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Biological control of parasitic sheep helminths



Key external stakeholders:

Sheep and Cattle producers, organic livestock producers, helminthologists, mycologists, veterinarians, agricultural advisors, pharmaceutical industry

Practical implications for stakeholders:

The evolution of anthelmintic resistance in nematode populations of economic importance in the livestock industry provides the impetus driving research on finding alternative and sustainable methods of parasite control. Among the alternative methods examined, biological control (BC) agents such as nematophagous fungi; predacious or endoparasitic, may offer a complementary control method in both conventional and organic farms thus reducing the reliance on anthelmintics and slow the development of anthelmintic resistance. In other countries, *Duddingtonia flagrans*, the predacious fungi which uses nets to trap the developing nematode larvae in faeces has been confirmed as a potential BC agent of endoparasitic nematodes of livestock. Information is limited on the potential of endoparasitic fungi as biological control agents of helminth parasites.

This study:

- Evaluated the potential of endoparasitic fungi to control endoparasitic helminths using *in vitro* and *in vivo* techniques. A number of endoparasitic fungi were identified as potential BC agents of endoparasites if methods other than oral administration of spores to animals to achieve efficacy or methods to protect the spores when passing through the gastrointestinal tract can be adopted.
- Identified a diverse range of predacious and endoparasitic fungi on Irish sheep pastures using conventional and molecular-based approaches and provided the first evidence for *D. flagrans* in Ireland.
- A simple PCR based diagnostic assay was developed for the accurate detection of *D. flagrans* which will be a valuable tool in the future evaluation of the bio control potential of this fungus

Main results:

- The study demonstrated significant potential for a number of endoparasitic fungi to be candidates for BC agents of parasitic nematodes if methods other than administering spores to animals, to achieve efficacy, can be adopted.
- The survey of habitats on Irish sheep farms highlighted a diverse range of predacious and endoparasitic nematophagous fungi and provided the first evidence for *D. flagrans* in Ireland.
- A PCR based diagnostic assay was developed for the accurate detection of *D. flagrans* which will be a valuable tool in the future evaluation of the bio control potential of this fungus. Using this assay *D. flagrans* was detected in 80% of farms surveyed as opposed to 10% using conventional methods.

Opportunity / Benefit:

A number of endoparasitic fungi with potential as BC agents of parasitic nematodes were identified. A simple PCR based diagnostic assay was developed for the accurate detection of *D. flagrans* which will be a valuable tool in the future evaluation of the bio control potential of this fungus.

Collaborating Institutions:

UCD

Teagasc project team: Dr. Barbara Good (PI)
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1. Project background:

Endoparasites cause major losses in sheep farming. Control of parasites has become heavily dependent on broad spectrum anthelmintics. The evolution of anthelmintic resistant parasites threatens this approach. Alternative methods of control are required so that a sustainable parasite control strategy is achieved. A biological control (BC) agent: such as nematophagous fungi would be an attractive option that could complement other methods of control. Found worldwide, these fungi are natural enemies of parasitic nematode and can be divided into two groups: predacious (use adhesive or non-adhesive trapping structures) and endoparasitic (produce spores that attach to cuticle or ingested by nematode) based on their nematode killing method. Research activities have focused on the predacious fungi, *Duddingtonia flagrans*, as a biological control of parasitic nematodes of livestock due to its ability to survive gastrointestinal passage and kill nematode larvae in the faeces. However, *D. flagrans* is not applicable to parasites that have prolonged egg stages e.g. *Fasciola hepatica* and *Nematodirus battus*. As the majority of work with egg parasitising fungi (endoparasitic) has concentrated on plant parasitic nematodes the project undertaken represents the first comprehensive attempt to evaluate the potential of endoparasitic fungi as BC agents of parasitic helminths of economic importance to the Irish sheep farming industry.

2. Questions addressed by the project:

- Nematocidal activity of endoparasitic fungi native to UK/Ireland and available from the Centraalbureau voor Schimmcultures Netherlands?
- Which fungi make good candidates as biological control agents?
- What nematophagous fungi are native to Ireland?
- Is *Duddingtonia flagrans*, the known nematophagous fungi, present in Ireland?
- The development of a rapid diagnostic test for *Duddingtonia flagrans*.

3. The experimental studies:

Experiment 1

- Assessed the ability of 20 endoparasitic nematophagous fungi (native to Ireland/UK, available from the Centraalbureau voor Schimmcultures, Netherlands) to parasitise *Teladorsagia circumcincta*, *in vitro* at 16°C and 24°C over 14 days (1,2,7 & 14)
- Six 'candidate' species were chosen based on their ability to significantly reduce larval development, to parasitise nematode eggs and produce resistant spores i.e. chlamyospore and their ability to parasitise *Nematodirus battus* eggs was determined

Experiment 2

- Assess the ability of the 'candidate' fungi to survive gastrointestinal passage in sheep and reduce larval development in faecal cultures. Chlamyospores were mass produced for each fungal species, lambs were administered fungal doses (5×10^5 chlamyospores per kg BW) and efficacy assessed following gastrointestinal passage.

Experiment 3

- Survey completed on 10 farms representing permanent sheep pastures to describe the most common nematophagous fungi present in a range of habitats. . Samples collected included soil, old and fresh ovine faeces.

Experiment 4

- Development of a PCR based assay for the detection of *D. flagrans*.

Experiment 5

- Molecular detection of nematophagous fungi with known potential as biological control agents, namely *Duddingtonia flagrans*, *Paecilomyces lilacinus* and *Verticillium chlamydosporium* in Ireland.

4. Main results:

Experiment 1&2

30 % and 55 % of the endoparasitic fungi tested were effective in significantly reducing *T. circumcincta* larval development over time in faeces incubated at 16 °C and 24 °C respectively. Five fungi namely, *Drechmeria coniospora*, *Fusarium incarnatum*, *Harposporium anguillulae*, *Haptocillium sphaerosporum* and *Lecanillium lecanii* were highly efficacious at both temperatures.

All six candidate fungi namely, *Fusarium coeruleum*, *Fusarium incarnatum*, *Harposporium anguillulae*, *Haptocillium sphaerosporum*, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, respectively, were capable of parasitising *Nematodirus battus* but their efficacy was affected following gastrointestinal passage. Alternative methods of fungal application or methods to overcome the deleterious effects of GI passage would need to be investigated if the potential of these endoparasitic fungi are to be exploited to their full potential in the future.

Experiment 3

The survey on farms highlighted a diverse range of predacious (n=12) and endoparasitic nematophagous fungi (n=16) and provided the first evidence for *Duddingtonia flagrans* in Ireland

Experiment 4

Developed a simple PCR based diagnostic assay was developed for the accurate detection of *Duddingtonia flagrans*. This will be a valuable tool in the future evaluation of the BC potential of this fungus.

Experiment 5

The PCR assay developed in this study detected *D. flagrans* in samples from 8 out of the 10 Irish farms surveyed. Likewise, *Paecilomyces lilacinus* was detected in material from 8 out of 10 farms surveyed while *Verticillium chlamydosporium* was confirmed in 5 out of 10 using PCR based assays. This represents the first record of this species in Ireland.

5. Opportunity/Benefit:

The study demonstrated significant potential for a number of endoparasitic fungi to be candidates for BC agents of parasitic nematodes if methods other than administering spores to animals, to achieve efficacy, can be adopted. The survey of habitats on Irish sheep farms highlighted a diverse range of predacious and endoparasitic nematophagous fungi and provided the first evidence for *D. flagrans* in Ireland. Moreover a simple PCR based diagnostic assay was developed for the accurate detection of *D. flagrans* which will be a valuable tool in the future evaluation of the bio control potential of this fungus.

6. Dissemination:

International conference

Presented at the 21st International Conference of the World Association for the Advancement of Veterinary Parasitology, Gent, Belgium, 19-Aug-2007,

National Conferences

Presented at the Agricultural Research Forum, Tullamore, Co. Offaly 15th March 2006 and 12th March 2007 and Irish Society for Parasitology annual meeting, CVRL, Co. Kildare 1st Dec 2006

Open Days

Presented at Sheep Open Days and Lab open days during Science Week

Main publications:

Kelly, P., Good, B., Hanrahan, J.P., Fitzpatrick, R. and de Waal, T. (2009). Screening for the presence of nematophagous fungi collected from Irish sheep pastures. *Veterinary Parasitology* 165: 345-349

Kelly, P., Good, B., Fitzpatrick, R., Hanrahan, J.P. and de Waal, T. (2008). Development of a PCR diagnostic test for the rapid detection of the nematophagous fungus *Duddingtonia flagrans*. *Mycological Research* 112: 1026-1030

Kelly, P (2008). Biological control of parasitic sheep nematodes. PhD thesis University College Dublin

Popular publications:

Kelly, P (2006). Biological control of sheep parasites TRResearch Teagasc IE p. 27-28

7. Compiled by: Dr. Barbara Good and Dr. Paula Kelly