Anti-parasitic benefit of nematophagous fungi and *Haemonchus contortus* vaccine in small ruminants

Lisa H. Williamson, DVM, MS, DACVIM
Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

Abstract

As anthelmintic resistance continues to escalate, the need for alternative parasite control methods in small ruminant has increased. Copper oxide wire particles and sericea lespedeza are commercially available in the United States. Several other novel control methods, such as feeding nematophagous fungal chlamydospores, and vaccination against *Haemonchus contortus*, hold great promise for control of gastrointestinal nematode infections in small ruminants. The *Haemonchus contortus* vaccine is licensed for use in sheep in Australia, but is not yet available in the United States. Experiments conducted around the world have demonstrated the excellent nematode controlling advantages of feeding *Duddingtonia flagrans* chlamydospores to grazing livestock. A commercially available product is expected to be available in the near future.

Key words: small ruminant, parasites, nematophagous fungi, haemonchus

Résumé

Comme antihelmíntico la résistance continue de s'aggraver, la nécessité de trouver d'autres méthodes de contrôle des parasites en petits ruminants a augmenté. Les particules d'oxyde de cuivre et fil lespedeza Sericea sont disponibles dans le commerce aux États-Unis. Plusieurs autres méthodes de contrôle des nouveaux, tels que l'alimentation des chlamydospores, champignons nématophages et vaccination contre Haemonchus contortus, très prometteuses pour le contrôle des nématodes gastro-intestinaux chez les petits ruminants. L'Haemonchus contortus vaccin est autorisé pour utilisation chez les moutons en Australie, mais n'est pas encore disponible dans les États-Unis. Des expériences menées dans le monde ont démontré l'excellent contrôle de nématodes avantages alimentation Duddingtonia flagrans chlamydospores à du bétail au pâturage. Un produit commercialement disponible devrait être disponible dans un proche avenir.

Introduction

Producers in many parts of the world struggle to manage parasitic nematodes such as *Haemonchus contortus* infection in sheep and goats. As anthelmintic resistance continues to rise in prevalence as well as magnitude, this undertaking is becoming more daunting.[12] Nontoxic biological control strategies, such as feeding the nematode-trapping microfungus *Duddingtonia flagrans*, have been shown to effectively reduce pasture larval contamination and worm burdens in many grazing species.[13,15] Efforts directed at developing a viable way to deliver the beneficial chlamydospores in a practical manner could soon result in release of a commercial product.[17] Another alternative parasite control strategy that has received research attention is the use of immunizing agents to induce protection against *Haemonchus contortus*. Currently a *H. contortus* vaccine[a] licensed in Australia is showing excellent protection in sheep under field conditions.[26]

Nematophagus Fungi

Nematophagous fungi occur naturally in soil and fecal material in many parts of the world.[15] Over 200 species are recognized, all of which utilize nematodes as an energy source. Of the known nematode-trapping varieties, *D. flagrans* has emerged as the most successful candidate for use in parasite control because it is the only nematophagous fungus studied thus far that significantly reduces infective trichostrongyle larvae in feces of grazing animals such as horses, cattle, pigs, small ruminants, giraffe, antelope, and gerenuk.[13,15,30] Sufficient numbers (approximately 10%) of the thick-walled chlamydospores survive transit through the digestive tract.[21] Once deposited in feces, the chlamydospores germinate, and form predacious traps on their hyphae, varying from sticky knobs, branches, 3-dimensional nets, to constricting rings.[13,15,17] The traps ensnare parasitic larvae as they migrate in feces, then fungal hyphae penetrate the cuticle and digest the larvae. As a result of this fungal activity, less larvae escape the fecal material, resulting in lower pasture infectivity. Nematode-trapping efficacy of *D. flagrans* is determined by measuring larvae in feces or in the herbage around feces, and by assessing acquired parasitic burdens in tracer animals.[17] Trap formation occurs at temperatures from 50 to 95 °F (10 to 35 °C), and gradually declines over 2 to 3 weeks.[11]

Several projects provided insights into dosage, and optimal treatment intervals of fungal spores in small ruminant systems. Peña et al evaluated doses of 50,000, 100,000, 250,000, 500,000, and 1,000,000 spores/kilogram body weight (kg BW) in feed for 7 days in adult sheep predominantly infected with *H. contortus*.[22] Larvae were reduced by 97.5% in feces from all treatment groups by day 3. In a second trial on lambs with significant parasitic burdens, 500,000

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spores/kg BW were fed daily for 7 days.[22] Fecal larvae were decreased in all fungus-fed groups by 95 to 96% on days 4 through 7. The effect was transient, however, as larval numbers rebounded once spore feeding ceased.[22] A dose titration experiment was conducted in Georgia on goats infected with *H. contortus*, *Trichostrongylus colubriformis*, and *Cooperia* spp.[29] Goats were fed spores each day at doses ranging from 500,000 to 50,000 spores/kg BW. By day 2 until day 8 (when treatment was stopped), larvae in feces were reduced by 93.6, 80.2, 84.1, and 60.8% in the 500,000, 250,000, 100,000, and 50,000 spores/kg BW treatment groups, respectively. Within 3 to 6 days, larval reduction was no longer apparent.[29] An experiment conducted in Malaysia on sheep and goats indicated that feeding spores at a daily dose rate of 250,000 spores/kg BW reduced fecal larval recovery by over 99%, compared to an 80 to 90% reduction when a lower dose was fed.[7] Waghorn et al also conducted a dose titration study using both goats and sheep.[31] The lambs and kids were experimentally infected with *H. contortus*, *Ostertagia* (*Teladorsagia*) *circumcincta* or *Trich. colubriformis.* Once the infections were patent, animals were dosed with 250,000 or 500,000 spores/kg BW for 2 consecutive days.[31] Average larval recovery was reduced by 78% in both sheep and goats; no species differences were noted. Interestingly, both the lower and higher dose were equally effective at reducing *H. contortus* and *Trich. colubriformis* larvae, but *O. circumcincta* reduction was higher when the 500,000 spores/kg BW dose was used.[31] In summary, doses of 250,000 to 500,000 spores/kg BW appeared to provide the most consistent and substantial broad spectrum benefit, but feeding 500,000 spores/kilogram BW improved efficacy against *O. circumcincta*. Terrill et al compared daily spore feeding with intermittent spore feeding, in goats. His work demonstrated that daily feeding was more effective at maintaining larval reduction than intermittent (every second or third day) feeding of *D. flagrans* spores.[29]

Fontenot et al studied the effect of feeding 500,000 spores/kg BW to grazing sheep that were naturally infected with *H. contortus.*[10] During the 18-week trial, infective larvae were reduced by 78.9 to 99.1% in feces, and gastrointestinal nematode burdens were reduced by 96.8% in tracer animals. No significant differences were noted in fecal egg count (FEC), packed cell volume (PCV), or animal weight between the naturally infected control and fungus-fed sheep.[10] These data illustrated that feeding *D. flagrans* provides benefit by reducing exposure to infective larvae, and not by reducing established parasitic infections. In an experiment where lambs were first cleared of their natural infections by anthelmintic treatment prior to going into a long-term feeding trial in Malaysia, fungal-fed lambs had lower FEC and better weight gain than untreated controls on pasture.[6] Similarly, animal benefit was noted in grazing sheep in Brazil that received anthelmintics prior to entering the trial.[25] Sheep that received *D. flagrans* spores in feed daily for a year had lower FEC and required fewer anthelmintic treatments than control sheep on pasture that received the same supplemental feed, but no spores.[25]

One of the biggest obstacles to practical application of nematophagous fungal spores in a field setting is finding a suitable delivery system. The ideal delivery method needs to be convenient, palatable, cost-effective, and have a reasonable shelf life. Most fungal spore trials were conducted by mixing spores in a feed supplement, or by using an oral dose syringe to deliver individual treatments. However, other delivery methods have been investigated, such as use of spore-impregnated feed or mineral blocks, and administration of spores in a slow-release device.[14] The main problems with feed or mineral blocks were 1) intake was too variable, and 2) the moisture content of the preparations limited shelf life to less than a week. Although sustained-release products were under investigation at one time, the Danish Company developing *D. flagrans* products stopped production about 10 years ago, so investigations ceased. Currently, daily feeding is the most feasible delivery option. Nutritional pellets containing *D. flagrans* chlamydospores were developed in Mexico for use in sheep.[9] Storage of the pellets under various conditions (refrigerated or room temperatures, within or outside of plastic bags) for 8 weeks did not reduce the larval trapping ability of the fungus.[9] Another promising recent development is that International Animal Health Products (IAHP) in Australia has established a *D. flagrans* strain that can provide larval control at a dose as low as 30,000 spores/kg BW, which will make the cost of commercialization reasonable.[17] International Animal Health Products is working with federal agencies in the United States to get the fungal feed additive registered for commercial use in zoos and small ruminant farms.[17]

Haemonchus contortus **Vaccine**

Studies have been conducted in sheep to investigate the immunoprophylactic potential of *H. contortus*-derived hidden gut antigens (H-gal-GP and H11),[18,27,28] cysteine proteinases,[23,24] and excretory-secretory products.[1,8] All these experimental vaccines caused a reduction in FEC and *H. contortus* worms in vaccinated animals. However, use of "hidden" somatic antigens in vaccines offer an advantage over soluble and exposed antigens, because the hidden antigens are not recognized by the immune system during natural infection.[20] As a result, no natural selection pressure is imposed, so blood-feeding worms should remain highly susceptible to antibodies in the vaccinated host's blood.[20] However, repeated vaccination within the same grazing season is necessary to maintain the protective effects when using hidden gut antigens.[16] Despite the remarkable success achieved by the experimental hidden gut antigen vaccine, commercialization was only thought feasible if a recombinant version of the antigen proved to be effective. Mass production of enough native antigen to meet market demand did not appear to be feasible. Unfortunately, recombinant antigens failed to achieve the reductions in FEC and worm counts at-

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tained by native antigen vaccines.[5,19] However, it was recently discovered that a much lower dose than what was used in the initial field trials also induced a protective response in sheep. This finding renewed interest in commercial vaccine development. Material obtained from 1 infected sheep could provide sufficient worm antigen to make several thousand doses.[2] In addition, a worm-harvesting machine was developed that could efficiently harvest worms from slaughtered lambs without reducing the carcass value.

A field trial was conducted in western Australia from October 2010 to January 2011 using the vaccine produced by Moredun Research Institute scientists to determine an optimal dosage and injection regimen in crossbred lambs.[2] The lambs were dosed with *H. contortus* larvae twice a week during the first half of the trial, then at weekly intervals for the remainder of the trial. The mean FEC of control lambs was 7,000 eggs/gram, despite the fact 7 of the 20 controls received anthelmintic treatment for haemonchosis. Treated sheep received a 2, 5, 10, or 50 microgram vaccine dose. A second dose was given 3 weeks later, and a third dose was given either 6 or 8 weeks after the second dose. The sheep that received the "early" 3rd dose at 6 weeks, received a 4th dose 6 weeks after the 3rd dose. A significant reduction in FEC was seen in sheep that received the 3rd dose of vaccine at 6 weeks compared to 8 weeks after the 2nd dose. The researchers concluded that a dose as little as 5 micrograms of *H. contortus* native gut antigen vaccine was sufficient to provide a high level of protection during periods of intense larval challenge.[2] The mean fecal egg count (FEC) was reduced by over 85% in vaccinates compared to controls.[2] Further, vaccinated animals gained more weight, had higher PCV, and fewer animals required anthelmintic treatment compared to controls.[2] Study results indicate that 2 priming doses should be given 3 weeks apart, and that vaccine boosters should be given at 6-week intervals to maintain protective effect during periods of high parasitic challenge.[2] Use of the vaccine confers between 75 and 95% protection.[3]

As a result of the successful collaboration between Moredun Research Institute scientists in Scotland and the Department of Agriculture and Food in western Australia, Barbervax® was released in October 2014 for use in lambs.[3] It is now approved for use in sheep of all ages.[3] The first batch of Barbervax® consisted of 30,000 doses, which were sold in 1 week by word of mouth, in Australia. Barbervax® is marketed in 250 mL containers, and unopened containers have a refrigerated shelf life of 2 years.[3] Each 1 mL dose consists of 5 *ug* native antigen and 1 mL of saponin adjuvant. Current recommendations in previously unvaccinated suckling lambs is to give 2, 1-mL doses subcutaneously, 3 weeks apart, followed by a booster 6 weeks later at weaning time. Re-vaccination (1 mL, subcutaneously) every 6 weeks is recommended during periods of high *H. contortus* exposure. An anamnestic response occurs in vaccinated sheep, so priming doses are not necessary the following grazing season. Vaccinations are given at 6-week intervals during "*Haemonchus* season" (warm, wet times of the year) in subsequent years.[3] The manufacturer plans to increase Barbervax® vaccine production over the coming years, and anticipates marketing the vaccine in other countries in the future.[3] Applications have been made to South African regulatory agencies to allow use of the vaccine in that country, marketed under the name, Wirevax®.[4] Barbervax® vaccine is currently undergoing 33 field trials in various species around the world.

Conclusions

Novel parasite control strategies will benefit producers by reducing reliance on conventional anthelmintics. Biological control methods such as nematophagus fungi, and use of *H. contortus* vaccines will not replace the need for judicious use of anthelmintics, sensible pasture management practices that break the parasitic life cycle, and genetic selection for traits such as resistance and resilience. Instead, these tools will need to be used as part of an integrated parasite control plan. Nematophagous fungi should provide a safe, nontoxic way to reduce infective larvae on pasture, but spores will need to be administered to each animal on a daily basis for maximum benefit. The cost could limit use of the product to farms with small numbers of animals, and to use in valuable collections such as grazing zoo animals. Experiences in the field with Barbervax in Australia have shown the vaccine reduces morbidity, the need for rescue anthelmintic treatments, and pasture infectivity when the recommended protocol is followed. Although the vaccine is not expensive, some producers will be unwilling or unable to vaccinate their animals themselves, or hire a veterinarian to vaccinate their animals every 6 weeks. However, in parts of the United States, and elsewhere around the world where total anthelmintic failure is currently threatening the future of the small ruminant industry, these new parasite management tools will be a welcome addition.

Footnotes

[a]Barbervax®, Albany Laboratory of the Department of Agriculture and Food, Western Australia

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