Veterinary Parasitology 253 (2018) 48–54

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

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

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

[a] International Animal Health Products Pty Ltd., P.O. Box 6199, Blacktown, NSW, 2148, Australia
[b] CSIRO F.D. McMaster Laboratory, Locked Bag 1, Armidale, NSW, 2350, Australia
[c] Invetus Pty Ltd., Trevenna Rd., Locked Bag 6865 West, Armidale, NSW, 2350, Australia

ARTICLE INFO

Keywords:
BioWorma
Duddingtonia
Nematophagous fungi
Parasitic nematodes
Nematode control

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

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

https://doi.org/10.1016/j.vetpar.2018.02.010
Received 30 June 2017; Received in revised form 25 January 2018; Accepted 2 February 2018

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