$. The same statistical methodology was used in all 4 studies.

Total parasitic burden for each tracer animal was measured by TWCs while for seeder sheep the parasite burden of each animal was estimated from FECs. Speciation was based on larval differentiation and overall TWCs following sacrifice. Treatment efficacy was calculated according to the formula:

$$\text{Efficacy} = [\text{Mean (Control)} - \text{Mean (Treated)}]/[\text{Mean (Control)}] \times 100 \quad (1)$$

3. Results

3.1. Consumption of supplements

The supplements offered (BioWorma and placebo) were both well-accepted by the animals in each trial. Over all four trials, 99.4% of each of the BioWorma and Livamol supplements that were offered were consumed.

3.2. Faecal egg count (FEC)

Group mean FECs of seeder sheep in Trials 1–4 are detailed in Table 2. FECs of seeder sheep in the Control and BioWorma groups in all trials were not significantly different ($P < 0.05$) at the time of allocation to groups (Week-1). In Trial 1, up to Week 6 there were no significant differences in FEC between groups, but by Week 8 the mean

Table 1
Overview of Trials 1–4.

Trial number	1	2	3	4
Trial dates	27/1/2009 to 24/3/2009	22/11/2011–20/3/2012	16/10/2012 to 21/2/2013	14/12/2012 to 18/4/2013
Location (Australia)	New England (Northern Tablelands)	Southern Tablelands	New England (Northern Tablelands)	South-east Queensland
Seasons	Summer/autumn	Spring/summer	Spring/summer	Summer/autumn
Pasture type	Improved and native grass species and legumes	Improved and native grass species, predominantly phalaris, fescue, white clover and native poa tussock	Improved and native grass species, predominantly phalaris, fescue and white clover	Native grass species, such as Red Grass and Pitted Blue Grass
Seeder sheep (breed, sex, age at start of grazing, number per group)	Merino, female, 6–18 months, 20	Merino, male castrate, 12–16 months, 30	Merino, male castrate and female, 6–24 months, 30	Merino, male castrate and female, 6–24 months, 30
Duration of grazing of seeder sheep (days)	56	120	120	125
Trigger threshold for salvage drench (epg)	N/A	2,000	1,000	1,000
Tracer lambs (breed, sex, age at start of grazing, number per group)	Merino, male castrate, 4–6 months, 23 (10 lightest sacrificed for TWC)	Merino, male castrate and female, 6–12 months, 10	Merino, male castrate and female, 6–12 months, 10	Merino, male castrate and female, 6–12 months, 10
Time of introduction of tracer lambs	End (following removal of seeder sheep)	Groups A, B: Midpoint Groups C, D: End	Groups A, B: Midpoint Groups C, D: End	Groups A, B: Midpoint Groups C, D: End
Duration of tracers in pens prior to sacrifice (days)	20	Groups A, B: 12 Groups C, D: 11	Groups A, B: 12 Groups C, D: 12	Groups A, B: 12 Groups C, D: 13

Table 2
Group mean FECs (epg) of seeder sheep in Trials 1–4 (Arithmetic means ± SE).

Trial	Treatment	Week								
		−1	0	2	4	6	8	11	13	15	End
1	Group 1 (Control)	598.0[1] ± 109.6	–	574.0[1] ± 107.1	1150.6[1] ± 218.0	322.2[1] ± 53.1	**1292.0[1]** ± 234.1	–	–	–	–
	Group 2 (BioWorma)	590.0[1] ± 111.8	–	798.0[1] ± 112.0	1219.0[1] ± 198.0	292.6[1] ± 62.5	**424.0[2]** ± 115.6	–	–	–	–
2	Group 1 (Control)	905.2[1] ± 99.3	466.7[1] ± 44.5	192.3[1] ± 23.7	**172.9[1]** ± 29.3	288.3[1] ± 35.3	409.3[1] ± 86.3	**608.3[1]** ± 79.3	**358.6[1]** ± 61.3	366.7[1] ± 60.6	**393.3[1]** ± 47.4
	Group 2 (BioWorma)	888.6[1] ± 102.8	413.3[1] ± 58.2	276.0[1] ± 45.5	**313.1[2]** ± 47.4	326.9[1] ± 40.4	408.6[1] ± 73.0	**281.4[2]** ± 37.3	**208.0[2]** ± 29.0	286.9[1] ± 40.1	**261.3[2]** ± 34.3
3	Group 1 (Control)	477.3[1] ± 86.1	**649.7[1]** ± 76.2	295.7[1] ± 47.7	408.0[1] ± 77.9	**176.9[1]** ± 32.2	224.6[1] ± 38.9	731.4[1] ± 112.9	620.8[1] ± 131.6	735.7[1] ± 215.3	682.7[1] ± 219.8
	Group 2 (BioWorma)	445.3[1] ± 68.3	**404.3[2]** ± 69.3	416.0[1] ± 46.9	314.7[1] ± 43.5	**296.6[2]** ± 30.4	276.0[1] ± 36.4	678.5[1] ± 109.4	945.2[1] ± 146.0	590.0[1] ± 83.5	616.0[1] ± 120.0
4	Group 1 (Control)	236.0[1] ± 39.9	**249.3[1]** ± 32.8	338.7[1] ± 60.5	284.0[1] ± 34.7	**789.3[1]** ± 85.3	414.3[1] ± 86.7	**326.7[1]** ± 89.7	354.7[1] ± 117.8	209.3[1] ± 79.1	76.0[1] ± 33.3
	Group 2 (BioWorma)	206.7[1] ± 28.5	**458.7[2]** ± 79.9	352.0[1] ± 39.2	238.7[1] ± 36.1	**312.0[2]** ± 49.3	278.7[1] ± 41.4	**1194.5[2]** ± 206.0	631.7[1] ± 167.0	247.1[1] ± 69.4	258.6[1] ± 96.1

[1, 2]Means within the same column and means type with the same superscript are NOT significantly different at $P < 0.05$.

Table 3
Group mean bodyweight (kg) of seeder sheep, Trials 1–4 (Arithmetic means ± SE).

Trial	Treatment	Week				
		0	4	8	13	End
1	Group 1 (Control)	29.3[1] ± 0.45	29.9[1] ± 0.47	**30.7[1]** ± 0.52	–	–
	Group 2 (BioWorma)	30.6[1] ± 0.71	31.5[1] ± 0.76	**32.9[2]** ± 0.67	–	–
2	Group 1 (Control)	36.0[1] ± 0.97	40.6[1] ± 0.91	40.1[1] ± 0.92	41.8[1] ± 0.91	42.5[1] ± 0.85
	Group 2 (BioWorma)	35.0[1] ± 0.76	38.7[1] ± 0.81	38.5[1] ± 0.79	41.4[1] ± 0.88	42.5[1] ± 0.87
3	Group 1 (Control)	27.5[1] ± 1.20	32.5[1] ± 1.14	35.4[1] ± 1.01	37.5[1] ± 0.86	37.9[1] ± 0.70
	Group 2 (BioWorma)	26.7[1] ± 0.85	31.0[1] ± 0.91	34.1[1] ± 0.86	36.9[1] ± 0.82	37.8[1] ± 0.78
4	Group 1 (Control)	**36.8[1]** ± 0.41	**40.9[1]** ± 0.49	41.7[1] ± 0.63	47.5[1] ± 0.64	48.0[1] ± 0.74
	Group 2 (BioWorma)	**34.6[2]** ± 0.58	**37.6[2]** ± 0.62	43.3[1] ± 0.69	47.5[1] ± 0.77	47.6[1] ± 0.68

[1,2] Means within the same column and means type with the same superscript are NOT significantly different at $P < 0.05$.

FEC for Group 1 (Control) was significantly higher than that for Group 2 (BioWorma). In Trial 2, the FEC for the BioWorma group was significantly lower ($P < 0.05$) from Week 11 onwards, despite the salvage drenches applied to the Control group. In Trial 3, significant differences were seen at Weeks 0 and 6, but not from Week 8 onwards and in Trial 4 significant differences were seen at Weeks 0, 6 and 11. It should be noted that in Trials 3 and 4 the data was impacted by the number of salvage drenches applied.

3.3. Bodyweight of seeder sheep

Group mean bodyweights are shown in Table 3. In Trial 1 there was a significantly higher weight gain in the BioWorma group by Week 8, which was consistent with their lower worm status as indicated by FECs. For Trials 2 and 3, no significant differences were seen between the treatment groups throughout the study. Bodyweights for Trial 4 sheep increased throughout the study for both groups with significant differences seen between treatment groups at Weeks 0 and 4. The Control group was significantly heavier at the commencement of the study as a consequence of the allocation to groups based on FEC. Since the animals were drawn from a single mob with a narrow age range, this may have introduced a slight unintended bias in favour of the Control group in terms of vigour. By the end of the study there was no significant difference between the groups.

3.4. Salvage treatments

The number of salvage treatments required in Trials 2, 3 and 4 are detailed in Table 4 (salvage treatments were not included in the design of Trial 1). In each trial the number of salvage treatments required by the BioWorma group was less than or equal to the Control group.

Table 4
Number of salvage treatments required in Trials 2–4.

Treatment	Trial 2	Trial 3	Trial 4
Group 1 (Control)	2	32	26
Group 2 (BioWorma)	0	26	26

Table 5
Group Mean Total Worm Counts for tracer sheep in Trial 1 (Arithmetic means ± SE).

Larval species	Group A (Control)	Group B (BioWorma)	Efficacy (% reduction)
Haemonchus spp.	**1869.6**[1] ± 397.1	**747.0**[2] ± 155.9	60
Teladorsagia spp.	1901.9[1] ± 483.1	1157.5[1] ± 280.9	39
T.axei	**119.9**[1] ± 46.7	**6.0**[2] ± 3.1	95
Trichostrongylus spp.	**5894.0**[1] ± 1206.6	**2084.0**[2] ± 661.2	65
Cooperia spp.	2.0[1] ± 2.0	8.0[1] ± 4.4	0
Nematodirus spp.	184.0[1] ± 76.4	250.0[1] ± 80.3	0
Oesophagostomum spp.	47.8[1] ± 12.9	37.9[1] ± 7.0	21
Total	**10023.9**[1] ± 2058.8	**4295.8**[2] ± 1110.3	57

[1, 2]Means within the same row and means type with the same superscript are NOT significantly different at $P < 0.05$.

Table 6
Group Mean Total Worm Counts for tracer sheep in Trial 2 (Arithmetic means ± SE).

Larval Species	Group A (Control)	Group B (BioWorma)	Efficacy (% reduction)	Group C (Control)	Group D (BioWorma)	Efficacy (% reduction)
Teladorsagia spp.	1700.0[1] ± 351.4	1438.0[1] ± 388.8	15	3556.0[1] ± 913.3	1663.3[1] ± 458.9	53
T.axei	28.01 ± 18.7	6.01 ± 4.3	79	**486.0**[1] ± 159.4	**103.9**[2] 40.8	78
Trichostrongylus spp.	426.0[1] ± 72.2	242.0[1] ± 66.7	43	**6053.9**[1] ± 800.4	**2454.0**[2] ± 443.1	60
Nematodirus spp.	146.0[1] ± 37.6	158.0[1] ± 54.8	0	192.0[1] ± 126.8	237.3[1] ± 100.0	0
Oesophagostomum spp.	**22.2**[1] **4.5**	**4.3**[2] ± 1.8	81	5.1[1] ± 4.5	21.6[1] ± 12.8	0
Total	2322.3[1] ± 427.8	1848.4[1] ± 476.4	20	**10295.1**[1] ± 1358.9	**4480.2**[2] ± 947.4	57

[1, 2]Means within the same row and means type with the same superscript are NOT significantly different at $P < 0.05$.

3.5. Total worm counts

Arithmetic group means for overall TWC and for each worm species in Trials 1–4 are presented in Tables 5–8. In Trial 1, significant reductions due to BioWorma were seen in overall TWC by 57%, as well as *Haemonchus* spp. (60%), *Trichostrongylus* spp. (65%), and *Trichostrongylus axei* (95%). In Trial 2, significant reductions due to BioWorma were seen in overall TWC (57%), as well as *Trichostrongylus* spp. (60%) and *Trichostrongylus axei* (78%) in those tracers introduced to trial paddocks at the end-point of the study (i.e. Groups C/D). In Trial 3, significant reductions due to BioWorma were seen in overall TWC (84%) as well as *Haemonchus* spp. (80%), *Trichostrongylus* spp. (87%), *Trichostrongylus axei* (92%) and *Teladorsagia* spp. (68%) in those tracers introduced at the end-point of the study (i.e. Groups C/D). In Trial 4 significant reductions due to BioWorma were seen in overall TWC (74%) and *Haemonchus* spp. (76%) in those tracer sheep introduced at the mid-point of the study (Groups A/B) and in overall TWC (75%), and *Haemonchus* spp. (76%) in those tracer sheep introduced at the end point of the study (Groups C/D).

3.6. Faecal egg count reduction tests

Results of FECRT's (Trials 2–4, Table 9) showed that multi-resistant nematodes were present for each trial.

4. Discussion

This series of four placebo-controlled studies carried out in Australia evaluated the effect of BioWorma (providing *D. flagrans* strain IAH 1297 at 3×10^4 chlamydospores/kg b.w./day) when administered to sheep harbouring naturally acquired worm burdens. Infections consisted predominantly of *Haemonchus* spp., *Trichostrongylus* spp. and *Teladorsagia* spp., including multi-resistant strains. The results demonstrated the ability of BioWorma to reduce the infectivity of pasture, as evidenced by 57–84% reduction in worm burdens in tracer sheep after grazing trial pastures. The results were consistent in sheep across a range of climatic zones and during different seasons.

Furthermore, results from Trials 1 and 2 indicate that BioWorma also reduced FECs of seeder sheep towards the end of the trials which is in accord with previous observations where FECs of sheep being fed *D. flagrans* reduced over time (Githigia et al., 1997; Knox and Faedo, 2001). In trial 1, where no salvage drenches were applied, there was also a greater weight gain in the BioWorma group. The greater requirement for salvage treatments in Trials 3 and 4 reduced the likelihood of similar observations in these trials. The rate of egg shedding by sheep that received salvage drenches was consequently reduced, however in each study the number of salvage drenches in the Control group was greater than or equal to the BioWorma group, so any bias in outcome would favour the Control group in terms of reduced contamination of their paddock. In addition, the overall results indicate that these drenches did not substantially impact on the nematophagous activity of the *D. flagrans*. Bodyweight gain was also greater in the seeder sheep in Trial 1, consistent with the reduced GIN burden as confirmed by FECs of those sheep administered BioWorma.

Tracer sheep used in these studies were young and recently weaned, and were considered to be the most appropriate assessment of pasture

Table 7
Group Mean Total Worm Counts for tracer sheep in Trial 3 (Arithmetic means ± SE).

Larval Species	Group A (Control)	Group B (BioWorma)	Efficacy (% reduction)	Group C (Control)	Group D (BioWorma)	Efficacy (% reduction)
Haemonchus contortus	488.7[1] ± 113.8	330.0[1] ± 56.5	33	**5348.0**[1] ± 904.9	**1060.0**[2] ± 201.3	80
Teladorsagia spp.	598.7[1] ± 117.1	392.0[1] ± 74.3	35	**322.0**[1] ± 71.8	**104.0**[2] ± 24.7	68
Trichostrongylus axei	14.0[1] ± 9.5	8.0[1] ± 4.4	43	**100.0**[1] ± 29.2	**8.0**[2] ± 6.1	92
Trichostrongylus spp.	1038.0[1] ± 176.1	658.6[1] ± 150.5	37	**8553.7**[1] ± 2355.9	**1126.0**[2] ± 255.8	87
Nematodirus spp.	144.0[1] ± 43.6	302.0[1] ± 131.1	0	136.0[1] ± 78.2	42.0[1] ± 37.8	69
Oesophagostomum spp.	5.5[1] ± 4.1	5.0[1] ± 1.5	9	9.0[1] ± 2.6	8.5[1] ± 3.0	6
Total	2288.9[1] ± 390.9	1696.1[1] ± 200.4	26	**14487.0**[1] ± 2814.3	**2356.9**[2] ± 354.5	84

[1, 2]Means within the same row and means type with the same superscript are NOT significantly different at $P < 0.05$.

Table 8
Group Mean Total Worm Counts for tracer sheep in Trial 4 (Arithmetic means ± SE).

Larval Species	Group A (Control)	Group B (BioWorma)	Efficacy (% reduction)	Group C (Control)	Group D (BioWorma)	Efficacy (% reduction)
Haemonchus contortus	**1848.3**[1] ± 179.1	**452.0**[2] ± 96.2	76	**2968.3**[1] ± 319.8	**715.3**[2] ± 137.7	76
Trichostrongylus spp.	24.0[1] ± 7.8	18.0[1] ± 8.1	25	47.5[1] ± 18.9	18.01 ± 3.6	62
Nematodirus spp.	4.0[2] ± 2.7	12.0[1] ± 4.4	0	20.0[1] ± 6.5	32.0[1] ± 14.4	0
Total	**1883.7**[1] ± 184.0	**485.3**[2] ± 102.9	74	**3036.5**[1] ± 334.8	**769.8**[2] ± 139.3	75

[1, 2]Means within the same row and means type with the same superscript are NOT significantly different at $P < 0.05$.

Table 9
FECRT results, Trials 2–4.

Trial	Worm species	Anthelmintic	Resistance level
2	*Trichostrongylus* spp. and *Teladorsagia* spp.	levamisole	Mid-level
		albendazole and naphthalophos/albendazole combination	Low level
		moxidectin.	No resistance
3	*Trichostrongylus* spp.	levamisole	High-level
		albendazole	Mid-level
		moxidectin and abamectin/albendazole/levamisole combination	No resistance
	Haemonchus contortus	moxidectin and albendazole	Complete resistance
		levamisole	High-level
		closantel	Low level
		abamectin/albendazole/levamisole combination.	No resistance
4	*Haemonchus contortus*	albendazole	High-level
		moxidectin and levamisole	Mid-level
		closantel/mebendazole and abamectin/albendazole/levamisole combinations	Low level
		naphthalophos and monepantel	No resistance

No resistance: > 95 % reduction in FECRT.
Low-level resistance: 75–95% reduction.
Mid-level resistance: 55–75% reduction.
High-level resistance: < 55% reduction.
Complete resistance: no reduction.

contamination in an on-farm situation. It is common knowledge that younger stock have no natural immunity to GINs and immunity develops with age and the level of exposure to GINs. Adult sheep in comparison have developed a level of immunity and although they expel most parasites they continue to carry a low burden. This however can revert without continued exposure to GINs or around parturition (Menzies et al., 2012). The age of the tracer sheep used in these studies is also reflective of the age of animals which requires the most anthelmintic intervention to maintain productivity.

A number of reports have been published by other groups, using various isolates of *D. flagrans* fed to sheep including studies utilising worm-susceptible tracer lambs to assess the degree of infectivity of the pasture. Reduction in the numbers of infective larvae on pasture of 67% for both ewes and lambs has been reported (Peart, 2002). In tracer studies, reduced infection levels were demonstrated in tracers by reductions in FECs of up to 74% (Epe et al., 2008) and up to 97% reduction in worm burdens (Fontenot et al., 2003). Similarly, clinical parasitosis was prevented (Chandrawathani et al., 2004), less worming treatments were required (Santurio et al., 2011) and improvement in weight gain (up to 14.8%) was reported in lambs reared by *D. flagrans* treated ewes (Gomez-Rincon et al., 2006). It should be noted that the dosage of *D. flagrans* spores used in most of the published trials (typically 1×10^6 spores/kg b.w./day) was much higher than the dose level of BioWorma (3×10^4 spores/kg b.w./day), however a similar low dose (1.25×10^4 spores/kg b.w./day) was also used by Hernandez et al., 2016) in horses. We suggest two possible reasons for this difference in effective dose rate for BioWorma. Firstly, the Australian isolate used (IAH 1297) has been observed to grow rapidly and have higher trapping efficiency compared to alternative isolates in conditions where parasite eggs hatch and larval development occurs (CSIRO F.D. McMaster Laboratory, unpublished data). Secondly, culturing methods have been refined for this particular isolate to maximise the durability of viable chlamydospores available for inclusion in BioWorma. This contrasts to many of previous studies where relatively crude preparations of *D. flagrans* on dried cereal grains were used. In addition, the presentation of BioWorma in a highly attractive and palatable feed supplement ensures that problems of variable consumption encountered in previous work (Knox and Faedo, 2001) can be avoided.

In consideration of the efficacy expectations for products providing novel and non-chemical helminth control strategies, these products cannot be evaluated using the same criteria as anthelmintics and Ketzis et al. (2006) proposed that evaluation be based on efficacy and economic benefit. Assessment of the impact of novel technologies of vaccination and nematophagous fungi to control worm populations in sheep was modelled by Barnes et al. (1995) where impact of treatment was determined in terms of predicted lamb mortality rates. Based on this modelling, use of *D. flagrans* instead of anthelmintics was predicted to result in mortalities over 20 years of 50 deaths per 2000 lambs even if administered for 30 days per year and having a treatment efficacy of only 50%. However, when administered for 90 days with a treatment efficacy of 75%, the predicted mortality rate was 8 deaths per 2000 lambs. Our trials indicate that treatment efficacy within this range is possible in sheep under Australian grazing conditions and it is anticipated that substantial production benefits and reduced requirement for intervention with anthelmintic chemicals would be attainable through the use of BioWorma.

5. Conclusion

In conclusion, these studies have shown that supplementation with BioWorma substantially reduced the infectivity of the pasture on which the recipient sheep grazed. Duration of treatment can affect efficacy as seen in Trials 2 and 3, where higher efficacy was observed at the end of the trials, by which time GIN numbers were higher in the tracer sheep. BioWorma provides a biological method to control worm burdens in grazing sheep by decreasing re-infection through pasture and will add another option for inclusion in integrated control programs to assist sheep producers to address this insidious problem. As this technology is most effectively used as a preventative means of GIN control in sheep, producers should plan BioWorma's use while continuing to apply recommended good practice with regular FEC monitoring, and judicious use of anthelmintic chemicals when necessary.

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Conflict of interest statement

Authors Healey and Lawlor declare their interest in the project, being Research and Development Manager and Chief Executive Officer, respectively, of International Animal Health Products Pty Ltd, the manufacturer of BioWorma and sponsor of these field studies. The sponsors contributed the study design, provided the test products, prepared and amended the draft manuscript and chose to submit it for publication. The other authors (Dr Knox, Dr Chambers and Ms Lamb) had primary responsibility for the study designs and between them were wholly responsible for the conduct of the field trials, analysis and interpretation of the data, and have approved the amended manuscript for release. Authors Knox, Chambers and Lamb have no commercial interest in BioWorma.

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