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Research paper

Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Pasture larval studies in horses, cattle and goats

Kevin Healey[a,*], Chris Lawlor[a,*], Malcolm R. Knox[b], Michael Chambers[c], Jane Lamb[c], Peter Groves[d]

[a] International Animal Health Products Pty Ltd, PO Box 6199 Blacktown, NSW 2148, Australia
[b] CSIRO F.D. McMaster Laboratory, Locked Bag 1 Armidale NSW 2350, Australia
[c] Invetus Pty Ltd, Locked Bag 6865, West Armidale, NSW 2350, Australia
[d] Zootechny Pty Ltd, 975 The Northern Rd, Bringelly, NSW 2556, Australia

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ABSTRACT

A series of placebo-controlled trials were conducted in horses, cattle and goats in different seasons and bioclimatic regions of New South Wales and Queensland, Australia, to evaluate the ability of BioWorma®, a feed supplement containing the spores of *Duddingtonia flagrans* IAH 1297, to reduce the larval development of parasitic gastrointestinal nematodes (GIN) and their subsequent migration from faeces onto the surrounding pasture.

In each trial, faeces were collected from animals harbouring a burden of nematode parasites following a period of supplementation with a placebo and again after supplementation with BioWorma. The faeces were manually placed onto pasture plots at one or two distinct geographical sites and the effect of treatment was determined by subsequent monitoring the numbers of parasitic larvae on the pasture surrounding the faecal pats at two weekly intervals over an eight week period. The results for these studies showed that administration of BioWorma at a minimum daily dose of 3×10^4 spores/kg bodyweight reduced parasite larvae in the pasture surrounding the faeces by 53–99 % over an eight week post treatment period in horses, cattle and goats in a range of bioclimatic zones and in different seasons.

Overall, the studies with BioWorma show substantial reductions in GIN infectivity of pasture surrounding faeces of treated horses, cattle and goats ($P < 0.05$). Results indicate that the use of BioWorma in these host species would lead to decreased levels of GIN infection in animals grazing pasture when this product is used and would provide an alternative means of controlling parasitic nematodes.

1. Introduction

Gastrointestinal nematodes (GIN) are important parasites of grazing animals worldwide, having a negative impact on productivity, reproductive performance and animal welfare. In extreme cases, parasitism can lead to death of the host animal. The problem has been exacerbated by the parasites' acquisition of resistance to the anthelmintic chemicals traditionally used to control them (Kaplan and Vidyashankar, 2012). The widespread problem of anthelmintic resistance has led to greater emphasis being placed on non-chemotherapeutic means of parasite control (Gill and Le Jambre, 1996; Knox et al., 2012). In addition, for meat and milk producing livestock, increasing numbers of consumers are requiring products that are derived from systems using minimal chemical interventions (Will, 2015).

One novel approach to controlling GIN is the potential to use nematophagous fungi to reduce free living larval stages. *Duddingtonia flagrans* has been widely studied and produces robust chlamydospores that can be added to animal feed (Mendoza de Gives et al., 2006). After being consumed in feed, the chlamydospores pass through the animal's digestive tract and inoculate the faeces where they germinate. *D. flagrans* then forms a network of hyphae throughout the faecal mass which inhibits the free living stage of larvae from completing their development by producing sticky traps which capture and destroy the larvae (Fontenot et al., 2003; Ojeda-Robertos et al., 2009; Paz-Silva et al., 2011). Therefore, *D. flagrans* reduces nematode populations on pasture surrounding the faecal mass and consequently lowers the incidence of

* Corresponding authors.
E-mail addresses: khealey@iahp.com.au (K. Healey), clawlor@iahp.com.au (C. Lawlor).

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Table 1
Summary of animal numbers, seasons, dates, trial sites and average daily temperatures at the trial sites.

Trial Code	Number of animals	Seasons (calendar months and year)	Locations of pasture phases	Average daily temperatures (°C) (Max. / Min.)
Horse Trial 1	5	Autumn (March – May 2009)	Armidale, NSW	20.8 / 9.2
Horse Trial 2	6	Spring (September – November 2010)	Armidale NSW Nimmitabel NSW	19.5 / 8.7 20.0 / 5.3
Horse Trial 3	6	Autumn (April – June 2011)	Armidale NSW Dayboro QLD	16.4 / 4.5 24.0 / 13.1
Cattle Trial 1	6	Spring (October – December 2010)	Armidale NSW Nimmitabel NSW	21.6 / 10.6 22.0 / 8.8
Cattle Trial 2	6	Autumn (April – June 2011)	Armidale NSW Dayboro QLD	15.3 / 3.9 22.9 / 12.3
Goat Trial 1	6	Spring (October – December 2010) =	Armidale NSW Nimmitabel NSW	21.5 / 10.3 21.8 / 8.2
Goat Trial 2	12	Autumn / Winter (May – July 2011)	Armidale NSW Dayboro QLD	13.6 / 2.2 21.6 / 10.0
Goat Trial 3	12	Spring/ Summer (November 2011 – January 2012)	Dayboro QLD Nimmitabel NSW	28.3 / 17.8 23.7 / 8.8

infection of animals grazing that pasture (Waller et al., 1994; Baudena et al., 2000).

A number of studies have been published where efficacy of *D. flagrans* has been demonstrated after being fed to horses (Larsen et al., 1996; Fernandez et al., 1999a; Hernandez et al., 2016), cattle (Grønvold et al., 1993; Nansen et al., 1995), goats (Wright et al., 2003; Sanyal et al., 2008) and sheep (Knox and Faedo, 2001; Fontenot et al., 2003; Healey et al., 2018). Efficacy was assessed by reductions in larval burdens on pasture or by reduced total GIN numbers in tracer animals after grazing.

Here we report results of a series of studies conducted to determine the effectiveness of BioWorma, a product containing the chlamydospores of *D. flagrans* strain IAH 1297, in reducing larval migration of GIN from faeces of horses, goats and cattle onto the surrounding pasture.

2. Materials and methods

2.1. Experimental protocol

For each trial, animals harbouring a burden of nematode parasites were selected from a larger group of animals on the basis of worm species present and individual faecal egg counts Larval differentiation (Thienpont et al., 1979; van Wyk et al., 2004) was conducted following group bulk coproculture (50 g sample size) and individual FECs were conducted in triplicate according to a modified McMaster method (Hutchinson, 2009) with sensitivity of 40 eggs per gram (2.5 g samples examined). Resistance status of natural infections was determined from the results of testing of the parent flock by the Diagnostic Services Laboratory of Invetus Pty Ltd. The animals were housed in individual pens with no access to pasture to prevent infection from pasture-based larvae and to assist with supplementation and faecal collection. In some cases the naturally-acquired infections were replaced by, or augmented with, artificial infections. The animals were fed placebo (Livamol®, a product made of molasses, protein and oilseed meals, fish oil, and vitamins and minerals, made by International Animal Health Products Pty Ltd) for 5–7 days then their faeces were collected. Each animal's faeces (Control samples) were kept separate and mixed until homogeneous and faecal egg counts (triplicate) were determined. Four "pats" per sample (matched by weight) were then transported within 24 h of collection by overnight transport for manual placement on day of arrival onto the centre of randomly-allocated 85 cm x 85 cm pasture plots at one or two distinct geographical sites, maintaining an 85 cm distance between plots. The pasture in all trials was typical of that used for grazing animals in the region and had not been grazed for more than 12 months to ensure freedom from infective larvae. The pasture was newly-cut to a height of approximately 10 cm prior to placement of the faecal pats.

The same animals were then fed an equivalent amount of Livamol containing BioWorma (Investigational Veterinary Product, manufactured by International Animal Health Products Pty Ltd) for 5–7 days, providing 3×10^4 chlamydospores *D. flagrans* strain IAH 1297/kg bodyweight (b.w.)/day, and their faeces were again collected and tested as above. The "treated" faeces (BioWorma samples) were then tested, prepared, transported and placed onto pasture plots as above at the same sites used for the Control samples. The total number of faecal pats deposited at each site was 48 = 6 animals x 2 treatments x 4 samples.

The trial dates, seasons and locations of the pasture phases in these trials are shown in Table 1. The trial sites and their bioclimatic zones (Taylor and Hodge, 2014) were Armidale, New South Wales (NSW) in the Northern Tablelands zone; Nimmitabel, NSW in the Southern and Central Slopes / Tablelands zone and Dayboro, Queensland (Qld) in the Subtropical Coastal Qld zone. Trials were conducted predominantly in the spring and autumn, as these are the times when parasite buildups are likely to occur (Donald et al., 1978; Barger et al., 1983).

At 2 weekly intervals from the date of placement (from week 2 through to 8 weeks post placement) for each animal at each trial site the herbage in a 40 cm circle under and around a randomly-selected Control and BioWorma faecal pat was collected down to the ground level using electric clippers. Pasture washings were conducted according to a method modified from Heath and Major, 1968. Briefly, the grass clippings were placed in a 350 µm mesh sealable bag within a pasture washer (metal conical vessel with gated valve at the bottom). The mesh bag was immersed in 90 L of warm water (30 °C) containing Pyroneg detergent (5 g, Diversey, Inc.) to facilitate separation of the larvae from the grass clippings. The contents were agitated for 5–10 min after 1, 3 and 5 h soaking time. After a total of 8 h, two 1 L aliquots from the sedimented washings were collected and cooled at 4 °C overnight. The supernatant was subsequently removed to reduce the volume to a level suitable for counting nematode larvae for each host species (enumeration performed by examination of 5 x 10 µL aliquots and with differentials performed as previously described). From the data the number of infective larvae in each grass sample was calculated.

Table 2
Details of artificial infections applied in goat trials.

Trial No.	Number of goats infected	Anthelmintic treatment for cleanout	Artificial infection applied (species, larvae per animal and resistance status)
1	3	Triton Multiphase Liquid for Sheep	Multi-resistant *Teladorsagia circumcincta* (10,000) and *Trichostrongylus colubriformis* (6000); *Nematodirus* spp. (3000) - recent field isolate.
	3	Triton Multiphase Liquid for Sheep	Multi-resistant *Haemochus contortus* (4000); *Cooperia* spp. (6000) – recent field isolate.
2	6	Triton Multiphase Liquid for Sheep	Multi-resistant *Teladorsagia circumcincta* (10,000) and *Trichostrongylus colubriformis* (6000); *Cooperia* spp. (6000) and *Nematodirus* spp. – recent field isolates
	6	–	(Natural infection – predominantly multi-resistant *Haemonchus contortus* and susceptible strains of *Trichostrongylus* spp. and *Oesophagostomum* spp.)
3	8	Pyrimide, followed next day by Rametin and Duocare LV	Multi-resistant *Teladorsagia circumcincta* (10,000) (n = 4 of 8 goats); OR Multi-resistant *Trichostrongylus colubriformis* (6000) and *Cooperia* spp. (6000) – field isolate (n = 4 of 8 goats).
	4	–	(Natural infection - predominantly multi-resistant *Haemonchus contortus* and susceptible strains of *Trichostrongylus* spp. and *Oesophagostomum* spp.)

Table 3
Anthelmintic products used for cleanout prior to artificial infection of goats.

Product	Manufacturer	Active Ingredients	Quantity used
Triton Multiphase Liquid for Sheep (Trials 1 and 2)	Merial Australia Pty Ltd	ivermectin 0.8 g/L, levamisole (as levamisole hydrochloride) 25.5 g/L, albendazole 20.0 g/L, selenium (as sodium selenate) 0.4 g/L and cobalt (as cobalt EDTA) 1.76 g/L.	1 mL/ 4 kg b.w.
Pyrimide 3-Way Combination Drench for Sheep (Trial 3)	Novartis Animal Health Australasia Pty Ltd	abamectin 0.8 g/L, levamisole (as levamisole hydrochloride) 25.5 g/L, albendazole 20.0 g/L, selenium (as sodium selenate) 0.4 g/L and cobalt (as cobalt EDTA) 1.76 g/L	15 mL per head
Rametin Sheep Drench (Trial 3)	Bayer Australia Ltd	naphthalophos 800.0 g/kg	10 mL of 15 % solution per head
Duocare LV plus Selenium Oral Anthelmintic for Sheep (Trial 3)	Virbac (Australia) Pty Ltd	levamisole 67.8 g/L (as levamisole hydrochloride), fenbendazole 50.0 g/L, selenium (as sodium selenate) 1.0 g/L	1 mL/ 10 kg b.w.

Trials were conducted according to VICH Good Clinical Practice (VICH, 2000) and WAAVP guidelines (Wood et al., 1995; Duncan et al., 2002) by an independent contract research organisation. Ethical approval was granted by the University of New England's Animal Ethics Committee for each trial.

2.1.1. Horse trials

Three horse trials were conducted, designated Horse Trials 1, 2 and 3. Horses were a mixture of female and male castrate, Australian Stock Horse, standard bred, thoroughbred, pony or crosses of these, two to eight years of age. Five horses were used in the first horse trial, six in the other two trials. Faecal pat sizes were 1 kg each. The animals carried naturally-acquired infections consisting principally of cyathostomes, plus some *Strongylus* spp. and *Trichostrongylus axei*.

2.1.2. Cattle trials

Two cattle trials were conducted, designated Cattle Trials 1 and 2. Cattle were a mixture of males and male castrate, mixed-breed (including Friesian, Hereford and Angus), 2.5–8 months of age. Six cattle were used in each trial, with naturally-acquired infections comprising *Cooperia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Ostertagia* spp. and *Haemonchus* spp. (including multi-resistant strains). Faecal pat sizes were 350–500 g each.

2.1.3. Goat trials

Three goat trials were conducted, designated Goat Trials 1, 2 and 3. Artificial infections of GIN were applied to some or all of the goats in these trials, as detailed in Table 2, in order to test the efficacy of Bio-Worma against multi-resistant nematodes. Infections were applied after "cleanout" treatment with a broad-spectrum, short-acting non-residual oral drench (Table 3) and infection rates were determined by the average liveweight of the goats and were in accordance with WAAVP guidelines (Wood et al., 1995).

Trial 1: six male castrate Boer goats, two years of age were used. Thirty-three days prior to commencement of faecal collections, all goats were artificially infected after cleanout, as detailed in Table 2. Faeces were collected over two consecutive days with the first day's collections stored in a cool place (8 °C–14 °C) with air excluded before combining with the second day's collections. Separate two-day collections were made for each trial site. Faecal pat sizes were 100–500 g each. Freezing conditions were experienced at the Nimmitabel site shortly after placement of the samples.

Trial 2: twelve young male castrate Boer goats aged 13 months were used, assigned into 6 pairs of 2 animals. All had naturally-acquired infections with predominantly multi-resistant *Haemonchus* spp. (61%) plus some susceptible *Trichostrongylus* spp. (31%) and *Oesophagostomum* spp. (8%). Twenty-six days before the first faecal collections, half the animals were further artificially infected after cleanout, as detailed in Table 2. The animals were paired, such that one animal in each pair had a natural infection and one had artificial infection and the faecal output of each animal in a pair was combined before testing and placement onto pasture. The faecal collections were conducted over five consecutive days, with each day's collections stored in a cool place (as above) with air excluded before combining with the other collections. Faecal pat sizes were 130–320 g each.

Trial 3: twelve young male castrate Boer goats aged 19 months were used, assigned into 6 pairs of 2 animals. Twenty-nine days before the first faecal collections, eight of the animals were artificially infected after cleanout as detailed in Table 2. The remaining animals had naturally-acquired infections of predominantly multi-resistant *Haemonchus* spp. plus susceptible *Trichostrongylus* spp. and *Oesophagostomum* spp. The animals were paired based on faecal egg counts, such that each pair had a similar FEC and mixture of parasite species. The faecal collections were conducted over six consecutive days, as above. Faecal pat sizes were 220–930 g each.

Table 4
Summary of trial results for horses over the eight week period showing impact of treatment on larval numbers on the herbage surrounding the faecal pats.

Mean number of horse parasite larvae per herbage sample (mean ± SE) over time								
Treatment	Trial No.	No. of horses	No. of trial sites	2 weeks	4 weeks	6 weeks	8 weeks	Overall mean, weeks 2-8
Control	1	5	1	921.4 ± 913.7	1541.1 ± 1449.5	3515.0 ± 2519.0	103.5 ± 80.4	1520.3 ± 756.5
	2	12	2	172.3 ± 121.7	4262.0 ± 2643.6	2953.0 ± 986.6	1866.7 ± 1208.0	2313.5 ± 774.6
	3	12	2	202.6 ± 93.8	1150.1 ± 449.9	2449.9 ± 665.2	7840.1 ± 2637.8	2910.7 ± 794.5
BioWorma	1	5	1	0.0 ± 0.0	5.2 ± 5.2	7.6 ± 7.6	379.0 ± 209.3	97.9 ± 60.8
	2	12	2	0.0 ± 0.0	326.7 ± 326.7	18.0 ± 18.0	247.3 ± 159.6	148.0 ± 90.5
	3	12	2	62.1 ± 33.1	1040.0 ± 679.4	442.4 ± 162.2	1828.6 ± 734.6	843.3 ± 263.9
Overall mean values for all horse trials combined								
Control				432.1 ± 164.8	2317.7 ± 1139.8	**2972.6**[1] ± 625.9	3270.1 ± 1321.8	**2248.2**[1] ± 476.1
BioWorma				20.7 ± 14.6	457.3 ± 313.8	**156.0**[2] ± 76.9	818.3 ± 337.1	**363.1**[2] ± 119.7

[a]mean values within the same column with different superscripts[1,2] differ ($P < 0.05$).

Table 5
Summary of trial results for cattle over the eight week period showing impact of treatment on larval numbers on the herbage surrounding the faecal pats.

Mean number of cattle parasite larvae per herbage sample (mean ± SE) over time								
Treatment	Trial No.	No. of cattle	No. of trial sites	2 weeks	4 weeks	6 weeks	8 weeks	Overall mean, weeks 2-8
Control	1	12	2	2783.3 ± 1345.0	25391.1 ± 21715.3	20701.5 ± 10681.8	8000.9 ± 4347.9	14219.2 ± 6104.7
	2	12	2	387.5 ± 214.7	7485.9 ± 3437.8	21846.0 ± 9109.3	41710.8 ± 11,471.0	17857.5 ± 4307.6
BioWorma	1	12	2	1022.0 ± 566.5	6849.5 ± 5129.1	3991.3 ± 2008.0	1253.7 ± 742.3	3279.1 ± 1394.9
	2	12	2	994.4 ± 554.7	956.0 ± 488.7	4444.2 ± 1701.6	4963.1 ± 2164.0	2839.4 ± 741.7
Overall mean values for all cattle trials combined								
Control				1585.4 ± 711.3	16438.5 ± 10912.1	21273.7 ± 6866.0	**24855.8**[1] ± 6952.5	**16038.4**[1] ± 3720.7
BioWorma				1008.2 ± 387.7	3902.8 ± 2593.4	4217.8 ± 1287.9	**3108.4**[2] ± 1183.7	**3059.3**[2] ± 786.0

[a] mean values within the same column with different superscripts[1,2] differ ($P < 0.05$).

Table 6
Summary of trial results for goats over the eight week period showing impact of treatment on larval numbers on the herbage surrounding the faecal pats.

Mean number of goat parasite larvae per herbage sample (mean ± SE) over time								
Treatment	Trial No.	No. of goats	No. of trial sites	2 weeks	4 weeks	6 weeks	8 weeks	Overall mean, weeks 2-8
Control	1	12	2	11342.7 ± 6998.3	50708.0 ± 29588.1	1187.7 ± 1032.4	2164.7 ± 1175.3	16350.8 ± 7933.3
	2	12	2	791.9 ± 341.7	3800.9 ± 2222.6	13382.9 ± 6790.0	8743.3 ± 3224.1	6679.7 ± 2022.8
	3	12	2	48918.1 ± 31251.0	11026.2 ± 6838.1	1339.8 ± 521.8	985.4 ± 521.4	15567.4 ± 8255.0
BioWorma	1	12	2	650.0 ± 394.8	9901.0 ± 4002.6	692.9 ± 282.3	0.0 ± 0.0	2811.0 ± 1144.2
	2	12	2	26.4 ± 23.3	145.3 ± 96.0	149.0 ± 86.5	79.0 ± 62.1	99.9 ± 35.9
	3	10[a]	2	8628.8 ± 6798.3	1583.0 ± 965.2	143.6 ± 77.9	10.4 ± 10.4	2591.4 ± 1741.5
Overall mean values for all goat trials combined								
Control				20350.9 ± 10938.2	21845.0 ± 10453.7	5303.4 ± 2429.6	3964.5 ± 1262.9	**12866.0**[1] ± 3865.7
BioWorma				3101.7 ± 2174.2	3876.4 ± 1555.3	328.5 ± 110.5	29.8 ± 21.8	**1834.1**[2] ± 676.7

[a] data for one pair of goats in trial 3 (BioWorma group, Dayboro site) was omitted from the analysis due to missing sample at week 2.
[b] mean values within the same column with different superscripts[1,2] differ ($P < 0.05$).

2.2. Statistical analysis

At each time point, at each site, the mean numbers of parasite larvae for all the control and BioWorma samples were calculated from the two sampled aliquots. In order to determine the treatment effect of BioWorma on larval numbers on pasture across trials, the data from each site for the multi-site trials were averaged and then, for each species of animal, the data from all studies were pooled for analysis by time period on pasture (*i.e.* for two, four, six, eight weeks and weeks two to eight combined). From this, the overall mean larval numbers (Control and BioWorma) at each time point and mean values for weeks two to eight were calculated.

The larval determinations at each time interval were not independent as for each trial the samples all came from a pooled composite from the same experimental animal. It was therefore appropriate to consider these were not completely independent and to use a repeated measure analysis of variance (ANOVA) for comparison of the results. Hence the data were analysed using repeated measure ANOVA, with non-treated faeces (Control) *versus* treated faeces (BioWorma) and trial number as the main effect variables, with $P < 0.05$ regarded as significant. A series of interactions were included in the analysis. For each species of animal, the sites differed between trials so only trial number was used as a variable in the data analysis.

3. Results

Statistical analysis showed that the FECs of the Control and BioWorma groups were not significantly different at a significance level of $P < 0.05$ at the time of collection in all trials except for the third horse trial, where the mean value was significantly higher ($P = 0.044$) in the BioWorma group due to natural fluctuations in the period between the time of collection of the Control and BioWorma samples. This posed an additional challenge for the test product to demonstrate a substantial reduction in pasture larval values compared to the Control group.

Mean larval counts in the herbage samples for each trial are shown in Tables 4, 5 and 6 for horses, cattle and goats respectively (average for both sites), by week and for weeks 2–8 combined, including the overall

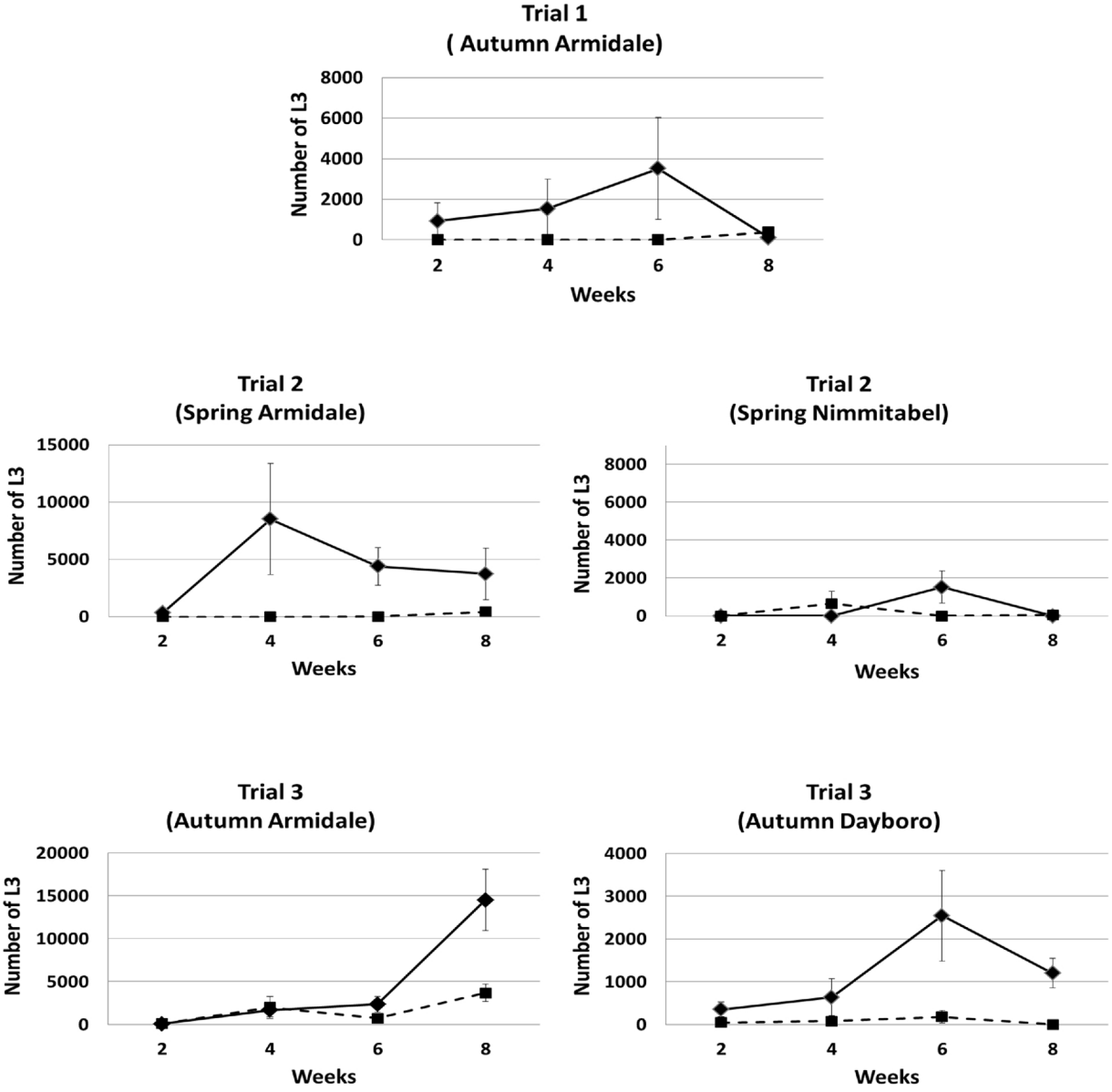

Fig. 1. Infective horse larvae numbers (L3) in herbage samples (mean ± SE) for all sites in the horse trials. Trial numbers, locations and seasons as indicated. Control (▬◆▬), BioWorma (- -■- -).

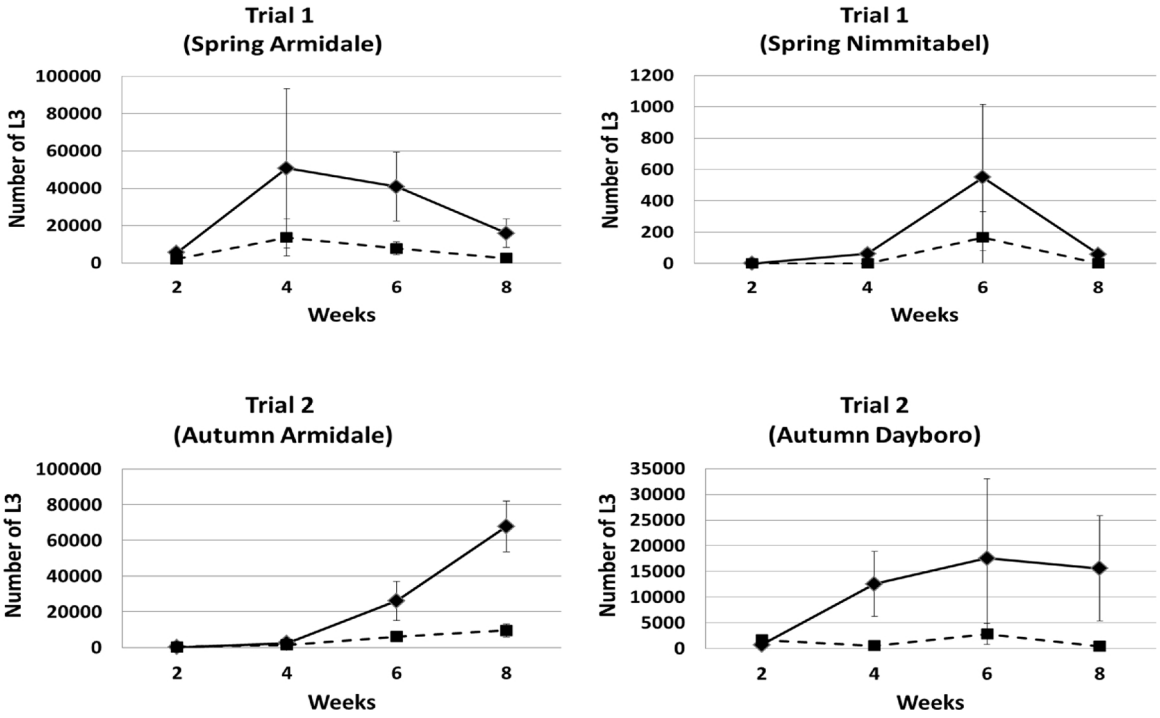

Fig. 2. Infective cattle larvae numbers (L3) in herbage samples (mean ± SE) for all sites in the cattle trials. Trial numbers, locations and seasons as indicated. Control (▬◆▬), BioWorma (- -■- -).

mean values (all trials combined). Results for each individual trial site in all of the trials are shown in Figs. 1–3 and summarised in Table 7. The pattern of larval emergence onto the pasture over the period of monitoring varied between trials and sometimes between locations within a trial. Generally, over the trial period the larval numbers increased to a maximum and then began to decline.

3.1. Horse trials

The reduction in number of horse worm larvae on pasture following BioWorma treatment compared to the Control (Table 4) showed that the effect of treatment over the eight week period was significant ($P < 0.05$), as was the week of sampling after treatment. There were significant interactions between the time after treatment and the trial and between time, trial and treatment.

The overall mean larval count for the BioWorma treated faeces over the eight week observation period was significantly reduced compared to the Control faeces (Table 4), with mean values of 2248 larvae for the Control samples compared with only 363 larvae for the BioWorma treated samples, a reduction of 84% ($P = 0.004$). Variability was marked across the trials but a significant difference was also observed at six weeks post treatment (Table 4).

3.2. Cattle trials

The reduction in the number of cattle worm larvae on pasture following BioWorma treatment compared to the Control (Table 5) showed that the effect of treatment over the eight week study period was significant ($P < 0.05$) and this varied over time. There was a significant interaction between the individual trials over time.

The overall mean larval count for the control samples over the eight week period for the BioWorma treated faeces was significantly reduced compared to the Control faeces (Table 5), with mean values of 16,038 larvae for the Control faeces compared to 3059 larvae for the BioWorma treated samples, a reduction of 81% ($P = 0.006$). Variability was marked across the trials but a significant difference was also seen at eight weeks post treatment (Table 5).

3.3. Goat trials

The reduction in the number of goat worm larvae on pasture following BioWorma treatment compared to the Control (Table 6) showed that the effect of treatment with BioWorma over the eight week period was significant ($P < 0.05$) and there was also a significant interaction between time and trial.

Variability was marked by week but the overall mean larval count for the BioWorma faeces over the eight week period was significantly reduced compared to the Control faeces (Table 6) with mean values of 12,866 larvae for the Control faeces compared with only 1834 larvae after BioWorma treatment, a reduction of 86% ($P = 0.01$).

3.4. All trials combined

Figs. 1–3 show that in most cases the larval numbers reached a maximum (typically at week 6) and then declined. Comparison of the mean numbers of parasitic larvae found on the herbage samples surrounding the Control and BioWorma faecal pats over the 8-week period allowed the calculation of the degree of reduction in pasture larval burden due to use of the test product. These values are summarised in Table 7. For each species of animal, substantial reductions were seen in each trial at each trial site, except for the Nimmitabel site of goat trial 1, where unseasonable freezing conditions were experienced shortly after the placement of the samples onto the pasture.

The difference in pasture larval counts between the groups demonstrated the ability of BioWorma to prevent the emergence of infectious larvae from the faeces of horses, cattle and goats onto the pasture. Average percent reduction (%) in larval numbers on pasture across all trials for horses, cattle and goats were 84%, 81% and 86% respectively (Tables 4–6).

4. Discussion

In these placebo-controlled studies carried out in Australia the effect of BioWorma (providing *D. flagrans* strain IAH 1297 at 3×10^4 chlamydospores/kg bodyweight daily) when administered to horses, cattle

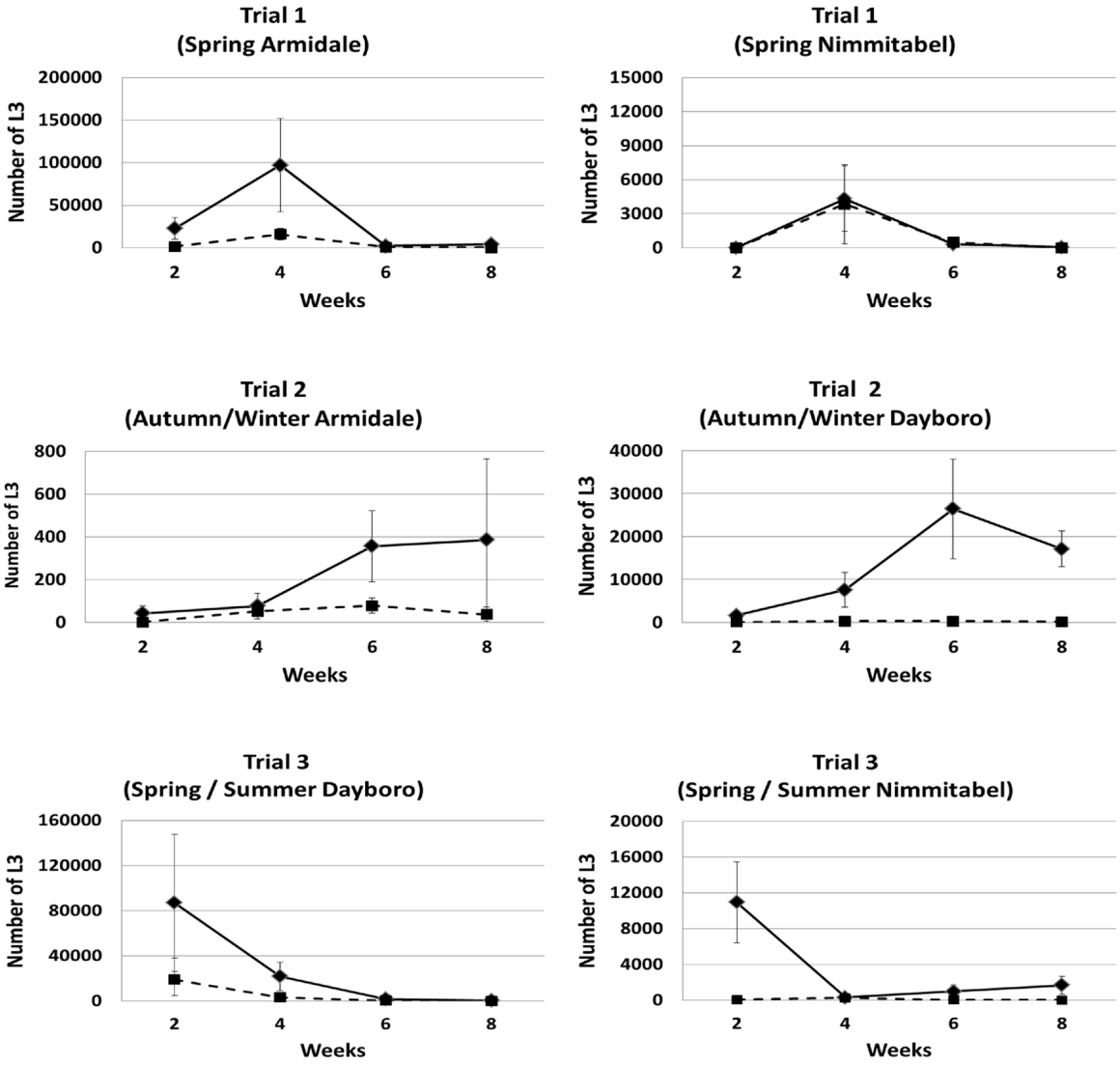

Fig. 3. - Infective goat larvae numbers (L3) in herbage samples (mean ± SE) for all sites in the goat trials. Trial numbers, locations and seasons as indicated. Control (–◆–), BioWorma (- -■- -).

Table 7
Mean % reduction of infective larvae on herbage surrounding the faecal pats after treatment with BioWorma for all trials, by location.

Trial code	Season	Mean % reduction of infective larvae on herbage surrounding the faecal pats over the eight week period		
		Dayboro	Armidale	Nimmitabel
Horse trial 1	Autumn	–	94	–
Horse trial 2	Spring	–	97	53
Horse trial 3	Autumn	94	65	–
Cattle trial 1	Spring	–	77	75
Cattle trial 2	Autumn	88	82	–
Goat trial 1	Spring	–	85	8[a]
Goat trial 2	Autumn / Winter	99	81	–
Goat trial 3	Spring / Summer	80	–	98

[a] unseasonable freezing conditions experienced shortly after placement of samples.

and goats was evaluated. The results showed substantial reductions in pathogenic GIN larvae for all three host species over an eight week period post treatment, across a range of climatic zones and in different seasons. However, for goats in Trial 1 at Nimmitabel in spring, freezing conditions were most likely responsible for destroying the GIN eggs and larvae in both BioWorma and Control group faeces. This was not observed at the duplicate site in Armidale. In most trials the larval numbers reached a peak (typically week 6) then decreased, which is thought

to result from natural decline (O'Connor et al., 2006) due to various effects depending on larval identity, local weather conditions and pasture microclimate, pasture type and length, migration beyond the herbage collection zone and predation (Barger et al., 1972).

In studies published by other groups in horses, reductions in infective larvae on pasture up to 99% were reported (Fernandez et al., 1999a). Braga et al. (2009) reported up to 73% reduction in FEC and Larsen et al. (1996) reported large reductions in worm burdens of tracer foals (88% reduction in arterial *Strongylus vulgaris* larvae, 96% reduction in *S. edentatus* larvae in flanks and kidneys, plus average 82% reduction in cyathostomes in the mucosa and 71% reduction in the dorsal lumen). Larsen et al. (1996) also reported reduced incidence of clinical parasitosis in tracer foals and more than doubled weight gain. Reductions in larval emergence from coproculture of up to 78% were also reported (Braga et al., 2009).

In cattle, the most common measure studied was the number of infective parasite larvae on pasture, where reductions of up to 90% were observed (Hertzberg et al., 2007). In addition, reduced infection levels in the grazing animals were demonstrated by means of: (1) the number of parasite eggs per gram (epg) in their faeces, where reductions of approximately 60% were obtained (Dias et al., 2007; Assis et al., 2012, 2013) and (2) total worm count, with up to 87% reduction achieved (Wolstrup et al., 1994). In a number of trials clinical parasitosis occurred in the control group but was prevented in the treatment group (Larsen et al., 1995; Nansen et al., 1995; Fernandez et al., 1999b; Sarkunas et al., 2000). Improved liveweight gain of up to 25% was observed during the grazing period, resulting from reduced loss of productivity due to parasitism (Nansen et al., 1995).

In goats, large reductions in infective larvae on pasture were demonstrated, including their "virtual elimination" in the trials of Sanyal and Mukhopadhyaya (2002). Reduced infection levels were demonstrated by reductions in FEC of grazing animals (58% reduction reported by Epe et al., 2009) and reductions in total worm counts of tracer animals of up to 87% (Vilela et al., 2012). Similarly, clinical parasitosis was prevented (Vilela et al., 2012) and the number of anthelmintic treatments required was reduced (Maingi et al., 2006; Vilela et al., 2012). In addition, reduction of larval emergence from coproculture of up to 99.5% was reported (Paraud et al., 2007).

In these published studies referenced above, a much higher dose of *D. flagrans* spores (typically 1×10^6/kg b.w./day) was required to achieve the effect, compared to the BioWorma trials reported here (3×10^4/kg b.w./day). As stated in Healey et al. (2018), reasons for this could be the use of a highly efficient isolate of *D. flagrans* and the methods used in culture and processing of chlamydospores of this isolate before inclusion in BioWorma.

5. Conclusion

Overall, the studies with BioWorma reported here show substantial and statistically-significant ($P < 0.05$) reductions in the emergence of infective nematode larvae from the faeces of horses, cattle and goats. It is suggested that use of BioWorma in these host species would, therefore, lead to decreased levels of GIN infection in animals grazing pasture where this product is used and would provide an alternative means of controlling parasitic nematodes.

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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 this draft report and chose to submit it for publication. Drs Knox, Chambers and Ms Lamb had primary responsibility for the study designs and between them were wholly responsible for the conduct of the field trials and interpretation of the data. Statistical analysis was conducted by Associate Professor Peter Groves. Authors Knox, Chambers, Lamb and Groves have no commercial interest in BioWorma.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2018.06.017.

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