A study of the pathogenicity and diagnosis of gastrointestinal parasites in young farmed deer

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Abstract

Gastrointestinal nematodes have become more of an issue in deer in recent years. This study investigated the pathogenicity of challenge with a mixed infection of gastrointestinal nematodes in young deer. In Phase 1, 45 weaner deer were divided into 3 groups of 11 and one of 12 animals. These were given a trickle infection three times a week with a mixed culture of only gastrointestinal nematodes. These were nominated as the Low Dose (LD), the Medium Dose (MD) (3 times as many larvae as the Low Dose Group) and the High Dose (HD) Groups (5 times as many larvae as the Low Dose Group). By Week 5 the HD and MD groups were showing clinical signs of parasitism and having reached the endpoint criteria, were euthanased at this point. The LD group was euthanased a week later. A feature of Phase 1 was the number of *Oesophagostomum radiatum*-like nematodes in the large intestine which had caused obvious pathological change with granulomatous nodules in the large intestinal mucosa/submucosa. There was a decline in serum albumin, liveweight gain and feed intake which was different between groups being dose-dependent with values declining as HD>MD>LD. Faecal egg counts also increased in proportion to the size of the challenge. Eosinophil counts increased over control animals but there were no significant differences between groups. There were no differences in plasma pepsinogen concentrations between any groups. As a consequence of the observations in Phase 1 the control group was split into 2 groups of 6 deer and one of these was given a trickle infection with a dose of the same mixed larvae source but at 30% of that given to the LD group. There were no obvious clinical signs of parasitism in these deer although these deer were older than those infected in Phase 1. There were no significant differences in voluntary feed intake or growth rates, no effect on serum albumin, eosinophil or plasma pepsinogen concentrations. Faecal egg counts did increase. This study has indicated the rapid reduction in weight gain with even modest worm numbers, and in particular the pathogenicity of this *Oesophagostomum radiatum*-like parasite in deer.

Key words. Deer, gastrointestinal parasites, control, diagnosis, faecal egg count, worm count, species, eosinophils, pepsinogen, albumin, weight, *Oesophagostomum radiatum*-like parasite.

Introduction

The impact of gastrointestinal nematodes on young deer is poorly understood and our ability to diagnose them in live animals is equally poor except for estimating faecal egg counts. As yet there is no robust quantitative marker for gastrointestinal worms in the live animal. We know deer are infected by a relatively limited range of species, most of which are concentrated in the abomasum. The most pathogenic species are considered to be deer-specific nematodes in the sub-family Ostertaginae (=*Ostertagia*-type), namely three related species, *Spiculopteragia asymmetrica*, *Spiculopteragia spiculoptera* and *Ostertagia leptospicularis*. Small burdens of the sheep species *Teladorsagia circumcincta* have also been reported from deer. Earlier studies have investigated the impact of internal parasitism in a field environment (Johnson et al in prep). It demonstrated subclinical losses caused by

internal parasites, identified a number of markers of potential use for identifying the need or otherwise for anthelmintic treatment, and identified the species of internal parasites present. That study evaluated the combined impact of both lung and gastrointestinal nematodes, thus was not able to partition the effects to either category of parasite. Similarly, other earlier studies have investigated the combined effect of both types of parasites given together including (Hoskin *et al.* 2000a) and (Hoskin *et al.* 2000b). In both those studies faecal egg counts peaked 5-6 weeks after infection and then declined. In the former, where the worm counts were estimated after 11 weeks, the total worm burden represented less than 1% of the number given. In the latter, where the animals were killed soon after the peak egg count, the abomasal worm burden represented 20% of the infective dose.

There has been one short-term study following a single infection of deer with lungworm but growth rates were not reported (Johnson 2003). To date no studies involving just infections with gastrointestinal nematodes have been reported. The range of dose rates to be used in this experiment, for the *Ostertagia*-type parasites are similar to those used for gastrointestinal nematodes by Hoskin et al (2000b) where an effect on growth rate was seen.

Since deer were first farmed in New Zealand, it has been appreciated that lungworm were an important pathogen but the impact of gastrointestinal parasitism appeared minimal. However, the impact of gastrointestinal parasites has likely been underestimated and recent experience suggests they may be more important that originally appreciated. A recent complication is the identification of apparent anthelmintic resistance in these *Ostertagia*-type parasites (Hoskin So *et al.* 2005). This development is likely to focus more attention on these parasites and also likely to increase their importance in the field. A further development is the report of *Oesophagostomum radiatum* from deer (McKenna 1999) where deer with mild to moderate burdens of *Oesophagostomum venulosum* and *Oesophagostomum radiatum* were reported.

The aim of the present project was to determine the relative importance of different challenges of gastrointestinal nematodes on productivity and to obtain additional information about the relationship between markers for parasitism, clinical pathology and worm burdens.
The purpose of this study was to investigate the impact of artificial infection with a mixed challenge of gastrointestinal nematodes in young housed deer. The relative importance of three levels of challenge on productivity was compared. An earlier project (Johnson et al in prep) evaluated the impact of lung and gastrointestinal nematodes under field conditions where the rate of challenge was unable to be controlled.

Materials and Methods

This experiment was run in 2 phases.

Phase 1.
This involved 45 deer divided into three treatment groups of 11 and one control group of 12 animals. Infections were with a mixed culture derived from weaner deer that spring. The deer comprised one group trickle infected three times per week with a high challenge(HD), a second a medium challenge (MD) and the third a low challenge LD) with the fourth as an uninfected control group. Infections were originally intended to proceed for 7 weeks with a further 4 weeks to allow parasites to mature. However, the endpoint criteria that were devised to ensure animals did not suffer unnecessary during the trial, including onset of clinical signs,

were met earlier than expected, shortening the time over which the trickle infection continued and advancing the date of termination of the trial by euthanasia. The high and medium dose rate groups were euthanased in Week 4 and the Low Dose Group in Week 5.

Phase 2. Following the observation of rapid onset clinical signs in Phase 1 it was decided to undertake a supplementary study utilising the uninfected Control Group from Phase 1, but administering a dose lower than the Phase 1 low dose (LLD). This was divided into two groups of 6 animals. This study used the methodology for the Phase 1 study, although at commencement these young deer were at least 2 months older.

Table 2: Treatment regime showing number of larvae per individual dose given three times per week

Group	Individual Dose of *Ostertagia*-type (L3) larvae	Individual Dose of *Oesophagostomum* (L3) larvae	individual Dose of *Haemonchus* (L3) larvae	Individual Dose of *Trichostrongylus* (L3) larvae
Phase 1				
Control (n=12)	0	0	0	0
Low Dose (LD) (n=11)	500	667	8	75
Medium Dose (MD) (n=11)	1500	2000	25	225
High Dose (HD)(n=11)	2500	3333	42	375
Phase 2				
Control (n=6)	0	0	0	0
Lower Dose (LLD) (n=6)	150	200	2	23

Source of Larvae

Young weaner deer that were already shedding eggs in faeces were given intramuscular dexamethasone sodium phosphate (0.25mg/kg twice a week) to increase larval and egg counts, hence minimising the time needed for sample collection. Faeces were mixed with vermiculite and cultured at 23°C for 2 weeks and larvae were harvested with a standard Baermann apparatus. Larvae were then pooled. Those used comprised 0.6% *Haemonchus*, 6% *Trichostrongylus*, 40% *Ostertagia*-type and 53.3% *Oesophagostomum*. The number of larvae given to each group was calculated for the *Ostertagia*-type component as the original view was that the *Oesophagostomum* were not likely to be that pathogenic.

Measurements

Measurements included:

- Faecal egg counts - estimated using a modified McMaster Technique where each egg counted represents 50 eggs/g;

- Blood parameters - total serum protein, serum albumin and globulin, white blood cell counts (including individual components; neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cells, haemoglobin and packed cell volume. These were measured in a commercial laboratory using equipment calibrated for deer samples;
- Serum pepsinogen levels were determined employing a modified method of Berghen et al. (1987);
- Average daily weight gains - weighing once a week;
- Voluntary feed intake measured by pen (5-6 animals/pen).

In addition, worm counts were estimated using guidelines described by WAAVP (Wood 1995). In brief organs were recovered and stored at -20°C until further processed. After thawing a 5% aliquot was removed and sieved through a 38µm sieve and then parasites present were counted. Individual male worms of each genus in the abomasum were recovered to identify to species. Adults of both sexes of *Oesophagostomum* were recovered to identify these to species. In addition the abomasum and large intestine were subject to a pepsin digest to recover immature larvae. This comprised a digest mixture of 0.4% Pepsin (Pepsin A, BDH 390324L) + 1.7%HCl with the tissue digested for 2 hours. To calculate the percentage of each nematode species in the abomasum and large intestine, 50 male nematodes of each genus were identified on morphological basis. If less than 50 males were recovered then all available male nematodes were examined.

Statistical Analyses

Repeated measures ANOVA was used to test the effect of treatment group on:

- Faecal egg count (log transformed data).
- Blood parameters including pepsinogen.
- Average daily weight gain.
- Voluntary feed intake.
- Worm counts (one-way ANOVA).

Results

Faecal Egg Counts

*Phase 1***.** Faecal egg counts increased in proportion to the size of the infective dose and were eventually significantly different from each other (Figure 1).
Phase 2. Similarly for this supplementary experiment the infected animals had positive faecal egg counts by Week 4 and these increased until the time of slaughter (Figure 2).

Figure 1: Mean faecal egg count from pre-treatment to week 5 after treatment for each treatment group.

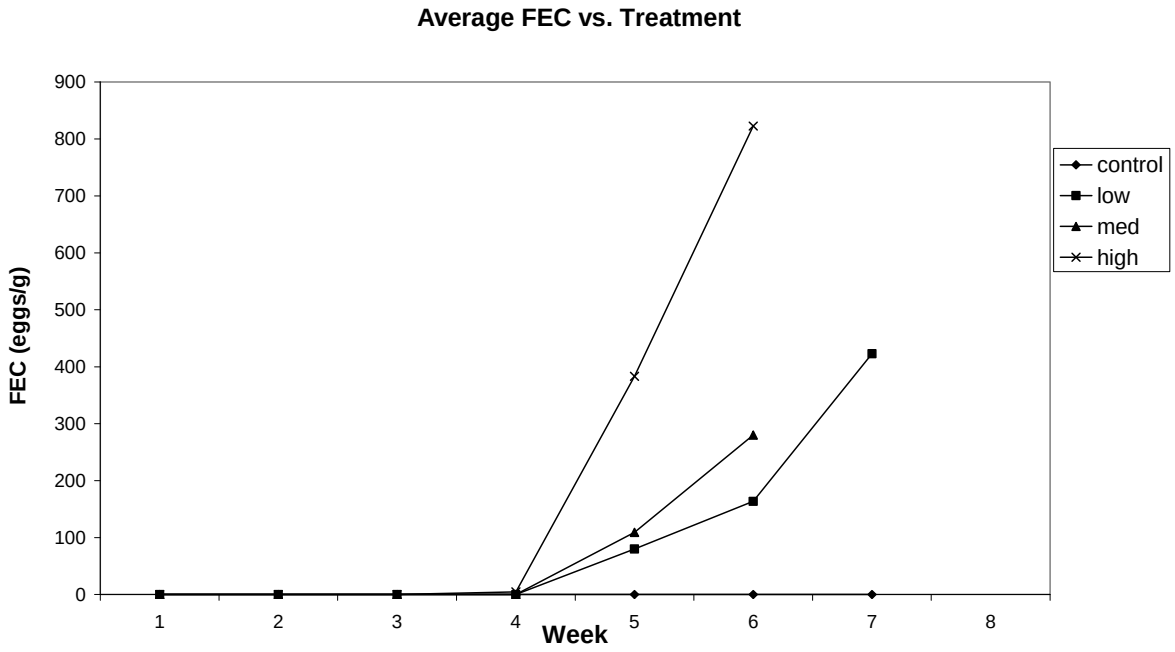
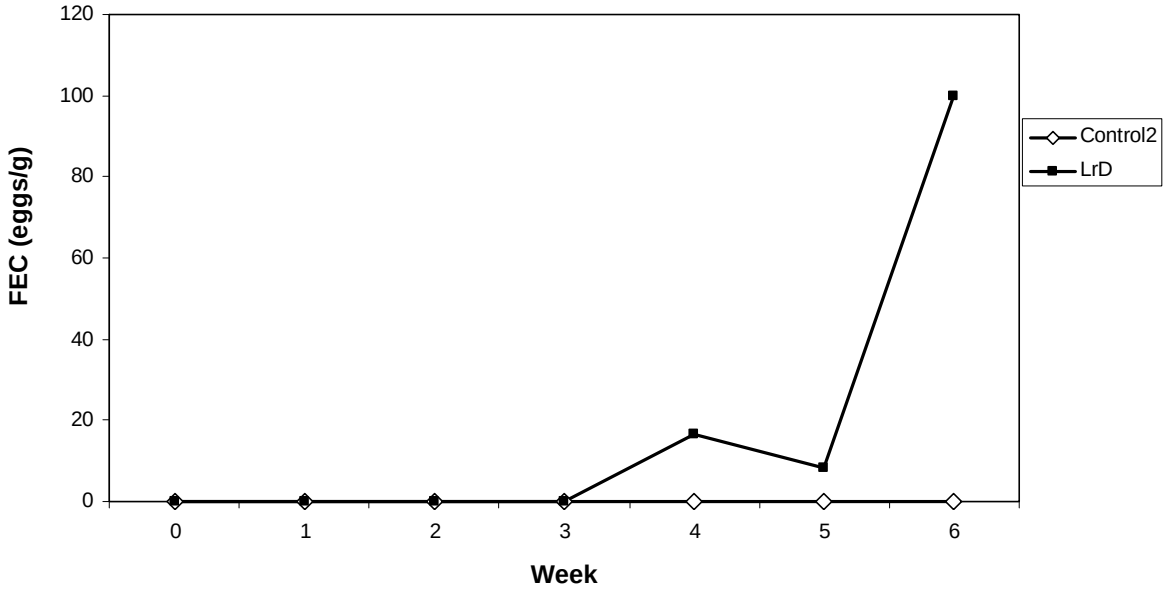


Figure 2. Mean faecal egg count from pre-treatment to week 6 after treatment



Worm Counts

Abomasum: Four species of the subfamily Ostertaginae were recovered from the treated deer, in order of frequency *S. spiculoptera* > *S. asymmetrica* > *O. leptospicularis* (including *O. kolchida*) > *O. circumcincta*. *O. kolchida* is a minor morph type of *O. leptospicularis* i.e. is the same species but just with a different morphological appearance. Negligible numbers of *Trichostrongylus* were recovered from any animal. Establishment rates were 35-37% for the

Ostertagia-like parasites, around 48% for *Haemonchus*. Establishment rates were <1% for *Trichostrongylus*.

Small Intestine: very few parasites were found in this organ in any animal.

Large intestine: Examination of the species of *Oesophagostomum* revealed the predominance of *Oe. venulosum* but some adult *Oe. radiatum*-like nematodes were also recovered. This latter observation is particularly significant given the extensive presence of nodules within the large intestinal mucosa which likely contributed to clinical signs of parasitism leading to early termination of the study. It is not possible to determine by simple morphological examination as to how many of either species were in the infective dose but use of molecular tools should allow that to occur in a retrospective study. Bisset (per com) investigated some of these adult parasites and has sequenced the ITS2 region, concluding that the *Oe venulosum* are consistent with others from sheep. However, the ITS2 of the *Oe radiatum* were similar to the genotype recorded on GenBank but there were 26 of 450 bases different (Bisset per com). Hence, these may be a deer-specific species. More research is needed to evaluate these findings.

Blood Parameters

Phase 1. Although total protein did not differ between groups there was a significant decline in circulating albumin in all treatment groups with levels declining in the same order as the number of larvae given. Thus the HD group declined rapidly but the LD group was slower to differ from the control group but was significantly different by Week 5.

There was a notable difference between control and treated groups in eosinophil counts with higher counts in infected groups ($p<0.05$) but no real difference between infected groups. There was also a difference with basophil counts with these being higher for infected groups compared to the controls ($p<0.05$). No significant differences were observed for other blood cell types.

There were no significant effects of treatment group on the pepsinogen values.

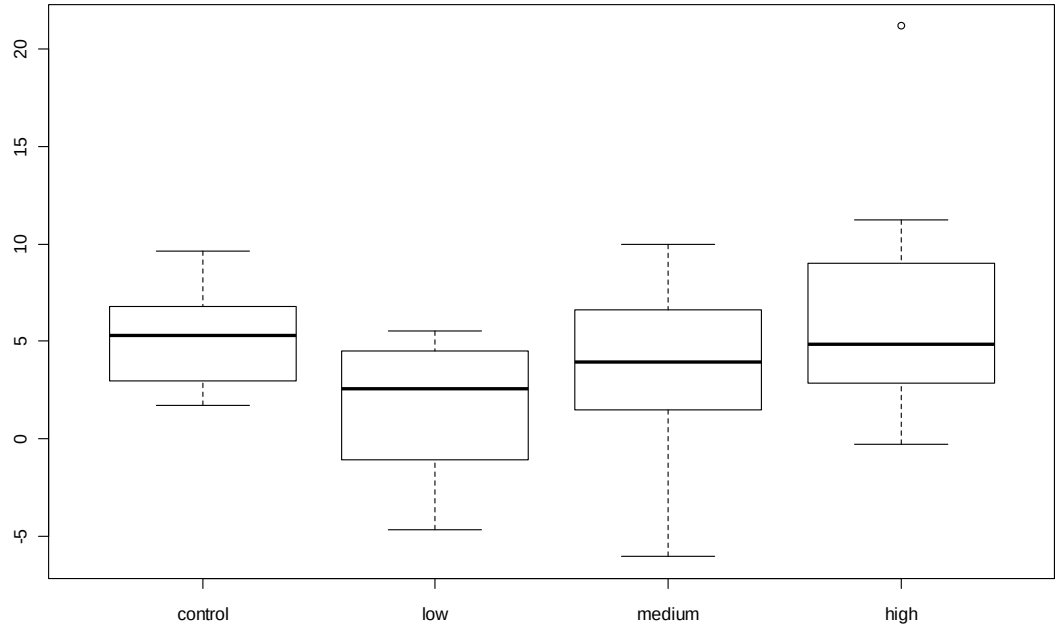


Figure 2. Box and whisker plot of pepsinogen values of all deer from Week 3 in Phase 1

There was a non-significant difference in the interaction between week and treatment group in both phases, **phase 1** (p= 0.2858) **phase 2** (p= 0.9348). There were no significant differences in eosinophil count between treatment groups (p=0.327). The control group eosinophil count was marginally non-significantly higher than that of the treatment group at Week 6 (p=0.057). There were no significant differences in basophil count between treatment groups within any given week.

Liveweight Gain and Feed Intake

Phase 1. There were a significant differences in feed intake between treatment group ($p<0.001$) with generally greater reduction in the higher dose groups. Those differences were mirrored by differences in weight gain.

Phase 2. There were no significant differences in growth rate between treatment groups within any given week.

Discussion

This study has shown that a high dose of a mixed culture of 2500 larvae/week led to the development of clinical signs of parasitism in Week 3, followed soon after by deer in the medium dose group (1500 *Ostertagia*- type larvae/week) in Week 4. Consequently both of these groups were euthanased at that time. The low dose group (500 *Ostertagia*- type larvae/week) were euthanased in Week 5 consequent to developing clinical signs in that group as well. However, the deer in the Lower Dose Group in Phase 2 did not display any clinical signs although there was a definite increase in faecal egg count in the infected animals.

The large intestines of all deer, but especially the high dose group, had numerous granulomatous nodules in the proximal large intestine accompanied by oedema of the mucosa and mesentery together with a general inflammatory response. This was almost certainly due to *Oesophagostomum* entering the mucosa and having a histotrophic stage. This has not been reported for *Oesophagostomum venulosum* infections in sheep but is a characteristic of other species of *Oesophagostomum* including *Oe. radiatum* in cattle. As the molecular evidence for this other species being *Oe. radiatum* is equivocal (Bisset pers com) assumptions about its life cycle need to be considered with care. In sheep *Oe. venulosum* is described as having a brief histotrophic stage in the small intestine as they were found as 3rd stage larvae in the mucosa 3 days after infection but by 4 days after infection had emerged as 4th stage larvae and proceeded to the large intestine with adults present after 13-16 days.

The prepatent period for *Oesophagostomum* at about 28-30 days is longer than for trichostrongyloid nematodes which is 2-3 weeks. *Oe. radiatum* L3 in cattle invade the mucosa of the small and large intestine where they develop to L4 larvae 5-7 days later before returning to the lumen 7-14 days after infection still as L4 larvae with the final moult about 17-22 days after infection. The minimum prepatent period for *Oe. radiatum* is reported to be 32-42 days, i.e. slightly longer than *Oe. venulosum.* Overall the development rate of *Oe venulosum* and *Oe. radiatum* is slower than for *Ostertagia*-type or *Haemonchus* species parasites. A key difference between these two *Oesophagostomum* species in their natural hosts is the propensity for inflammation to occur around the developing larvae of *Oe. radiatum* in the mucosa but not around those of *Oe. venulosum*. Although larvae of *Oe. venulosum* initially establish in the small intestine and *Oe. radiatum* may also invade the small intestinal mucosa, no *Oesophagostomum* have been found in the small intestine in this present study.

The establishment rate of *Oesophagostomum* overall was low as indicated by counts of adult parasites but consistent between the 3 infected groups. This count doesn't reflect that numerous larvae that were likely to be still within inflammatory nodules in the large intestine. A pepsin digest of the large intestine was not effective in releasing these larvae. Counting these overlaying nodules was also not possible. Whether this observed establishment rate is reflective of parasites that are not well adapted for deer or that they were undergoing their histotrophic stage in the large intestine more slowly remains to be determined. The low establishment rates reported for *Oesophagostomum* in this study may partly reflect a parasite not readily adapted for deer but is also distorted by the longer development time for this genus and the fact that the experiment was terminated early.

The observation that a *Oesophagostomum radiatum*-like parasite was found in these deer is consistent with an earlier report of *Oe. radiatum* found in hinds by (McKenna 1999). Previous studies involving worm counts in animals raised on the Massey University Deer Unit have only recorded *Oe venulosum* as being present (Hoskin et 2005; Hoskin et al 2000b) and obvious nodule formation in the large intestine has not been observed previously. In sheep *Oe. venulosum* is generally considered to be a parasite of minimal significance as it causes no obvious pathology, which is consistent with these earlier reports.

The source of larvae for this study were young naturally-infected weaner deer raised on the Massey University Deer Unit with faeces collected by attached faecal collection bag. This suggests recent introduction of this *Oe radiatum*-like species. Whether this is a finding of some significance or just a peculiarity of the design of this particular experiment remains to

be seen. There is no doubt these parasites were very pathogenic for these young deer. *Oe radiatum* are a species that are generally considered to prefer a warmer climate and consequently although found in New Zealand cattle are not considered to be an important species as the burdens are generally small. Whether these restrictions apply to this *Oe. radiatum*-like species is not known.

A study currently underway, which is investigating the parasites recovered from faeces of deer on >40 farms in New Zealand, will shed some further light on the prevalence of this nematode species in deer. It is also necessary to recover *Oe. radiatum* from cattle to investigate how similar the ITS2 region from those of deer are to others obtained directly from New Zealand cattle.

Overall establishment rates were remarkably similar between the high, medium and low dose groups for other types of worms. Immature adult *Ostertagia*-type parasites would be expected to emerge into the lumen of the abomasum at about 18 days after infection so the establishment rate needs to consider this time lag. Nevertheless, the establishment rate of about 35% for the *Ostertagia*-type and 48% for *Haemonchus contortus* is consistent with that expected from infections of young sheep with sheep-specific species (*Teladorsagia circumcincta*) and *Haemonchus contortus*.

The Ostertaginae species recovered were mostly deer-specific species with *S. spiculoptera* the most common species found. *S. kolchida* was the only minor morph species (of *O. leptospicularis*) found. The relationship between major and minor species in the sub-family Ostertaginae is still being determined but to date no difference in pathogenicity have been reported for related species in cattle and sheep.

There was a marked serum albumin decrease over the course of the study in Phase 1 with a clear relationship to the dose rate of parasite larvae (e.g. high dose group albumin decreased more over time than medium dose group which decreased more over time than the low dose group). This reflects a classic parasitology response related to damage to the gut and a rapid turnover rate of cells. In Phase 2 such a response with the lower dose was not seen and this may have been attributable to the older age at challenge, allowing deer to develop innate resistance to parasites.

Differences in Pepsinogen values were not significant between Groups and/or weeks indicating no likely change in abomasal mucosal histology occurred.

Voluntary feed intakes decreased rapidly over time with a classic “dose-rate” response. It is commonly accepted that gastro-intestinal parasitism reduces voluntary feed intake and the efficiency of feed utilization. The extensive pathology in the large intestine would almost certainly have influenced voluntary feed intake. The lower dose group in Phase 2 showed no significant reduction in intake but a trend downwards was starting in the infected animals.

There was no clear relationship evident in white blood cell counts but in terms of the constituents of white blood cells several showed a relationship with time. Eosinophils increased in Phase 1 infected groups but not in an obvious dose-dependent fashion in Phase 1 and no effect was seen in Phase 2. Interestingly, basophils also increased with time in infected groups in Phase 1 with a dose response evident.

Conclusion

This study has shown a rapid reduction in feed intake and weight gain in recently weaned deer even at the low dose given. Clinical signs of diarrhoea occurred within 5 weeks of commencement. A dose-related increase in faecal egg count was observed. Blood albumin and white blood cell changes were consistent with previous results in deer. Plasma pepsinogen was not diagnostic in parasite infection or worm count. This study identified what may be a deer-specific species of *Oesophagostomum*, which warrants further research, given its apparently pathogenicity in this study.

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