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An Investigation into the Role of Dung Beetles in the Control of Gastrointestinal Nematode Larvae Numbers

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ABSTRACT

This study investigated the effects of dung beetles on the development of infective gastrointestinal nematode larvae. This was performed by way of a field trial involving the construction of experimental dung pats using strongyle infected equine dung, and the manipulation of dung beetle colonisation. Nematode larvae were extracted from cuttings of herbage surrounding the dung pats on three dates and the larvae numbers were then counted. While the results of this study did not find evidence that nematode larvae numbers decreased due to the activity of dung beetles, the experiment was relatively short. If the experiment had been continued it is possible that a relationship between larval numbers and dung beetle activity would have been observed. Performing this study also highlighted some interesting areas for future research.

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1. INTRODUCTION

The control of gastrointestinal nematodes is essential for the health and wellbeing of equines and other livestock. High worm burdens can negatively affect health and production and thus result in a significant cost to herd owners [1]. Anthelmintics are in general very effective at killing nematodes. However, resistance can develop as has been demonstrated in sheep and cattle herds across Ireland [2] [3]. Early indications of developing anthelmintic resistance have also been seen in Irish horse herds [4]. Therefore, we must be careful when using these drugs and employ them only when necessary to limit the development of resistance. Since the beginning of 2022, an EU regulation (Regulation (EU) 2019/6) has been in effect in Ireland, which means that antiparasitic drugs are now prescription only medications. This is one measure that will hopefully slow the development of resistance.

There are other things that may help to lower the parasite numbers on pasture, thus limiting the risk of parasite infection. The activity of dung beetles is one of them. Parasitic nematodes are excreted in dung, and dung pats serve as the habitats where they develop into larvae that can infect livestock. However, dung beetles may be effective in inhibiting this development by changing the dung environment, so the conditions are no longer hospitable to the development of these parasites.

It has also been demonstrated that the widespread usage of common antinematodal agents like macrocyclic lactones has a negative effect on the biodiversity of farmland [5]. These agents have a broad insecticidal effect and can kill non-target species like dung-dwelling beetles and other invertebrates. This may have a knock on effect on pasture health, and on insectivorous birds and mammals who could lose their food source [6]. These are important factors to consider when choosing worming strategies in livestock. If there are potentially effective, non-toxic means to reduce parasite numbers these should be fully explored.

There have been studies worldwide looking into the effect of dung beetles on gastrointestinal parasites, but these have mainly looked at cattle dung and cattle parasites. However, in Ireland there has been very little research in this area in any livestock animals. Globally, there is a lack of research into the interaction between dung beetle larvae and gastrointestinal nematodes. Dung beetle larvae may have a different effect than adults on nematode development. This project investigated the effect of dung beetle activity on the emergence of infective strongyle larvae, using equine dung exposed to differing levels of dung beetle colonisation, and recording dung beetle larval numbers in the sampled dung pats.

2. LITERATURE REVIEW

2.1 Dung beetles

2.1.1. Terminology and life cycle

Dung beetle is a term that can be used to describe a number of beetles that dwell in dung, encompassing seven different families. However, in its strictest definition, referring to those who are entirely coprophagous through all life stages, it includes just two families (in Ireland): the Scarabaeidae and Geotrupidae families (Table 1). The *Aphodius* genus, of the Scarabaeidae family, are the most widely occurring coprophagous beetles in Ireland [7], with 27 species recorded (Table 1). These beetles are endocoprid beetles meaning that they live in the dung. The females of the *Aphodius* species lay their eggs within the dung pat or superficially within the soil; this varies according to the species. The larvae once hatched feed on dung [8]. The beetles in the Geotrupidae family are much larger in size and not as commonly encountered [9]. In the Southeast of Ireland the most commonly encountered Geotrupidae are two species of *Geotrupes* (Dor beetles). They are paracoprid beetles, named because they dig tunnels below or near dung pats in order to bury dung. This dung is referred to as a brood mass as they lay their eggs in it and it is then used as a food source for the dung beetle larvae as they develop [10]. The larvae of both *Aphodius* and *Geotrupes* undergo three larval instars and then enter the pupal stage. The length of each developmental stage varies between species. The life cycle of *Geotrupes* is completed in one or more years [7]. Most *Aphodius* are univoltine (one generation per year). Some may be bivoltine (two generations per year) [11]. The adult dung beetles colonise fresh dung and feed on it. The dung beetles locate dung by the detection of volatile organic compounds which are emitted by the dung [12]. Adults are fluid feeders but the larvae feed on solid dung matter [13].

Table 1. The Irish dung beetle fauna

Group	Trophic group		Family	Irish species	
	adult	larvae		all species	dung associated
Scarabaeids	coprophagous	coprophagous	Geotrupidae	5	5
			Scarabaeidae (*Aphodius*)	27	26
			Scarabaeidae (*Onthophagus*)	2	2
Hydrophilids	coprophagous	predatory	Hydrophilidae (*Sphaeridiinae*)	26	18
Predators	predatory	predatory	Histeridae	21	13
			Staphylinidae	641	?
Fungus beetles	mycophagous	mycophagous	Cryptophagidae	59	?
			Ptilidae	43	?

Reproduced from Gittings 2020 [12].

2.1.2. Anthelmintic effect on dung beetles

Veterinary anthelmintics are drugs that are used to control parasitic worm infections in livestock. Among the most commonly used in equines are the macrocyclic lactones (ML). The ML are used due to their broad spectrum of action and wide availability. They are active against many nematodes and arthropods [15]. The ML are important when talking about dung beetles as their primary route of excretion is the dung, and it has been widely observed that residues of these chemicals can persist for months in dung [16]. These residues have been demonstrated to have a detrimental effect on the coprophilic fauna of dung pats. Lumaret *et al.* [17], gives a good overview of the recorded effects of ML in many different invertebrates. The effects vary according to the specific chemical, the concentration and the route of administration. In dung beetle adults a wide range of effects have been reported including reduced activity, which is commonly reported, increased mortality and decreased fecundity. Interestingly, changes in dung beetle attraction to the dung pats have also been demonstrated. Dung attractiveness to beetles can be increased or decreased by the presence of ML residues. This varies according to the specific chemical [18]. The ML are particularly toxic to dung beetle larvae. In one Australian field trial it was shown that larvae feeding on treated dung had an increased rate of mortality and a slower rate of development then those feeding on untreated dung [19].

The impact of ML on dung beetles can have a knock on effect on pasture health. Due to the toxic effects in beetles, the degradation and break down of dung can be slower. This can lead to a build-up of dung on pasture, leading to the loss of productive grazing land [20]. The impact on beetle eating insectivores like birds, bats and rodents is also something to consider. While the biological accumulation of ML in vertebrates is not thought to be an issue due to the low toxicity of ML to these creatures [17], the decrease in dung fauna (a potential food source) could have a negative effect [21].

2.2 Nematode classification and life cycle

The strongyles, of the Strongylidae family, are very important intestinal nematode parasites of equines. They can be divided into two major groups: the subfamily *Strongylinae* (large strongyles) and the subfamily *Cyathostominae* (small strongyles).

The large strongyles that most often occur in horses are those of the *Triodontophorus* genus and the *Strongylus* genus of which, *Strongylus vulgaris*, *Strongylus edentatus* and *Strongylus equinus* most commonly occur in Europe [22]. The adult large strongyles measure between 1.5-5 cm long and have a large globular or funnel-shaped buccal capsule [23][24], which allows for easy attachment to the large intestinal mucosa.

The small strongyles or Cyathostomins are made up of many different genera, but the four main ones that infect horses are *Cyathostomum, Cylicocyclus, Cylicodontophorus*, and *Cylicostephanus* [25]. The adults are between 0.5 and 2 cm in length and have a small, cylindrical buccal capsule [23][24], which allows for a weaker attachment to the intestinal mucosa.

The life cycle of all the strongyle species is direct. The adult nematodes live in the lumen of the caecum and colon producing fertilized eggs, which are shed in the faeces. These eggs are indistinguishable between the subfamilies and are referred to as strongyloid eggs. The eggs then hatch in favourable environmental conditions producing three consecutive larval stages: first stage (L1), which molts to second stage (L2), which then molts to third stage (L3) larvae [22]. The L3 are the infective larvae as they are able to migrate from the dung pat into the surrounding herbage where they may be ingested [26]. The L1 and L2 larvae can feed on organic matter. The L3 larvae have a protective cuticle (sheath), which provides the larva with protection but has no oral opening so they cannot feed [22]. Once inside the horse, the L3 larvae exsheath and invade the intestinal mucosa.

The main difference between the large and small strongyles is that the L3 larvae of the *Strongylus* genus can migrate extraintestinally, inducing a serious pathological condition known as strongylosis. The L3 larvae of *Strongylus vulgaris* migrate from the intestines towards the cranial

mesenteric artery, the L3 of *Strongylus edentatus* migrate to the liver, peritoneum and abdominal wall, and the L3 of *Strongylus equinus* migrate towards the liver and pancreas [25]. The larvae continue to develop to fourth (L4) and fifth (L5) stage larvae and then return to the lumen of the caecum and colon where they emerge as adults. The L3 of the Cyathostomins stay within the intestinal tract. They encyst within the mucosa of the large intestines and may lie dormant for many years or continue their development to L4 and then L5 larvae and then to adults [27].

2.3 Dung beetle and nematode interaction in dung pats

The interaction between dung beetles and nematodes is a complex one that is not fully understood even though it has been studied for many years. One early study from 1960 found that fewer nematode larvae were recovered from cow dung pats that had been broken down by dung beetles [28]. In a 1979 Australian study on equine dung, reductions in strongyle larval output of the order of 60% were observed during the peak activity months of one genus of tunnelling dung beetles [39]. The reasons for this reduction in nematode larvae were attributed to the dung beetle activity within the dung pats.

Nematode larvae development is influenced by environmental conditions within the dung pat. The interactions between weather, environment and larvae are complex due to the number of variables [30]. The optimal temperature of strongyle development has been demonstrated in a laboratory study to be 28ºC and changes away from this temperature led to a slower and lower recovery of infective larvae. This study found that changes to the moisture content of the dung also affected larval development [31].

The presence of endocoprid beetles in cattle dung pats for 12 days has been demonstrated to initially increase the number of infective larvae when compared to dung beetle free pats. However, after an additional 12 days the L3 numbers recovered from beetle colonised pats were significantly less than that of the beetle free pats [32]. This result was attributed to the increased aeration provided by the beetle activity within the pat. The hatching of strongyle eggs is dependent on oxygen levels and thus the initial aeration of the dung pat caused by beetle activity may aid them to hatch more quickly [33].

The physical disturbance to the dung caused by paracoprid beetles is more extreme than that caused by endocoprid species. The burying behaviour of paracoprid beetles increases the rate of fragmentation of the dung pat and can thus lead to increased, rapid, desiccation if the weather conditions are warm and dry [34]. This may result in an initial decrease in larval numbers, as due to the intensive desiccation of the dung, the L1 and L2 larvae will

die. However, since the L3 are ensheathed they can protect themselves from desiccation by moving to the surrounding soil where they can stay for months [35]. This means that once there is enough moisture to provide a protective film for migration these infective L3 can emerge and migrate to the surrounding herbage [30]. So, over a longer time period there may in fact be more infective larvae available [35].

The ingestion of parasite eggs by dung beetles has been proposed as a potential cause of parasite mortality. However, the maximum size of the particles ingested by *Geotrupes* are 40-65 μm [36] and by *Aphodius* are <5-50 μm [37], and the normal size of strongyle eggs is 70-90 μm long [38]. So, there is no real possibility for ingestion even by the larger *Geotrupes.* However, dung beetle larvae ingest solid dung, and thus are likely to ingest larger particles then the fluid feeding adults. Though not much is known about larval feeding there is the potential that they may be capable of ingesting nematode eggs. This is an interesting area of potential research.

There are also other interactions at play here: other coprophilous insects and earthworms colonise the dung pats along with the dung beetles and the different types and relative numbers of these may also have an impact on L3 development. Also of note is the activity of birds who predate on the dung fauna and mechanically break up the dung pats. Soil type is also important when it comes to paracoprid beetle activity as stony, sandy soil is unattractive for *Geotrupes* because it inhibits the formation of tunnels.

3. AIM OF RESEARCH

The aim of this study was to investigate the effects of dung beetle activity on the emergence of infective gastrointestinal nematode larvae using equine dung.

I performed a field trial by constructing experimental dung pats made from strongyle infected equine dung. The dung beetle colonisation was manipulated to compare parasitic nematode numbers between four treatments: enhanced colonisation by endocoprid beetles, enhanced colonisation by paracoprid beetles, natural dung beetle colonisation and no dung beetle colonisation. The experiment also investigated how parasitic nematode numbers changed over time as the dung pats aged.

I also examined the occurrence of dung beetle larvae in the experimental dung pats, to investigate whether the presence of these larvae affected nematode larval numbers.

4. METHODS AND MATERIALS

Dung Beetles

Dung beetles were collected over a one week period in August 2021 in East Cork, Ireland, in a location around 15 km away from the experimental location. The beetles were collected from dung from horses that had not been treated with an anthelmintic for over six months. The beetles were collected by a combination of pitfall traps baited with fresh equine dung, and manual hand searching of pats. 34 paracoprid beetles were collected, namely *Geotropes spiniger*. 134 endocoprid beetles were collected, of the *Aphodius* genus; these were mainly *A. rufipes*, which is the largest Irish *Aphodius* species. As the beetles were collected over a weeklong timeframe, they were stored in plastic, well-ventilated containers, with fresh horse dung, which was replaced daily to prevent fungus from forming.

Faeces

Faecal egg counts (FEC) were performed on fresh dung from horses that had not been treated with anthelmintics for a period of at least six months. FEC were performed using the McMaster technique. This confirmed a strongyle infection in these animals.

In August 2021, 60 kg of dung was collected from these animals over a period of three days. The dung was stored in plastic containers in a dark, cool, space. Once all the dung was collected it was mixed and a sample was taken from the dung to recheck the FEC.

Larval culturing was performed to confirm the presence of strongyles. This was done by taking three samples of faeces, each of 50 g. Each sample was then mixed with sawdust, and water was added, until the mixture had a jam like consistency. This mixture was then placed into a shallow Petri-dish and covered loosely with a lid in order to allow air to circulate in and out. This mixture was stored for eight days at room temperature and the lid was opened daily and water applied to the mixture to prevent drying. The larvae were then harvested from these dung mixtures by using a modified Baermann technique (see below).

Experimental Field Trial

Once all the dung had been collected it was transported to the experimental site. This was a 70 x 3 m strip of old grassland in Harper's Island Wetlands (51.910465, -8.315303), which is a nature reserve in East Cork, Ireland. This grassland had not had grazing livestock on it for at least four years. 60 artificial dung pats were formed using the collected horse dung. These pats were formed using 1 kg dung and a plastic bucket of 21 cm in diameter.

Four different treatments involving the manipulation of dung beetle colonisation were applied to the dung pats: (1) natural colonisation, (2) no colonisation, (3) enhanced paracoprid beetle colonisation, and (4) enhanced endocoprid beetle colonisation. Fifteen pats were assigned at random to each of the four different treatments.

In the no colonisation treatment, insects were excluded using metal cages of 25 x 25 x 25 cm lined with 0.5 mm insect mesh. These were pegged securely over the pats immediately after forming them.

In the enhanced paracoprid beetle treatment, 2 or 3 *Geotrupes* beetles were added randomly to each of the 15 pats. Metal cages with insect mesh (as above) were also applied for 12 hours to try and prevent the beetles from leaving the pats.

For the enhanced endocoprid beetle treatment, 9 or 10 *Aphodius* beetles were randomly allocated to each of the 15 pats. Metal cages with insect mesh (as above) were also applied for 12 hours.

For the natural colonisation treatment, no beetles were added.

Metal cages measuring 25 x 25 x 25 cm of 2.5 x 2.5 cm wire mesh were constructed. These were placed above each pat as soon as they were constructed and secured with bamboo poles for the entirety of the experiment to protect the pats from destruction by wildlife. Since the no colonisation pats already had a protective cage, an additional cage was not needed.

Grass Sampling

On day 14, day 23 and day 29 after pat construction, five pats were randomly sampled from each treatment. On the day prior to sampling, 1 litre of water was poured on each of the pats to be sampled. Sampling was then done the following day by cutting all herbage within a 15 cm radius of each pat and placing them into clean plastic Ziplock bags.

Searching of Dung Pats

At the same time as the herbage was cut the appearance of the dung pats was also recorded, noting the state of degradation of the dung pat. The pats were then collected in clean plastic containers and the area underneath was checked for signs of *Geotrupes* tunnelling. If tunnels were discovered they were dug out to look for brood masses and larvae. The collected pats were then stored in a cool, dark shed. Over the next three days the dung was hand searched in order to check for dung beetle larvae.

Nematode Extraction from Foliage

The nematode extraction methods were based on those used by Sands and wall [39].

Nematodes were extracted from the herbage using a modified Baermann technique method to extract the L3 stage. Plastic funnels of 18 cm diameter were set up and silicone tubing measuring 20 cm long with an internal diameter of 2 cm was attached to these funnels. Grass samples were wrapped in 30 x 30 cm muslin cloth pieces and secured with rubber bands. The grass samples were then secured to a metal rod by the rubber bands and suspended over the funnels. The funnels were then filled up to 0.5 cm below the rim with water containing 1 ml of detergent per 6 litres of water, ensuring that the entire muslin bag was submerged in the water. The samples were left submerged for 24 hours; they were agitated after 12 hours to encourage larval migration from the samples. After they had been left for 24 hours, the fluid at the end of the tubing was carefully collected and placed in the refrigerator for 1.5 hours.

After the fluid collection, the water at the top of the tubes was siphoned off using a pipette leaving only 5 ml of sediment. The sediment was then transferred to a clean test tube with the washings from the original tube. These were then centrifuged for 2 mins at 1500 rpm. The supernatant was siphoned off leaving 1 ml of sediment which was mixed and transferred to specimen tubes along with the washings from the tube. The specimen tubes were then made up to 10 ml. These larval suspensions were then stored in the fridge until counting was performed.

Nematode Identification and Counting

A pipette was used to take 1 ml aliquots from each larval suspension. They were then mixed with 1 drop of Lugol's iodine and put into a Sedgewick Rafter nematode counting chamber and examined at 30x magnification in order to count the third stage larvae. Each sample was counted three times and the mean was used for the analyses. I used the following criteria to differentiate the parasitic larvae from the free-living larvae: the presence of a sheath, the presence of a tail filament and the staining of the intestinal cells.

5. RESULTS

Faecal egg counts

The FEC performed on the dung prior to the experimental field trial recorded a mean of 250 strongyle eggs per gram. This is classified as a moderate egg count.

Nematode larval numbers

The nematode larval numbers recorded were highest across all treatments on the first count date (Aug 24th; Figure 1). The larval numbers then decreased across all treatments in the second count (Sept 2nd; Figure 1). The change varied in each treatment on the final count date (Sept 8th; Figure 1), with three treatments decreasing and one increasing relative to the second count. But the larval numbers had still decreased relative to the first count in all four treatments. The decrease in larval numbers was the least in the no colonisation treatment.

A two factor analysis of variance was performed using Excel to test for a difference between the treatments and a difference between the days and any interactions between treatment and day. This showed that there was a statistically significant difference in nematode larval numbers between the days but no significant difference between the treatments and no significant interactions (Table 2).

Dung pat degradation

Three out of the 60 dung pats had signs of *Geotrupes* activity. Signs of *Geotrupes* activity were tunnels below the dung pats and excavated soil within the pats. These were one from the *Geotrupes* treatment, one from the natural colonisation treatment and one from the *Aphodius* treatment. In the pat from the *Geotrupes* treatment, three brood masses were found inside the tunnels.

Nine of the dung pats were significantly broken down by the time of sampling: four from the *Geotrupes* treatment, two from the *Aphodius* treatment, two from the natural colonisation treatment and one from the no colonisation treatment. Significantly broken down pats were classified as

pats where much of the dung had been removed and what was left was dry, fragmented and had a fibrous texture.

Aphodius larvae were found in two dung pats: one from the *Aphodius* treatment and one from the natural colonisation treatment.

Table 2. Results of Anova testing the effects of sampling day and treatments on larval numbers.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatment	12.54815	3	4.182716	0.201038	0.895163	2.798061
Sampling day	199.8926	2	99.9463	4.803827	0.012539	3.190727
Interaction	70.81852	6	11.80309	0.567305	0.754188	2.294601
Within	998.6667	48	20.80556			
Total	1281.926	59				

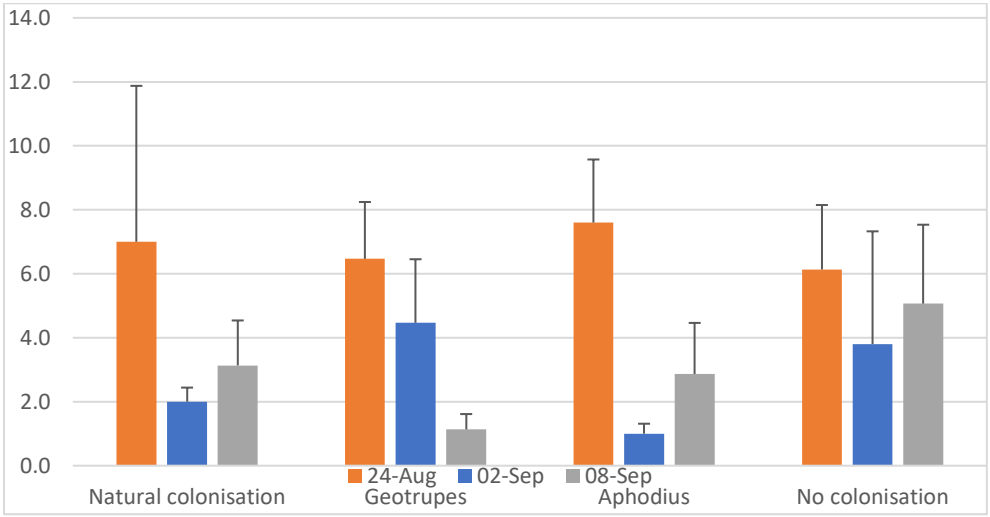

Figure 1. Change in larval numbers over time in the four treatments.

6. DISCUSSION

The development of L3 strongyles and their emergence from dung pats is complex and is known to be influenced by a number of climatic and environmental variables [39]. Dung colonisation by beetles and other coprophagic fauna is also influenced by weather, location, dung pat size, individual beetle preference and dung pat succession [40][41] [42]. These are important factors to bear in mind while looking at these results.

In this experiment the numbers of L3 decreased over time. In a much longer study performed in the UK using cattle dung, the numbers of *C. oncophora* and *O. ostertagi* L3 emerging from colonised dung pats were significantly higher than that of uncolonised dung pats in the first two weeks, but by eight weeks the pattern was reversed; L3 numbers in that study increased over time. That study also found no significant difference between beetle colonisation and treatment at 4 or 6 weeks [39]. Due to time constraints the present study was limited to 29 days. However, Figure 1 shows that the numbers of larvae recovered from the no colonisation treatment did not decrease to the same extent as it did in the other treatments. The other treatments all had larger decreases in the nematode larval numbers over the experimental period, although the differences from the no colonisation treatment were not statistically significant. It would have been very interesting to see in what way the larval numbers changed over a longer period.

One Australian study found that dung beetle activity reduced the period of potential infectivity of pasture. Pats not attacked by dung beetles provided nematode larvae for 202 days and the time was reduced to 173, 128, and 111 days for pats suffering minimum, moderate and intense dung beetle attack, respectively [43]. It seems that if the experiment had run for longer significant differences might have been observed in nematode larval numbers between the treatments.

There was evidence of dung beetle colonisation of the experimental dung pats. Three of the dung pats had tunnelling and/or brood masses upon examination. This means that they were inhabited by *Geotrupes*. *Aphodius*

larvae were found in two pats. One of these was a pat that was significantly broken down. The fragmented dung pats were most likely due to the activity of *Aphodius* [44]. The low number of *Aphodius* larvae found in the pats suggests that most of the colonising beetles were not reproductively mature. *A. rufipes* would have had high proportions of immature females in early August, as was demonstrated in an Irish study by Gittings and Giller [8]. Earthworms were also found when hand searching the pats. It has been shown that they can help with dung removal and may influence nematode larvae numbers too [45]. Woodlice were also found, but there is no literature available on whether they have an impact on parasite larval numbers.

It is difficult to know if all the dung beetles remained in the pats for a sufficient period of time. Though insect netting was placed over the beetle enhanced pats for 12 hours, which should have been enough time for beetle colonisation, there is the possibility that after it was removed the beetles left the pat. This would be a very difficult thing to protect against, especially with the tunnelling beetles as it would require the construction of underground wire cages.

The FEC that were performed seem accurate and indicated a strongyle infection in the sampled animals. The recovery of L3 larvae also indicated that a strongyle infection was present in these animals. The FEC value and the parasite recovery levels from pasture seem to correlate well with similar research performed by Sands and Wall [39].

Though it may seem that the results suggesting any effect on L3 parasite larvae numbers by dung beetles were inconclusive, the techniques used to confirm gastrointestinal nematode infection in the horses, catch and store the dung beetles and extract and recover the nematode larvae were effective. This means they could be used in other, related areas of research. Performing this research increased my interest in the biologic control of parasitic nematodes and highlighted some potential areas for further study. One is research into dung beetle larvae and how they affect nematode larvae numbers. While there has been much research on dung beetle adults and their effect on parasites there is a lack of research looking at the effect of

larvae which could potentially be more pronounced as they may be capable of ingesting nematode eggs. Another potential area is the effect of bird predation on the parasite larvae numbers. Birds, especially corvids predate on the dung fauna and in doing this mechanically break open and fragment the pat to search for insects inside [46], which leads to rapid desiccation of the dung pat. These are topics that would be very interesting to study in the future.

7. SUMMARY

The control of gastrointestinal parasites in livestock is very important. Anthelmintics are widely relied on to treat and control nematode infections, but resistance to these drugs is increasing. Some of these drugs have also been demonstrated to have a negative effect on farmland biodiversity as they can harm coprophilic fauna and reduce prey resources for the animals that predate the coprophilic fauna. Dung beetle activity may change the dung environment in a way that inhibits the development of infective nematode larvae. Therefore, it is important to investigate whether dung beetles could be incorporated into pasture management programs to control gastrointestinal parasite numbers.

In this study, my aim was to investigate the effects of dung beetles on the development of infective gastrointestinal nematode larvae. This was done by constructing experimental dung pats using strongyle infected horse dung. The infective nematode larval numbers were compared between four treatments that manipulated dung beetle colonisation: enhanced colonisation by endocoprid beetles, enhanced colonisation by paracoprid beetles, natural dung beetle colonisation and no dung beetle colonisation. Grass sampling was performed on three dates and nematode larvae were extracted and counted. The dung beetle larvae numbers were also looked at to see if they had an effect on nematode larval numbers.

This experiment found that infective nematode larvae numbers were highest across all treatments on the first sampling date. They then decreased across all treatments on the later sampling dates. There was least decrease in the larval numbers in the no colonisation treatment. However, the differences in nematode larval numbers between the treatments were not statistically significant. *Aphodius* larvae were found in three dung pats and *Geotrupes* larvae were found in one dung pat, but these numbers were not high enough to properly investigate the effects of dung beetle larvae on the nematode larvae.

Although the results of this study did not find statistically significant evidence that gastrointestinal nematode larvae numbers decreased due to the

activity of dung beetles, the trends in the data were in line with other, longer studies that found more conclusive evidence suggesting this. So, if the experiment had been continued it is possible that a relationship may have been observed.

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