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Prevalence and Distribution of Gastrointestinal Nematode Parasites and Dung Beetles Associated with Upland and Lowland Grazing of Irish Native Dexter Cattle

By

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A Thesis Submitted in Fulfilment of the Requirements for the

Degree of Master by Research

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Abstract

Prevalence and Distribution of Gastrointestinal Nematode Parasites and Dung Beetles Associated with Upland and Lowland Grazing of Irish Native Dexter Cattle by Noel Dineen

Diseases such as parasitic gastroenteritis are very common in cattle and can have detrimental economic consequences on agricultural productivity. This study was conducted to establish the prevalence and distribution of gastrointestinal nematode parasites associated with Dexter cattle under an organic lowland grazing regime and an extensive upland system. A secondary aim to the study was to investigate dung beetle assemblages in upland and lowland sites and their interactions with gastrointestinal nematode parasites as they offer an alternative method of natural control to organic farming systems in particular.

Cattle weights were recorded and faecal samples were collected at the beginning and end of the study. Faecal egg counts (FEC) were performed using the McMaster method and pasture infectivity was monitored by transect twice monthly. Standardised artificial dung pats were used to monitor dung beetle assemblages, larval migration and nematode–beetle interactions. Dung samples were analysed for Nitrogen and Carbon across the grazing season and more exhaustive subsampling was performed across the month of August.

The level of parasite burden was low in both in the upland and lowland cohorts. Younger animals had higher FEC compared to adults which is characteristic of herd infections. Mean FEC were below subclinical levels indicating that cattle were healthy with minimum risk of production loss. Pasture larval levels were low on all sites with a typical peak observed in late summer. The predominant species of nematode larvae in both sites were *Ostertagia ostertagi* and *Cooperia oncophora.* Cattle weights increased in the lowlands but were sustained in the uplands. Dung analysis showed higher levels of nitrogen and lower dry matter in the lowland dung. Major differences between populations were the dominance of *Cercyon* genus in the lowlands. *Aphodius rufipes* and *A. fimetarius* were found in similar numbers in both sites and *A. fasciatus* was found exclusively in the uplands. Dung beetle numbers were higher in the lowlands compared to the uplands, 1,660 and 301 respectively, with species richness similar in both sites. However, species diversity and evenness was greater in the upland site.

This study has found that there was a minimum risk to animal health by nematode parasites in the upland grazing regime suggesting that upland pastures can be utilised in the summer months to alleviate lowland grazing pressure by removing non-fattening stock. Low levels of gastrointestinal parasites in the organic lowland site were achieved by good management practices. A novel finding of this study points to the Hydrophilidae beetle family as potential nematode control agents. Grazing management strategies and biological control methods should form an integral part of any grazing management plan.

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List of Abbreviations

°C	Celsius
DH	Dry herbage
DWG	Daily weight gain
EPG	Eggs per gram
FEC	Faecal egg counts
g	g-force
g	Gram
IPM	Integrated parasite management
KG	Kilogram
L1	First stage larvae
L2	Second stage larvae
L3	Third stage larvae
LTA	Long term average
MAFF	Ministry of Agriculture, Forestry and Fisheries
PGE	Parasitic gastroenteritis
TST	Target selected treatment

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Chapter 1: Introduction

Gastrointestinal nematode parasites are a major concern in grazing livestock of both conventional and organic agricultural enterprises worldwide (Perry and Randolph, 1999; Waller, 2006). Parasitic gastroenteritis (PGE) is a disease commonly found in cattle of all ages caused by gastrointestinal nematode parasites. Effects of the disease are poor thrive and live weight gain, poor health, reduced growth rate and decreased milk yield which lead to poor production and financial losses (Animal Health Ireland, 2012).

Improvements in animal husbandry, grazing management and the use of anthelmintic drugs mean that clinical parasitic infections in cattle are quite rare in developed countries. The biggest losses in production are now attributable to sub-clinical parasite infection (Dimander, Höglund, Spörndly and Waller, 2000). These infections can be difficult to detect and therefore the farming sector may be unaware of the related production loss (Animal Health Ireland, 2012).

In conventional farming, farmers depend on anthelmintic drugs for parasite control but this method of control is now becoming a serious concern, due to the widespread and rapid development of resistance to chemotherapy (Sutherland and Leathwick, 2011). In organic systems, the use of anthelmintic drugs is highly regulated, with a preferential emphasis on parasite disease prevention through regulation of stocking densities and complimentary grass management methods (Stromberg and Gasbarre, 2006).

Nematode ecology is highly complex and nematode interactions with different grazing systems poorly understood (Morand and Guegan, 2000; Neher, 2010). To date, researchers been predominantly focused on intensive systems. There is little evidence examining the link between upland farming or traditional organic farming practices

with nematode infection. Such grazing systems have been reported to encourage an increase in invertebrate biodiversity including dung-beetles (Beynon, Peck, Mann and Lewis, 2012). Invertebrates including dung beetles have a strong role in controlling the nematode population through competition for dung resources and in this way can reduce the parasitic burden of cattle dung facilitating the faecal-oral mode of transmission for these parasites (Nichols *et al.*, 2008; Beynon *et al.*, 2012)

Many parasitic nematodes use dung as a medium to disperse eggs and juvenile larval stages as the adult nematodes do not multiply in the cattle host but must pass as eggs into the environment via the faecal route to continue the parasite life cycle. These include nematode species of *Ostertagia*; *Cooperia*; *Haemonchus*; *Trichostrongylus*; *Nematodiurs*; *Strongyloides* and *Oesophagostomum*. These parasites share the same resources as a number of other biological groups including insects and fungi, offering the possibility for biological control of nematodes and other parasites at many levels (Larsen, 1999). The extremely high plasticity of nematode parasites, shown in their increasing resistance to different classes of anthelmintic drugs serves as a warning, namely that a single approach to biocontrol of nematodes is unsustainable in the long term. Thus there is increasing support for an integrated biological control strategy targeting the free-living parasite stage and involving a number of interventions at the level of grazing management as well as the use of more than one biocontrol agent (Waller and Faedo, 1996; Waller, 2006)

Invertebrates like dung beetles have a strong role in controlling the nematode population through competition for dung resources and recycling nutrients back into the soil (Nichols *et al.*, 2008). Classical coprophagous dung beetle families such as Scarabaeidae, Aphodiinae and Geotrupidae are intrinsically involved with parasite

ecology affecting dung parasite populations by competing with them for resources, by removing the dung from the land surface and acting as potential vectors for their spread (Beynon *et al*., 2012; Chirico, Wiktelius and Waller, 2003)

Other dung-inhabiting beetles are predatory, omnivorous or opportunistic (in particular the Staphylinidae, Histeridae and Carabidae families) and can thus directly remove nematodes and other prey items from dung.

Independent economic assessments have revealed that losses in cattle productivity due to gastrointestinal nematode infections can be considerable (Hawkins, 1993 cited in Höglund, Svensson and Hessle, 2001).

Organic farming is rapidly expanding in the European Union. It can therefore be expected that an increasingly larger proportion of cattle farmers will require parasite control programmes that exclude the use of anthelmintics, and it is therefore important to assess how grazing management practices affect the pattern and efficacy of parasitic nematode distribution and dung beetle development over the grazing season (DG Agriculture and Rural Development, 2013).

1.1 Study Objectives

This research project aimed to investigate the prevalence and distribution of nematode parasites and associated dung beetle populations in two different grazing regimes in native Irish cattle that are raised according to the confines of organic practices and bred for the food-tourism market. The two management systems converge on the same herd of Dexter cattle on the Dingle peninsula in County Kerry, Ireland. The grazing regimes consist of a lowland organic-grassland management system, while the alternate is a free-ranging summer upland system in the Brandon Mountains in County Kerry.

1.1.1 Objectives

The projects specific objectives were to;

1. To establish and compare the distribution and prevalence of gastrointestinal nematode and dung beetle populations in upland and lowland management systems

2. Compare and assess how cattle grazing management practices affect the pattern of parasitic nematode distribution and dung beetle development over the grazing season.

3. To establish and compare the distribution and prevalence of gastrointestinal nematode and dung beetle populations in upland and lowland management systems.
4. To identify the serial changes in beetle species composition in lowland versus upland sites.

5. To examine the physic-chemical differences in dung in upland versus lowland sites.

6. To identify measures that will minimise the parasitic burden of cattle dung in upland environments while maximising dung-beetle diversity.

Chapter 2: Literature Review

2.1 History of Dexter Cattle

The Dexter cattle are one of two cattle breeds' native to Ireland and both originate in the south west of the country. Native Irish cattle are descended from those that were first introduced to the island in prehistoric times and the Kerry breed is regarded as the most ancient of the Irish breeds. Early breeds of cattle are synonymous with the colour black, as old Celtic breeds were known to be black in colour. Both the Dexter and Kerry breeds share this trait (Figure 1). The histories of these two cattle breeds were investigated by Curran (1990) and were found to be heavily entwined with each other having once been regarded as the same breed.

Figure 1. Dexter cow grazing in the Brandon uplands

In the nineteenth century, both were reared together in the same herd book and it was only relatively recently that the two breeds were separated. At a Royal Dublin Society Show in 1863 the first separation of the true Kerries from Dexters-Kerries in separate livestock classes took place. After 1863 mixed Dexter and Kerry groupings still

appeared but it wasn't until 1876 that consistent attempts were made to separate the two breeds into distinct classes. In September 1890 the first volume of the Kerry herd book was issued with a total of 1,297 animals: 237 Dexters 118 Kerry cows and Kerry bulls, 942 (Curran, 1990).

Both the Dexter and Kerry breed were hugely popular and successful in agricultural shows in Ireland and Britain. Reasons for their popularity were due to the natural beauty of the breeds as well as attributes including frugality, hardiness, productiveness, longevity and disease resistance (Curran, 1990).

However, as the nineteenth century drew to an end, the Dexter and Kerry breeds declined in popularity. In the early twentieth century Kerry cattle classes temporarily subsided in the Royal Dublin Society (RDS) Shows, this was attributed to the on-going war at the time as numbers rose again after 1918.

The Kerry and Dexter Cattle Society of Ireland was formed in July 1917 and laid out a number of objectives to maintain, protect and promote the breeds. However after only two years it was decided to rename the Society as "*The Kerry Cattle Society of Ireland"* because Dexter cattle had practically ceased to exist in Ireland. This was in part attributed to difficulties in breeding pure Dexters due to an inherited trait that resulted in deformed aborted offspring known as Bulldog calves. Bulldog calves are said to have arisen when the Kerry and Dexter breeds were separated in the herd book as they were once interbred to avoid this problem (Curran, 1990). Fortunately the Dexter breed remained popular in Britain with the establishment of the Dexter Cattle Society in 1892.

Dexter cattle are considered to be among the smallest breeds of cattle in the world. They stand at 92 – 107 cm tall at the shoulder and cows typically weigh between 300 and 350 kg. Two types are recognised, short legged and non-short legged (The Dexter Cattle Society, 2016). Dexters show black, red and dun colouration but black is the dominant. White horns with black tips are generally present which grow outwards with an upward curve in females and forward curve in males.

Despite their small stature, Dexters are a hardy, productive, dual-purpose breed (used for both milk and beef) and are adaptable to a range of environments. Their small size and agility helps to minimise poaching making them well suited to upland environments. The breed's ability to adapt to various climatic conditions and management systems is one of its greatest attributes and this helped to establish the breed worldwide (The Dexter Cattle Society, 2016).

2.2 Gastrointestinal nematode parasites

Gastrointestinal nematode parasites are one of three major groups of parasitic worms known as helminths, with the other two groups being the Trematodes (flukes) and Cestodes (tapeworms) that are of great veterinary importance (Figure 2). Nematode parasites belong to class Nematoda in phylum Nemathelminthes, which is the only class in this phylum containing worms of parasitic significance (Taylor, Coop and Wall, 2007).

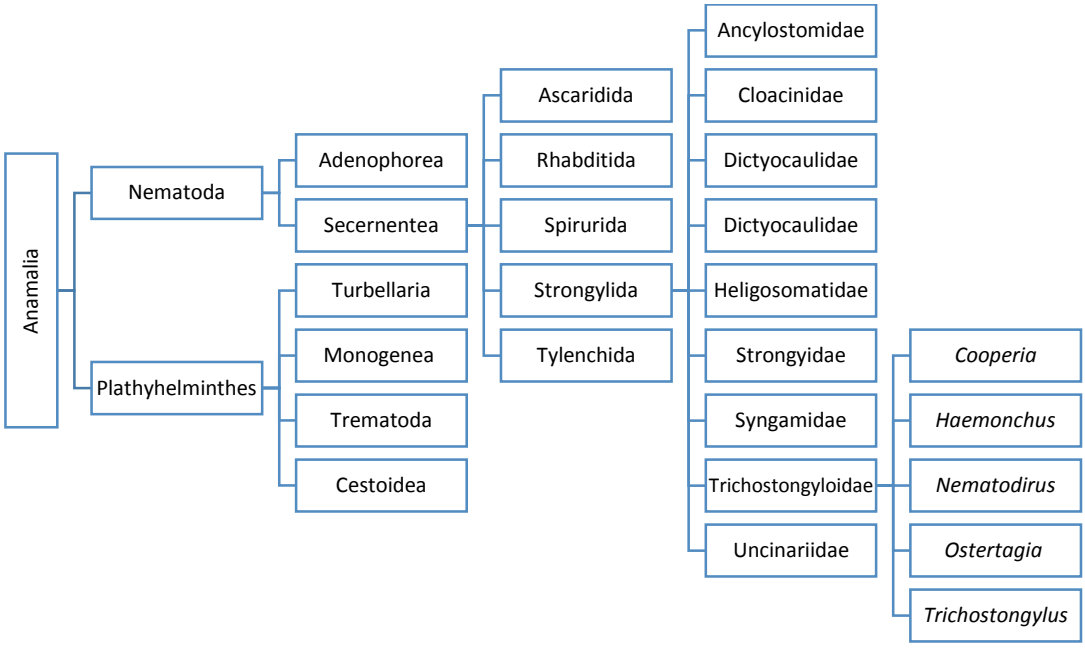

Figure 2. Classification of common gastrointestinal nematode parasites of ruminants. Kingdom, Phylum, Class, Order, Family, Genus.

Nematodes, commonly known as round worms, have a long un-segmented cylindrical or tube shaped body, with a tapering head and tail. Their fluid-filled body covered in a protective cuticle acts a hydrostatic skeleton and longitudinal muscle provides locomotion in a whipping or trashing motion. The digestive system is relatively simple with a mouth opening at the anterior end that leads directly into the oesophagus. The intestine is longitudinal, running almost the entire length of the body and ending in the anus. Adult worms form separate sexes and reproduce sexually. Life cycles are commonly direct in which one host is required, but may also be indirect where an intermediate host, such as a mollusc, is required for complete development (Taylor, Coop and Wall, 2007). In the common direct life cycle, eggs passed in faeces hatch and larvae undergo two moults before becoming infective L3 larvae which are ingested by the host. In the indirect life cycle, larvae require an intermediate host, such

as a mollusc or annelid and infection of the definitive host occurs by ingestion of the intermediate host containing the infective larvae (Bowman, 2014).

2.3 Parasite life cycle

Gastrointestinal nematode parasites typically have a direct life cycle with an internal parasitic stage inside the host and an external free-living stage in the environment (Fiel et al., 2012). The external stage of the life cycle is where the majority of the population can be found. A typical nematode parasite life cycle is depicted in Figure 3.

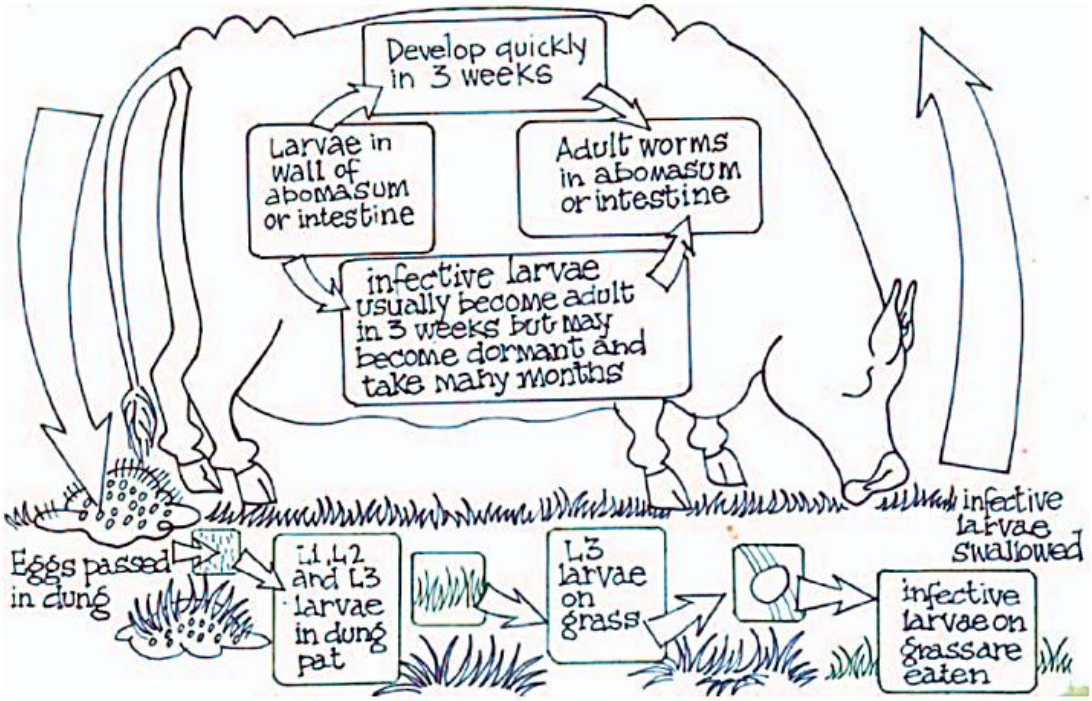

Figure 3. Typical nematode parasite life cycle in a ruminant (Animal Health Ireland, 2012)

Nematodes have separate sexes (dioecious) with the females generally being larger than the males (Taylor, Coop and Wall, 2007). Mature females produce typical strongyle eggs that are ellipsoidal in shape, with a smooth surface and contain an embryo in the morula stage of development when passed in faeces (Bowman, 2014).

During development nematodes moult on four occasions by shedding their outer cuticle as they grow. The four successive larval stages are L1, L2, L3, L4 and the final adult stage is L5 (Taylor, Coop and Wall, 2007).

Nematode eggs are shed in the faeces and deposited onto pasture where they hatch and develop into first-stage larvae (L1) within a day or two under optimum conditions (25°C) (Bowman, 2014) but this can take longer depending on environmental factors.

Figure 4. Gastrointestinal nematode parasite egg, approximately 80µm in length

First stage larvae undergo their first moult and develop into second stage larvae. Free living first and second stage larvae are microbivorous and feed on bacteria in the faeces. Second stage larvae continue to feed until they moult and develop into infective third stage larvae. After the second moult, third stage larvae retain the cuticle of the second stage which acts as a protective sheath and is not shed until they are ingested by a suitable host (Bowman, 2014). With the third stage larvae sealed off from the

environment they are unable to feed and must survive on energy stores acquired in the previous stages (Taylor, Coop and Wall, 2007). This forces the sheathed third stage larvae to migrate out of the faecal mass and onto the surrounding soil and vegetation (Bowman, 2014). The process by which eggs hatch and become infective third stage larvae on pasture where they are available to grazers is known as translation (Rose, 1961).

2.4 Host infection

Grazing animals are infected when free-living L3 larvae are ingested. After ingestion, L3 larvae exsheath by shedding their protective cuticle in the rumen of the host. Within minutes of exposure to carbon dioxide or a mild hypochlorite solution L3 larva shed their protective sheath (Stromberg and Gasbarre, 2006). Exsheathed larvae then move to their desired site depending on the species e.g. the abomasum (*Ostertagia*) or small intestine (*Cooperia*). Generally, L3 develop into L4 in a matter of days and after two to three weeks L4 develop into adult L5. Immature adults develop into sexually mature adults and after copulation a further life cycle is initiated (Taylor, Coop and Wall, 2007). The time required for development from the ingested infective L3 larvae to the mature egg producing parasitic adult worm is known as the pre-patent period.

The pre-patent period is three weeks but, under certain circumstances, the parasite can arrest its development for several months (Chaparro, Canziani, Saumell and Fiel, 2011). Developing L3 larvae can cease development at the early fourth larval stage (EL4) and enter a state of arrested development known as hypobiosis (Taylor, Coop and Wall, 2007). Arrested development of parasitic larvae is of great biological and epidemiological importance. This strategy allows parasites to avoid adverse climatic conditions by remaining sexually immature in the host until conditions become more

favourable. In Ireland hypobiosis usually takes place late in the grazing season, when ingested larvae enter a state of dormancy that can last for up to six months. Development resumes at the end of the winter when temperatures begin to rise and environmental conditions are suitable for the free-living larval stage (Taylor, Coop and Wall, 2007).

Translation of gastrointestinal nematode larvae from faecal pats onto herbage is an extremely complex process and can be influenced by both environmental and biological factors (Pandey, 1974).

When L3 larvae are ingested by the host they shed their protective cuticle retained from the L2 stage within the alimentary tract of the host animal. The host provides the stimulus for exsheathment and in response to this the larva releases a fluid with an enzyme which dissolves the protective sheath allowing the larva to free itself (Taylor, Coop and Wall, 2007).

2.5 Disease

The most economically important gastrointestinal nematode parasite which infects cattle is *Ostertagia ostertagi*. The epidemiology and life cycle of *O. ostertagi* is similar to that of other gastrointestinal nematodes parasites but their predilection site varies.

O. ostertagi attaches to the abomasum of the host where, in heavy infections, 40,000 or more adult worms can develop. When parasites emerge from the gastric glands most harm is done to the host. The acidity of the abomasal fluid can increase from pH 2.0 up to 7.0. This prevents pepsinogen from changing to pepsin. Also, permeability of the abomasal epithelium is increased. This results in pepsinogen leaking into the circulation leading to increased plasma concentrations, loss of plasma proteins and eventually hypoalbuminaemia. Leakage of endogenous protein into the

gastrointestinal tract accounts for substantial weight loss in the host, more so than reduced feed intake and diarrhoea as previously thought (Taylor, Coop and Wall, 2007)

Two forms of infection occur in cattle; Type I, or summer, ostertagiosis usually occurs in first season grazing calves approximately three to four weeks after ingesting infective larvae on pastures. Morbidity is typically high but mortality is rare once animals are treated early. Type II, or winter, ostertagiosis typically occurs in yearlings in late winter or spring when larvae that arrested their development in autumn reach maturation. Type II infections are usually less severe than type I as less animals tend to become infected (Taylor, Coop and Wall, 2007; Bowman, 2014).

2.6 Host resistance

Gastrointestinal nematodes evoke a wide variety of immune responses in ruminants. Responses may vary from those that give strong protection from reinfection after high exposure to those that are weak (Gasbarre, 1997). Resistance to parasitic infections are typically split into two groups. The first, innate resistance, includes species resistance, age resistance and breed resistance. Secondly, acquired immunity depends on antigenic stimulation and humoral and cellular responses (Taylor, Coop and Wall, 2007)

Many species of nematode parasite are highly host specific and do not develop if ingested by another host. Other species, such as the cattle parasite *O. ostertagi*, can develop partially in sheep but typically does not reach the adult stage (Taylor, Coop and Wall, 2007).

The majority of animals develop a resistance to infections with age. Resistance to *O. ostertagi* is slow to develop, with high immunity taking two to three grazing seasons to develop. While immunity to *Cooperia oncophora* typically takes only twelve months (Taylor, Coop and Wall, 2007). A study by Ploeger, Kloosterman, Borgsteede and Eysker (1990) suggested that immunity built up in calves during the first year of grazing had a positive effect on growth performance in the second year. Acquired immunity develops over time, therefore, mature cows can retain small populations of nematode parasite infections resulting in a continuous shedding of a small number of eggs, providing a source of infection for young calves (Corwin, 1997).

Certain breeds of ruminants may be more resistant to parasitic infections than others (Taylor, Coop and Wall, 2007). A study by Golding and Small (2009) investigated nematode parasite resistance in three different breeds of sheep; and demonstrated that two primitive breeds, Manx Loaghtan and Shetland sheep had a greater resistance to gastrointestinal parasites.

Oliveira *et al.* (2009) investigated infection resistance in purebred Nelore cattle (*Bos indicus*) and Nelore crosses (Nelore/Senepol and Nelore/Aberdeen Angus). The level of infection was determined in each group using faecal eggs counts and faecal cultures. The study found no significant difference between the genetic groups, however, levels of infections were lowest in the Nelore crosses.

It has been suggested that ancient breeds of cattle have a higher degree of disease resistance and are described as “hardy” (Curran, 1990). The Dexter breed is an ancient Irish breed and their hardiness and disease resistance has been reported (Curran, 1990).

2.7 Influence of environmental factors on development of parasitic free-living stages

Environmental conditions have a significant impact on parasite populations especially at the free-living stage. These conditions include meteorological factors such as drought, rainfall, humidity, temperature, cloud cover and sunlight as well as biological factors such as animals and fungi (Pandey, 1972; Stromberg, 1997). Different weather conditions, due to geographical location, can influence the behaviour of the free-living stages of gastrointestinal nematodes (Fiel *et al.*, 2012). Of all the meteorological conditions, the most important environmental factors influencing translation of the free-living stages of nematode parasites are temperature and moisture (Pandey, 1972; O'Connor, Walkden-Brown and Kahn, 2006; Taylor, Coop and Wall, 2007; Chaparro *et al.*, 2011). Seasonal variation in weather conditions has an impact on development rate of nematode parasites. Development from egg to infective larvae can take between 1 to 6 weeks under field conditions depending on the time of year (Pandey, 1974).

Nematode eggs are deposited in faecal pats that provide the ideal environment for egg hatching and larval development. In dry weather the ambient humidity and temperature in the faeces provide sufficient conditions for larvae to develop (Taylor, Coop and Wall, 2007). Cattle faeces can remain virtually intact for much longer periods of time compared to faeces of other grazers such as sheep. Thus, the centre of cattle dung pats can remain moist for long periods of time, providing a refuge for developing larvae (Boom and Sheath, 2008)

2.7.1 Temperature

Temperature is one of the most important factors in the development of free-living stages (Pandey, 1972). The eggs and larvae of most trichostrongylid parasitic nematodes develop at similar rates at various temperatures. In general, there is an inverse relationship between the development time from egg to infective larvae and air temperature, the higher the temperature the shorter the development time (Fiel *et al.*, 2012).

Eggs excreted in the dung hatch and develop into free-living larvae quickly under ideal conditions. Hatching of eggs is mainly influenced by temperature but moisture is also necessary to prevent desiccation. The optimal temperature for egg development is 18 – 26°C (Taylor, Coop and Wall, 2007), however development of *O. ostertagi* eggs at temperatures ranging from 4°C to 35°C have been observed (Pandey, 1972).

Once hatched, larvae develop to the infective L3 stage at temperatures between 10°C and 35°C but the optimal temperature is 25°C (Ciordia and Bizzell, 1963; Pandey, 1972). Generally, at temperatures below 10°C development from egg to L3 larvae is not possible. Above optimal temperatures increase larval development but they become hyperactive and deplete their lipid reserves, increasing the mortality rate (Taylor, Coop and Wall, 2007).

2.7.2 Moisture

Though temperature is regarded as the most important factor influencing the hatching and development of nematode parasites, moisture is also a key factor. It is known that temperature controls the rate of development, but without moisture development is not possible (Stromberg, 1997). Moisture is present in the environment in different forms (rain, dew, and humidity) and the smallest quantities can facilitate larval development.

Even in dry weather the microclimate in dung can remain sufficiently moist for the development of larvae (Taylor, Coop and Wall, 2007). Optimal humidity for larval development is 100%, however, development can take place as low as 80% relative humidity (Taylor, Coop and Wall, 2007). As well as hatching, moisture plays an important role in larvae development and migration. L1 and L2 larvae are particularly vulnerable to desiccation and moisture is essential for completion of translation to the L3 phase.

If the faecal mass becomes dry and crusty larvae are unable to leave, becoming trapped inside (Pandey, 1974). However, after the pat is sufficiently moistened larvae can emerge in high concentrations (van Dijk and Morgan, 2011). Dry conditions may reduce or even completely stop migration onto herbage but generally it will not stop larval development (Boom and Sheath, 2008).

Though moisture is important for the development of nematode larvae, high rainfall can cause increased mortality to eggs and pre-infective larvae due to dung washing. Dung washing removes eggs and pre-infective larvae from faeces and distributes them in the pasture where conditions are appropriate for development (Chaparro *et al.*, 2011). Silva *et al.* (2008) recorded low numbers of larvae after heavy rains occurred and the effects of dung washing was also observed by Santos, Silva and Amarante (2012) when larvae recovery from pasture was low.

2.7.3 Survival of eggs and L3 larvae

Factors effecting survival of L3 larvae are mainly environmental, especially seasonal changes in climate. Changes in climate may have an effect on the development and survival of the infective stages of many parasites. Warmer and wetter weather favours the development of most nematode parasites and may lead to an increase in prevalence

in temperate regions. Desiccation is considered the biggest threat to the survival of larvae. A study by Fiel *et al.* (2012) showed that nematodes highly prevalent on pasture were those that were better adapted to cold temperate climates (*Ostertagia*, *Cooperia* and *Trichostrongylus* species).

Nematode eggs and L3 larvae are much more capable of surviving adverse conditions such as desiccation and freezing than the pre-infective L1 and L2 larvae. Hence, it is only the eggs and infective L3 larva stages than can survive for long periods on pasture.

Eggs of trichostrongylid species are hardy and can survive through harsh conditions for long periods due to the protection of the thick egg wall. Below 10°C development generally stops which is favourable for survival. When conditions are not suitable for development, eggs can remain dormant until conditions are more favourable (Taylor, Coop and Wall, 2007). Despite all these survival strategies, only a small proportion of nematode eggs in faecal pats develop into infective larvae. In a review by Stromberg and Averbeck (1999), it was reported that less than 33% of the eggs in faecal pats developed to the infective L3 stage.

Infective L3 larvae cannot feed due the presence of a cuticle and must survive on its lipid stores acquired in the pre-parasitic phases. Though the cuticle prevents L3 larvae from feeding, it is very important for larval survival as it provides protection. At temperatures below 5°C metabolism slows and increases survival rates. Stromberg and Averbeck (1999) reported that most infective L3 larvae can survive on pasture over the winter months and be available to infect cattle in the spring (Stromberg and Averbeck, 1999). *O. ostertagi* have the ability to migrate into soil and back onto

herbage (Krecek and Murrell, 1988) meaning that there is potential to survive in the soil when conditions on the surface are less favourable.

2.7.4 Larval Migration

Migration of infective third-stage larvae from dung pats to the surrounding herbage is a key point for parasite transmission. Larvae must migrate from the faeces onto the adjacent herbage where they are available for ingestion by grazing animals (Silva *et al.*, 2008). Migration of L3 larvae from faecal pats onto herbage is mostly influenced by water in form of rain.

Migration distance is crucial because cattle naturally tend to avoid grazing in close proximity to faecal depositions (Goldberg, 1970). L3 larvae are capable of migrating distances as far as 90 cm from the edge of dung pats with the help of splashing water but most larvae do not migrate more than 30 cm from the edge of the pat (Boom and Sheath, 2008).

Boom and Sheath (2008) investigated the lateral migration of infective gastrointestinal nematode larvae from artificially placed dung pats on pastures within a temperate region of New Zealand. Faecal material collected from naturally infected calves was formed into standardised pats 15 cm in diameter and deposited on pastures in summer, autumn and winter. At each collection period herbage was harvested from areas adjacent to the faecal pats in three radial zones from the centre of the faecal mass. Results showed that the majority of larvae were recovered from the zone nearest to the dung pat (0 – 20 cm from the centre of the pat).

Previous studies that investigated lateral migration recorded similar results. Pandey (1974) rarely found larvae more than 12 cm from the edge of dung pats in significant numbers. The majority were recovered from herbage up to 6 cm from the edge of pats.

Any larvae that migrated further than 12 cm where in such low numbers and were regarded as insignificant. These results agreed with Goldberg (1970) who observed little larvae migration with 88% of the larvae recovered within 13 cm of the pats. Similarly, Langrová, Jankovská, Borovský and Fiala (2003) found that most larvae remained very close to faecal samples stating that 89% of larvae were collected within 10 cm of faeces.

Not only do nematode larvae migrate laterally in vegetation, they also migrate vertically along blades of grass. Silva *et al.* (2008) investigated the vertical migration of the sheep parasite *Haemonchus contortus* on grass. Faeces were acquired from naturally infected sheep and deposited on soil in the middle of herbage on four occasions (spring, summer, autumn and winter). Faeces were exposed to environmental conditions for seven days and samples were collected from three different herbage strata throughout the day (sunrise, mid-day, sunset and mid-night). The investigation found that, diurnal periods had no influence on vertical migration of *H. contortus* larvae indicating that there was no specific time of day when grazing animals were more susceptible to infection.

A similar study by Santos, Silva and Amarante (2012) found larvae predominantly at the base of herbage which agree with those of Silva *et al.* (2008). Goldberg (1968) also found no correlation between herbage height and larval recovery.

2.7.5 Pasture contamination

Gastrointestinal nematode infections in grazing animals are caused by ingestion of infective L3 larvae on herbage. Overwintered larvae provide a source of infection for first season grazers on pastures in spring. A large number of L3 larvae can survive the winter in soil and on pastures. In temperate areas, eggs deposited in winter may result

in large numbers of larvae being available to grazing animals in spring, depending on climatic conditions (Barger, 1999).

In temperate regions overwintered infective L3 larvae provide a source of infection for first season grazers on pastures in spring. Eggs deposited by infected animals in the spring develop slowly due to low temperatures. As the temperatures rise in mid-summer, developmental rates increase and eggs deposited from April to June are infective L3 stage by mid-July. If sufficient numbers of L3 are ingested disease occurs in late summer to early autumn. This gives rise to a late summer peak in egg development. Development of eggs to infective larvae slows in autumn as temperatures fall and ingested L3 can enter a state of arrested development at the early fourth stage (EL4). In early spring L4 resume development and type II disease occurs (as described previously). Infection in the spring is less common in beef herds where immune adults graze side-by-side with young calves. This is because immune adults produce low numbers of eggs and overwintered L3 die before suckling calves begin grazing. However, when calving occurs in autumn and winter, calves grazing the following spring can acquire high levels of L3 (Taylor, Coop and Wall, 2007).

2.8 Effects on animal production

Gastrointestinal nematode parasites are a serious cause of production loss in both dairy and beef enterprises worldwide. Productivity losses are attributed to the health effects associated with gastrointestinal parasitism (Armour, 1989; Corwin, 1997; Gasbarre, 1997; Perry and Randolph, 1999; Dimander et al., 2000; Charlier et al., 2009; Fiel et al., 2012; Stromberg et al., 2012; Piekarska et al., 2013; O'Shaughnessy et al., 2015). The most common and economically important species of gastrointestinal nematodes responsible for disease and production loss worldwide are those of the family

Trichostrongylidae, in particular *O. ostertagi* and *Cooperia oncophora* (Armour, 1989; Corwin, 1997; Dimander *et al*., 2003; Larsson *et al.*, 2006; Taylor, Coop and Wall, 2007; Fiel *et al.*, 2012; Rehbein *et al.*, 2013).

The economic loss associated with gastrointestinal nematodes in cattle is widely accepted but there is lack of study estimating the financial costs of production losses or the costs and benefits of control measures (Charlier *et al.*, 2009). The costs associated with animal disease can be loosely divided into two categories; direct and indirect costs. Direct costs are the immediate impact on livestock and agriculture while indirect costs include mitigation and control measures, losses in revenue, effect on human health and impact on the environment.

It is difficult to calculate the total cost associated with gastrointestinal parasite infections because both production losses and the cost of treatment need to be factored in. Gasbarre (1997) reported that losses incurred as a result of gastrointestinal nematodes in the Unites States could potentially exceed two billion US dollars annually while in Australia costs associated with parasitic disease in both sheep and cattle has been estimated at AUD$1 billion (Roeber, Jex and Gasser, 2013). While in Europe costs for anthelminthic treatment and veterinary consultation is similar to that of the Unites States and Australia, amounting to approximately €1 billion annually (Vercruysse, Charlier, Dorny and Claerebout, 2006)

The effects of parasitic gastroenteritis, caused by gastrointestinal nematodes, on the health of cattle are poor thrive, reduced live-weight gain, ill health, reduced feed consumption, decreased milk yield, poor production, depressed reproductive potential, and of course financial loss (Perry and Randolph, 1999). Heavy infections in young animals can even cause death, though infections are rarely lethal, however, substantial

losses in productivity are associated with subclinical infections (Svensson, Hessle and Höglund, 2000).

Sub-clinical infections can be difficult to identify with associated production losses (Dimander *et al.*, 2000). The main symptoms associated with sub-clinical infections are poor appetite and reduced feed intake. The combination of these two symptoms reduces weight gain. Increased weight gain is the most often observed benefit of gastrointestinal nematode control in young cattle (Charlier *et al.*, 2009). Weight gain in relation to the administration of anthelminthics has been well studied (Shaw, Vercruysse, Claerebout and Dorny, 1998; Dimander *et al.*, 2000; Larsson *et al.*, 2006). However, the use of anthelminthics is highly regulated and generally prohibited in organic cattle production as a prophylactic measure. This makes organic beef production more susceptible to parasite infections than conventional beef enterprises.

Cattle most at risk of disease are typically first-season grazers (Armour, 1989; Fiel *et al.*, 2012) grazing on pastures contaminated the previous year, although cattle of all ages are susceptible to infection.

In developed countries, clinical parasitic infections in cattle are extremely rare due to the development and availability of highly effective anthelmintic drugs and improved pasture utilisation and management (Corwin, 1997; Dimander *et al.*, 2000). Therefore, clinical parasitism of cattle often indicates poor pasture management, especially in the modern livestock industry (Corwin, 1997).

2.9 Control methods for gastrointestinal nematode parasites

"There is no single requirement more crucial to the rational and sustainable control of helminth parasites in grazing animals than a comprehensive knowledge of the epidemiology of the parasite as it interacts with the host in a specific climatic, management and production environment" (Barger, 1999).

Gastrointestinal nematode parasites are a global economic disease (Perry and Randolph, 1999). Controlling nematode parasites in conventional grazing livestock systems has relied on the use of synthetic chemotherapeutic drugs, better known as anthelminthics. Due to the efficacy, broad spectrum use, safety and relative low cost, anthelminthics have become the most popular means of controlling parasites, with the livestock industry relying almost exclusively on their use (Waller, 2006). The heavy dependency on their use has led to increased drug resistance and changes in consumer preference for organically produced livestock. Alternative parasite control measures have been developed including methods for reducing and controlling nematode parasite infections through pasture management, controlling stocking densities and biological control methods.

In organic farming systems the use of anthelminthics is generally prohibited and can only be used in extenuating circumstances. Because of this, the organic industry relies on good knowledge of the epidemiology of parasites and the use of pasture management strategies for the control of parasites. The goal in parasite control is not to eradicate parasites completely as a low level of parasitism is necessary for a positive immune response in the host animal (Corwin, 1997)

2.10 Control using Anthelminthics

Anthelminthics are used in all ruminant livestock species, however the largest market for anthelminthic drugs is the cattle market. Millions of pounds are spent each year to control parasite infections in cattle (Taylor, Coop and Wall, 2007). The three major classes of broad spectrum anthelminthics are benzimidazols, imidazothiazoles and macrocyclic lactones (Waller, 2006). There are two basic methods of anthelminthic administration; mass medication and therapeutic treatment. Mass medication in the form of prophylactic and metaphylactic treatment is designed to suppress or prevent a disease from occurring in an animal while therapeutic treatment is concerned specifically with the treatment of a disease. The first of these two approaches is the most effective in the short term and most commonly used, however, it has been shown to cause drug resistance in parasites. Therapeutic treatment on the other hand is less likely to lead to drug resistant in parasites but the risk of disease and production loss is much higher (Barger, 1999).

2.10.1 Effects on non-target species

Ivermectin (member of the macrocyclic lactone groups) is a highly effective anthelminthic and efficiently controls a broad range of parasites including gastrointestinal and pulmonary nematodes as well as various ectoparasites. It has been widely studied in ecotoxicology due to its persistence, toxicity and wide distribution. However, growing concerns on the unintended side-effects of the widespread use of anthelmintic chemicals (particularly the macrocyclic lactone class), have been expressed amongst the scientific community. Most antiparasitic agents are excreted to varying degrees in the faeces of treated animals, creating concern for fauna that inhabit animal dung (Lumaret and Errouissi, 2002).

Numerous studies have shown strong negative impacts on dung invertebrate fauna from the use of anthelminthics (particularly ivermectin) and these have been thoroughly reviewed by Beynon (2012). Dung invertebrates such as beetles and earthworms provide important ecosystem functions and services in terms of dung decomposition and nutrient cycling on pastures.

Beynon, Peck, Mann and Lewis (2012) showed that ivermectin dramatically alters insect assemblages by reducing Diptera biomass and dung beetle abundance. Additionally, dung decompositions rates were also much lower from ivermectin-treated cattle.

Similar results were observed by Römbke et al. (2010) wherein a significant decrease in the abundance of adult dung beetle in dung pats containing ivermectin was noted. The abundance of dung fly larvae was significantly reduced indicating that they were the most sensitive dung fauna group. This study supported previous findings that dung from ivermectin-treated cattle degraded much more slowly than control pats free of ivermectin.

2.11 Grazing management

Grazing management strategies as a control method were reviewed by Waller (2006). Three management strategies were described: preventative, evasive and diluting strategies.

Preventive strategies involved turning worm-free animals onto clean pastures at the beginning of the grazing season. Evasive strategies involved moving animals onto new pastures before infective larvae emerge in high numbers on the previously grazed pasture, thus avoiding infection. Finally, diluting strategies used older animals that

have acquired natural immunity to graze alongside young vulnerable animals in an effort to reduce herbage infestation.

However, it has also been shown that the use of different livestock species in a mixed grazing system, either grazing together or alternately, is effective at reducing pasture contamination due to host specific parasites that are unable to survive when ingested by non-target hosts (Rocha et al., 2008; Fraser, Moorby, Vale and Evans, 2014).

Parasites are acquired by young animals when grazing on pastures contaminated with parasitic larvae from previous periods of grazing, typically from the previous season. Svensson, *et al*. (2000) compared methods of nematode control in both organic and conventional dairy farming in Sweden through a questionnaire survey. The investigation found that most conventional farmers used prophylactic anthelminthic treatment for parasite control while in organic production, parasite control methods used a range of grazing management procedures integrating grazing management and nutritional supplementation with concentrates. The most commonly used grazing management procedure was the turnout of first season grazers onto pastures that were not grazed by cattle in the current or previous season, showing that good management practices using parasite safe pastures and supplementary feeding at the time of turnout can help control nematode parasite infections. This grazing method seems to be the most popular method used in organic farming and has proven effective in other similar studies (Höglund, Svensson and Hessle, 2001; Larsson *et al*., 2006, 2007).

Despite an increased awareness in various worm control strategies, organic farmers in general have greater problems with parasitic infections in comparison to conventional farmers (Svensson, Hessle and Höglund, 2000).

2.12 Biological control

Biological control methods differ from most other control methods in that they target the free-living stages of the parasite outside of the host. Conventional control methods generally target parasites inside the host. Free-living stages of nematode parasites are particularly vulnerable to environmental conditions and it is this weakness that biological control methods exploit. A number of biological agents have been shown to reduce parasite numbers on pasture, including nematophagous fungi, earthworms and dung beetles.

The efficacy of the nematode trapping fungus *Duddingtonia flagrans* has been investigated in a number of studies with mixed results (Larsen, 1999; Fernández et al., 1999; Waghorn et al., 2002; Waghorn, Leathwick, Chen and Skipp, 2003; Fontenot et al., 2003; Gómez-Rincón, Uriarte and Valderrábano, 2006). While some studies found *D. flagrans* to be effective in controlling nematode parasite numbers (Dimander *et al.*, 2003) others did not. Faessler, Torgerson and Hertzberg (2007) found that *D. flagrans* performed well in the laboratory, reducing the number of infective larvae in faecal cultures by up to 93%. It performed poorly in field experiments by failing to significantly reduce L3 levels.

Studies have shown that dung beetles are capable of reducing the number of larvae of nematode parasites on grazing pastures. Through processes such as feeding and nesting, adult and larval dung beetles can reduce or control the abundance of dung-breeding parasites such as flies, nematodes and protozoa (Nichols *et al.*, 2008).

Fincher (1973) examined the effects of burrowing dung beetles on .*O. ostertagi* larvae. Naturally infected cattle faeces was used to make 1 kg dung pats which were placed on pastures. Beetles were introduced to one of the pastures at 5 times the normal

density. Dung beetles buried 100% of the dung within 72 hours and herbage samples collected form the pasture had 14.7 times less *O. ostertagi* larvae than beetle free pastures.

Dung beetles play an indispensable role in reducing the number of L3 larvae ingested by grazing cattle as shown in an experiment where parasite-free calves were grazed on infected pastures with varying numbers of dung beetles. It was found that calves grazing on pasture with lower than normal dung beetle population acquired nine times more parasites than calves grazing on pasture with higher concentrations of dung beetles (Fincher, 1975)

A study by Grønvold et al. (1992) demonstrated that dung beetles greatly reduced artificially produced faecal pats by 62% and furthermore diminished the effect of splash dispersal of faeces on soil by 70-90%. Additionally, the dung burying activity of beetles was found to be responsible for the destruction of parasite eggs in the soil.

A wealth of similar literature is available on the effects of paracobrid or tunneller beetles on parasitic L3 larvae of trichostrongylids. However, the beetle population of Ireland and other cool temperate regions are dominated by endocopbrids or dung dwellers. Bergstrom, Maki and Werner (1976) showed that *Aphodius* beetles (dwellers) reduced the number of trichostrongylid eggs in faeces by over 90% in a short period of time (1 – 2 days) demonstrating that smaller coprophagous beetles such as *Aphodius* spp. can be effective biological control agents for gastrointestinal parasites.

In contrast to these results a study by Chirico, Wiktelius and Waller (2003) investigating the activity of dung beetles on the development of trichostrongylid eggs found that a greater number of L3 larvae were recovered from dung pats subjected to

beetle activity compared to control pats without dung beetles. This result suggested that the activity of *Aphodius* beetles in faeces can optimise conditions for nematode development from egg to infective larvae under favourable environmental conditions due to aeration of the dung pats. However, unlike the studies mentioned previously which were carried out under field conditions, this study was conducted under artificial laboratory conditions, thus making these results difficult to translate to field conditions.

2.13 Integrated parasite management

Parasite drug resistance is an ever growing problem in the livestock industry due its overwhelming dependency on preventative anthelminthic treatment. This has driven research to discover new ways of controlling parasites. Integrated parasite management (IPM) is a method that uses a combination of both chemical and non-chemical methods for the control of parasites. Implementation of IPM reduces the use of anthelminthics in livestock and in turn reduces the amount of chemicals entering the food chain and the environment. Molento (2009) outlined some effective strategies that may be implemented in parasite control programmes including, target selected treatment (TST), animal-tree-crop integration, drug combination, move and dose and natural remedies. Target selected treatment (TST) is a therapeutic treatment where anthelminthics are only administered to animals that are in need of treatment based on evidence of parasitism and animal production. Faecal egg counts (FEC) are a useful way of determining animal infections that may incur production losses. The use of FEC has been well documented (Ward, Lyndal-Murphy and Baldock, 1997; Agneessens *et al.*, 2000; Borgsteede *et al.*, 2000; Höglund, Svensson and Hessle, 2001; Dimander *et al.*, 2003; Uriarte, Llorente and Valderrábano, 2003; Cringoli *et*

al., 2004; Gruner, Sauvé, Boulard and Calamel, 2006; Bricarello *et al.*, 2007; Larsson *et al.*, 2007; Rocha *et al.*, 2008; Golding and Small, 2009; Oliveira *et al.*, 2009; Piekarska *et al.*, 2013; McMahon *et al.*, 2013; O'Shaughnessy *et al.*, 2015) and remains the most popular diagnostic method for gastrointestinal nematode infections.

2.14 Dung Beetles

Dung beetles belong to the taxonomical order Coleoptera which includes all beetles and comprises more species than any other taxonomical order worldwide (Skidmore, 1991). Although Coleoptera is numerically the largest order of insects, the number of beetles that inhabit and utilise dung is quite low (Skidmore, 1991).

The term dung beetle applies to coprophagous beetles that feed partially or exclusively on animal dung. Dung beetles are found in almost every habitat globally from cool temperate regions in northern Europe to the tropics. True dung beetles are comprised of three families in the superfamily Scarabaeoidea: Scarabaeidae, Geotrupidae and Aphodiidae (Hanski and Cambefort, 1991) but beetles that use dung as a resource are also found in other families.

Dung beetles can be classified into three groups based on behaviour and function. These are: dwellers (endocoprids), tunnellers (paracoprids) and rollers (telecoprids) (Figure 5). Dwellers live within the dung pat and both adult and larval stages feed on dung. Eggs of most species are deposited in the dung without the construction of a nest chamber. Tunnelers dig a vertical tunnel bellow the dung pat and into the soil. Dung is transported to the base of the tunnel for either feeding or breeding purposes. The final group, the rollers, form a dung ball (food ball or brood ball) from faeces taken from the pat and roll it away before burying below the soil surface (Hanski and Cambefort, 1991).

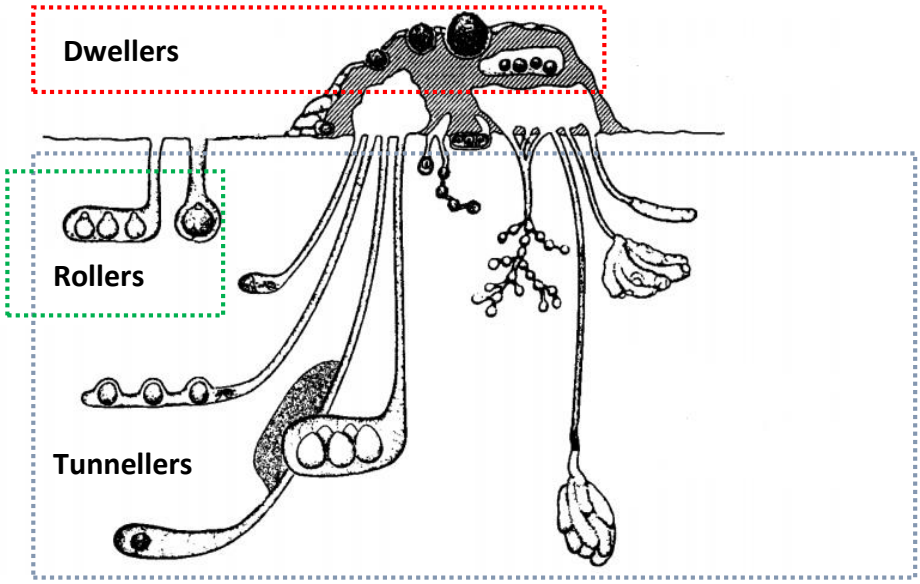

Figure 5. Dung beetles are grouped according to three functional groups: dwellers, tunnellers and rollers (Floate, 2011)

2.15 The Dwellers

The majority of coprophagus species that make up the dwellers are from the genus *Aphodius* in the Aphodiidae family. The majority of these species are relatively small, measuring less than 15 mm in length. They are the most common dung beetles in north temperate regions (Hanski and Cambefort, 1991).

Adults feed on liquid within the dung pat and depending on the species lay their eggs in various parts of the dung. The entire development of *Aphodius* takes place in the dung pat. When the eggs are deposited in the dung patch, the larvae undergo development through three larval stages and a pupal stage before metamorphosing into an adult beetle (Hanski and Cambefort, 1991; Finn and Giller, 2002).

Aphodius species typically prefer to utilise large dung deposits, especially bovine dung. Adults of most species are usually found in fresh dung (or relatively fresh) while

larvae are typically found in dung that is two to three weeks old after the majority of other insects have left (Hanski and Cambefort, 1991). Most species are believed to lay single eggs, but some lay small clutches of eggs in various parts on the dung and soil (Gittings and Giller, 1997)

2.16 North temperate dung beetles

Dung beetle assemblages can be described based on their geographical locations. The communities of dung beetles found vary widely from northern temperate regions to the tropics (Hanski and Cambefort, 1991).

North European temperate coprophagous dung beetles are usually dominated by *Aphodius* species (family Scarabaeidae) (Figure 6), but contain other important beetles including Geotrupidae (dor beetles), Histeridae (clown beetles), Hydrophilidae (water scavenger beetles) and Staphylinidae (rove beetles). Geotrupidae are usually present in low numbers and are typically less abundant in Britain and Ireland (Skidmore, 1991).

Geotrupes (family Geotrupidae) are larger beetles approximately 30 mm in length and are paracoptids, tunnelling beneath the dung forming nests (Figure 6). The adults are fluid feeders and the larvae feed on fibre material within the dung. Hyrophilidae are represented mostly by *Sphaeridium* (mainly *S. lunatum* and *S*, scarabaeoides) and *Cercyon* species (Finn, Gittings and Giller, 1999). *Sphaeridium* species are relatively small, measuring less than 10 mm in length. Adults are dung-feeders and the larvae are predatory. *Cercyon* species are very small, typically less than 4 mm in length. They are amongst the commonest beetles found in British dung (Skidmore, 1991). Dung beetles rarely occur by themselves in dung but are part of a complex community

of other beetles groups, flies and organisms including mites and nematodes (Hanski and Cambefort, 1991).

One of the main characteristics of dung beetle assemblages from north temperate and other temperate regions is the lack of Scarabaeidae species (rollers). Despite the lack of these species, these regions are just as species rich as tropical and sub-tropical communities (Hanski and Cambefort, 1991).

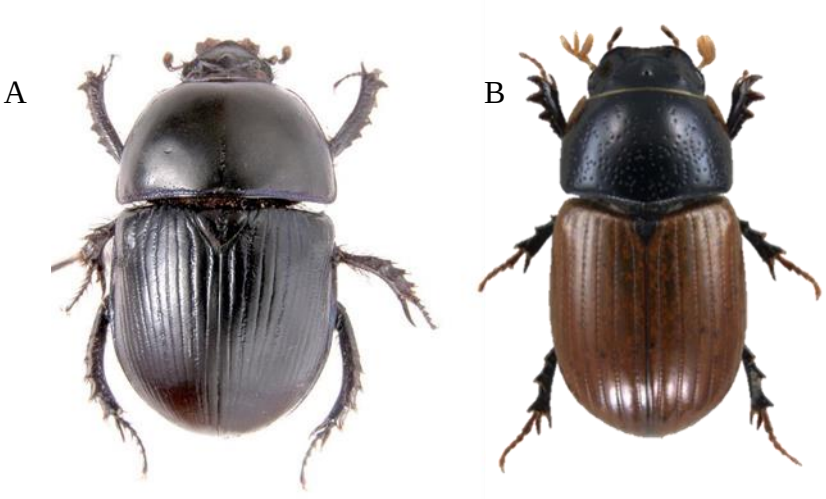

Figure 6. Two species of dung beetle found in Ireland that differ in their nesting behaviours: A: *Geotrupes spiniger* (Sukhenko, 2016), B: *Aphodius fimetarius* (Mann, 2015)

2.17 Ecosystem functions and services of dung beetles

Dung beetles provide important ecosystem functions and services, particularly in the agricultural sector (Beynon, Wainwright and Christie, 2015). Important ecosystem functions provided by dung beetles include dung burial, nutrient cycling, improved plant growth, soil mixing and aeration, secondary seed dispersal and parasite suppression (Nichols *et al.*, 2008). Functions that benefit humans directly or indirectly are also known as ecosystem services. Ecosystem services are the benefits provided by the ecosystem to human life and wellbeing. These services provided by dung beetles are of particular value in the livestock industry (Hanski and Cambefort, 1991; Nichols *et al.*, 2008; Beynon *et al.*, 2012; Beynon, Wainwright and Christie, 2015). Many studies have shown the efficacy of parasite control by dung beetles (Fincher, 1973, 1975; Bergstrom, Maki and Werner, 1976; Grønvold *et al.*, 1992).

Species richness and species diversity is important for the performance of ecosystem functions and services by dung beetles (Yoshihara and Sato, 2015). Studies have investigated the effects different species assemblages have had on ecosystem functions.

Tixier, Bloor and Lumaret (2015) investigated the effects of dung beetle abundance in dung removal and leaf litter decomposition in soil beneath dung pats. The study indicated that dung removal and reduction in leaf litter were positively correlated with dung beetle biomass and abundance. Dung removal rates increased with increasing beetle abundance up to 23%.

A British study carried out by Manning, et al (2016) investigated ecosystem services provided by functionally rich dung beetle assemblages. The study consisted of a field experiment on standardised cattle dung pats and measured three main functions

including dung removal, soil surface aeration and plant litter decomposition. Three dung beetle assemblages of equal biomass were used to represent these functions. A fourth assemblage of all three together was also used for comparative purposes. The presence of individual dung beetles species increased all three ecosystem services. However, it was shown that no single-species assemblage was the most efficient for all measured services. This suggest assemblages with low diversity were less likely to maximise function across a range of ecosystem services (Manning *et al.,* 2016).

Beynon *et al*. (2015) estimated the economic value of dung beetles to the U.K. cattle industry based on four ecosystem services including reduced pest flies; reduced livestock gastrointestinal parasites; reduced pasture fouling and increased nutrient cycling. The study suggested that dung beetles at present may be saving the U.K. cattle industry £367 million annually; £354 million in conventional systems and £13 million in organic systems. Furthermore, it was suggested that if dung beetles were protected under agri-environmental schemes the cattle industry could save a further £40.2 million each year and protecting dung beetles under organic schemes could save the industry £387 thousand annually.

Chapter 3: Methodology

3.1 Overview

The study was conducted during the 2014 grazing season on two sites on the Dingle Peninsula in West Kerry. The two sites used were a coastal lowland commercial organic beef farm and an upland site which comprised of a mosaic of habitats in the Mount Brandon Nature Reserve. The nematode control regime was a lowland organic grassland management system and a free-ranging summer upland system. Initially the entire cattle herd grazed together on the lowland pastures and during this time data was collected on animal live weights and faecal egg counts (FEC). The herd was split in July into two groups, lowland and upland, whereby 61 cattle remained in the lowlands and 39 cattle were allocated to the uplands site to graze for the remainder of the season. Cattle were split into two groups as decided by the farmer based on commercial interests. The study was conducted under real life farming conditions and therefore randomly selected cattle groups could not be used. Animals selected for grazing the uplands were in-calf cows and their calves, in-calf heifers and non-fattening stock. Cows and heifers that were not in-calf were not permitted to go to the uplands as they were to remain with the bull for insemination. Cattle in the lowland group was comprised of cows and their calves, heifers, and fattening stock intended for slaughter during the study period.

Between the months of July and October, information on the prevalence and distribution of nematode parasites and dung beetle populations associated with the two grazing regimes was gathered. Data was collected on pasture infective third-stage (L3) larvae levels, development and migration in cattle dung, (FEC) and dung beetle populations. Vegetation and dung samples were collected and analysed for nutrient and mineral content.

3.2 Study Area

3.2.1 Lowland Site

The lowland site is a 21 hectare commercial organic beef farm located at The Paddock on the eastern edge of Ventry Bay on the southern side of the Dingle Peninsula, approximately 5 km west of Dingle town in County Kerry (Figure 7). The farm is located on the coast, perched on sea cliffs overlooking the Atlantic Ocean. The farm is organically certified and managed in accordance with the standards developed by organic associations recognised by European Union (EU) law (Regulation EEC No.2092/91 and 1840/99). The topography is relatively flat with few distinguishing features with the exception of a ring fort close on the eastern boundary of the farm. The soil is a combination of well-draining fertile acid brown earths/brown podzolics and thin dry lithosols/regosols sitting on top of the underlying sandstone bedrock. Pastures were comprised mostly of perennial rye grass and red clover. Cattle grazed on the fresh pasture throughout the grazing season. A rotational mob grazing system was implemented where high numbers of cattle grazed a relatively small area of pasture for a short period of time. Younger animals were housed for the winter while cows over wintered on turnips or swedes until January when all cattle were housed. Indoors, cattle are fed a whole crop silage containing oats, peas and red clover. Pastures were reseeded in rotation with approximately 10% of the land reseeded annually and silage ground was rotated annually.

3.2.2 Upland Site

The upland site is a 462 hectare Mount Brandon Nature Reserve located in the Brandon Mountains in the townland of Arraglen on the northern edge of the Dingle peninsula approximately 40 km west of Tralee in County Kerry (Figure 7). The site is nestled within the Mount Brandon Special Area of Conservation and straddles a

section of the Dingle Peninsula Special Protected Area. Elevation in the site ranges from almost sea level to the peak of Más an Tiompán, the highest point in the reserve standing at 760 metres (Appendix C). The site is characterised by steep slopes and deep valleys with a mosaic of habitats. The underlying geology is old red sandstone of the Devonian period and the soils are mostly peaty podzols. The dominant habitats in the reserve are Active Blanket Bog, Dry Heath, Northern Atlantic Wet Heath and Wet Grassland (Appendix C) which are listed on Annex I of the E.U. Habitats Directive (National Parks and Wildlife Service, 2013) and are of high conservation value. Cattle had the freedom to roam and graze the full expanse of the reserve but spent much of their time in the Arraglen valley and its adjacent slopes.

The Mount Brandon Nature Reserve was established in 1986 by, the Minister for Tourism, Fisheries and Forestry at the time (Liam Kavanagh) and the reserve has been under the ownership and management of the National Parks and Wildlife Service (NPWS) since its inception. In the past the site was predominantly grazed by sheep, goats and small numbers of cattle. A resident herd of feral goats currently graze the site while sheep have been largely excluded with the erection of a perimeter fence. Grazing trials using Kerry/Highland crosses occurred during the 1990's but it is unclear how many animals were used. Since 2011 approximately 30 Dexter cattle have been grazing the site each summer.

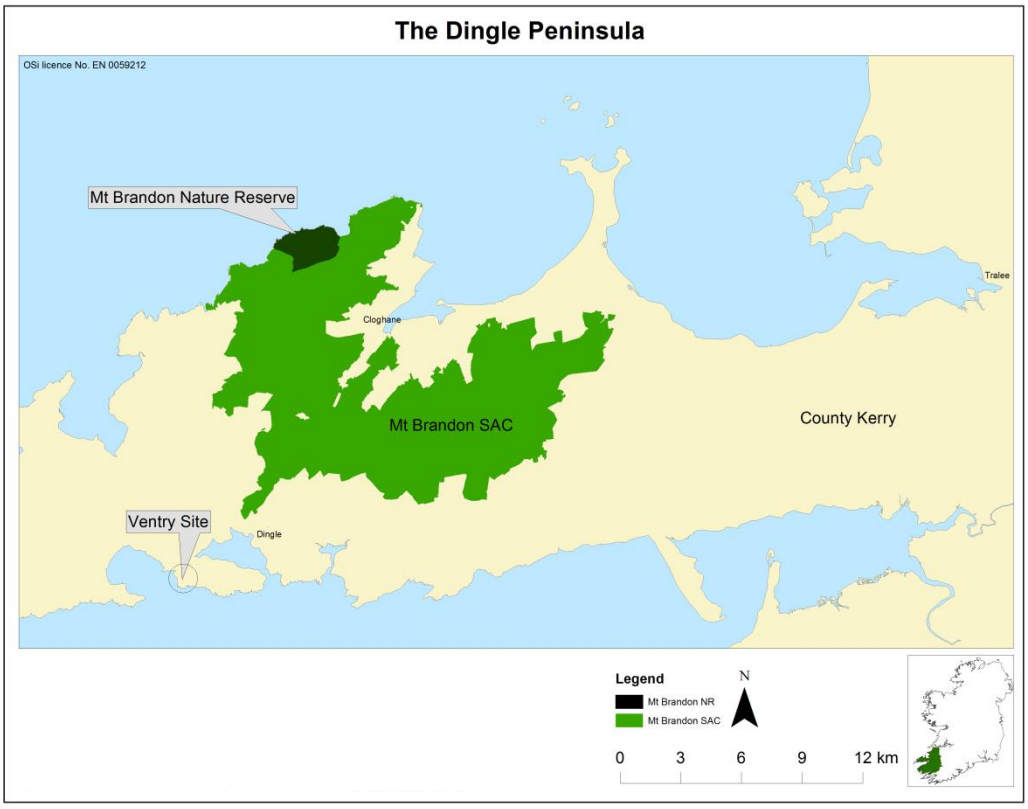

Figure 7. Map of Dingle Peninsula and locations of study sites; Lowland (Ventry) and Upland (Brandon)

3.3 Dexter cattle

Ninety Dexter cattle of mixed age (1 – 15 years) from a total of 100 cattle were used in the study. Natural nematode infection was confirmed by faecal analysis in the form of faecal egg counts (FEC). The herd contained a small number of non-Dexter or Dexter cross breeds which were omitted from the study but did graze alongside the Dexter cattle during the entirety of the season. All animals originated from the same commercial organic beef farm in Ventry, Co. Kerry in 2014. The animals were divided into two groups in July based on the commercial interests of the farm and under the supervision of the owner (Appendix A). This project is part of an upland grazing agreement between the National Park and Wildlife Service and the farm owner. Approximately 30 cattle grazed the Mount Brandon Nature Reserve for the past five years as part of a conservation management study.

The lowland group consisted of 61 cattle of which 52 were pure-bred Dexter and of mixed age. This group grazed improved grassland and were managed in accordance with organic farming practices in Ireland. The upland group comprised of 39 cattle (38 pure-bred Dexter) of mixed age in a grazing system that was extensively managed.

Cattle being fattened for slaughter were retained in the lowlands for ease of access as were heifers/cows that were not in calf at the time. Due to the remote and treacherous nature of the upland location, cows with previous experience of the site were selected along with their calves. Other cattle included in the group were young heifers and steers that were not ready for slaughter and some in-calf cows.

3.4 Cattle Weighing

Cattle were weighed on a lightweight aluminium weighing platform (O'Donovan Engineering) fitted with two TRU-Test MP600 Multipurpose Loadbars with a total capacity of 2000kg each. The load bars were secured to the concrete floor with bolts to prevent the platform from moving. A TRU-Test® ID3000 indicator was connected to the load bars and used for recording cattle weights.

3.5 Parasites burdens in cattle

3.5.1 Faecal Sample Collection

Faecal samples were collected directly from the rectum of each animal. The samples were stored in plastic disposable gloves that were used for faecal collection and labelled with the animal's identification number. Samples were placed in a cooler box for transportation to the lab.

Faecal samples were collected twice during the study. The first samples were collected on July 11th 2014 before the cattle were split into upland and lowland groups and again at the end of the grazing season on November 14th 2014 when cattle returned from the upland site. It was not possible to obtain faecal samples from 5 animals during the first collection in July (at the beginning of the study when the animals were split) as the rectum of these animals was void of faeces. On the second faecal collection in November (at the end of the study), 18 cattle were unable to provide a sufficient faecal sample.

The first faecal egg counts were conducted 6 days after faecal collection. Due to the large numbers of samples, the second faecal egg counts were conducted 16 days after faecal samples were collected. Faecal samples were stored at 4^{o}C until egg counts could be performed.

3.5.2 Faecal Egg Counts

3.5.2.1 McMaster Method

Faecal egg counts were performed using the McMaster method (The Ministry of Agriculture, Fisheries and Food, 1986). Three grams of faecal material was weighed (Shinko Denshi Vibra® HG series Tuning Fork scales) into a glass beaker and 42 ml of tap water added. The mixture was allowed stand for a 10 minutes to assist faecal dispersion. The sample was mixed thoroughly until a homogenous consistency was obtained. The suspension was passed through a 1mm fine mesh sieve to remove debris and the filtrate was collected in a beaker. Debris remaining on the sieve was discarded. Filtrate was transferred to a 15 ml centrifuge tube (Sarstedt, Dublin) and centrifuged at 950 *g* for 2 minutes using a Sigma 6K15 laboratory centrifuge (rotor 11650, bucket 17660). Supernatants were siphoned using a Pasteur pipette aided by a water pump and disposed (autoclaved). The pellet was re-suspended in flotation solution (NaCl and sucrose) up to the 15 ml mark and mixed thoroughly by inverting the tube several times taking care not to create air bubbles. A Pasteur pipette was used to transfer the sample to both chambers of a clean dry McMaster slide (Chalex Corporation). Both chambers were filled to capacity without air bubbles. Here, timing was critical to prevent eggs floating within the pipette. The slide was set aside for 10 minutes during which time intact eggs rose to the surface where they could be counted within the grid area of both chambers. The McMaster slide was placed on a compound microscope (Nikon Eclipse E200) and both chambers were examined at 100x magnification for the presence of eggs. The top layer of the McMaster slide was examined. Gastrointestinal nematode eggs were counted and identified according to Taylor, Coop

and Wall (2007). Egg counts in each grid were summed and multiplied by 50 to obtain the total eggs per gram (EPG) for each sample.

3.6 Flotation Solution

The flotation solution used for faecal egg counts (FEC) was a general purpose sodium chloride and sucrose solution with a specific gravity (SG) of 1.28.

Sodium chloride 400g (Panreac AppliChem) and 500g of sucrose (Panreac AppliChem) were weighed out using a balance (Shinko Denshi Vibra® HG series Tuning Fork scales) and added slowly to 1000 ml of deionised water. Twenty four hours of continuous magnetic stirring was required for the full dissolution of the solids.

3.7 L3 herbage sampling

Pasture sampling was performed every two weeks throughout the grazing season. Samples were collected from 50 x 50 M plots, five in the lowlands and eight in the uplands. In the later site, two plots were located in each of the dominant habitat types: wet grassland, wet heath, dry heath and blanket bog. The position of the upland plots were based on cattle ranging behaviour in the upland site as determined by an on-going study in the Institute of Technology Tralee (Kilian Kelly, personal communication, 2014). In the lowlands five plots were selected in five separate fields as cattle had access to all fields at various times of the years, as per a rotational grazing system.

Herbage samples were collected using a method first described by (Taylor, 1939). Pasture were sampled along a pre-determined “W” shaped transect across each plot. This provided an index of abundance in a manner that could be replicated with minimal bias. Herbage was sampled every 2 meters, collecting a pinch of foliage from 4 points; at the toe, to the left, the right, and straight in front of the collector (Figure

8). As each line of the "W" was approximately 50 meters in length, the total length of the "W" transect was approximately 200 meters. Sampling herbage every two meters from four points provided 400 subsamples across the plot.

To collect the samples the vegetation was pinched with the thumb and fore finger at its base and cut as close as possible to the ground with a knife. Approximately 500 to 1000 g of herbage was collected from each plot. Cutting of the herbage was favoured over pulling because roots and soil can be inadvertently taken which can cause problems when processing in the laboratory. Samples taken at each point were pooled in polythene bags. A uniform sampling pattern was implemented, with all volunteer samplers trained prior to herbage collection to ensure consistent collection.

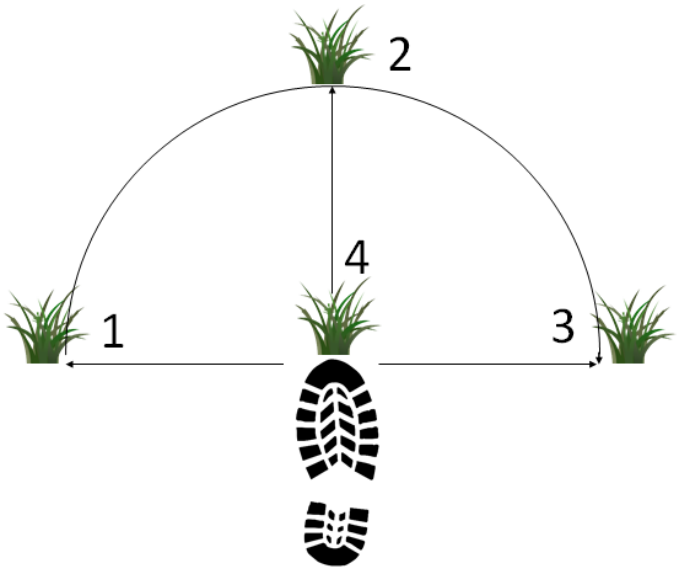

Figure 8. Technique used for collecting pasture samples. Samples were collected in an arc from position 1 to position 3 and in front of the toe at position 4.

3.8 Recovery and isolation of infective L3 larvae

Recovery and isolation of infective larvae was by the MAFF method (The Ministry of Agriculture Fisheries and Food, 1986). The freshly collected herbage samples were placed in 20 litre buckets for washing. Buckets were filled with water and a few drops of the non-ionic surfactant (Tween® 80 BioChemica, PanReac AppliChem) added to reduce surface tension. Contents were agitated by hand to free the L3 larvae from the herbage taking care not to over-fill the buckets to prevent spillage. For the first three hours, the herbage was agitated and churned several times and set aside for one hour to sediment. Washed herbage was removed in handfuls and wrung over the bucket to extract excess water. Drained herbage was then spread on foil trays, weighed and placed in an oven at 100°C for 24 hours until fully dried. When fully dried the herbage weight was recorded and the samples stored in paper bags for further analysis.

The content of the bucket was passed through a 2 mm screen that removed large coarse material such as plant matter. The filtrate was then passed through a second sieve with a mesh size of 212µm. The second stage filtrate was finally passed through a 37µm sieve in order to collect the L3 larvae. The final stage, described above, required the application of a gentle jet of water over the sieve which was held at an angle of 45° to enable larval concentrate. All larvae were removed (by washing) and placed in a beaker. All screens were washed between samples to prevent sample carry over.

Larvae were separated from the water by vacuum filtration. A 15 cm Büchner funnel was fitted to a vacuum flask. The flask was connected to a water jet pump to provide suction. A 150 mm diameter (Whatman™ Grade 1 Qualitative) filter paper was placed in the flask and moistened sufficiently to create a seal. The larvae concentrate from

the last sieving stage was slowly poured into the Büchner funnel and remaining sediment was washed.

The filter paper was placed sediment-side down on single-ply paper towel in the Baermann funnel (Figure 9). The paper towel was porous enough to allow the nematode larvae to pass through but retain the unwanted sediment (Fiel, Steffan and Ferreyra, 2011). The paper towel and filter paper were submerged in the Baermann apparatus for 24 hours to allow larvae to settle to the bottom. After 24 hours a sample was taken from the Baermann apparatus in a 15 ml centrifuge tube and stored at 4°C.

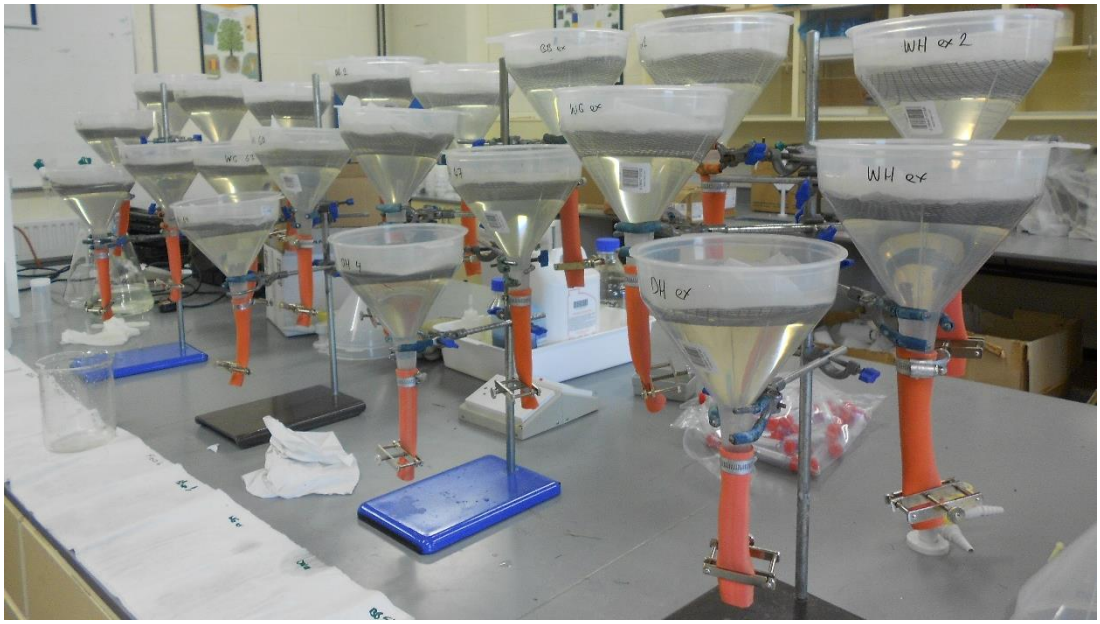

Figure 9. Multiple Baermann funnels used for isolating L3 larvae

3.9 L3 counts and identification

Samples were centrifuged at 240 *g* for 2 minutes and the supernatant discarded leaving behind approximately 1 ml of the larval concentrate. The larval concentrate was placed on several microscope slides to which a drop of Lugol's iodine (Riedel-de Haën) was added. Lugol's iodine was used to kill the larvae and stain any free-living nematodes yellow (The Ministry of Agriculture Fisheries and Food, 1986), while the parasitic L3 larvae remained unstained for up to one hour. Microscope slides were examined using a microscope (Nikon Eclipse E200) at 100x magnification. Photographs of specimens were taken with a high resolution microscope camera (Nikon Digital Sight DS-Fi1). Infective L3 larvae were counted and identified to species level by morphological features using the identification key by van Wyk and Mayhew (2013).

3.10 Larval migration from dung pats

Field experiments investigating the migration of infective third-stage (L3) larvae from dung pats onto herbage were carried out in the lowland and upland sites from August to October. The experiment consisted of replicate standardised dung pats placed in a grid on pasture in both locations at the beginning of each month. Only fresh faecal matter from cattle was used to make the dung pats. The pasture plots in which the pats were placed were cordoned off using electrified fence to prevent cattle interfering with the experiment. Dung pats were formed using a mould 16 cm in diameter and weighed 1 kg each. The dung was homogenised to standardise all samples.

On each sampling day herbage was collected from two radial zones around the perimeter of 5 replicate dung pats. Zone 1 was 10 cm from the edge of the dung pat and zone 2 was 10 cm from the edge of zone 1.

Herbage samples were carefully cut as close as possible to the ground in each zone at four positions around the pat, avoiding the collection of dung or soil, and placed in polythene bags. Herbage samples were collected on days 5, 10, 15, 20, 25, 30 and 35 after dung pat placement.

Herbage samples were transported to the laboratory and stored at 4°C until L3 larvae could be recovered. The procedure was repeated each month until the end of the season.

3.11 Recovery of L3 larvae from herbage of dung radial zones

Recovery and isolation of infective larvae was achieved by following the method described by Pandey (1974). Herbage samples were taken from the polythene bags and placed on a sheet of paper towel and suspended by wire mesh in Baermann funnels. Funnels were filled with tap water, ensuring herbage samples were completely submerged. Samples were at incubated room temperature for 24 hours to allow larval sedimentation.

After 24 hours, the bottom 15 ml of the suspension was taken from the funnel by loosening the tube clamp and filling a 15 ml centrifuge tube. The tube was centrifuged at 240 *g* for 2 minutes. The supernatant was removed from the tube to approximately the 1 ml mark. Infective nematode larvae were identified and counted as described previously.

3.12 Dung beetle collections

Field experiments were conducted to identify serial changes in dung beetle species composition and investigate dung beetle colonisation of dung pats in two different geographical locations under different grazing regimes.

3.12.1 Dung collection for artificial dung pats

Dung for artificial dung pats was collected from both the lowland and upland sites. Only fresh dung could be used for the artificial dung pats to ensure it was free of dung beetles initially. Dung was pooled and stored in buckets for transport. It was important to keep lids on the buckets to prevent dung beetles flying into the buckets during collection. Collected dung was pooled and manually homogenised. This was repeated monthly for the duration of the study.

3.12.2 Dung pat placement

Replicate standardised dung pats were placed in a grid system at the beginning of each month from August to October, on pastures in open areas. Artificially deposited dung pats were 16 cm in diameter and weighed 1 kg each. Dung pats were standardised by weight so as to hold approximately 1 kg of dung. This was achieved by using a dedicated scoop.

Thirty five dung pats were placed in a grid, spaced 1 metre apart with electrical fence erected around the perimeter of the area to prevent cattle from interfering with the experiment.

3.12.3 Collecting the samples

Dung pats were recovered on days 5, 10, 15, 20, 30, and 35 after deposition. On each sampling day, five dungs pats along with underlying soil were collected in 20 litre buckets and sealed with lids. The dung pat and soil were removed to a depth of

approximately 10 cm to entrap tunneler dung beetles that are known to be paracoprid. All samples were placed in individual buckets for transport.

3.13 Dung beetle collection

3.13.1 Extracting the beetles from dung and soil

Beetle isolation protocol was based on the method described by (Krell, 2007). Water was added to each dung sample, filling it almost to the top. The bucket was set aside for approximately 10 minutes to allow the beetles float to the surface. Floating beetles were skimmed from the surface of the water using a 1 mm mesh sieve and put into a labelled plastic collection container. A forceps was used to collect some smaller species. Once surface beetles were removed the softened dung pat and soil were gently agitated and broken apart freeing trapped beetles. The sample was set aside again to allow beetles to float to the surface and beetles were skimmed as described previously.

A novel method was designed to prevent debris floating to the surface (making collection difficult). A 10 mm mesh screen was inserted into the bucket below the surface of the water. The mesh size was large enough to allow beetles to pass through while holding back pieces of debris. This modification was particularly useful in the upland site due to peat soil. A strainer with a 1 mm mesh was used to recover smaller beetles.

When all beetles were collected the water was slowly emptied from the bucket and passed through a sieve to recover any beetles that were missed. Finally, the sediment in the bottom of the bucket was searched for any remaining beetles. Beetles were stored in 96% industrial methylated spirits (Lennox) for identification at a future date.

Dung beetles were removed from their containers and placed on a paper towel sheet to dry. Beetles were examined using a dissecting microscope (Nikon SMZ 745) and

identified by their morphological features using identification keys (Jessop, 1986; Foster, Bilton and Friday, 2014)

To verify beetle species identification, specimens of each species identified were sent to Mr. Mike Hackston (210 St Peters Road, Basingstoke, RG22 6TJ, U.K.), a leading U.K. taxonomist, for confirmation. Species of unconfirmed identification were sent to Mr. Darren Mann, Head of Life Collections at the Oxford University Museum of Natural History in the United Kingdom for further identification.

3.14 Dung and forage analysis

3.14.1 Dung Quality

Dung subsamples were taken from the homogenised dung collected in lowland and upland sites at the beginning of each month (day 0). Subsamples were also taken from dung pats on days 5, 10, 15, 20, 30 and 35 of each month during the study. Dung samples were sent to The Agri-Food & Biosciences Institute (AFBI) in County Down, Northern Ireland for carbon, nitrogen, and dry matter analysis. Only dung subsamples collected in August were analysed as this was the peak larval period.

3.14.2 Forage quality

Herbage samples (from "W" transects) collected during the month of July for the analysis of infective third-stage (L3) larvae were dried and stored in paper bags for forage analysis. Herbage samples from each of the 5 main habitats; improved grass land (lowland), wet grassland, wet heath, dry heath and blanket bog (upland), were sent to The Agri-Food & Biosciences Institute (AFBI) in County Down, Northern Ireland for mineral analysis.

3.15 Meteorological Data

Meteorological data related to both lowland and upland study sites was collected during the study period. Meteorological data for the lowland site was supplied by Ventry Weather, which is located approximately 8 km west of the lowland study site. The privately owned weather station was a Davis Vantage Vue ® and the data was downloaded via an open port and uploaded into Microsoft Excel.

A Davis Vantage Vue ® weather station was positioned in the upland sited to gather meteorological data. The weather station was positioned in the centre of the wet heath enclosure (GPS: Q 47912 15265) which approximated the centre of the upland site. This location was chosen because it was open, easy to access, the cattle were unable to interfere with it and it was out of view from the public. Weather data was stored on a data logger connected to the Davis Vantage Vue ® console. The console was stored in a waterproof box in close proximity to the weather station. The data was uploaded to a computer using Davis Weather Link ® software.

3.16 Statistical Analysis

Raw data was entered into spread sheets and descriptive statistics were calculated using Microsoft Excel® 2013.

Data were evaluated statistically with Graphpad Prism 6. Kruskal Wallis and Mann-Whitney *U*-test were used to analyse FEC abundance, intensity, L3 migration, and pasture larval levels. Chi-squared and Fisher's exact test were used to analyse FEC prevalence between sex and age groups.

Microsoft Excel® was used to perform Shannon-Weiner diversity index, Pielou's evenness index and to create Whittaker plots. Shannon-Weiner diversity index was used to calculate dung beetle species diversity and Pielou's evenness index was used to calculate dung beetle species evenness. Species evenness was presented using Whittaker plots.

No statistical analyses were performed on the analytical parameters of the dung, as these measurements were only used to describe the dung quality.

In all cases a *p*-value of < 0.05 was taken to indicate significance.

Chapter 4: Results

4.1 Host parasite burden

Faecal egg counts (FEC) were performed on faecal samples collected from cattle to determine the burden of gastrointestinal nematode eggs in the host. Samples were collected twice during the study (Appendix A).

The first FEC were performed on July samples at the beginning of the study before the cattle were split into two groups for the duration of the grazing season. A total of 82 cattle (24 calves, 28 weanlings and 30 adults) were sampled from the 87 Dexter cattle in the herd. Faecal samples were collected again in November 2014 at the end of the grazing season when the cattle were reunited. By the end of the study the number of cattle was reduced in size from 87 to 71 due to farming practises. Of the 71 cattle remaining faecal samples were collected from 53 animals (20 calves, 9 weanlings and 24 adults).

4.2 Host parasite distribution

The distribution of gastrointestinal parasite eggs in cattle faeces in July and November is shown in Figure 10. On both sampling sessions the majority of cattle did not excrete detectable levels of nematode eggs (< 50 EPG) in the faeces. Most of the FEC came from a relatively small number of individuals. As with most parasite infections the majority of cattle harbour little or no parasites, but a small proportion of individuals carry most of the parasites present within the population. The distribution of parasites was over-dispersed on each occasion resulting in an aggregated (right skewed) distribution (Figure 10).

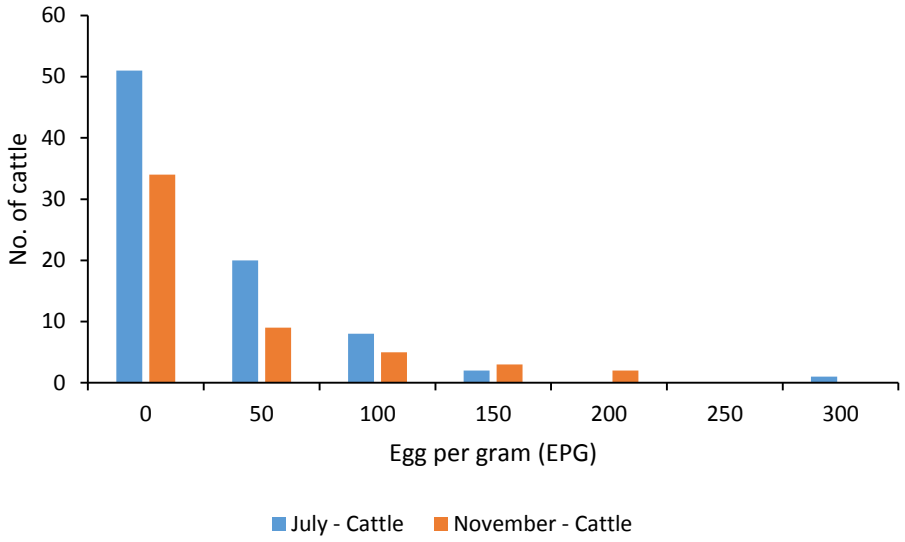

Figure 10. The distribution of nematode eggs per gram (EPG) of faeces in pooled faecal samples collected from all cattle in July and November 2014

Mean FECs were similar in July and November with no significant difference ($p > 0.05$). The mean FEC at the start of the study in July were 29.27 EPG and this rose to 33.96 EPG by the end of the study in November. Individual faecal egg counts ranged from 0 -300 EPG and 0 – 200 EPG for July and November FEC's respectively.

Distribution of nematode eggs from FECs between the three cattle age groups in July and November 2014 is shown in Figure 11 and Figure 12 respectively. In both July and November the younger cattle; calves and weanlings, excreted higher numbers of nematode egg in faeces compared to adults (Figure 11 and 12)

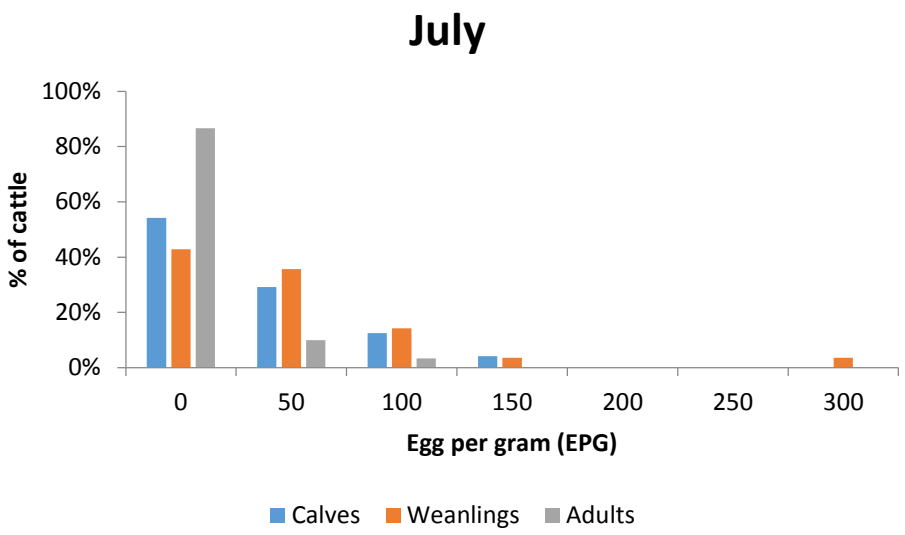

Figure 11. The distribution of nematode eggs per gram (EPG) of faeces in faecal samples collected from the calf, weanling and adult age groups in July 2014

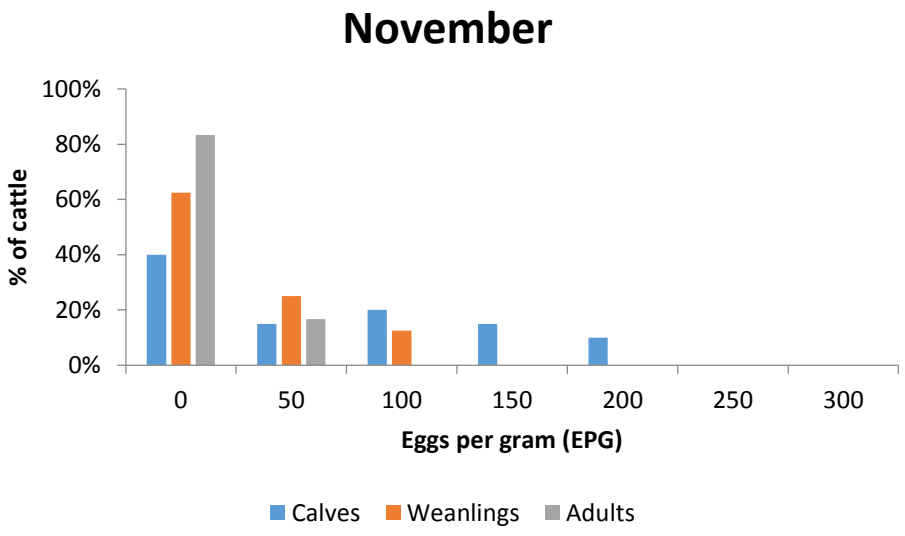

Figure 12. The distribution of nematode eggs per gram (EPG) of faeces in faecal samples collected from the calf, weanling and adult age groups in November 2014

4.3 Parasite prevalence in hosts over grazing season

Faecal egg counts recorded at the beginning of the study were compared to counts at the end of the study to investigate if parasite burden changed over the course of the study.

Three measures were used to describe the gastrointestinal nematode parasite infection in the cattle used in the study. These were prevalence, mean intensity and mean abundance as defined by Bush *et al.* (1997).

*"**Prevalence** is the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species."*

*"**Mean intensity** is the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite."*

*"**Mean abundance** is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of the species examined (including both infected and uninfected hosts).*

A total of 135 faecal samples were collected from Dexter cattle from the beginning of the study in July to the end in November and of these, 50 (37 %) of the samples tested positive for gastrointestinal nematode parasite eggs.

The prevalence of gastrointestinal nematode parasites in cattle did not differ significantly between July to November ($p = 0.857$).

Parasite egg prevalence in cattle sampled in July and November is shown in Table 1. Nematode eggs were found in the faeces of all three cattle age groups and in both males and females. There was no significant difference in prevalence between males and females in age groups ($p > 0.05$) (Appendix B).

There was a significant difference in relation to age. Over both sampling periods there were statistically significant differences in prevalence between cattle age groups. Both calves and weanlings had a significantly higher prevalence of parasitic nematode eggs than adults in July ($p < 0.05$). In November, calves had a significantly higher prevalence than adults ($p < 0.05$).

Table 1. The mean prevalence (%), mean abundance (EPG) and mean intensity (EPG) of nematode eggs in cattle of different age groups sampled in July and November 2014 (± Standard error)

Time	Age Group	n	Prevalence	Abundance	Intensity
July	Calves	24	45.83	33.33 ± 8.86	72.77 ± 10.37
	Weanlings	28	57.14	48.21 ± 12.19	84.38 ± 16.28
	Adults	30	13.33	8.33 ± 4.21	62.5 ± 12.5
	Overall	82	37.80	29.27 ± 5.41	77.42 ± 9.23
November	Calves	20	60.00	70 ± 15.98	116.67 ± 15.49
	Weanlings	9	33.33	22.22 ± 12.11	66.67 ± 16.67
	Adults	24	16.67	8.33 ± 3.88	50
	Overall	53	35.85	33.96 ± 7.59	94.74 ± 12.03

4.3.1 Lowland versus upland

By the end of the study in November, the number of cattle in each group had reduced due to farming practices. Of the 52 lowland cattle 38 remained in November as 14 animals (13 weanlings and one adult) were slaughtered. The number of cattle in the upland group was reduced from 38 to 33 as four animals were sold and one animal went missing and was never recovered. Faecal samples were collected from 31 of the 38 upland cattle and 22 of the 33 lowland cattle (Table 2). A third of the lowland cattle were unable to provide a faecal sample as their rectums were void due to the long journey from the upland site in Brandon to the farm in Ventry where weighing and faecal sampling was performed. Only seven of the 38 lowland cattle were unable to provide a faecal sample due to empty rectums (Table 2).

Faecal egg counts recorded from cattle that grazed in the lowlands and uplands were compared to determine if the grazing site had an effect on parasite burden.

Overall, there was no significant difference in prevalence between cattle that grazed the lowlands and those that grazed the uplands ($p = 0.385$), but there were significant differences between age group. Across both the lowland and upland sites calves and weanlings had a higher prevalence than adult cattle, as shown in Table 2. Calves had the highest prevalence in each site with adults having the lowest.

Despite the difference in prevalence between calves and adults across both sites, in the uplands this difference was not statistically significant ($p = 0.235$) but there was a statistically significant difference between the calves and adults in the lowlands; calves had a significantly higher prevalence than adults ($p = 0.025$).

Table 2. The mean prevalence (%), mean abundance (EPG) and mean intensity (EPG) of nematode eggs in cattle of different age groups sampled in November 2014 from lowland and upland groups

Site	Age Group	n	Prevalence	Abundance	Intensity
Lowland	Calves	14	64.29	82.14 ± 20.71	127.78 ± 18.84
	Weanlings	2	50	-	-
	Adults	15	20	10 ± 5.35	50
	Overall	31	41.94	43.55 ± 11.53	103.85 ± 16.47
Upland	Calves	6	50	41.67 ± 20.07	83.33 ± 16.67
	Weanlings	7	28.57	21.42 ± 14.87	75 ± 25
	Adults	9	11.11	-	-
	Overall	22	27.27	20.45 ± 7.83	75 ± 11.18

4.4 Mean abundance and intensity of parasite in host

Mean abundance and intensity could not be calculated for lowland weanlings as only two animals remained in this age group at the end of the study due to farming practices (slaughter). Similarly, mean abundance and intensity could not be calculated for upland adults. Of the nine upland adults only a single animal was infected, hence means could not be calculated.

4.4.1 Mean Abundance

The overall mean abundance for July and November as well as the age groups is shown in Table 1. Mean abundance was similar for both July and November with no significant difference between periods, however there was a statistically significant difference among the cattle age groups ($p < 0.05$), with calves and weanlings having a higher abundance than adults in July and calves having a higher abundance than adults in November, as shown in Table 1.

The mean abundance for the lowland and upland sites is shown in Table 2. The lowlands had the highest overall mean abundance but it was not significantly higher than the uplands ($p > 0.05$). There was a significant difference in age groups ($p < 0.05$) with calves having a significantly higher abundance than adults ($p = 0.005$) in the lowland site.

4.4.2 Intensity of infection

Table 1 and Table 2 show the mean intensity of nematode eggs in cattle in July and November and upland and lowland sites, respectively. There was no significant difference in intensity between the sampling times (July and November) and site location (upland and lowland) ($p > 0.05$). In the uplands, calves had a significantly

higher intensity than adults (p = 0.029). There was a similar trend in the lowlands, with calves having a higher intensity than adults (p = 0.059).

4.5 Cattle weight gain

Cattle were weighed to at the beginning of the study to acquire a baseline weight before they were split into the lowland an upland cohorts (Appendix A). At the end of the study in November cattle were reweighed and weight gain between groups were compared. The mean weight for each animal group is shown in Table 3. Overall the lowland cattle had a higher daily weight gain than the upland group. All three age groups in the lowland site gained more weight than their counterparts in the upland site. Calves gained the most weight, followed by weanlings, and adults gained the least amount of weight. Results showed upland adults had a reduced weight and negative weight gain.

Table 3. Number of cattle (n) and mean cattle live weights (kg) recorded in July and November. Mean daily weight gain (kg) (DWG)

		July		**November**	
Lowland	**n**	**Weight (kg)**	**n**	**Weight (kg)**	**DWG**
Calves	16	114.5 (±8.5)	16	191.7 (±13.2)	0.61
Weanlings	14	241.8 (±16.7)	4	244.5 (±45.9)	0.22
Adults	18	326.2 (±16.2)	15	327.8 (±17.4)	0.08
Overall	**48**	**231.0 (±15.3)**	**35**	**256.1 (±15.2)**	**0.34**
		July		**November**	
Upland	**n**	**Weight (kg)**	**n**	**Weight (kg)**	**DWG**
Calves	9	82.5 (±10.7)	9	129.6 (±12.9)	0.37
Weanlings	15	211.0 (±14.2)	12	217.5 (±10.3)	0.18
Adults	14	292.0 (±10.3)	12	278.5 (±8.6)	-0.14
Overall	**38**	**210.4 (±14.9)**	**33**	**215.7 (±11.9)**	**0.12**

4.6 Pasture L3 larvae level

The levels of L3 larvae on pastures in lowland and upland sites throughout the study are shown in Figure 13. The highest L3 levels on pasture were measured in late August in both the lowland and upland sites, with 262.8 and 47.6 L3 per kg dry herbage, respectively. There was significantly greater level of L3 larvae on the lowland pastures than the upland pastures (p = 0.003).

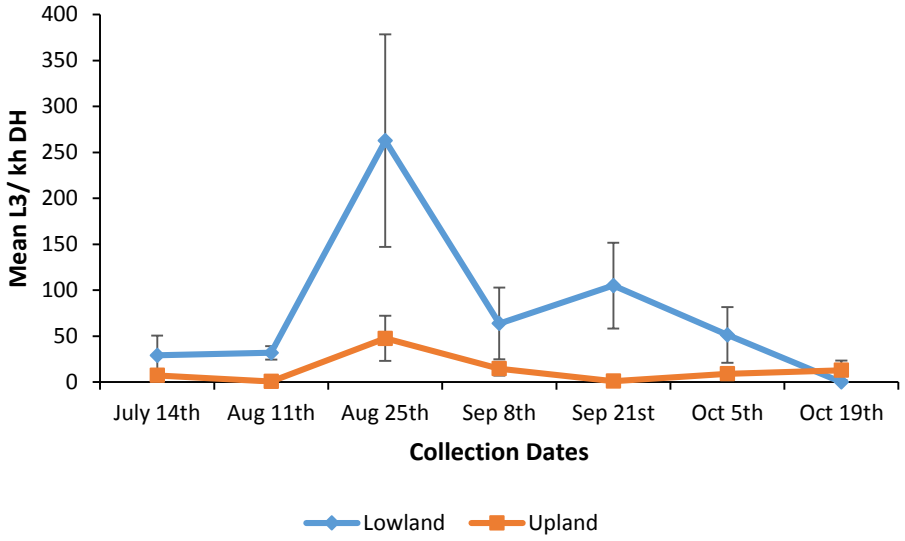

Figure 13. Pasture burden of infective third-stage (L3) larvae in the lowlands and uplands throughout the study period

Data from the grass samples showed mixed parasite populations on the pasture in both the lowland and upland sites. The most prevalent nematode larvae species found in both the uplands and lowlands were *O. ostertagi* and *C. oncophora*. Other species recorded were *T. axei*, *Strongyloides sp.*, *Oesophagostomum radiatum* and *N. battus* but these were present in low numbers on a few occasions (Table 4).

Table 4. Prevalence (%) of third-stage (L3) larvae recovered from lowland and upland pastures over the study period

	Mean prevalence (%)	
Species	**lowland**	**upland**
Ostertagia ostertagi	63.72	42.85
Cooperia oncaphora	31.79	30.13
Trichostrongylus axei	2.99	16.45
Strongyloides sp.	0.83	0.59
Oesophagostomum radiatum	0.53	9.98
Nematodirus battus	0.14	0.00

The mean infective L3 larvae levels of parasitic nematodes on the pasture of each habitat sampled is shown in Figure 14. Of the four habitats that were sampled in the upland site, the highest infective third-stage larvae (L3) levels were found on the dry heath with 133.9 L3 larvae per kg of dry herbage recorded on the second collection period in August. Infective L3 larvae were recovered on all five habitats sampled during the study period.

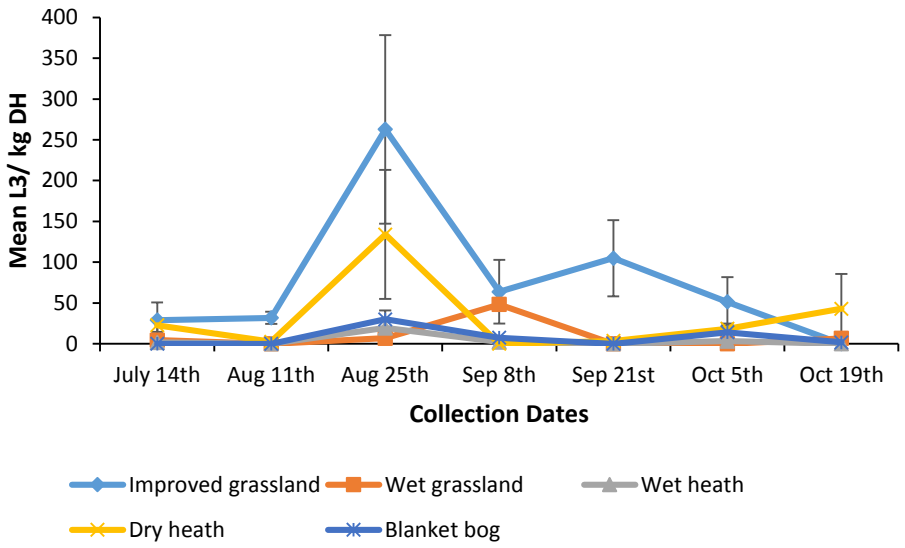

Figure 14. Pasture levels of infective third-stage (L3) larvae on each of the habitats sampled throughout the study period

4.7 Migration of third-stage (L3) larvae from artificial dung pats

To investigate the migration of L3 larvae from dung pats on to pasture in the lowlands and uplands, herbage was collected adjacent to dung pats deposited on pasture each month. Samples were collected five days after deposition and every five days thereafter, with seven collection days in total for each set of dung pats.

4.7.1 Larvae species

Table 5 shows the L3 larvae species that migrated from dung pats onto herbage in the lowland and upland sites. Third-stage larvae recovered from herbage adjacent to dung pats in both uplands and lowlands showed a similar mix of species. Both sites were dominated each month by the same two species, *O. ostertagi* and *C. oncophora*, with *T. axei*, *O. radiatum* and *N. battus* found in low numbers on a few occasions.

Table 5. Mean percentage of *C. oncophora* (% Coop), *O. ostertagia* (% Oster), and other nematode species (% Other) extracted from herbage harvested within 20 cm of artificial dung pats deposited in July, August, September and October

		July	**August**	**September**	**October**
	% Oster	56.5	49.2	39.3	50
Lowland	% Coop	38.8	50.3	60.7	49.7
	% Other	4.6	0.5	0.0	0.3
	% Oster	-	37.8	69.3	64.9
Upland	% Coop	-	61.7	30.4	29.73
	% Other	-	0.5	0.3	5.4

Table 6 shows the mean number of infective third-stage (L3) larvae recovered each month from herbage around the dung pats.

In the months August and September a higher number of larvae were recovered in the uplands compared to the lowlands, however it was not statistically significant ($p > 0.05$). There was a significant difference between the L3 larvae numbers in the uplands and lowlands in October with the lowlands having significantly higher counts ($p = 0.0001$).

Table 6. Mean number of infective third-stage (L3) larvae recovered from herbage around dung pats placed each month (± Standard error)

Month	**Lowland**	**Upland**
July	6.8 ± 2.2	-
August	27.7 ± 12.5	34.0 ± 18.9
September	10.5 ± 4.4	29.6 ± 16.0
October	20.8 ± 6.3	1.1 ± 0.5

4.7.2 Distance L3 larvae migrated from dung pats

Migration of L3 larvae from artificial dung pats in both the upland and lowland sites was measured throughout the grazing season. Table 7 shows the proportion of larvae found in each radial zone in each month during the study. The majority of L3 larvae were found in the herbage inner zone (0 -10 cm) around the dung pat on both sites.

Table 7. Percentage of third-stage larvae recovered from herbage adjacent to dung pats in both inner and outer radial zones

	Lowland			
	July	**August**	**September**	**October**
Inner zone	83.97%	90.63%	73.77%	69.92%
Outer zone	16.03%	9.37%	26.23%	30.08%
	Upland			
	July	**August**	**September**	**October**
Inner zone	-	86.88%	73.62%	62.16%
Outer zone	-	13.12%	26.38%	37.84%

Dung pats were not placed in the uplands in July

4.8 Monthly weather data

The mean monthly temperature and cumulative monthly precipitation during the study period are presented in Figure 15. Monthly cumulative rainfall was higher for the upland site than the lowland site in all months. It was observed that mean monthly temperatures were higher in the lowlands than the uplands.

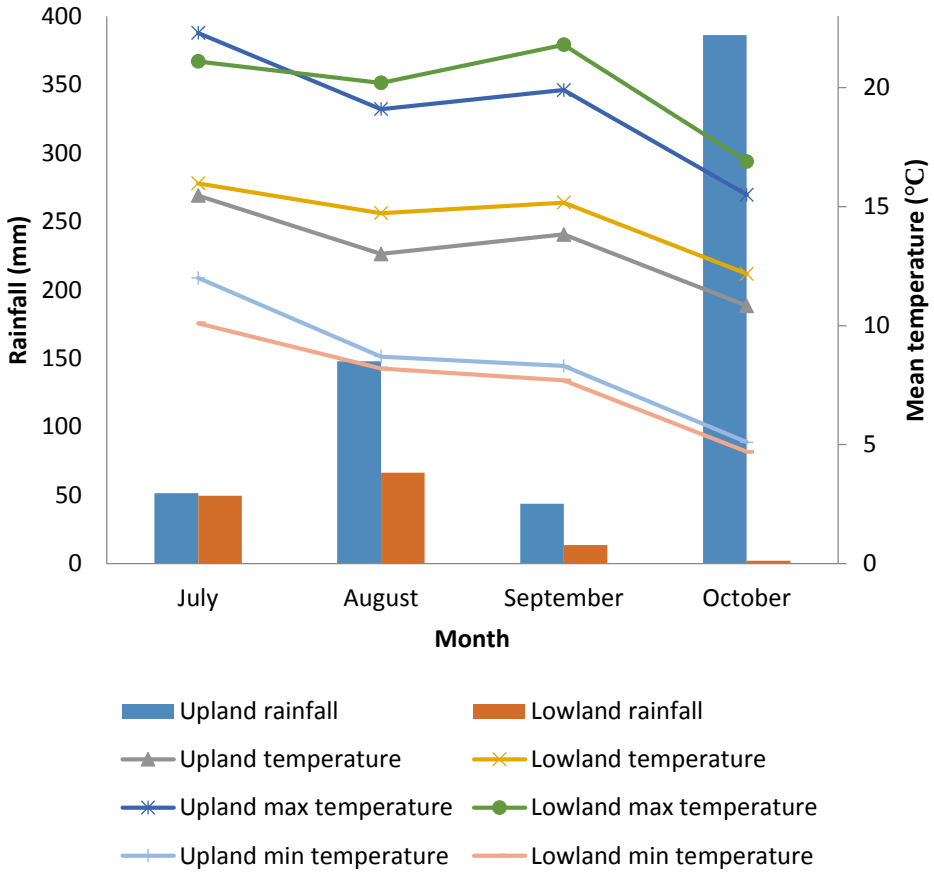

Figure 15. Monthly rainfall and maximum, minimum, and mean monthly temperatures in the lowlands and uplands during the grazing season

The mean daily temperature in the lowlands was higher than the long term average (LTA) (Appendic D), in July, September and October whereas temperatures were lower than the LTA in August. Monthly cumulative rainfall in the lowlands in July,

August September and October were lower than the LTA. September and October considerably lower with rainfall of 13.6 mm and 2 mm respectively (Appendix E)

The mean daily temperatures in the uplands were lower than LTA in August, September and October while July was higher. Monthly cumulative rainfall in the uplands was lower than the LTA in July and September. August and October experienced more rainfall than the LTA with October being exceptionally wetter with approximately 209 mm more than the LTA (Appendix E)

As weather influences the development and migration of nematode larvae meteorological conditions were looked at in more detail. Appendix D shows the total mean temperature, total rainfall and relative humidity between each collection period.

4.9 Dung Beetles

4.9.1 Species list

Table 8. Number of each beetle species collected from artificial dung pats in both study sites

			Lowland	**Upland**
Family	**Genus**	**Species**	***n***	***n***
Scarabaeidae	*Aphodius*	*ater*	0	3
Scarabaeidae	*Aphodius*	*depressus*	1	7
Scarabaeidae	*Aphodius*	*fasciatus*	0	25
Scarabaeidae	*Aphodius*	*fasciatus transitus*	0	1
Scarabaeidae	*Aphodius*	*fimetarius*	24	14
Scarabaeidae	*Aphodius*	*fossor*	1	0
Scarabaeidae	*Aphodius*	*prodromus*	1	0
Scarabaeidae	*Aphodius*	*rufipes*	18	27
Scarabaeidae	*Aphodius*	*sphacelatus*	1	0
Hydrophilidae	*Cercyon*	*haemorrhoidalis*	0	2
Histeridae	*Margarinotus*	*carbonarius*	4	0
Hydrophilidae	*Cercyon*	*impressus*	53	23
Hydrophilidae	*Cercyon*	*lateralis*	908	8
Hydrophilidae	*Cercyon*	*melanocephalus*	39	19
Hydrophilidae	*Cercyon*	*pygmaeus*	285	83
Hydrophilidae	*Coelostoma*	*orbiculare*	0	19
Hydrophilidae	*Cryptopleurum*	*minutum*	189	21
Hydrophilidae	*Cryptopleurum*	*subtile*	3	0
Hydrophilidae	*Megasternum*	*concinnum*	130	47
Hydrophilidae	*Sphaeridium*	*lunatum*	1	2
Hydrophilidae	*Sphaeridium*	*scarabaeoides*	2	0

4.10 Species richness

A total of 1,961 beetles, nine species of *Aphodius*, five species of *Cercyon*, two species of *Sphaeridium* and *Cryptopleurum* and a single species of *Megasternum*, *Margarinotus* and *Coelostoma* were recorded between August and October in the lowland and upland study sites (Table 8).

These beetles belonged to three families of Coleoptera which were; Scarabaeidae (scarab beetles), Hydrophilidae (water scavenger beetles) and Histeridae (clown beetles). Of the 21 species identified, 16 species were recorded in the lowlands and 15 in the uplands. Six species (28.6%) were found exclusively in the lowland site, five (23.8%) species were found exclusively in the upland site and 10 species shared (47.6%) both sites.

Hydrophilidae was the richest family in both sites with eleven species, followed by Scarabaeidae with nine species. The Histeridae family was represented by only one species, *Margarinotus ventralis*, which was present in the lowland site only. The majority of beetles were members of the Hydrophilidae family which made up 93.5% of the total catch. *Aphodius* was the richest genus with nine species recorded across both sites. Of these, only single specimens of *A. depressus, A. fossor, A. prodromus* and *A. sphacelatus* were recorded.

The lowland site had the highest number of beetles recorded with 1,660 individuals comprising 84.7% of all beetles recorded (Table 8). Of these, 1610 individuals belonged to the family Hydrophilidae. *Cercyon lateralis* was the most common hydrophilid, followed by *Cercyon pygmaeus*, *Cercyon minutum* and *Megasternum concinnum*. Other hydrophilids were present in relatively low numbers (Table 8). *Aphodius* was the only genus of Scarabaeidae present. *Aphodius fimetarius* and

Aphodius rufipes were the most common scarabids in the lowland site with all other *Aphodius* species appearing only once in the season (Table 8). Common *Aphodius* species are shown in Appendix F.

The upland site had a much lower number of beetles recorded than the lowland site, with a total of 301 beetles (15.3%) recorded (Table 8). *Cercyon pygmaeus* was the most abundant species overall followed by *Megasternum concinnum*. *Aphodius rufipes, Aphodius fasciatus transitus* (sub species of *Aphodius fasciatus*) and *Aphodius fimetarius* were the most common scarabids.

Table 9. Characteristics of beetle assemblages in both sites over the season. Columns indicate the total number of beetles (n) collected, Simpson's Index of Diversity, maximum possible value of H^I (H^Imax), number of species (SR = species richness)

Lowland Site

	n	**SR**	**Diversity**	**H^I_{max}**	**Evenness**
August	658	13	0.75	2.3	0.32
September	859	13	1.6	2.61	0.61
October	147	11	2.00	2.4	0.83
Season	**1660**	**16**	**1.44**	**2.77**	**0.52**

Upland Site

	n	**SR**	**Diversity**	**H^I_{max}**	**Evenness**
August	47	13	1.77	2.4	0.74
September	217	14	2.02	2.64	0.76
October	37	10	1.95	2.3	0.84
Season	**301**	**15**	**2.26**	**2.71**	**0.83**

Overall species richness was similar in both the upland and lowland sites over the entire season and across each month (Table 9). Sixteen species were found in the uplands and 15 in the lowlands.

Species diversity was generally highest in the uplands each month. In the lowland site, diversity was lowest in August due to the dominance of *C. lateralis*. The uplands showed little fluctuation in diversity across the season.

Though species richness was similar across both sites, species assemblages differed. Less than 50% of beetles shared both lowland and upland sites. *C. laterlis* was found in very high numbers in the lowlands but very few in the uplands. Similarly, *A. depresses* and *A. fasciatus* were found in higher numbers in the uplands.

Dung beetles succession was observed in the month of August in both sites (Figure 16 and 17). Figure 16 shows that the mean number of dung inhabiting beetles was highest in the lowland site on the first collection day (5 days after deposition) with. Beetle numbers quickly dropped after day 5 to a recover of zero beetles on day 35. Dung beetle numbers in the uplands were low throughout the month (Figure 17).

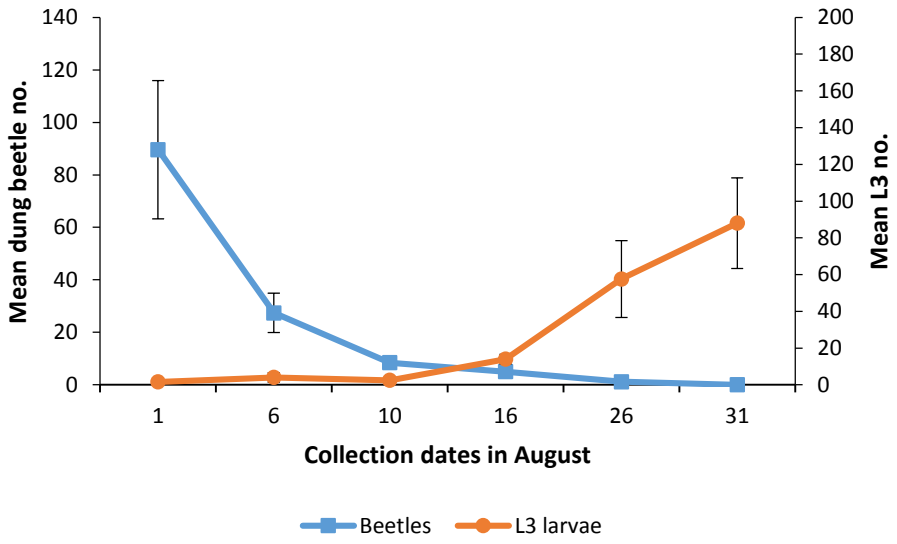

Figure 16. Number of dung beetles and L3 larvae collected in the lowland site in August

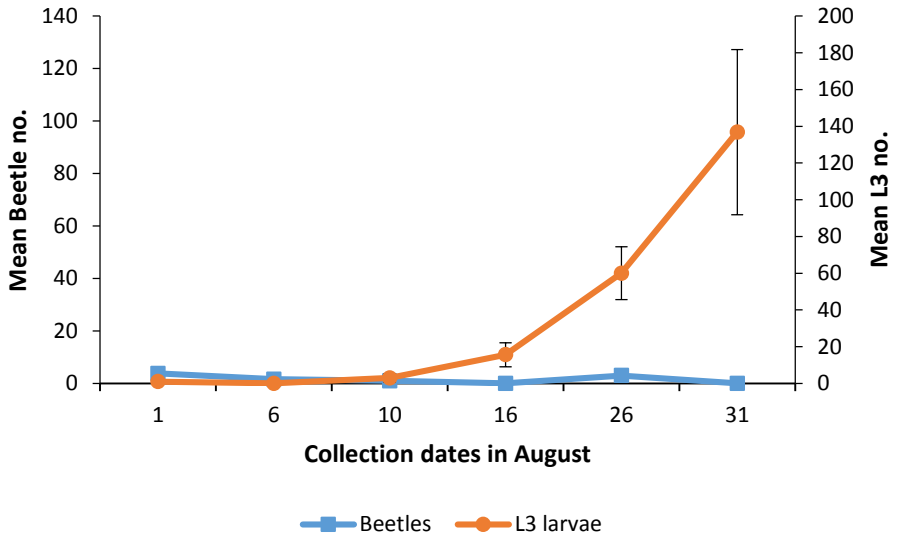

Figure 17. Dung of dung beetles and L3 larvae collected in the upland site in August

4.11 Rank Abundance

Whittaker plots were used to graph species evenness across each month and the entire grazing season. Species diversity was highest in all months in the upland sites. Species evenness was also highest in the uplands across the season.

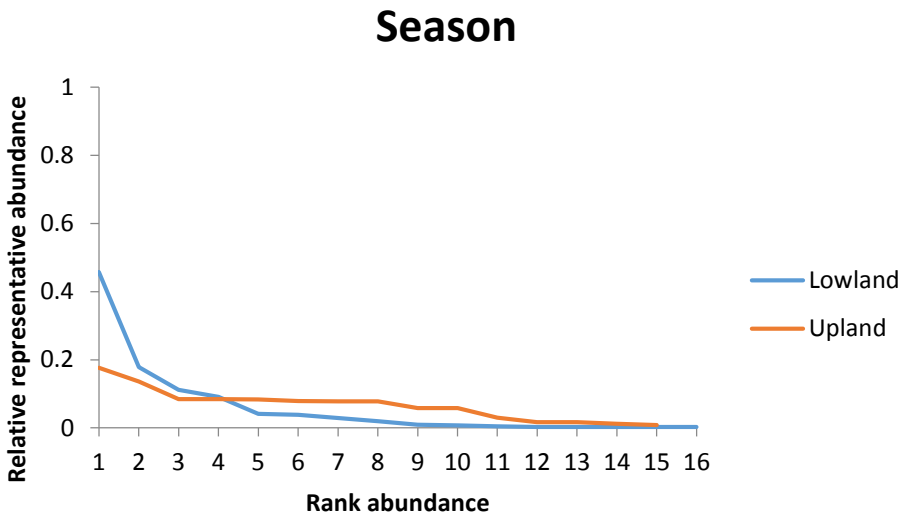

Figure 18. Species evenness in the lowland and upland sites across the entire season

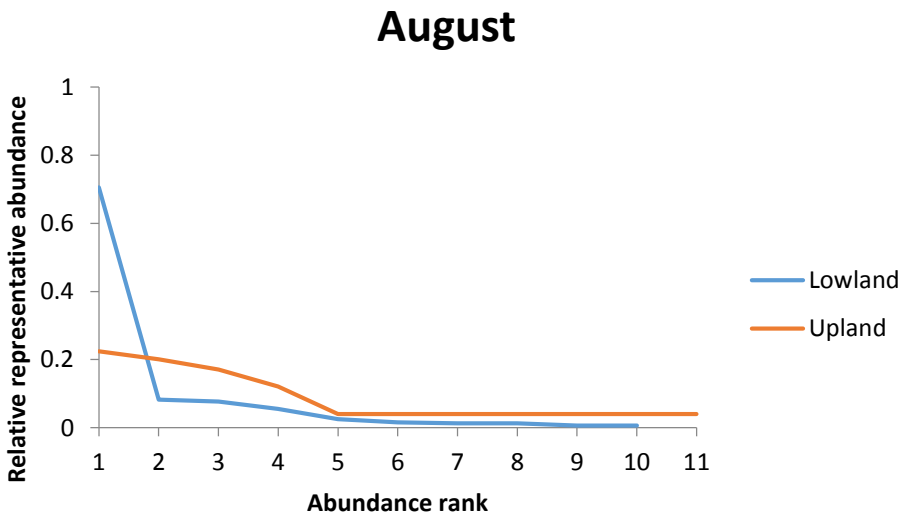

Figure 19. Species evenness in the lowland and upland sites in August

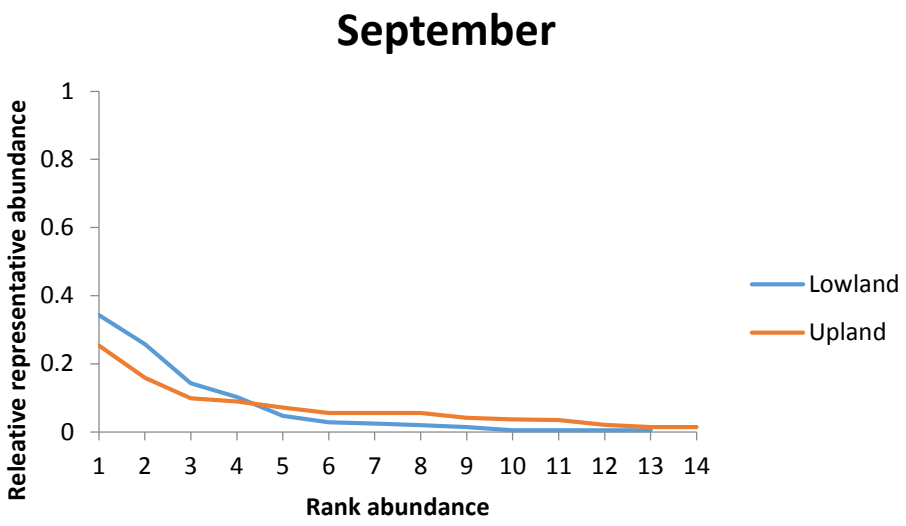

Figure 20. Species evenness in the lowland and upland sites in September

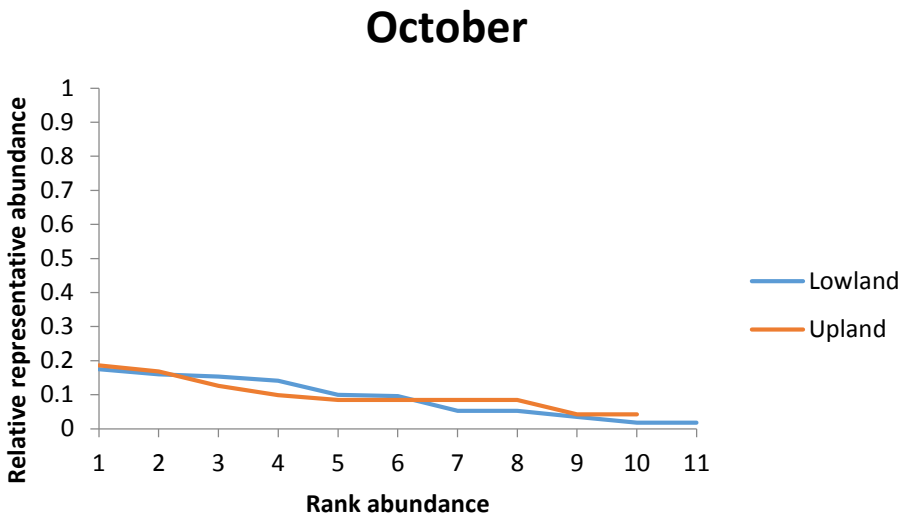

Figure 21. Species evenness in the lowland and upland sites in October

4.12 Dung beetle and nematode parasite interaction

Mean dung beetle numbers and mean nematode larvae numbers from dung pats sampled throughout the month of August in upland and lowland sites are shown in Figure 16 and 17, respectively. The lowland site had higher numbers of beetles and lower numbers of larvae compared to the upland site which had low numbers of beetles and higher numbers of larvae.

4.13 Monthly dung analysis

Dung subsamples were taken from the homogenised dung collected in lowland and upland sites on day 0 of each month: August, September and October. Dry matter, carbon and nitrogen levels are presented in Table 10. Lowland dung had a lower carbon/nitrogen ratio in each month and lower dry matter levels than upland dung.

Subsamples were also taken from dung pats on days 5, 10, 15, 20, 30 and 35 from upland and lowland sites during the month of August. Samples were tested for carbon, nitrogen and dry matter. Dry matter, carbon and nitrogen levels from lowland and upland sites are presented in Tables 11 and 12 respectively. Lowland dung had a lower carbon/nitrogen ratio on each collection day.

Table 10. Analysis of dung collected at day 0 and the beginning of each month

	Lowland Site			Upland Site		
	August	September	October	August	September	October
DM (g/kg F)	114.90	90.10	107.10	146.80	128.90	147.10
C (g/kg F)	50.44	44.70	49.62	76.03	60.65	70.73
N (g/kg F)	2.75	3.45	2.74	2.98	2.63	2.74
C:N Ratio	**18.34**	**12.96**	**18.11**	**25.51**	**23.06**	**25.81**

Table 11. Lowland dung analysis from collection days in August

Day	**5**	**10**	**15**	**20**	**30**	**35**
DM (g/kg F)	126.10	144.80	131.60	146.40	159.50	163.80
C (g/kg F)	50.27	61.91	56.90	63.72	65.51	66.77
N (g/kg F)	2.50	2.85	2.62	3.05	3.39	3.46
C:N Ratio	**20.11**	**21.72**	**21.72**	**20.89**	**19.32**	**19.30**

Table 12. Upland dung analysis from collection days in August

Day	**5**	**10**	**15**	**20**	**30**	**35**
DM (g/kg F)	152.80	185.00	164.90	177.90	150.90	139.10
C (g/kg F)	73.37	76.48	71.79	75.41	72.62	67.61
N (g/kg F)	2.78	3.11	2.55	2.83	2.63	2.59
C:N Ratio	**26.39**	**24.59**	**28.15**	**26.65**	**27.61**	**26.10**

Chapter 5: Discussion

At the beginning of this study, baseline infection levels were established in the herd of Dexter cattle using FEC prior to portioning them between the two grazing sites. Cattle were sampled again at the end of the study to compare parasite distributions from each site. No difference in parasite distribution between the two groups was found. The majority of cattle showed little or no parasitic burden while a small proportion of individuals carried higher burdens. FEC's showed that the distribution of the parasite population was over dispersed which is typical in cattle populations. This is in agreement with Borgsteede et al. (2000) who saw a similar distribution in a dairy cow herd. Over dispersed or aggregated distributions are a result of previous exposure to infections and the varying degrees of immunity to nematode parasites. There is a general consensus within the literature that an aggregated distribution pattern is characteristic of a wide range of parasite populations (Roberts and Dobson, 1995). Dexter cattle have been described as a long living, hardy breed with a resistance to disease. A review by Curran (1990) of historical agricultural books from the late nineteenth century highlighted a number of attractive characteristic associated with the Dexter breed. Two of these characteristics which made the breed popular at the time were their "*longevity"* and their "*freedom from disease"*, though it does not specify any disease in particular. Results from the study support this observation as evidenced by the low parasite levels (< 100 EPG) detected by FEC in the majority of cattle sampled. Previous studies have highlighted that genetic disease resistance is present in some breeds of cattle (Frisch, O'Neill and Kelly, 2000).

FEC were performed on faecal samples collected from cattle to determine the burden of gastrointestinal nematode eggs in each grazing group (lowland and upland) and also each age group (calves, weanlings and adults). In this study mean FEC ranged from 0 – 300 EPG in all cattle in July and also in November (18 weeks later). Similar results

were found in organic dairy calves in Sweden where levels ranged from 50 – 300 EPG (Höglund, Svensson and Hessle, 2001). Shaw, Vercruysse, Claerebout and Dorny (1998) reviewed a number of European studies (including Irish) and found that first-grazing season calves with no signs of infection had a mean FEC below 250 EPG. Most of these studies were conducted on dairy farms so care must be taken when comparing results. There is little information on the parasitology of beef cattle and practically nothing on upland cattle grazing. This makes drawing comparisons difficult but highlights the novelty of the study.

This study found a difference in parasite burden between age groups. Calves and weanlings had higher nematode burdens compared to older adult cattle. First season grazers typically have little or no immunity to nematode infections and for that reason are more susceptible to infections (Armour, 1989). Cattle generally develop a resistance to nematode infections with age. Resistance to *O. ostertagia* is slow to develop, with high immunity taking up to three grazing seasons to develop. Immunity to *C. oncophora* is generally achieved after the first year of grazing (Taylor, Coop and Wall, 2007).

Weanlings showed a lower FEC abundance and intensity in July compared to November, at the end of their second grazing season. It is possible that weanling immunity increased during the course of the grazing season due to exposure to low levels of gastrointestinal nematode parasites. Natural immunity develops in cattle when exposed to gastrointestinal nematode parasites in their first two grazing seasons (Ploeger *et al*., 1990; Vercruysse and Claerebout, 1997). Weanling cattle are considered to be immune to most nematode parasites when they are turned out for their second grazing season but resistance to *O. ostertagia* can take longer to develop

(Armour, 1989). Another possible reason for low FEC in weanlings at the end of the season may be due to the slaughter of 14 animals during the grazing season. These animals could possibly have contributed to a higher FEC in November as they carried an average of 58 EPG in July (Appendix A). It has been reported (Corwin, 1997) that culling has been used as a means of controlling nematode parasites by removing parasitised animals from the herd.

Adult cattle showed the lowest prevalence, abundance and intensity of nematode eggs in all age groups. This is in agreement with an Irish study by Murphy *et al*. (2006) who investigated helminth parasites in dairy cows. Results in this study showed that a small proportion of adults had low numbers of nematode eggs in their faeces. It is known that egg counts in adult cattle are generally low (approximately 10 EPG), as was previously shown in studies with dairy cows (Agneessens et al., 2000; Borgsteede et al., 2000; Höglund, Svensson and Hessle, 2001). Mature cows that retain small populations of nematode parasite provide a source of infection for young calves (Corwin, 1997). This source of low infection can have a positive effect on growth performance in the second year of grazing (Ploeger *et al*., 1990; Corwin, 1997), since the goal in parasite control is not to eradicate parasites completely because a low level of parasitism is necessary for a positive immune response in the host animal.

Individual cattle weights were recorded twice over the study; once at the beginning of the study before cattle were split and again at the end when cattle returned from the hill. This was an attempt to measure effects on production loss by either parasite infection or grazing location. It was clear from the results that lowland cattle gained more weight than upland cattle. However, FEC analysis showed that lowland cattle had higher egg counts than the upland cohort; therefore the level of infection did not

appear to affect production. A reason for reduced weight gain may have been due to poor fodder quality in the uplands. It is well known that upland pastures are less productive than lowland pastures at this is a key reason for decline in upland farming (Tubridy, 2013). The important cattle weights were those of the calves and the weanlings as they were raised for beef production where weight gain is paramount. Weanling weight gain was almost identical in each site, though calf performance was much better on lowland pasture. Weight gain was not as important in adult cattle as they were not raised for beef production however; weight loss was not desirable either. Adult cattle in the lowlands maintained their body weight throughout the study while upland cattle lost weight. This weight loss may be attributed to the length of the grazing period in the upland site. Throughout the study cattle in the uplands appeared to thrive but a noticeable change appeared in adult cattle towards the end of the grazing period. Adults appeared to lose weight in the final two to three weeks of the study as vegetation began to die back and this may provide a plausible explanation for their weight loss. Further study would be required to provide evidence for this as cattle weights were only recorded at the beginning and the end of the study. Perhaps with the implementation of shorter grazing period upland grazing may be an effective means of maintaining cattle weights and sustaining suckler herds during the summer months.

The levels of larval infectivity on pastures during the period of this research was observed to be very low. The mean larval levels on pastures grazed by cattle were 80 L3/kg DM and 9 L3/kg DM in the lowland and upland site respectively. Despite low larval numbers, larval pasture levels followed a similar pattern in both sites throughout the season. The lowland site generally had higher larval levels on every collection date except on the last collection day in October when levels dropped below detectable

levels. Overall pasture larval levels remained low for most of the season but a peak was observed in late August. This is in agreement with Claerebout *et al.* (1998) in Belgium who reported very low pasture larval counts (< 100 L3/kg DM) for most of the season but larval levels rose to approximately 2000 L3/kg by September. Similarly a peak in pasture larval levels was observed in July by Uriarte, Llorente and Valderrábano (2003) on pastures grazed by sheep in Spain. While in New Zealand, Vlassoff (1973) reported two peaks, a small peak in spring and a large peak in autumn. These results indicates that cattle may be at a higher risk of infection late in the summer when pasture larval levels are highest.

There was a significantly higher level of larvae on the lowland pastures. The higher larval levels in the lowlands may be due to higher numbers of cattle grazing in a smaller area. Increased stocking rates are associated with for greater parasite contamination on pastures (Corwin, 1997; Waller, 2006). Lowland cattle grazed in a rotation system meaning they grazed on pastures that were previously fouled. These unclean pastures would normally result in increased larvae ingestion and therefore higher infections.

In comparison, low larval levels were detected in the upland site. Reduced larval levels in the uplands are possibly due to the extensiveness of the site. Cattle had access to approximately a 420 hectares area and it is possible that areas sampled were not used very frequent by the grazers. Though it was known that the cattle typically remained confined to a smaller proportion of the site (Kilian Kelly, personal communication, 2014), the area used was still very large. Another reason for low larval levels was due to the late turnout of cattle on the site. Unlike the lowland site were cattle grazed prior to the split in July, the upland site was not grazed since the previous season. The

rationale for late turn out to the uplands is that sufficient grazing pasture is not available until later in the season as there tends to be a delay in growth due the lower temperatures and higher rainfall associated with upland environments. The benefits of delayed turnout have been described by Nansen, Jørgensen, Henriksen and Foldager (1987) where pasture larval levels were markedly reduced by postponing turnout onto pasture by just four weeks.

Environmental conditions may also have had an effect on the low larval levels in the uplands as rainfall in July and September was lower than average. Low precipitation can affect the development and survival of parasitic larvae. If dung pats become dry a crust will form preventing larvae migrating onto pasture (Pandey, 1974). Herbage length was not measured in the study but it was observed to be much taller in the uplands Tall herbage can conserve moisture near the ground and reduce the effects of direct sunlight on larvae (Goldberg, 1968).

Of the four habitats that were sampled in the upland site, the highest pasture larval levels were found on dry heath. This result suggests that cattle spend more time, either grazing or perhaps resting, on dry heath than the other three habitats. This cannot be confirmed as cattle observation data were not recorded. It has been reported that dry heath habitat is the principle source of forage for sheep (Tubridy, 2013). Based on this evidence it may be a main source of grazing for cattle.

Such low parasite numbers was somewhat unexpected in the study as the cattle used were from an organic herd. In organic farming system the use of anthelminthics is prohibited and because of this, the organic industry relies on good knowledge of the epidemiology of parasites and the use of pasture management strategies for the control of parasites. Despite this organic farmers in general have greater problems with

parasitic infections in comparison to conventional farms where prophylactic anthelminthics are used widely (Svensson, Hessle and Höglund, 2000). However, it was not the case in this study.

In this study the migratory distance of L3 larvae from dung pats onto herbage was measured to determine if there was a difference between the lowland and upland sites. The results showed that there was no difference in migration distance from dung pats between sites. Concentrations of L3 larvae were highest on herbage in zone 1 closest to the pat with fewer larvae found in zone 2. This agrees with the results of other researchers (Goldberg, 1970; Pandey, 1974; Langrová *et al*., 2003; Boom and Sheath, 2008) who all reported L3 larvae congregating close to the edge of dung pats.

The number of migrating larvae from dung pats and onto herbage was examined in both sites during August. Larvae numbers on herbage were ientified in very low number in the first few collection days but numbers began to increase dramatically after day 15 in both sites at similar rates, though more larvae appeared in the uplands. Development time from egg to L3 larvae generally takes one to two weeks in summer (Pandey, 1974; Taylor, Coop and Wall, 2007) which concurs with the results in this study though significant numbers were not seen until after three weeks.

Dung pats in the lowland site were observed to dry-out and form a crust, possibly trapping larvae inside (Pandey, 1974), while upland dung showed almost no signs of degradation. Dung pats appeared intact and retained their form from the beginning to the end of the study which was not the case in the lowland site. This could play an important role in the survival of nematode larvae in upland areas. Higher rainfall recorded in the uplands may have created more favourable conditions for nematodes as moisture is important for migration and survival (Taylor, Coop and Wall, 2007).

This result suggests that upland conditions are more favourable for the survival of nematode larvae, meaning grazing animals may be more at risk. However this did not agree with results from pasture samples which showed low levels of larvae on the herbage.

In this study the two most common gastrointestinal nematode parasite species found in both sites were *O. ostertagia* and *C. oncophora*. These two parasites are considered to be the two most common and important species found in first season grazers in temperate regions (Dimander *et al*., 2000). These two genera are typically found in cool temperate regions as they are adapted to survive in cold conditions more so than other nematode parasites (Fiel *et al*., 2012). The dominance of these two nematode parasites in both young and old animals is in agreement with other studies in Europe (Shaw *et al*., 1998; Borgsteede *et al*., 2000; Larsson *et al*., 2007) and Ireland (Murphy *et al*., 2006).

This study also investigated dung beetle assemblages in lowland and upland sites. Species were identified and counted from both sites and species richness, species diversity and species evenness were calculated. The number of individual beetles recorded was much higher in the lowland site with more than three times the abundance found in the uplands.

The obvious difference between the two sites was the dominance of the Hydrophilidae family in the lowlands, in particular *Cercyon* genus. *Cercyon* are small beetles ranging in size from 1 mm to 5 mm. Adults are coprophagous and larvae carnivorous (Koskela and Hanski, 1977). Most studies in temperate regions focus on *Aphodius* species but little work is done on the Hydrophilidae and there possible roles in the dung pat

community. For this reason it is not possible to draw any comparisons between results in this study and others and this appears to be a novel finding in this study.

A. rufipes and *A. fimearius* were common to both sites and are known to be a common widespread species (Jessop, 1986). *Aphodius fasciatus* was found exclusively in the uplands in relatively large numbers. However, this species is associated with uplands and moorlands (Jessop, 1986) so this result is not unusual. The reason for higher beetle numbers in the lowland site may be due to dung quality. Dung analysis showed that the lowland dung had a lower carbon/nitrogen ratio than upland dung indicating nitrogen content was higher in lowland pats. Gittings and Giller (1998) showed that dung quality affected the structure of dung beetle assemblages in Ireland. Moisture and nitrogen are associated with reproductive success of dung inhabiting beetles so it can be suggested that dung with a combination of these characteristics is more attractive to dung beetles. In this study lowland dung was found to harbour more dung beetles that dung in the uplands. The reason for this may be due to the differences between the two dung types. Lowland dung was wetter (low dry matter content) and higher in nitrogen compared to upland dung. These characteristics are desirable for dung beetles. Dung with higher nitrogen content is seen to me more attractive to beetles and this was reported in a study by Holter and Scholtz (2007).

Despite the difference in beetle numbers, species richness was similar in both sites. However, species diversity and evenness was greatest in the upland site indicating the uplands supports higher diversity.

Dung beetles succession was observed during the month of August in both the lowland and upland sites. The lowland sites had the highest numbers of dung beetles in the first collection day after which there was a very steep decrease in numbers throughout the

month until no beetles were recorded on the last day. The pattern of succession seen in this study is a typical heterotrophic succession where energy sources are largest at the beginning and decrease continually over time. In parallel, a study by Koskela and Hanski (1977) in which they saw a peak in species numbers at day two and this quickly decreased thereafter. This pattern of succession was only observed in the lowlands in this study as there was no obvious pattern of succession in the upland site since low numbers of beetles were recorded in the uplands on each occasion across the month.

Dung beetles have been shown to reduce nematode larval levels on grazing pastures by destroying nematode eggs in faeces therefore reducing nematode survival (Fincher, 1973, 1975; Bergstrom, Maki and Werner, 1976; Grønvold *et al*., 1992; Nichols *et al*., 2008).

In this study the interaction between dung beetles and gastrointestinal nematode larvae was investigated during the month of August in both sites. The data indicated that a higher number of early-colonising dung beetles in the lowland site reduced the number of L3 emerging from the dung pat. While in the upland site higher levels of larvae appeared after little beetle activity. This result suggests that dung beetles and in particular Hydrophilidae may play a role in controlling gastrointestinal nematode larvae.

Chapter 6: Conclusion & Recommendations

This study was undertaken to investigate the prevalence and distribution of gastrointestinal nematode parasites in upland and lowland grazing regimes using organic Dexter cattle. A secondary aim of the study was to investigate dung beetle assemblages in upland and lowland sites and their interactions with gastrointestinal nematode parasites.

Parasite numbers were low in all age groups both in the upland and lowland cohorts. However, there was a difference between age groups. Younger animals had higher FEC compared to adults which is typical in herd infections. Mean FEC were below subclinical levels (> 200 EPG) indicating that cattle were healthy and there was minimum risk of production loss due to gastrointestinal nematode infections. Pasture burden was low on all sites with a typical peak in late summer. Cattle weights increased in the lowlands but were maintained in the uplands.

There was minimal risk to animal health by nematode parasites in the upland grazing regime suggesting that upland pastures can be utilised in the summer months to alleviate lowland grazing pressure by removing non-fattening stock. Evidence is provided in this project that cattle breed may have been a factor in relation to low infection levels. Dexter cattle are a hardy breed well designed for upland grazing due to their small stature and ability to thrive on poor quality sward (The Dexter Cattle Society, 2016).

Dung beetle species richness was similar in both sites but species diversity and species evenness was greater in the uplands illustrating the importance of maintaining upland habitats. Dung beetles provide important ecosystem services by increasing rates of dung decomposition, nutrient cycling and parasite control. The negative effects of anthelminthic treatments on invertebrates and in particular on dung beetle composition

and richness have been well publicised (Beynon, 2012; Beynon et al., 2012), therefore, the replacement of anthelminthics with biological control agents to increase dung beetle diversity and reduce parasite infections should be a major goal for parasite ecologists and parsitologists. Grazing management strategies and biological control methods should form an integral part of any grazing management plan.

This project is ideally positioned to link Irish research to emerging Irish policy on the best-approach to manage upland farmland in agri-environmental schemes (Bullock, Kretsch and Candon, 2008). Therefore replication of this study over several grazing seasons and multiple sites would be necessary to gain a better understanding on gastrointestinal parasite populations and dung beetle assemblages in upland environments.

Studies to investigate genetic resistance to disease in traditional cattle breeds could provide important information for policy makers and farmers on cattle breeds that are best suited for grazing in an Irish upland environment. Due to the low precision of the McMaster method a multi-faceted approach is recommended to examine Dexter nematode resistance involving a combination of other diagnostic tools such as coprocultures and blood parameters providing a more sensitive option to identify levels of parasitism, particularly subclinical infections.

Studies analysing dung pat degradation in the uplands could provide important information on nematode larvae survival. In this study it was observed that dung pats remained relatively intact for the duration of the study period (5 months). These pats could provide a refuge for nematode parasites which could be a potential risk to grazing animals.

The results from this study provide some information on dung beetles diversity, species richness and evenness in upland and lowland habitats. However, on the basis of the results presented here, the following recommendations are offered for the design of future studies. A combination of pitfall and dung pat trapping may help record more species in the area. Trapping on multiple sites may provide a clearer picture of dung beetle assemblages on a regional scale.

Future research on the Hydrophilidae family and their relationship with nematode larvae is warranted based on their presence in the lowland site in association with a marked reduction in dispersing larvae.

To conclude, the current study shows that gastrointestinal nematode parasites may be kept at low to moderate levels by good management practices and without the use of prophylactic anthelminthic treatments. This agrees with studies by Dimander et al. (2003), Waller (2006) and Larsson et al. (2006). Understanding the risks and benefits of diversifying into upland grazing systems could assist in augmenting animal productivity and improving animal welfare. Using the information gained from this project, farmers would be able to make informed choices through adjustment in pasture management and thus increase added-value to upland farm income. This research provides useful information on the impacts of organic upland grazing on the biodiversity of dung beetles in this environment.

Chapter 7: References

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Appendices

Appendix A: Faecal egg counts (FEC) and Cattle weights

Table A.1 FEC and cattle weights from July and November 2014

M = male, F = female, DX = Dexter, UL = upland, LL = lowland										
No.	**ID no.**	**Year of birth**	**Sex**	**Breed**	**Site**	**EPG July**	**EPG Nov**	**Weight July (kg)**	**Weight Nov (kg)**	**Weight Gain (kg)**
1	229	2014	F	DX	UL		100	47.1	91.6	0.35
2	224	2014	F	DX	UL	0	50	70.8	103.5	0.26
3	230	2014	F	DX	UL	0		51.4	104	0.42
4	226	2014	F	DX	UL	0	0	62.8	117.5	0.43
5	219	2014	M	DX	UL	0	100	70.4	100	0.23
6	209	2014	F	DX	UL	150		116.5	160.5	0.35
7	205	2014	M	DX	UL	100	0	107.5	168	0.48
8	221	2014	M	DX	UL	0		74	117.5	0.35
9	215	2014	M	DX	UL	50	0	142	204	0.49
10	249	2013	F	DX	UL	0	0	183.5	197.5	-0.06
11	251	2013	F	DX	UL	50		174.5	203	0.13
12	14	2013	M	DX	UL	50		142	168	0.21
13	201	2013	F	DX	UL	0		148.5	159	0.08
14	202	2013	M	DX	UL	100	100	176.5	206	0.23
15	12	2013	F	DX	UL	100	0	216	242	0.21
16	11	2013	M	DX	UL	50	0	215	234	0.15
17	195	2013	F	DX	UL	0		182.5		
18	190	2013	F	DX	UL	0	50	178	199.5	0.1
19	188	2013	M	DX	UL	0	0	245	260	-0.01
20	182	2013	F	DX	UL	0		321		
21	183	2013	F	DX	UL		0	244	262	0.14
22	184	2013	F	DX	UL	100		230	267	0.16
23	180	2013	F	DX	UL	0		183	212	0.23
24	178	2013	F	DX	UL	0		325		
25	166	2012	F	DX	UL	0	0	256	246	-0.08
26	2	2011	F	DX	UL	0	0	256	233	-0.18
27	154	2011	F	DX	UL	0		317	251	-0.17
28	147	2011	F	DX	UL	0		302	395	
29	146	2011	F	DX	UL	0	0	316	305	-0.09
30	647	2011	F	DX	UL		0	303	295	-0.06
31	8	2010	F	DX	UL	0		253	274	0.13
32	140	2010	F	DX	UL	0		237	298	-0.41
33	60	2009	F	DX	UL	50	0	275	261	-0.11
34	149	2009	F	DX	UL	0	50	260		
35	136	2009	F	DX	UL	50	0	350	339	0.15
36	4	2009	F	DX	UL	0		273	267	-0.05
37	15	2006	F	DX	UL	0	0	339	341	0.02

38	26	2005	F	DX	UL	0	0	351	291	-0.48
39	217	2014	M	DX	LL	0	150	92.8	162.5	0.55
40	227	2014	M	DX	LL	0	150	59.2	107.5	0.38
41	228	2014	M	DX	LL	0		56.8	103	0.37
42	218	2014	F	DX	LL	50	100	144	218	0.59
43	223	2014	F	DX	LL	50		74.8	134.5	0.47
44	214	2014	F	DX	LL	100	200	89.8	145.5	0.44
45	216	2014	M	DX	LL	50	100	135.5	225	0.71
46	213	2014	M	DX	LL	0	0	115	197	0.65
47	210	2014	F	DX	LL	100	50	136.5	207	0.56
48	211	2014	M	DX	LL	50	150	159.5	271	0.88
49	204	2014	F	DX	LL	0	50	151.5	244	0.73
50	220	2014	F	DX	LL	0	200	113	209	0.76
51	206	2014	F	DX	LL	0	0	152.5	260	0.85
52	207	2014	M	DXX	LL	0	0	152	251	0.79
53	222	2014	M	DX	LL	50	0	97.6	169	0.57
54	269	2014	F	DX	LL	50	0	102	163.5	0.49
55	250	2013	M	DX	LL	100		96.4	110	0.11
56	203	2013	M	DXX	LL	50		311		
57	10	2013	M	DX	LL	150	0	228	264	-0.04
58	192	2013	M	DX	LL	0		256		
59	198	2013	M	DX	LL	0		336		
60	194	2013	M	DX	LL	50	50	257	292	-0.25
61	193	2013	M	DXX	LL	50		305		
62	196	2013	M	DX	LL	50		196.5		
63	197	2013	F	DX	LL	50		286	312	-0.29
64	191	2013	M	DX	LL	300		212		
65	189	2013	F	DX	LL	0		183.5		
66	186	2013	F	DX	LL	50		224		
67	187	2013	F	DX	LL			311		
68	185	2013	F	DX	LL	50		284		
69	179	2013	F	DX	LL					
70	177	2013	F	DX	LL	0		210		
71	175	2012	F	DX	LL	0	0	272	244	-0.22
72	171	2012	F	DX	LL	0	0	272	301	0.23
73	163	2012	M	DX	LL					
74	157	2011	M	DX	LL	0		459		
75	5	2011	F	DX	LL	0	0	285		
76	3	2011	F	DX	LL	100	0	440	438	-0.02
77	155	2011	M	DX	LL	50		359	280	-0.29
78	150	2011	F	DX	LL	0	50	272	251	-0.07
79	7	2010	F	DX	LL	0	0	292	334	0.33
80	6	2010	F	DX	LL	0	50	181.5	176	-0.04
81	605	2009	F	DX	LL	0	50	296	325	0.23

82	130	2008	F	DX	LL	0	0	320	221	
83	125	2006	F	DX	LL	0	0	399	410	0.09
84	36	2006	F	DX	LL				307	
85	448	2005	F	DX	LL	0		293	307	0.11
86	117	2005	F	DX	LL	0	0	302	334	0.25
87	396	2004	F	DX	LL	0	0	309	324	0.12
88	22	2004	F	DX	LL		0	366	385	0.15
89	155	2003	F	DX	LL	0	0	397	375	-0.17
90	72	1999	F	DX	LL	0	0	357	374	0.13

*** Blanks cells appear when it was not possible to acquire an animals weight or retrieve a faecal sample**

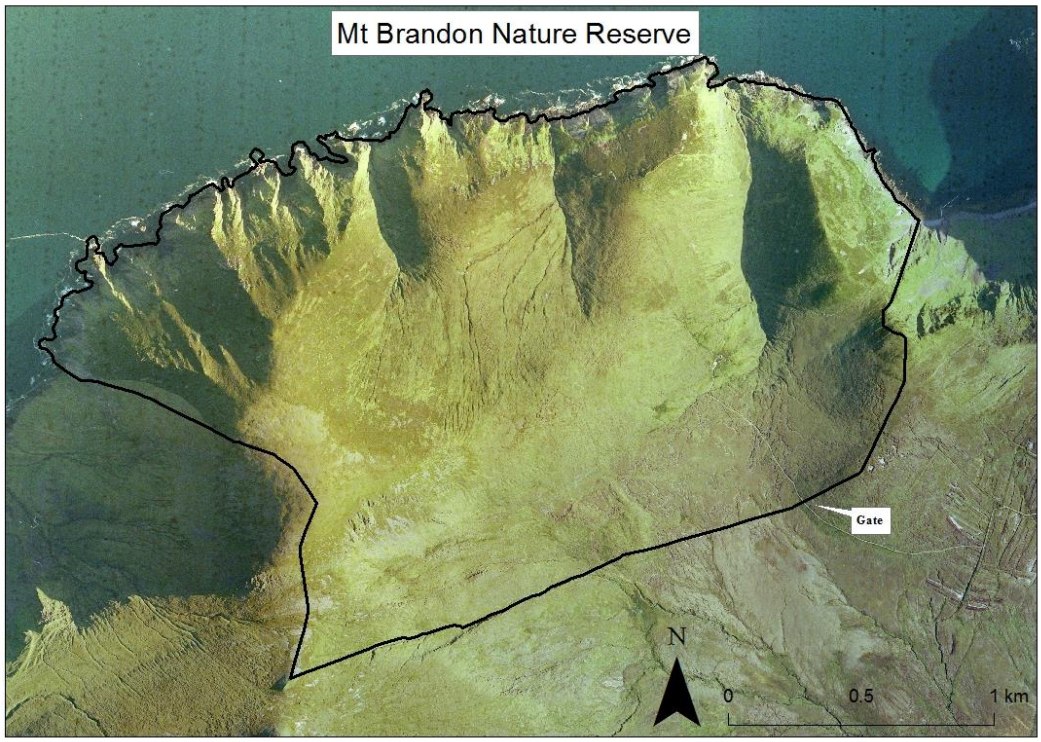
*** Highlighted animals were slaughtered during the 2014 grazing period**

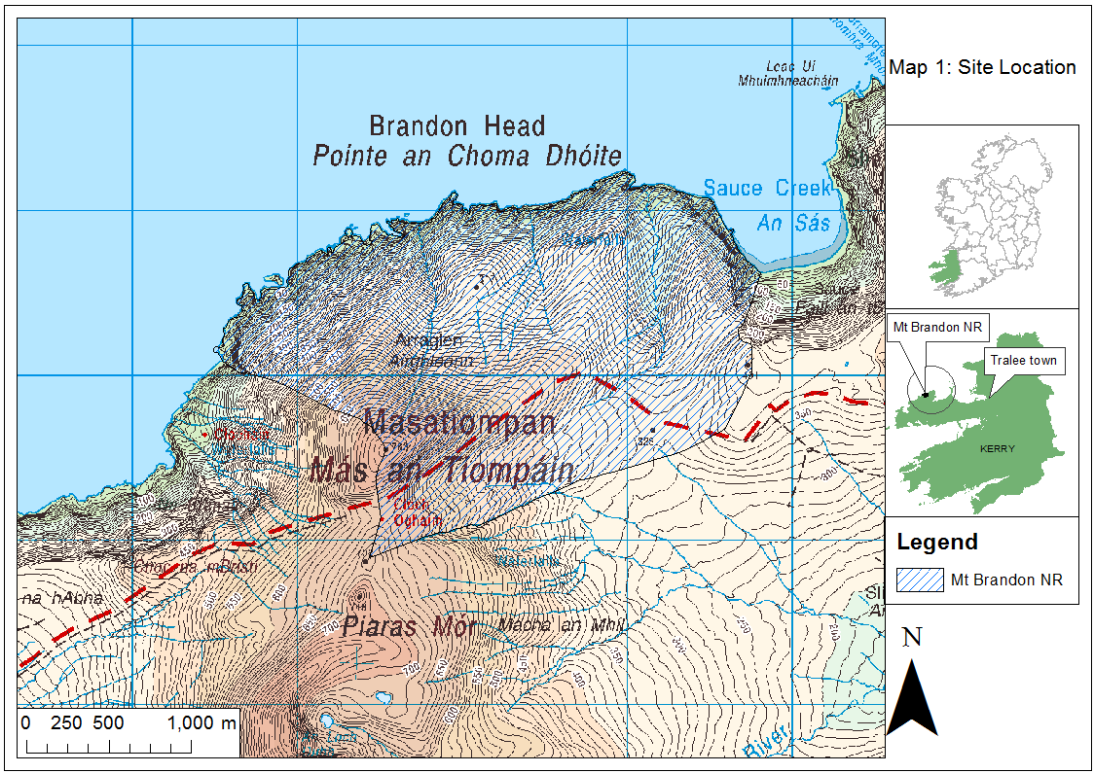
Appendix B: Parasite prevalence in Male and Female cattle

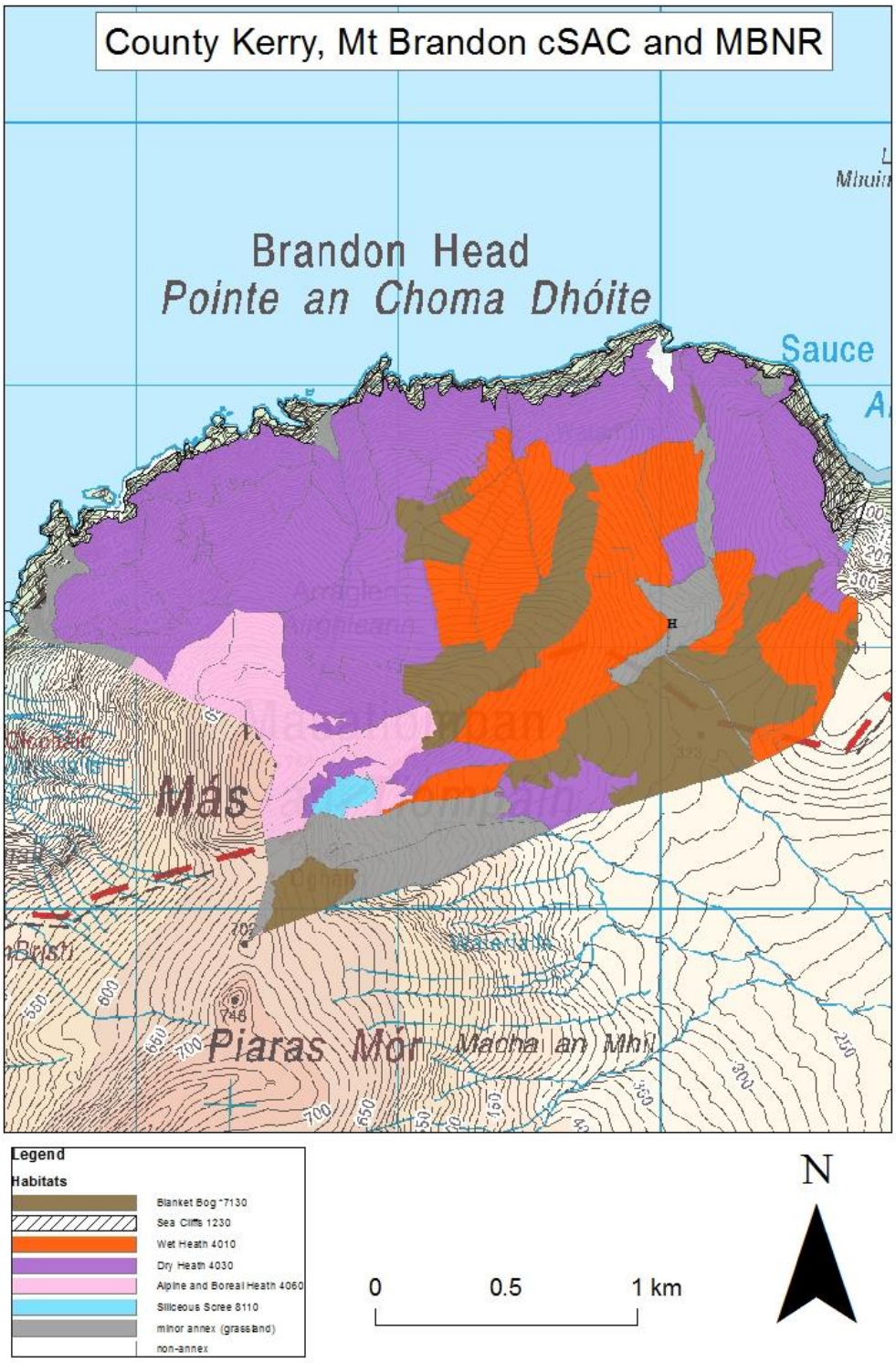
Table B. The mean prevalence (%) of nematode eggs in male and female cattle of different age groups sampled in July and November 2014

Time	Age Group	Sex	n	Prevalence
July	Calves	Male	12	58.33
		Female	12	50.00
	Weanlings	Male	13	23.08
		Female	15	60.00
November	Claves	Male	10	50.00
		Female	10	30.00
	Weanlings	Male	5	60.00
		Female	4	75.00

Appendix C: Maps






County Kerry, Mt Brandon cSAC and MBNR
Brandon Head
Pointe an Choma Dhóite
Sauce
Más
Piaras Mór
Legend
Habitats
Blanket Bog *7130
Sea Cliffs 1230
Wet Heath 4010
Dry Heath 4030
Alpine and Boreal Heath 4060
Siliceous Scree 8110
minor annex (grassland)
non-annex
0
0.5
1 km
N

Appendix D: Weather Data from 2014

Table C: Mean temperature, total rainfall and relative air humidity between collection periods

Period	Lowland			Upland		
	Mean temperature	Rainfall (mm)	Relative Humidity (%)	Mean temperature	Rainfall (mm)	Relative Humidity (%)
02 July	16.31	1.8	81.1			
07 July	14.56	7	88.82			
12 July	14.92	5.8	90.54			
17 July	15.52	14	91.08			
22 July	16.9	10.8	92.38			
27 July	17.54	5.8	94.7			
01 August	16.25	6.2	88.96			
01 August	16.25	6.2	89.38	14.57	13.2	90.55
06 August	15.61	3.6	88.76	13.88	17.6	88.1
10 August	15.74	2	84.3	13.9	12.4	84.89
16 August	14.63	25	85.63	12.56	36.4	89.3
21 August	13.47	1.4	83.64	11.81	8	85.66
26 August	14	8.8	87.24	12.52	19.2	87.58
31 August	15.2	16.8	90.46	13.46	33.6	92.42
05 September	14.96	10.2	85.53	13.39	0	84.75
10 September	13.73	0	87.06	12.36	0.6	89.64
15 September	15.84	0	82.86	15.15	0	79.28
20 September	16.39	9.4	86.98	14.69	10	89.87
24 September	13.82	0.2	88.23	12.55	7.4	87.88
30 September	15.65	2.6	91.1	14.29	18.8	86.79
4 October	13.9	0	87.24	12.75	16.4	86.36
03 October	14.79	1.2	90.14	13.38	24.2	86.79
08 October	11.27	0	84.2	9.18	68.6	85.13
13 October	10.69	0.8	89.58	9.55	18.2	88.28
18 October	12.46	0	90.4	11.29	28.4	87.62
22 October	12.99	0	88.53	11.27	74.6	89.86
28 October	12.82	0	90.05	11.24	70.2	90.89
2 November	11.36	1.2	93.42	11.29	142.6	92.79

Appendix E: Long Term Weather data

Long term averages of monthly mean, maximum and minimum temperatures, relative humidity and monthly rainfall for the south-west of Ireland

Month	Temp Av.	Max temp*	Min temp*	Hum Av.	Rainfall (mm)
July	15.4	18.1	12.7	84.10	99
August	15.4	18.2	12.6	84.30	114.9
September	14.1	17	11.2	84.10	125.4
October	11.7	14.3	9	84.70	177.1

*Max temp = mean daily maximum
*Min temp = mean daily minimum

Monthly mean, maximum and minimum temperatures, relative humidity and total monthly rainfall in the lowlands

Month	Temp Av.	Max temp	Min temp	Hum Av.	Rainfall (mm)
July	15.98	18.55	13.63	90.56	62.20
August	14.73	17.53	12.05	87.05	66.40
September	15.17	18.57	11.93	87.27	13.60
October	12.18	14.54	9.53	89.37	2.00

*Max temp = mean daily maximum
*Min temp = mean daily minimum

Monthly mean, maximum and minimum temperatures, relative humidity and total monthly rainfall in the uplands

Month	Temp Av.	Max temp	Min temp	Hum Av.	Rainfall (mm)
July	15.47	18.32	13.59	90.86	51.40
August	13.01	15.54	11.15	88.28	147.80
September	13.83	17.07	11.15	83.95	43.80
October	10.83	13.05	8.77	89.02	386.20

*Max temp = mean daily maximum
*Min temp = mean daily minimum

Appendix F: Common dung beetles found during the study

Common dung beetles found in during the study. (Left) *Aphodius rufipes*, (middle), *Aphodius fimetarius* and (right) *Aphodius fasciatus* (Mann, 2015)

Aphodius rufipes

One of the lager *Aphodius* species, measuring 9 – 13 mm in length, *A. rufipes* is a common and widespread beetle found in dung of large herbivores. They appeared in June and were the dominant species by early August.

Aphodius fimetarius

A common and widespread species found in various types of dung and decaying vegetation. Measuring 5 – 8 mm in length, it is one of the smaller *Aphodius* species. Peak abundance was in May and September.

Aphodius fasciatus

A. fasciatus is generally small measuring 3.5 – 4.5 mm in length. They are found in various types of dung in shaded habitats and in uplands.