Gastrointestinal nematodes in deer – summary of a PhD programme

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Introduction

In New Zealand parasites are an important economic and clinical problem (Audigé et al. 1998) and they have been acknowledged as a problem since the commencement of deer farming (Mason 1977, Watson and Charleston 1985, Audigé et al. 1998). The most important parasites in farmed deer are the lungworm *Dictyocaulus eckerti* that can cause severe disease in naïve animals (Charleston 1980, Mason 1985) and gastrointestinal nematodes (GIN). Due to the initial view that GIN were of limited importance, few studies were undertaken with these parasites alone. However, it is known that the most important GIN are located in the abomasum including the deer-specific nematodes of the subfamily Ostertagiinae (=Ostertagia-type; *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*) and *Trichostrongylus axei* as well as the recently identified *Trichostrongylus askivali*. Deer can be also infected with some GIN of sheep and cattle including; *Haemonchus contortus*, *Oesophagostomum venulosum*, *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *Nematodirus spp.*, *Chabertia ovina*, *Cooperia oncophora* and *Cooperia punctata* (McKenna 2009). The aim of this PhD was to better understand different aspects of GIN parasite infection in red deer, including pathogenicity, diagnosis and control.

Pathogenicity study

To date most studies (Wagner and Mackintosh 1993, Waldrup and Mackintosh 1993, Waldrup et al. 1994, Hoskin et al. 2000a, Hoskin et al. 2000b) have examined the combined effects of GIN and lungworm infections and thus it has not been possible to separate the effects of these two groups of parasites. The aim of this study was to understand the pathogenicity of gastrointestinal parasites in young deer. The effect of artificial infection with a challenge of GIN in young housed deer was investigated in two phases. Phase 1 compared one Control Group (n=12) and three infected groups (n=11). The latter comprised groups given a low dose (LD), medium dose (MD) or a high dose (HD) which were trickle infected three times per week with a mixed culture of deer-origin infective larvae obtained from naturally infected young weaner deer at Massey University. The infection comprised a number of species as follows in order of magnitude: *Oesophagostomum spp.* > *Ostertagia-type* > *Trichostrongylus* > *Haemonchus*. Weekly live weights, voluntary feed intake, faecal egg counts and blood samples were taken. Groups were euthanized after 10, 11 and 13 individual doses in the LD, MD and HD groups respectively. This was earlier than originally planned but was in response to many deer developing clinical signs. Faecal egg counts began to be positive at four weeks post-infection. Voluntary feed intake in the infected groups decreased rapidly after the first week and was followed by a decrease in live-weight gain from the second week. The serum albumin decreased over time in the three infected groups (HD>MD>LD) and serum globulin levels in the infected groups increased after a week of the first dose and remained high over the course of the study. Serum albumin to globulin ratios were significantly lower (p<0.001) in the infected groups than the Control Group from the second week onwards. The establishment rates of *Ostertagia-type* were similarly high in all infected groups. In the abomasum the worm
burdens from highest to lowest were: *Spiculopteragia spiculoptera > Spiculopteragia asymmetrica > Ostertagia leptospicularis > Teladorsagia circumcincta*. There were almost no worms in the small intestines. In the large intestine the overall establishment rates of *Oesophagostomum* spp. were low. Two species were recognized. *Oesophagostomum venulosum* was the predominant species with a small proportion of *Oesophagostomum sikae* also being identified (Patrelle et al. 2014). *O. sikae* has not previously been recognised in New Zealand although it does resemble *Oesophagostomum radiatum* which has been reported once previously. The cause of the clinical signs was due to the occurrence of numerous inflammatory nodules in the mucosa of the terminal small intestine and large intestine together with oedema and a general inflammatory response in the large intestine. It is most likely that *O. sikae* was the cause of this damage although there are previous reports of nodule formation in sheep with large infections of *O. venulosum*. This latter species is generally a non-pathogenic parasite and does not induce nodule formation in natural infections but in experimental infections they have been observed together with diarrhea (Goldberg 1952, Clark et al. 1978). When comparing infected versus control groups, the voluntary feed intake at week four was significantly lower for the infected groups (HD<MD<Control) and in week five the LD group was lower than the control group (LD<Control). Daily growth rates in weeks 3–4 were significantly lower in the infected groups (HD<MD<Control) and weeks 4–5, LD was lower than the control group (LD<Control).

Phase 2 of this study was undertaken to further explore the pathogenicity of these nematodes using lower infection rates than in Phase 1. It utilised the animals in the control group from Phase 1. The Infected Group (n=6) was given a trickle infection with a dose equivalent to 30% of the LD group of the same source of larvae as in Phase 1 whilst the remainder of the deer were in the uninfected Control Group (n=6). There were no obvious clinical signs of parasitism in these deer, although they were older than those infected in Phase 1. There were no significant differences in voluntary feed intake (p=0.326), or growth rates (p=0.075), no significant effect on serum albumin (p=0.053) and serum globulin (p=0.055). Faecal egg counts were observed in all animals in the infected group indicating that worm burdens had established.

In summary this study indicated there was a rapid reduction in weight gain and voluntary feed intake with even modest worm numbers and this was most likely due to the pathogenicity of *Oesophagostomum* spp. in deer.

Identification and distribution of nematodes in New Zealand

There is some knowledge about the most common GIN in red deer in New Zealand but there is little specific information on their prevalence, including species for which the preferred hosts are sheep or cattle. There is also no information on the prevalence of the newly recorded GIN in New Zealand including *Trichostrongylus askivali* and *Oesophagostomum sikae*. The aim of this study was to investigate the prevalence of different nematode species of deer around the country.

For the identification of GIN infective larvae (L3) in deer, a PCR-based method was used following a similar protocol to that described by Bisset et al. (2104), who developed species-specific DNA primers based on sequences of the internal transcribed spacer 2
(ITS-2) region of ribosomal DNA in sheep GIN. Additional primer sets were required to be developed for deer-specific GIN. To obtain the L3, faecal samples from 59 farms around the country were collected in conjunction with a Johne’s disease study (Verdugo et al. 2014). The faecal samples from each farm were bulk cultured for the development from eggs to L3. For this study 24 larvae from each farm were isolated and their DNA extracted and identified by PCR. In order of prevalence the results were O. venulosum > S. asymmetrica > O. leptospicularis > S. spiculoptera > C. oncophora > H. contortus > T. askivali > T. axei > T. vitrinus > O. sikae > T. colubriformis > T. circumcincta > C. curticei. The order of prevalence is similar to the proportions found in the L3 recovered from deer at Massey University for the pathogenicity study.

Infectivity of sheep and cattle gastrointestinal nematodes in deer

Red deer can be infected with some GIN of cattle and sheep, but the level of infectivity is unknown. Two indoor studies were conducted to determine the establishment rate of sheep and cattle GIN in young deer. Group sizes were n=5 for both studies. All animals were effectively treated when housed and then infected two weeks later. After four weeks they were killed for total worm counts. Establishment rates were assessed comparing worm counts to the infective dose where the L3 were identified morphologically.

Sheep-origin GIN: The establishment rates (%) in sheep and deer respectively were Haemonchus contortus (18.6, 10.5, p <0.05), Teladorsagia circumcincta (35.5, 1.0, p <0.001), Cooperia curticei (30.7, 0.1, p<0.01), Trichostrongylus spp. (74.9, 1.0, p<0.01) and Oesophagostomum + Chabertia spp. (19.9, 4.8, p<0.01). No T. colubriformis or T. vitrinus were seen in deer but were present in all sheep. No C. ovina were seen in any deer but were present in four of five sheep in low numbers.

Cattle-origin GIN: The establishment rates (%) in cattle and deer respectively were H. contortus (8.0, 19.0, p=0.18), Ostertagia ostertagi (31.0, 0.7, p<0.001), Cooperia spp. (72.0, 2.3, p<0.001) and Trichostrongylus spp. (19.0, 25.3, p=0.12). The majority (>98%) of Trichostrongylus spp. were Trichostrongylus axei in both hosts. In cattle >98% of Cooperia were Cooperia oncophora but in deer there were similar proportions of Cooperia oncophora, Cooperia punctata and C. curticei. A small number of Oesophagostomum venulosum were present in cattle and deer, but if comparing the establishment from the total number of larvae given they established better in deer than in cattle (p=0.016).

These results indicate that some sheep- and cattle-origin GIN can establish in red deer. The establishment of H. contortus and T. axei could allow sufficient burdens to build up to be clinically significant. O. venulosum is able to establish reasonably well in deer although still less successfully than in sheep, but it is considered of limited pathogenic significance. Importantly, almost no Teladorsagia/Ostertagia spp. of common sheep/cattle species or small intestinal sheep/cattle species established in deer.
Cross grazing with sheep or cattle for gastrointestinal and pulmonary nematode control in deer

The aim of this trial was to determine the effectiveness of an organised cross-grazing system between deer and sheep or cattle in controlling deer nematode parasitism. This was a replicated study in two locations and over two years for each location. In both years the study commenced at weaning at the end of February/beginning of March and ran for sixteen weeks. There were four treatment groups of 19–20 deer at each location: deer cross-grazing with calves (DC); deer cross-grazing with lambs (DS); deer-only grazing (DD); and deer grazing on their own being ‘suppressively treated’ with anthelmintics (SP). The decision to treat deer was based on ‘trigger’ criteria including: faecal egg counts ≥250 eggs/g; or faecal larval counts ≥100 larvae/g; or when the individual growth rate was <80% of the mean of the SP group. The key outcome was the number of treatments (NT) given to deer in each treatment group. In addition, two sets of three parasite-free ‘tracer’ deer per treatment were introduced in weeks three and eleven and killed five weeks later to quantify the species of parasites cycling in each treatment. The NT for the DS and DD groups was significantly greater than for the DC group (p>0.0001). In tracer animals there were significantly fewer abomasal *Trichostrongylus* spp. from the DC, DD and SP (p<0.0001) than in the DS group, and significantly fewer *Ostertagia*-type nematodes in the DS (p=0.017) and SP (p=0.004) than in the DD group. There were significantly fewer *Dictyocaulus* in DS, DC and SP (p<0.0001) than in DD treatment group. The DC and SP groups had significantly higher daily liveweight gain (p<0.001) than the other two groups. Results in this study demonstrated that cross-grazing with alternative ruminant species offers some advantages over a monoculture of young deer. However, the advantages varied between the use of sheep or cattle with regards the level of control of different species of parasites. Using the criteria of this study some animals in each treatment group still required treatments in both years on both properties suggesting that cross-grazing alone would not be sufficient to control GIN parasitism but was effective in controlling lungworm infection.

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