THE UNIVERSITY *of* EDINBURGH

Investigating interactions between coinfecting gastrointestinal nematodes and host immunity using meta-analytic, observational and experimental approaches

Michael John Evans

THE UNIVERSITY *of* EDINBURGH

Submitted for the degree of Doctor of Philosophy

Institute of Ecology and Evolution

School of Biological Sciences

University of Edinburgh

October 2025

Declaration

[Student Declaration removed for online published version]

Thesis Abstract

Gastrointestinal nematodes (GIN) have enormous global impacts in humans, wildlife and grazing livestock. Natural infections are composed of co-infections with multiple GIN species, forming an ecosystem of interacting organisms within the host. In this thesis I used meta-analytic, observational and experimental methods to explore this ecosystem, the interactions within it, and the impacts it has on host health. The work focuses on GIN coinfections in sheep, both as a model for the evolutionary ecology of host-pathogen relationships, and to inform decision-making in sheep farming, where widespread anthelmintic resistance necessitates a holistic understanding of GIN coinfection epidemiology.

I first conducted a systematic review and meta-analysis of experimental GIN coinfections, which demonstrates that coinfecting GIN tend to interact antagonistically. This means that on average, fewer worms of one species establish when a coinfecting species is also present. This effect is dependent on the infectious dose and is more pronounced in sequential coinfections. The effect occurs across sites within the gastrointestinal tract, suggesting a common mucosal immune mechanism may drive the interactions.

I then applied an ITS2-sequence based nemabiome speciation technique to over 3000 faecal samples collected across four years from over 500 sheep living in an unmanaged population on St Kilda. The results show how parasite community diversity and the species-corrected faecal egg counts (sFEC) vary with season, reproductive status and age, in the absence of human intervention. This includes descriptions of the seasonal epidemiologies of relatively understudied species (*Trichostrongylus axei*, *Chabertia ovina* and *Bunostomum trigonocephalum*). In particular I found that *Teladorsagia circumcincta* and *Nematodirus* spp. conform to epidemiological patterns previously described in domestic sheep, whereas

Trichostrongylus vitrinus is more abundant in lambs in the summer than domestic studies would predict. This epidemiological difference may be a consequence of the antagonistic interaction between *Te. circucmcincta* and *Tr. vitrinus*, combined with variation in sFECs in late pregnant females and co-grazing between multiple age classes.

I then go on to show that the epidemiology of *Tr. vitrinus* has important implications for the health and fitness of wild sheep in this system, with significant negative associations between *Tr. vitrinus* and both the bodyweight and overwinter survival of lambs. This demonstrates the power of the nemabiome approach, with these species-specific measures explaining more variation in bodyweight and survival than are explained by unspeciated faecal egg counts (FEC) or summary measures of parasite alpha- and beta-diversity. The approach also identified a robust, positive association between *Tr. axei* and August bodyweight, that suggests the impacts of parasites in natural coinfections may be more complex than simply the addition of their individual pathological effects.

Given that cross-protective immunity between sites within the gastrointestinal tract may be a key driver of the interspecies interactions in this GIN ecosystem, I then performed an experimental infection to identify the mechanism. This study demonstrated that infection with the intestinal GIN *Trichostrongylus colubriformis* induces IgG within intestinal and abomasal mucus that cross-reacts the abomasal-dwelling parasite *Teladorsagia circumcincta*, whereas cross-reactive IgA is restricted to the site of the primary infection. Proteomic analysis indicates that the cross-reactive IgG recognises conserved structural and metabolic proteins unlikely to be exposed in live nematodes and hence unlikely to be effective targets *in vivo*. However, *Tr. colubriformis* infection results in upregulation of other immune pathways within

the abomasum that are associated with anti-parasite T-helper type-2 (Th2) immunity and are a more likely mechanism for inter-GIN interactions.

Together, these findings demonstrate that coinfecting GIN tend to interact antagonistically, most likely via cross-protective immunity that is mediated by immunological changes within the mucosa rather than cross-reactive antibodies. These interactions may be interrupted in domestic systems, with different seasonal epidemiologies for some GIN species observed in wild sheep. These differences may help guide management decisions in domestic flocks and the development of multispecies anti-GIN vaccines. The individual species within the GIN ecosystem also have specific significant impacts on health and fitness of their wild hosts, with demographic feedback on the epidemiology potentially driving host and pathogen co-evolution.

Lay Summary

Gastrointestinal nematodes are parasitic worms that live in the stomach or gut of many people, wild animals and farmed animals, causing a range of health problems. Most of the time, animals are host to more than one species of parasite at once. These parasites can then interact with each other and with the host animal, forming an ecosystem. In this thesis I used a variety of different scientific methods to discover more about how these parasites interact with each other and with the host animal's immune system, and what impacts this has on the host animal's health. I did this with the hope of learning more about how animals and their parasites evolve together. I focussed on sheep as host animals in particular, with the additional hope of learning more about these parasites in order to improve how they are managed. This is particularly important in sheep because drug resistance is wide-spread on sheep farms, making the parasites particularly challenging to deal with.

I first carried out a detailed search of previous studies that had used experiments to look at infections with two species of worm. I then used the data from those studies and analysed it all together. This new analysis showed that the interactions are negative, that is, when one species of worm is living in a sheep, another species of worm will find it harder to infect the sheep. This effect is greater if there are more worms present in the first infection. The effect also occurs even if one species of worm lives in the gut and the other species of worm lives in the stomach, suggesting a defence mechanism that works across both sites.

I then looked at how the balance of different worms changes with age, season and reproduction in a population of wild sheep that are never treated with anti-parasite drugs. I did this by counting the number of parasite eggs in sheep stool samples and adjusting that number according to the proportion of each worm species present in each sample. The eggs

from different worm species all look alike, so to find out the proportion of each species I extracted DNA from the eggs and used the DNA sequence to identify them. This allowed me to described the changes associated with age, season and reproduction, including some species that have had very limited descriptions in the past. In particular I found that some species of worms show similar seasonal patterns to what happens on farms, but one species was at much higher levels among lambs in summer than would be expected on farms. This may be due to differences in how the animals are separated from each other on farms, the level of immunity among pregnant female sheep on farms, and the interactions between worms that I identified in the first chapter.

I then found out that the worm that is at higher-than-expected levels in lambs is linked with poor growth and a higher risk of the lamb dying over winter. In contrast, having high levels of a different species of worm was linked with better growth. This shows how important it is to be able to tell the different species of worms apart from one another.

I then performed an experiment to test if the immune system could be responsible for the interactions between worm species. This experiment showed that when sheep are infected with a parasitic worm in the gut, the cells in the stomach respond, turning up parasite sensors and specific anti-parasite defences. It is by this mechanism that I believe some interactions between gut worms and stomach worms are controlled.

Together, these findings show that multiple species of parasitic worms interact negatively with each other due to the host animal's immune system. These interactions may be interrupted in domestic animals, leading to different patterns of disease from wild animals. These differences affect the survival of wild sheep and as a result will affect their evolution.

Understanding these differences may help improve the treatment of domestic flocks and the development of vaccines to protect against parasitic worms.

Acknowledgements

Foremost I must acknowledge the sheep: both the wild Soay sheep on St Kilda from whom we have learned so much by simply observing their natural lives, and the domestic sheep used in the experimental infections in Chapters 2 and 5.

After that there are an enormous number of people to thank. The work presented in this thesis would not have been possible without the collaborative effort of a great number of people at both The University of Edinburgh and Moredun Research Institute, plus a huge body of work performed by volunteers and team members from many institutions and organisations as part of the St Kilda Soay Sheep Project. It is impossible to name everyone but in particular I would like to thank:

My PhD supervisors Dan Nussey, Fiona Kenyon and Tom McNeilly for their ideas, encouragement, guidance and feedback throughout this process;

Yolanda Corripio-Miyar – I would have been lost in the lab without your help and support throughout the PhD;

Xavier Bal for the huge amount of dedicated work running the fieldwork on St Kilda in recent years, in particular collecting all the samples used in Chapter 3;

Adam Hayward for your invaluable advice on the data analysis and feedback on multiple manuscripts;

Josephine Pemberton, Jill Pilkington and Ian Stevenson for contributing so much to the sheep project for so long;

All members of the Ecology Within Project for your feedback on my ideas and manuscripts, particularly Adam Hayward, Amy Pedersen, Amy Sweeny, Andy Dean, Andy Fenton, Dan Nussey, Fiona Kenyon, Tom McNeilly and Yolanda Corripio-Miyar;

All the members of the Moredun Bioservices team for their help running the experiment in Chapter 5.

The many colleagues at Moredun who have helped with and provided advice regarding Chapter 5, plus the organoid work that didn't come to fruition: Al Nisbet, Ali Morrison, Dan Price, David Frew, David Smith, Dorota Androscuk, Hannah Peaty, Katie Hildersley, Kevin McLean, Marc Faber, Margaret Oliver, Miriam Garner, Phil Steele, Tom McNeilly, Will Anderson, Yolanda Corripio-Miyar and Yvonne Bartley;

All the Sheepies and NTS folk I had the pleasure to share August catches with: Amelie, Anthony, Brian, Clare, Craig, Dan, Dianne, Ellis, Erin, Gina, Hannah, Ian, Isabel, Joey, Jon, Josephine, Kasha, Kirsty, Libby, Liz, Lizzie, Lucy, Matt, Megan, Michael, Robin, Sam, Sanjana, Sienna, Sue, Tilly, Xavier and Yolanda, and also all those I missed catching with whilst my knee was healing in 2023!

Dan Nussey and Kathryn Watt for introducing me to the Soay Sheep Project all those years ago;

Richard Mellanby whose support was crucial to my joining the ECAT programme;

Neil Sargison for starting me on the road of exploring gastrointestinal nematode coinfections;

My friends and family for their constant support;

And Otter and Lucy for being such good dogs.

Dedication

Finally, and above all, thank you to Helen. None of this would have been possible without your constant love, care and confidence. This thesis is dedicated to you, as am I always.

Contents

List of Figures

Chapter 1 - General Introduction

The Evolutionary Ecology of Coinfections and Immunity

Infectious diseases have often been studied in isolation, as a single interaction between host and pathogen; however, the vast majority of naturally-occurring infections are likely to be co-infections (A. J. McArdle, Turkova, and Cunnington 2018). These co-infecting species can be considered an ecosystem within the host, with interactions between co-infecting pathogens occurring via competition for resources, alteration of the environment within the host (niche modification), and stimulation or modulation of the host immune system (Cox 2001; Pedersen and Fenton 2007). Priority effects are a well-established concept in ecology (Stroud et al. 2024) and can also influence pathogen community assemblage within hosts (Debray et al. 2022), especially when there is seasonal variation in pathogen exposure (Halliday et al. 2020). However, there remains a lack of understanding regarding the regulation of coinfection dynamics across host and pathogen species, and how the resultant variation feeds back onto host-pathogen coevolution.

The animal immune system has co-evolved with this ecosystem and has many non-specific defences to counter this multitude of pathogens, commonly grouped together as the innate immune system (Gerardo, Hoang, and Stoy 2020). However, non-specific immune responses are a disruptive selective force that may drive pathogens to diversify and escape these defences (Murall et al. 2017). Likely in response, jawed vertebrates also possess adaptive immune components, which are able to recognise specific pathogens and mount more targeted responses (Cooper and Alder 2006; Flajnik and Kasahara 2010). T lymphocytes drive differentiation of adaptive immune responses, principally via cytokines produced by CD4+ cells (T helper cells). These cells have classically been divided into one subpopulation that

produce Th1-type cytokines that trigger inflammatory responses targeted at intracellular pathogens (micro-parasites), and another subpopulation that produce Th2-type cytokines that target extracellular pathogens (multicellular macro-parasites) (Berger 2000). These responses require resource investment by the host, therefore where resources are limited, polarisation of the immune system towards one adaptive response may entail a trade off against another adaptive response (A. L. Graham 2008).

The situation is actually much more complex than this, with many subtypes of CD4+ now defined, and greater plasticity of these subtypes recognised (Rogozynski and Dixon 2024). However, research in wild hosts supports the concept of specific of T-helper cell phenotypes associated with micro- and macro-parasite infection intensities (Corripio-Miyar et al. 2022). In addition, immune responses can cause pathology of their own, and pathogens may suppress host immune responses, leading to complex outcomes when micro- – macro-parasite coinfections are experimentally perturbed (Ezenwa and Jolles 2015; Seguel et al. 2023; A. R. Sweeny et al. 2020).

Gastrointestinal Nematode Coinfections

Gastrointestinal nematodes (GIN) are probably the most numerous class of macro-parasite, infecting the majority of the human population (Stepek et al. 2006; Brooker 2010) with enormous impacts on health and wellbeing (Mathers, Ezzati, and Lopez 2007; Chan 1997). They are also ubiquitous in grazed livestock, where they impact animal welfare, farm productivity and carbon emissions (Charlier et al. 2020; Mavrot, Hertzberg, and Torgerson 2015; Kenyon et al. 2013), and wildlife where they affect host fitness and demography (Tompkins and Begon 1999; Coulson et al. 2018). GINs are commonly found in parasite communities not only with micro-parasites but also with multiple other GIN species

(Avramenko et al. 2015; Desai, Diamond, and Thackray 2021; Ezenwa and Jolles 2011; Hananeh et al. 2022). These coinfecting GIN species can occupy the same region within the gastrointestinal (GI) tract, where they may interact directly with each other, as well as via competition for resources (e.g. space and nutrients) and by niche carving (Pedersen and Fenton 2007). Alternatively, they may occupy different regions within the GI tract, with the aboral flow of digesta potentially leading to unidirectional interactions, for example the alteration of pH in the abomasum influencing the enteric environment (Jackson et al. 1992).

As for coinfections between micro-parasites and macro-parasites, the host immune response is a key component of this ecosystem, with potential interactions via immunosuppression, cross-protective immunity and trade-offs between immune responses (Cox 2001; Pedersen and Fenton 2007). However, when coinfecting parasites are ecologically similar (as many GIN species are), ecological theory predicts that cross-protective immune responses are more likely to drive inter-parasite interactions than immune trade-offs are (Pedersen and Fenton 2007; A. L. Graham 2008). This theory has been supported by a combination of observational and experimental approaches in trematode–trematode coinfections in frogs (Johnson and Buller 2011). However, those trematode parasites all invade the tissue parenchyma and are subject to systemic immune responses. In contrast, GIN coinfections may occur within and between compartments of the GI tract. Communication between immune tissues across mucosal sites is well described (Iijima, Takahashi, and Kiyono 2001), however there is a degree of compartmentalization in immunity within the gastrointestinal (GI) tract (Brown and Esterházy 2021; Mowat and Agace 2014; Agace and McCoy 2017). It is unclear to what extent interactions between coinfecting GIN are confined within regions of the GI tract, and to what extent this is driven by compartmentalisation of immune responses, versus other ecological interactions. Further, it is unclear whether immune-mediated interactions between

coinfecting parasites at different mucosal sites are effected by antibodies that bind common antigens (Harrison, Pulford, Hein, Barber, et al. 2003) or the activation of cellular responses in the mucosae (Yacob et al. 2002).

Methods for Investigating GIN Coinfections

Coinfections are commonly investigated by one of three methods: controlled infection experiments, experimental perturbation of existing coinfections, or observations of natural coinfections. Controlled infection experiments provide the ability to demonstrate causal interactions between co-infecting GIN (Jackson et al. 1992); however, two-species experiments do not reflect the complexity of many naturally occurring GIN coinfections. In addition, the nature of interactions in controlled experiments may also be influenced by specific aspects of the life histories of the species selected (Lello et al. 2018), potentially limiting their generalisability. That study is part of a larger body of literature using pairwise controlled infections to explore interactions between coinfecting species (Christensen et al. 1987), many of which utilised sheep, presumably due to both their tractability as a model organism and the large economic impacts of GIN coinfections in domestic sheep (Nieuwhof and Bishop 2005; Charlier et al. 2020). Meta-analysis offers the opportunity to both summarise these findings and to explore the impact of experimental design factors that may provide insights into underlying mechanisms (Anello and Fleiss 1995), but this technique has not yet been applied to the GIN coinfection literature.

Perturbation experiments are another very powerful technique that have provided strong evidence of coinfection interactions in micro- – macro-parasite coinfections (Ezenwa and Jolles 2015; Seguel et al. 2023; A. R. Sweeny et al. 2020). However, the broad-spectrum activity of most anthelmintics makes it extremely challenging to target perturbation at a

specific GIN species within a GIN community. Further, these experiments may be unsuited to examine longer-term impacts of coinfection that may persist after the perturbation (for example host condition and cross-protective immune responses).

Simulation suggests that in the absence of experimental approaches, longitudinal observations offer the most power for identifying coinfection interactions (Fenton et al. 2014). Longitudinal studies are better able to identify these effects than cross-sectional studies, as repeated measures from the same individuals can overcome the challenge of collinearity between coinfection status and host condition (Thumbi et al. 2013; A. R. Sweeny et al. 2022; Karvonen, Bagge, and Valtonen 2007). This collinearity between coinfection status and host condition emphasises the idea that the fitness of hosts, pathogens and symbionts are fundamentally entwined and may even be considered a single unit of selection (the *superorganism* or *holobiont*) (D. S. Wilson and Sober 1989; Zilber-Rosenberg and Rosenberg 2008). Therefore, if we wish to understand the evolution of the *holobiont*, it is essential that observational studies are performed in populations without human interventions interrupting dynamics within individuals and the impacts on their fitness. From an applied perspective, such unmanaged populations also provide a model baseline for disease ecology, against which to compare the impact of specific management interventions.

Molecular Speciation of GIN

The most accurate way of assessing GIN burdens is via post-mortem worm counts, however this naturally precludes longitudinal study designs. Therefore minimally-invasive faecal egg counts (FECs) have commonly been used to provide estimates of GIN burden (McKenna 1981; Roberts and Swan 1981; Amarante et al. 1999; Gulland 1992). Unfortunately, the eggs of many GIN species are morphologically indistinguishable and traditional morphological

methods for identifying the species of GIN present in a faecal sample are highly-skilled and labour-intensive, limiting their availability and the number of samples than can be examined (Gasser et al. 2008; Van Wyk and Mayhew 2013). As a result, a large body of research has been dedicated to developing molecular methods for speciating GIN, often utilising species-specific variation in the relatively well-conserved ITS-2 ribosomal DNA regions (Gasser et al. 2008; Roeber et al. 2017; Powers et al. 1997). However, those methods are only able to detect the specific species for which they were designed and are still of relatively limited through-put.

The field of nematode speciation was revolutionised however by the application of barcoded amplicon sequencing to the ITS-2 region by Avramenko et al. (2015). In this technique, DNA is first extracted from the GIN within faecal samples, either directly or from isolated eggs, first-stage larvae (L_1) or third-stage larvae (L_3). The choice of DNA source is influenced by time constraints and risks of variation in larval development during coproculture. However, if coprocultures environments are well-controlled, all methods provide very similar estimates for GIN in cattle, sheep and goats (Redman et al. 2019; Francis and Šlapeta 2022). Once DNA has been extracted, the ITS-2 rDNA locus is amplified using primers conserved across the Clade-V nematodes (Gasser et al. 1993). A second PCR is then performed using barcoded primers that allow hundreds of samples to be sequenced in a single run (Wong, Jin, and Moqtaderi 2013). Each amplicon sequence variant (ASV) is then assigned to a species by comparing against a reference database, or to a higher taxonomic rank if the ASV is not classifiable to species level. Three bioinformatic pipelines have been developed for this process with very little difference in their results (Baltrušis, Halvarsson, and Höglund 2022). This process allows the identification of any Clade-V GIN within the sample, with very low detection limits, without *a priori* knowledge of the GIN community (Davey et al. 2023).

Insights from Molecular Speciation of GIN

Anthelmintic resistance is an enormous challenge across host species and many studies have used molecular GIN speciation to diagnose anthelmintic resistance in individual species within a multispecies coinfection (Kaplan et al. 2023; Antonopoulos, Gilleard, and Charlier 2024; Charlier et al. 2022; Nielsen et al. 2022; Avramenko et al. 2017; McIntyre et al. 2018; Krücken et al. 2024; Bull et al. 2025; Queiroz et al. 2020; Halvarsson and Höglund 2021; Eranga et al. 2023; Leathwick et al. 2025). Given that those studies identified variation in anthelmintic susceptibility between species, it is not surprising that anthelmintic treatment has been associated with decreases in alpha diversity (species richness and evenness within a subpopulation or individual host) (Avramenko et al. 2017; Halvarsson and Höglund 2021; Brachmann et al. 2025). This effect has also likely driven observations that livestock with less intensive management and wildlife have higher alpha diversity than more managed livestock (Sinclair et al. 2016; Avramenko et al. 2018; Beaumelle et al. 2022). Molecular speciation has also revealed overlap in GIN species between co-grazed hosts of different species (Kelly et al. 2025; Tombak et al. 2021; Halvarsson et al. 2022; Ilík et al. 2023), raising the question of what role wildlife and their diverse nemabiomes may play as *refugia* from anthelmintics. Beaumelle et al. (2022) demonstrated that GIN can circulate between farmed and wild ruminants that share space, which is necessary to function as *refugia.* However, it may also allow them to act as conduits for gene flow between farms, and the high levels of anthelmintic resistance genes in feral goats suggests wildlife's role as refugia may be limited (Francis and Šlapeta 2023).

This overlap between host species highlights the importance of the free-living larval stages and their dependence on external environmental conditions as drivers of GIN epidemiology (T. Wang et al. 2022). The influence of these external larval stages is likely a major driver of

widely observed seasonal variation in both alpha-diversity and beta-diversity (differences in species abundances between subpopulations or individual hosts) within a location (Geddes et al. 2024; Evans et al. 2021; Jouffroy et al. 2025; Waghorn et al. 2025; Sargison et al. 2022; T. Wang et al. 2021; Ahn et al. 2024) and between geographical regions (Avramenko et al. 2017; Poissant et al. 2021; Redman et al. 2019; Beaumelle et al. 2021; De Seram et al. 2022; Kipp et al. 2025; Gravdal et al. 2024).

However, host factors are clearly also important, with Sargison et al. (2022) showing in an unmanaged population of horses there were consistent differences between individuals in both alpha-diversity and in the magnitudes of the seasonal trends in beta-diversity. That study also found the oldest and youngest horses to have the greatest GIN species richness but did not have sufficient individuals to statistically test the effect of age. Other studies have shown complex relationships with age, with beta-diversity analyses indicating species compositions may differ between adults and young animals (Redman et al. 2019; Halvarsson et al. 2024; Evans et al. 2021). Studies in domestic livestock have suggested seasonal patterns may differ between age classes (Waghorn et al. 2025) and that alpha-diversity is lower in young animals than in mature animals (Kipp et al. 2025; Jouffroy et al. 2025; Evans et al. 2021); however, they may have all been influenced by more regular anthelmintic treatment of youngstock. In contrast, studies in unmanaged horses and deer have suggested that maturation of the host leads to reductions in alpha diversity with age (Ahn et al. 2024; Beaumelle et al. 2021), emphasising the need for fundamental studies of GIN epidemiology to be performed in unmanaged populations. However, those studies sampled hosts at a single time point and hence were unable to describe the dynamic progression in GIN community within individuals. Further, Evans et al. (2021) indicated that differences between age classes varied between cohorts. There is therefore a need for longitudinal studies of individual, unmanaged hosts of

a range of ages, recruiting young individuals in multiple years to differentiate cohort-specific effects.

Sex and reproductive status are also clear individual drivers of variation in GIN community, with species-level differences associated with host sex and female reproduction in horses (Ahn et al. 2024; Abbas et al. 2024) and alpha- and beta-diversity differences associated with reproduction in female sheep (Evans et al. 2021; E. G. Williams et al. 2024). Sheep are a good model species for studying the effect of reproduction, given the well-defined reduction in anti-GIN immunity among periparturient females associated with dietary protein limitation (known as the periparturient relaxation in immunity (PPRI)) (Kidane et al. 2010; Houdijk 2008). The strength of this effect appears to vary between GIN species (Jackson, Jackson, and Williams 1988) and E.G. Williams *et al.* (2024) showed increased dominance of one GIN species during the PPRI among a less resistant breed of sheep. This suggests host immunity may increase alpha-diversity by preventing single species dominance and buffering against seasonal perturbations. However, all these experiments were performed within relatively narrow nutritional ranges and may not encapsulate the variation in nutrition experienced by wild hosts, therefore further studies in wild sheep would be particularly valuable. Most research has focussed on the impact of reproduction on female hosts, but studies in wild sheep (*Ovis aries* and *Ovis canadensis*) have also shown that seasonal reproduction is associated with elevated unspeciated FECs in males (Coltman et al. 2001; Rijal et al. 2024; A. R. Sweeny et al. 2022). However, it is unclear whether seasonal reproductive effects in males are also associated with specific GIN species.

GIN Coinfections in Domestic Sheep

As well as being a useful model for ecological research, GIN coinfections in sheep place large constraints on agricultural production (Nieuwhof and Bishop 2005; Charlier et al. 2020). These have been historically managed by regular anthelmintic treatment but that approach is no longer sustainable due to the widespread development of anthelmintic resistance (Rose Vineer, Morgan, et al. 2020). Management strategies developed in response to anthelmintic resistance centre around building resistance through exposure to the parasites and selective breeding for resistant phenotypes, whilst minimising costs by monitoring host condition, breeding for tolerant phenotypes, and targeted-selective treatments with anthelmintics (Stubbings et al. 2022; Charlier et al. 2024). Understanding the epidemiology of the parasites is integral to these approaches, both to ensure adequate exposure to stimulate immunity and to avoid the grazing of high-risk pastures by vulnerable groups (Gascoigne et al. 2018).

In this context, mechanistic models of nematode epidemiology are likely to prove fundamental, both for the ability to forecast long-term changes to species distributions with climate change (Rose Vineer, Wang, et al. 2015) and to predict the intensities of infectious pressures for individual GIN species at field level within a farm (Rose Vineer, Verschave, et al. 2020; McFarland et al. 2022). However, these models currently do not account for changes in host resistance with changes in host physiology (e.g. development/ageing and reproductive status), or potential interactions between coinfecting GIN species.

In addition, agricultural management practices – particular regular anthelmintic treatment – profoundly impact parasite epidemiology (Morgan and van Dijk 2012). Therefore, research within specific agricultural systems may not generalise well when management practices vary, or if anthelmintic use becomes increasingly ineffective. Recently there has been renewed

interest in utilising knowledge of unmanaged ungulate populations to inform parasite evasion strategies in grazed livestock, often referred to as holistic or regenerative grazing (Hawkins 2017; Newton et al. 2020). Studies in unmanaged or wild host systems may therefore provide a more fundamental archetype against which to plan and assess management interventions.

Furthermore, there is very little knowledge regarding how GIN coinfections go on to impact host condition, with a very limited number of experimental studies showing mixed impacts of coinfections on bodyweight (Steel, Jones, and Symons 1982; Coop et al. 1988; Sykes, Poppi, and Elliot 1988; A. R. Williams et al. 2010). These impacts on bodyweight may then in turn influence the epidemiology via the impact of host condition on immunity (Houdijk 2008). These factors become of even greater importance in evolutionary studies of wild hosts, given impacts on condition will likely impact fitness. This may then influence demography which will feedback onto the epidemiology (e.g. via altered host density and age-structure), potentially driving the co-evolution of the host and the multiple co-infecting parasites.

Soay Sheep and their GIN parasites

Populations of unmanaged, wild sheep present a unique opportunity to advance our understanding of the evolutionary ecology of co-infections whilst simultaneously informing veterinary and agricultural management practices. Soay sheep (*Ovis aries*) are one such population, having lived wild on the island of Soay in the remote St Kilda archipelago since the Bronze Age. In 1934, 107 sheep were transferred from Soay to the depopulated island of Hirta. This population have since expanded across the island and remained unmanaged. Since 1985, the sheep living in Village Bay area of Hirta (around a third of island's 628ha area), have been part of a long-term individual-based study in which neonates are captured and ear-

tagged within a few days of birth allowing them to be identified and monitored throughout life (Clutton-Brock and Pemberton 2004).

As part of this long-term study, corral traps are constructed each August by a team of fieldworkers. Approximately 50-60% of the Village Bay population are caught in these traps and their bodyweights recorded (Clutton-Brock and Pemberton 2004). Previous work has shown that bodyweight is positively associated with both survival (Clutton-Brock et al. 1992) and reproductive success (Clutton-Brock et al. 1996; Preston et al. 2003) and therefore annual fitness (Milner et al. 1999).

Faecal samples are also routinely collected from animals captured in August and the unspeciated strongyle-type FEC obtained from those samples have been negatively associated with bodyweight (K. Wilson et al. 2004; Craig et al. 2008), survival (Illius et al. 1995; Coltman et al. 1999) and annual fitness (Hayward et al. 2011). Further, treatment with anthelmintics has been shown to reduce mortality overwinter, strengthening the evidence that these relationships are causal (Gulland 1992). The impact of these GIN infections on overwinter mortality mediate selection against inbred individuals (Coltman et al. 1999; Stoffel et al. 2021) and mediate the fitness costs associated with reproduction (Leivesley et al. 2019). Resistance against GIN has also been shown to be heritable (Sparks et al. 2019) with evidence of selection upon both resistance to and tolerance of them (Hayward et al. 2010, 2014) and maternally transferred passive immunity passing survival benefits to offspring (Sparks et al. 2020).

The majority of that work was performed using unspeciated strongyle-type FECs which comprise the eggs of *Teladorsagia circumcincta*, *Trichostrongylus axei, Trichostrongylus vitrinus, Chabertia ovina and Bunostomum trigonocephalum* (Gulland and Fox 1992; Craig

2005; Chambers 2020). However, the relative roles of these species in mortality in this system are unclear: post-mortems by Gulland (1992) showed *Te. circumcincta* to be the most abundant parasite in adult sheep and lambs that died in the winter of 1988/1989 (75% *Te. circumcincta*), whereas Craig et al. (2006) found are more diverse mixture in lambs that died in the winter 2001/2002 (c.17% *Te. circumcincta*, c.40% *Tr. axei* and c.35% *Tr. vitrinus*). Further, *Tr. vitrinus* counts showed the strongest association with weight-loss in animals that died over that winter (Craig et al. 2009). However, worm burdens at post mortem are not necessarily reflective of causal effects, and may instead reflect increased susceptibility of animals in poor condition. Given their strong predictive value for future survival, the unspeciated FECs from lambs in August are likely to have a causal relationship, and the FECs in that group have been assumed to be largely *Te. circumcincta* due to its predominance in domestic lambs of that age in epidemiological surveys in the UK (Boag and Thomas 1977; Waller and Thomas 1978; Paton, Thomas, and Waller 1984; Reid and Armour 1975); however, that assumption remains as yet untested.

GIN Life Histories

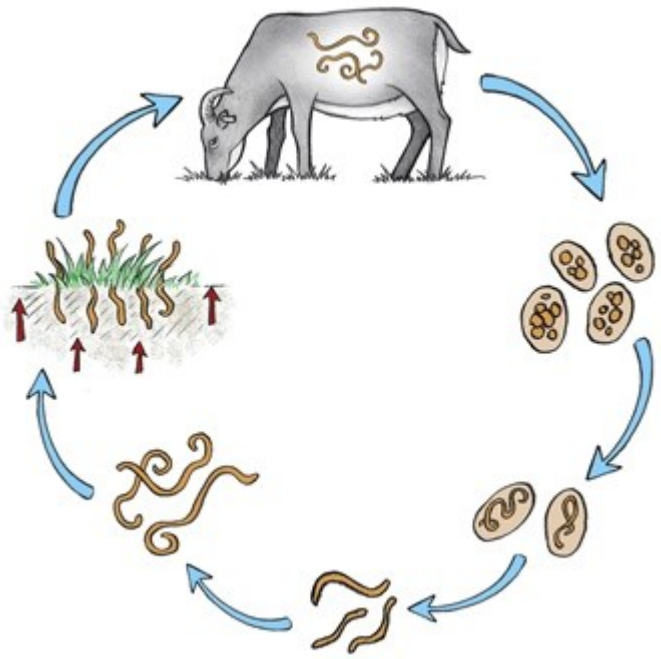


Figure 1: The archetypal life cycle of the Clade V GIN parasites of ruminants. Image created by Becky Lister-Kaye after Abbott, Taylor, and Stubbings (2012)

The GIN present in the sheep on St Kilda have broadly similar life cycles conforming to the Clade-V archetype (Lok and Unnasch 2018) (Figure 1). Eggs are passed in faeces; these develop in the faeces through first (L_1) and second (L_2) larval stages until the infections third stage larvae (L_3) emerge onto the pasture. These L_3 are then consumed and develop via the fourth larval stage (L_4) into adults within their predilection site in the GI tract. In order to aid understanding and interpretation of the subsequent chapters, the life histories of the major GIN on St Kilda are briefly outlined below and summarised in Figure 2.

Teladorsagia circumcincta

Eggs are shed into faeces which develop into L_3 on pasture. The optimum temperature range for development is 6.6-11.1°C (Callinan 1978a). Following ingestion these L_3 moult within 48h into fourth-stage larvae (L_4) which invade the gastric glands of the abomasum. These then develop into adults and emerge into the lumen of the abomasum where they reproduce sexually. The prepatent period is approximately 17 days (H. D. Crofton 1957). High levels of exposure in naïve animals cause inflammation of the abomasum with consequent anorexia and intraluminal protein loss. Naïve animals develop immunity following 8-12 weeks consistent exposure, comprising both an IgE-mediated immediate hypersensitivity reaction associated with worm expulsion and an IgA-mediated inhibition of worm growth and fecundity (McRae et al. 2015). In northern climates the majority of larvae on pasture die during the winter months, however L_4 are able to undergo hypobiosis in the abomasum, with subsequent emergence in the spring. Where dietary protein is limited the anti-*Te. circumcincta* immune response is limited; this is particularly true for peri-parturient ewes where IgA production in the mammary gland is prioritised over GI mucosal production (Kidane et al. 2010). This relaxation in immunity allows the development of emerging L_4 in spring, leading to an increase in *Te. circumcincta* egg production from approximately 2 weeks prior to lambing until around 6 weeks post-lambing. These larvae develop on the pasture and infect lambs, leading to relatively early infections compared to other GIN species, classically leading to peak in *Te. circumcincta* infections in lambs around midsummer, with a subsequent decline as the lambs develop immunity (H. D. Crofton 1957; Boag and Thomas 1977). The use of FECs as a proxy for *Te. circumcincta* worm numbers is often debated due to evidence of density dependent fecundity in this species (Bishop and Stear 2000), however there is no evidence of

such density dependence within the St Kilda context (Gulland 1991; Craig 2005; K. Wilson et al. 2004).

Trichostrongylus vitrinus

The life cycle of *Tr. vitrinus* is similar to *Te. circumcincta*, except that rather than invading the abomasal glands, they invade between the small intestinal villi and form sub-epithelial tunnels. Emergence of adult worms causes enteritis with haemorrhage and protein loss. This species may also able to undergo hypobiosis in the host, although this has been debated (Eysker 1978; Reid and Armour 1972). There is also a less significant peri-parturient relaxation of immunity than for *Te. circumcincta* (Jackson, Jackson, and Williams 1988) and field studies show more modest increases in this species in peri-parturient ewes (Reid and Armour 1975; D. J. Wilson et al. 2008; Evans et al. 2021). This, in addition to antagonistic interactions between species (Jackson et al. 1992), is thought to lead to a delayed epidemiology in lambs with egg counts and worm numbers in lambs increasing in late summer through into winter and early spring (Reid and Armour 1975; Parnell et al. 1954; S. M. Taylor and Pearson 1979; Geddes et al. 2024), despite a slightly higher optimum temperature range for larval development than *Te. circumcincta* (Callinan 1979). The prepatent period is similar to *Te. circumcincta* and adult females produces approximately half as many eggs per day as adult *Te. circumcincta* (H. D. Crofton 1957).

Trichostrongylus colubriformis

Tr. colubriformis is not present on St Kilda but was used in experimental infections in Chapter 5. It has a life history very similar to *Tr. vitrinus* but with a higher range of temperatures optimal for development, leading to greater prevalence and intensities than *Tr. vitrinus* in

warmer areas (Beveridge et al. 1989; Callinan 1979; Bailey, Kahn, and Walkden-Brown 2009; O'Connor, Walkden-Brown, and Kahn 2006).

Trichostrongylus axei

This species is a generalist parasite capable of infecting multiple host species (although no hosts other than sheep are present on St Kilda) and can invade either abomasal glands and intestinal crypts, inserting the head but leaving the body protruding onto the mucosal surface (Ross et al. 1967; Kates and Turner 1960b). It can undergo hypobiosis within the host (Reid and Armour 1972) and a PPRI has been described in this species but at lower magnitude than *Te. circumcincta* (Reid and Armour 1975; Evans et al. 2021). Although the optimal larval development conditions are almost identical to *Te. circumcincta* (Callinan 1978b), infections in lambs are relatively delayed, tending to rise steadily through autumn and winter (Brunsdon 1970; Reid and Armour 1975; Parnell et al. 1954). This is presumably a product of the lower magnitude PPRI and potentially interactions between coinfecting species that establish sooner (Turner, Kates, and Wilson 1962). It has been arguably less well studied than intestinal *Trichostrongylus* spp., as it may be outcompeted in mono-species grazing systems, but can cause significant clinical impacts in multi-species grazing systems (Bairden, Armour, and Duncan 1995). The prepatent period is similar to *Te. circumcincta* and *Tr. vitrinus* and female fecundity is similar to *Tr. vitrinus* (H. D. Crofton 1957).

Nematodirus spp.

Three species of *Nematodirus* (*N. battus*, *N. filicollis* and *N. helvetianus*) are present on St Kilda but they have similar lifecycles, *N. battus* predominates, and they are difficult to distinguish (even with molecular techniques, due to difficulties extracting DNA), hence they are considered together in this system (Gulland and Fox 1992; Craig 2005; Chambers 2020).

The *Nematodirus* lifecycle is largely similar to *Te. circumcincta* and the *Trichostrongylus* spp. except that the development to L_3 stage occurs within the egg. Climatic triggers (warming after a sustained cool period) cause mass hatching of the infectious L_3 which then complete their lifecycle in the small intestines (van Dijk and Morgan 2008). The L_3 have relatively short survival time on pasture with recovery rates dropping to 20% within one month and close to zero after 2 months (Thomas 1959; Boag and Thomas 1975; Gibson and Everett 1963; E. G. Graham, Harris, and Ollerenshaw 1984). This epidemiological pattern causes the clinical impact to depend upon the timing of the hatch in relation to the lambing date: if the hatch is very early, the majority of L_3 will have died before lambs begin to graze leading to low exposure; if the hatch is very late, the older lambs tend to be relatively resilient to the challenge; however if the hatch occurs when lambs are just starting to graze significantly, it can lead to severe, potentially fatal diarrhoea (Boag and Thomas 1975; D. M. Taylor and Thomas 1986). Immunity is relatively strong within approximately 6 weeks of first infection, therefore the vast majority of transmission occurs from lambs in one year, to lambs in the subsequent year, although a proportion may hatch in the autumn leading to within-year transmission (Tetley 1935; van Dijk and Morgan 2008; D. M. Taylor and Thomas 1986).

Chabertia ovina

The lifecycle is broadly similar to the other species, but rather than remaining within a single GI site, the larvae migrate distally as they mature, with L_3 found in the small intestine, L_4 in the caecum, and L_5 and adults in the colon (Herd 1971). This migratory lifecycle requires a longer time to complete with the prepatent period approximately 63 days, with female fecundity approximately 1.5 times that of *Te. circumcincta* (H. D. Crofton 1957). It is uncommonly seen on commercial farms in the UK, presumably as its long prepatent period

precluded survival in the face of regular anthelmintic treatments. Although levels have been shown to peak in lambs in late summer in New Zealand (Brunsdon 1970), in Scotland it has been described increasing more gradually to a peak in late winter/early spring (Parnell et al. 1954). There is little information regarding a PPRI in *C. ovina* in domestic sheep, although (Chambers 2020) suggested one may occur in young female sheep on St Kilda.

Bunostomum trigonocephalum

Unlike the other species described so far, *B. trigonocephalum* is a hookworm and has the typical lifecycle for that family. L_3 principally invade through the skin (although they may also be ingested), before migrating to the lungs where they develop to L_4 (Kamenov and Kanchev 2022; Bhatt, Srivastava, and Subramanian 1969; Habermann 1946). Those L_4 are then coughed up and swallowed, before passing to the jejunum where they develop into and feed on plugs of mucosa, leading to anaemia and hypoproteinaemia associated with blood loss from the resultant lesions (Westen 1967; Lucker and Neumayer 1944; Kamenov and Kanchev 2022). They complete their life cycle in the small intestines after a prepatent period of approximately 70 days, with fecundity comparable to *C. ovina* (Ortlepp and Du Toit 1939; H. D. Crofton 1957). Similarly to *C. ovina*, it is now rarely seen in commercial farming systems in temperate regions, although it remains a clinical problem in a global context (Khare et al. 2018; Naem and Gorgani 2011; Aragaw and Gebreegziabher 2014; Atayev et al. 2022; Ma et al. 2014). As such there is limited information about its epidemiology in northern temperate climates, although Cameron (1923a) and Parnell et al. (1954) reported no seasonal variation in it, and Brunsdon and Vlassoff (1971) reported that it did not express a PPRI. There is similarly limited information in the ecology of the free-living stages, although hatching temperatures of isolates from Northern India ranged from 10 to 34°C (Premvati and Narain 1969), and no over

winter survival of larvae on pasture was reported in studies in Canada, in the eastern USA and in India (Swales 1940; Kates 1943; Cameron 1923b).

Other species

Trichuris ovis and *Capillaria longipipes* are at extremely low prevalence and are therefore not discussed further, and *Strongyloides papillosus* is also not discussed given challenges enumerating it and its low clinical significance (M. A. Taylor, Coop, and Wall 2015).

Teladorsagia circumcincta

Site of infection: Abomasum
Pathology level: High
Pathogenesis: L_4 distend gastric glands, leading to hyperplasia, hyperaemia, oedema and sometimes sloughing of the mucosa.

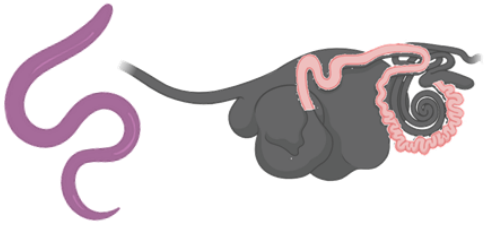
Trichostrongylus axei

Site of infection: Abomasum
Pathology level: Medium
Pathogenesis: Similar to *Te. circumcincta.* Causes irregular raised plaques, which may ulcerate in heavy infections.

***Nematodirus* spp. (esp. *battus*)**

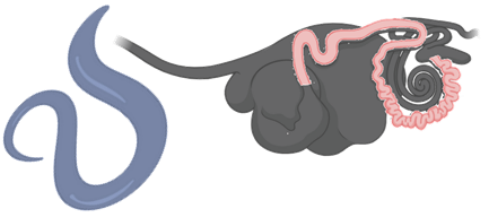
Site of infection: Small Intestine
Pathology level: High
Pathogenesis: Development from L_3 to L_5 occurs both within the mucosa and on the mucosal surface. This causes villous atrophy, impacting fluid and nutrient absorption.

Trichostrongylus vitrinus & colubriformis



Site of infection: Small Intestine
Pathology level: Medium
Pathogenesis: Larvae burrow through the epithelium and develop in subepithelial tunnels. Emergence of the adults causes haemorrhage, oedema and protein loss.

Bunostomum trigonocephalum



Site of infection: Small Intestine
Pathology level: Medium
Pathogenesis: Adult worms attach to the mucosa and feed on blood.

Chabertia ovina

Site of infection: Large Intestine
Pathology level: Low
Pathogenesis: L_5 and adults attach to the intestinal mucosa and then ingest plugs of tissue, resulting in local haemorrhage and protein loss.

Figure 2: Sites of infection, pathology level and pathogenesis for the six major GIN species present in the sheep on St Kilda. Trichostrongylus colubriformis is not present on St Kilda but has a very similar life history to Trichostrongylus vitrinus and was used in the experimental infection in Chapter 5. Sites of infection, pathology level and pathogenesis taken from Taylor, Coop and Wall (2015) and Stubbings et al. (2020). Images produced in Biorender. N.B. parasite images are illustrations only and do not represent the shape or anatomy of each parasite species.

Immunity against Gastrointestinal Nematodes

The protective immune response against gastrointestinal nematodes is largely driven by Group 2 Innate Lymphoid Cells (ILC2s) and T helper type 2 (Th2) cells (Figure 3). These responses are characterized by increases in the levels of interleukins (IL) 4, 5, 6, 9, 13, 21 and 25, which trigger activation and expansion of CD4+ Th2 cells, plasma cells secreting IgE and IgA, eosinophils, mast cells, basophils and alternatively activated macrophages (Anthony et al. 2007).

These Th2-type cytokines, particularly IL-13, also stimulate increased mucus cell hyperplasia and mucus production (Gossner et al. 2013; McKenzie et al. 1998), which provides a physical barrier against GIN as well as being the carrier medium for inhibitory/immune mediators (Douch et al. 1984; Harrison et al. 1999). At the same time, IgE-mediated degranulation of mast-cells releases vasoactive mediators the increases immune cell recruitment to the submucosa, and the leakage of fluid from capillaries into the mucus (which carries with it serum IgG). In addition, these Th2-type responses (especially IL-4 and IL-13) increase GI motility via direct effects on smooth muscle contractility (Wang et al. 2024; Zhao et al. 2003) and via alternatively activated macrophages (M2) (Zhao et al. 2008). Together, this increase in mucus volume, decrease in mucus viscosity and increase in contractility form a response commonly referred to as 'weep and sweep', which may dislodge and remove helminths within the GI lumen (Anthony et al. 2007).

This response may also be aided by impacts of IgA on helminth motility (McRae et al. 2015; Ramos et al. 2022; Aboshady et al. 2020) and by the release of the neuromuscular transmitter acetylcholine (ACh) into the lumen by tuft cells (Ndjim et al. 2024). Cellular responses effected by eosinophils, mast cells (including intra-epithelial mast cells also referred to as globule

leukocytes) and M2s are also important in the immune response against helminths, especially against tissue-dwelling/migrating immature stages, although this appears to vary between parasite and host species (Kreider et al. 2007; McKean and Pritchard 1989; Metz and Maurer 2007; Vannella et al. 2016; Meeusen and Balic 2000).

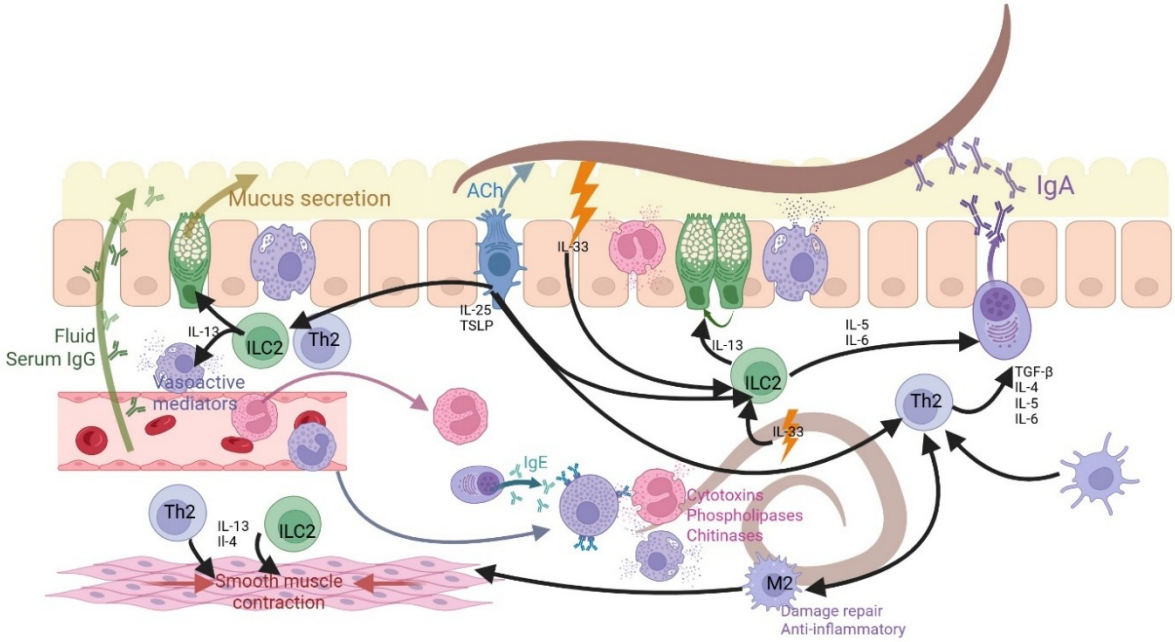


Figure 3: Schematic diagram representing selected key pathways and effector mechanisms in the Th2- type response against gastrointestinal nematodes. Produced in Biorender.

In temperate climates, sheep face the challenge of multiple GIN species in sites across the GI tract, including *Te. circumcincta* in the abomasum, and *Tr. colubriformis* and *Tr. vitrinus* in the small intestines (O'Connor, Walkden-Brown, and Kahn 2006). Immunity against the intestinal GINs *Tr. colubriformis* and *Tr. vitrinus* is characterised by an initial reduction in the number of incoming L_3 that become established (Seaton et al. 1989a; Dobson, Waller, and Donald 1990a). This is mediated by IgG and IgA responses against L_3 somatic antigens (Harrison et al. 2008) which can be detected in serum from 4 weeks post-infection (Cardia et al. 2011). This is followed by arrested worm development, reduced worm fecundity and the eventual expulsion of the established worm population (Dobson, Waller, and Donald 1990b, 1990c;

Eysker 1987; Sykes, Coop, and Angus 1979). In contrast, the initial response against the abomasal GIN Te. circumcincta is an IgA-mediated reduction in worm length and via that fecundity (Stear, Strain, and Bishop 1999). This is followed by increases in mast cells, eosinophils, globule leukocytes and IgA plasma cells in the both the pylorus and fundus with an immediate-type hypersensitivity reaction against incoming L_3, reducing further parasite establishment (W. D. Smith et al. 1984; Seaton et al. 1989b; Stear et al. 1995).

Potential mechanisms for cross-protective immune responses include cross-reactive antibodies, which due to the common mucosal immune response, may be induced at one site within the gastrointestinal tract (GIT) and be produced at other sites within the GIT. The glycolipid known as CarLA (carbohydrate larval antigen) has been a focus of much research, and animals with higher salivary IgA against CarLA have lower parasite faecal egg count (FEC), lower breech soiling and improved weight gain in field grazed sheep (presumably facing a multi-species challenge) (R. J. Shaw, Morris, and Wheeler 2013; R. J. Shaw et al. 2012). Further, anti-CarLA antibodies produced during experimental *Tr. colubriformis* infections cross-react with similarly sized antigens in somatic extracts of multiple species of ovine GIN (Harrison, Pulford, Hein, Severn, et al. 2003a). Harrison et al. (2008) found that preincubating *Tr. colubriformis* L_3 with these antibodies then provided passive protection against infection by surgical implantation, but cross-species passive protection was not provided when the antibodies were incubated with *Te. circumcincta* or *H. contortus* L_3. Similarly, serum levels of *Te. circumcincta* L_3 somatic antigen (*Te.c*-L_3-SA)-specific IgG are associated with protection against GIN, being negatively associated with FEC and positively associated with both adult and lamb survival in wild sheep (Sparks et al. 2020, 2018; Froy et al. 2019). Interestingly, in these studies, serum IgG responses to *Te. circumcincta* L_3 were highly correlated with responses against L3 antigen prepared from other sheep and mouse GIN (and even non-

parasitic soil-dwelling nematodes), suggesting the antibodies largely targeted conserved pan-nematode antigens (Froy et al. 2019). However, it is currently unclear what antigen targets these cross-reactive antibodies recognise, and whether they contribute to immune protection.

In order for an immune response to be triggered, the pathogen must first be detected. In addition to IL-33 released in response to parasite-mediated damage, mouse models indicate that intestinal tuft cells sense and initiate the Th2-type response against GIN infection via the secretion of IL-25 and thymic stromal lymphopoietin (TSLP) (Gerbe et al. 2016; Howitt et al. 2016) (Figure 3). These cells increase in number in the ovine abomasum in response to *Te. circumcincta* infection and possess conserved signalling pathways with murine tuft cells, suggesting they play a similar function in parasite sensing (Hildersley et al. 2021). Subepithelial dendritic cells may also play an important role in the recognition of GIN infection within the abomasum, with glycan moieties potentially influencing T-cell polarization (McNeilly, Devaney, and Matthews 2009). Communication between immune tissues across mucosal sites is well described (Iijima, Takahashi, and Kiyono 2001), however there is a degree of compartmentalization in immunity within the gastrointestinal (GI) tract (Brown and Esterházy 2021; Mowat and Agace 2014; Agace and McCoy 2017) and it is unclear to what extent the ability to detect and generate responses against co-infecting GIN are compartmentalized within the GI tract.

Thesis Aims

In this thesis I aimed to explore these important GIN coinfections in sheep using meta-analytic, observational and experimental methods. In chapter 2 I performed a systematic review of GIN co-infection experiments in sheep and then conducted a meta-analysis of

parasite establishment to test for evidence of interactions between species. The meta-analysis was aimed at answering the following questions in order to narrow down likely mechanisms for interactions:

1. Do GIN co-infections tend to be synergistic (indicating possible immunosuppression) or antagonistic (via resource competition, niche modification or triggering cross-immunity)?
2. Does the time-course of these co-infections impact the strength of any interactions? (Resource competition might be immediate whereas immune stimulation and niche modification would take time.)
3. Are interactions between coinfecting GIN shared between compartments of the GI tract? (Resource competition and niche modification would be unlikely to have effects 'upstream' of their GI compartment.)

In chapter 3 I applied ITS-2 amplicon sequencing (nemabiome) to samples taken longitudinally from over 500 individual sheep from an unmanaged population of wild sheep present on St Kilda. I used those data to derive species-corrected faecal egg counts (sFECs) and then answer the following questions regarding community composition in the absence of management interventions:

1. How does GIN community composition change as lambs age through their first year of life?
2. How does GIN community composition vary between adults and lambs?
3. Is there seasonal variation in the GIN community composition in adults, and is this affected by sex and reproductive status?

In chapter 4 I then used the nemabiome data from lambs to answer the following questions regarding the impact of GIN community composition:

1. Do total unspeciated FEC, GIN alpha diversity, GIN beta diversity or species-corrected FECs best predict future bodyweight?
2. Do total unspeciated FEC, GIN alpha diversity, GIN beta diversity or species-corrected FECs best predict mortality?

For chapter 5 I conducted an experimental infection trial exploring the effects of infection with the intestinal GIN *Trichostrongylus colubriformis* upon the mucosal immune response 'upstream' in the abomasum that might provide cross-protection against the abomasal GIN *Teladorsagia circumcincta*. Specifically, I aimed to answer the following questions:

1. Does *Tr. colubriformis* infection induce the production of mucosal antibodies upstream in the abomasum, that cross-react against *Te. circumcincta* antigens?
2. What are the targets of these cross-reactive antibodies?
3. Does *Tr. colubriformis* infection induce changes in abomasal gene expression indicative of a Th2 immune response?

Chapter 2 - Antagonism between co-infecting gastrointestinal nematodes: A meta-analysis of experimental infections in sheep

Publication of this chapter

This chapter was published as:

Evans MJ, Corripio-Miyar Y, Hayward A, Kenyon F, McNeilly TN, Nussey DH. Antagonism between co-infecting gastrointestinal nematodes: A meta-analysis of experimental infections in Sheep. Vet Parasitol. 2023 Nov;323:110053. doi: 10.1016/j.vetpar.2023.110053.

The content of the chapter is the Author's Accepted Manuscript, with the only alteration being figure references, which have been updated for consistency across the rest of the thesis. The PDF version of the published article is included as Appendix I.

Abstract

Gastrointestinal nematodes (GIN) have enormous global impacts in humans, wildlife and grazing livestock. Within grazing livestock, sheep are of particular global importance and the economics and sustainability of sheep production are greatly constrained by GIN infections. Natural infections are composed of co-infections with multiple species, and while some past work suggests species can interact negatively with one another within the same host, there is wide variation in reported patterns. Here, we undertook a systematic literature search and meta-analysis of experimental GIN co-infections of sheep to determine whether these

experimental studies support the hypothesis of antagonistic interactions between different co-infecting GIN, and test whether aspects of parasite biology or experimental design influence the observed effects.

A systematic search of the literature yielded 4848 studies, within which, we identified 19 experimental sheep studies comparing *post-mortem* worm counts across two co-infecting GIN species. Meta-analysis of 67 effects obtained from these studies provides strong evidence for interactions between GIN species. There was wide variation in the strength and direction of these interactions, but the global effect was significantly antagonistic. On average, there was a decrease in the number of worms of one species when a co-infecting species was also present, relative to a mono-infection with that species alone. This effect was dependent on the infectious dose and was rapidly lost after anthelmintic treatment, suggesting that live worms are required for the effect to occur. Individual parasite species varied in the extent to which they both exerted, and were subject to, these interspecies interactions, and these differences are more complex than simply co-localisation within the gastrointestinal tract.

Antagonistic interactions between co-infecting GIN may feedback into their epidemiology as well as potentially affecting the clinical impacts of infection. Furthermore, the consequences of these interactions may be heightened when clinical interventions affect only one species within the co-infecting network. Whilst it was not possible to identify the causes of variation between GIN species in the impact of co-infection, these findings point to new avenues for epidemiological, clinical and mechanistic research on GIN co-infections.

Introduction

Gastrointestinal nematodes (GIN) infect over half the world's human population (Chan 1997; Horton 2003) and are near ubiquitous parasites in wildlife and grazing livestock. Within grazing livestock, sheep are the most numerous species globally (Gilbert et al. 2018) and are highly important to rural economies both in higher income countries and in lower income countries, where, alongside goats, they are particularly relied upon by people living in poverty, especially women (Sinn, Ketzis, and Chen 1999). However, the economics and sustainability of sheep production are greatly constrained by GIN infections (Nieuwhof and Bishop 2005; McLeod 1995; Fitzpatrick 2013; Mavrot, Hertzberg, and Torgerson 2015; Charlier et al. 2020) and are further threatened by the widespread development of anthelmintic resistance (Kaplan and Vidyashankar 2012; Rose Vineer, Rinaldi, et al. 2015). These challenges have pushed the livestock industry to develop host genetics and farm management techniques that are less dependent on anthelmintics, and to invest in research that has generated enormous advances in GIN epidemiology, vaccines and immunology (Morgan et al. 2019). However, most research considers GIN species in isolation, whereas most natural infections are in fact complex co-infections of multiple species, affecting multiple sites within the GI tract. In temperate climates, the composition of these communities vary seasonally (Boag and Thomas 1977) and geographically (Redman et al. 2019); however, variation in GIN community composition may also occur between age classes and between years even within a single farm (Evans et al. 2021).

Epidemiological models of livestock GIN continue to improve (McFarland et al. 2022; Rose Vineer, Wang, et al. 2015; Rose Vineer, Verschave, et al. 2020) but an understanding of interactions between co-infecting GIN species is vital for the construction of holistic multi-

species models of GIN. Between-species differences in the ecologies of GIN parasites' free-living larval stages will undoubtedly contribute to observed variation in co-infection composition (O'Connor, Walkden-Brown, and Kahn 2006). However, interactions between species may also contribute to epidemiological patterns, as was postulated by Jackson et al. (1992) for *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* - two species with similar free-living ecologies, but markedly different seasonal epidemiologies.

Interactions between co-infecting GIN could also have impacts on the effectiveness of clinical interventions, and the evolution of anthelmintic resistance. Lello et al. (2004) demonstrated in rabbits that the removal of a single species from a network of interacting parasites can have unexpected effects on the remaining species. Such situations may readily arise in veterinary practice, for example targeted treatment of *Haemonchus contortus* with salicylanilide drugs, use of monovalent anti-nematode vaccines, or use of broad spectrum anthelmintics where anthelmintic resistance is present in only some of the co-infecting species. If such interactions are antagonistic they could also have impacts on the evolution of anthelmintic resistance, via both competitive release (increased fecundity or survival of those worms surviving treatment) and subsequent competitive exclusion (reduced establishment of susceptible worms from *refugia*).

Interactions between GIN of sheep are therefore of clear importance, and there has been an increase in scientific interest in the composition of ovine GIN communities following the development of a metabarcoded ITS-2 sequence-based GIN speciation platform ('the Nemabiome') (Avramenko et al. 2015; Redman et al. 2019). However, this platform has almost exclusively been applied to cross-sectional studies utilising samples pooled from multiple individuals, which are ill-suited to identifying inter-specific interactions (Fenton et al.

2014). Experimental approaches are more powerful in that regard but the results can be hard to extrapolate beyond the specific experimental conditions. The review of co-infection experiments by Christensen et al. (1987) showed that interactions between ovine GIN were predominately antagonistic, but examples of synergistic interactions do exist (Turner, Kates, and Wilson 1962; Kates and Turner 1960a; Turner and Colglazier 1954; Lello et al. 2018). We therefore aimed to perform a meta-analysis of GIN co-infection experiments in sheep to test the hypothesis of antagonistic interactions between different co-infecting GIN, and determine whether the broad range of experimental results reported were affected by the parasite species, or by experimental design details.

Pedersen and Fenton (2007) described how co-infecting parasites may interact with each other negatively (via competition for space, consumption of resources, or stimulation of non-specific host responses) or positively (via mechanical facilitation, immunosuppression, or immune-polarisation). In general, interactions are predicted to be strongest for species occupying similar ecological niches, and Lello et al. (2018, 2004) and Lello and Hussell (2008) showed that by defining the ecological niches of GIN as a combination of their feeding habit and their predilection site, the strength and direction of co-infection interactions could be predicted. However, within the GIN species routinely studied in sheep, *H. contortus* is unique in feeding on blood, with the other species all considered 'mucosal browsers'. Due to the lack of replication across feeding habits, we therefore chose to also test whether the co-infection interactions were affected simply by the relationship between the parasites' anatomic predilection sites.

Methods

Systematic Literature Review

Our systematic literature review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (Page, Moher, et al. 2021; Page, McKenzie, et al. 2021) (see Figure 4 for PRISMA diagram). The systematic literature search was performed in September 2022 (last search 2022-09-22) using CABabstracts, CABabstracts archive, MEDLINE, SCOPUS and Web of Science databases, searching in all fields. The search string was composed of four elements:

Synonyms for sheep:

Sheep OR Lamb* OR Ovine OR "Ovis aries"

Synonyms for co-infections:

"co-infect*" OR "co infect*" OR "coinfect*" OR "concomitant*" OR "concurrent*" OR synerg* OR antagonis* OR compet* OR interact* OR interspecific OR influenc* OR heterologous OR "cross-resistan*" OR "cross resistan*" OR "cross-immun*" OR "cross immun*"

Genera of species reported to infect sheep (including historic names) (M. A. Taylor, Coop, and Wall 2015), truncated in order to find references to both the genus and the associated clinical syndrome (e.g. *Haemonchus* and haemonchosis), or a truncation of the word 'nematode'. Following a scoping search specific exclusions were added to the truncation of '*Capillaria*' in order to avoid irrelevant references to 'capillary' and 'capillaries':

Nematod* OR Bunostom* OR Camelostrongyl* OR (Capillar* NOT capillary NOT capillaries) OR Chabert* OR Cooper* OR Gaiger* OR Gongylone* OR Haemonch* OR Marshallag* OR Mecistocirr* OR Monodont* OR Nematodir* OR Oesophagostom* OR Ostertag* OR Oxyur*

OR Parabrone* OR Skrjabine* OR Strongyloid* OR Teladorsag* OR Trichocephal* OR Trichostrongyl* OR Trichur*

Synonyms for nematode community diversity (adjacency operators dependent on database):

Nemabiome* or (Nematod* adj4\NEAR4\W/4 diversity) or (Nematod* adj4\NEAR4\W/4 community)

These four terms were linked to create the complete search string:

Sheep_synonyms AND ((Co-infection_synonyms AND Nematode_synonyms) OR Nematode_diversity_synonyms)

This search strategy yielded a library of 4157 studies. Backward and forward citation searching (using SCOPUS, Web of Science and Google Scholar) of all final eligible included papers yielded an additional 690 studies which were fed back into the library to give a total of 4847 studies. These were uploaded into the online Covidence application, which screened for duplicates automatically (Veritas Health Information 2022). After final manual curation, this resulted in the removal of 2210 duplicate studies. The remaining 2637 studies were screened for relevance against their title and abstract (including any study that reported GIN species composition from co-infected sheep), resulting in the exclusion of 2496 studies. The full texts for the remaining 141 studies were then assessed for eligibility. 82 of these studies reported natural co-infections rather than experimental infection; 8 were secondary reports or conference abstracts without data, and 25 did not have the right study design. Those studies

were excluded from the meta-analysis, but their meta-data were recorded, in order to assess the temporal and geographical representativeness of the included studies relative to the wider literature. This left 24 eligible studies that compared *post-mortem* worm counts of sheep infected with two GIN species (referred to hereafter as the 'principal species' and the 'co-infecting species') against control sheep mono-infected with just a single species (i.e., the principal species) (Figure 4).

Data Extraction, Effect Size Calculation

The standardized mean differences (SMD or Hedges' *g*) in *post-mortem* (principal species) total worm count (all worm life-stages) between the co-infected sheep and the mono-infected sheep were calculated from the mean, standard deviation (SD) and number of sheep (n) in each experimental group using the 'escalc(..., measure = "SMD")' command in the 'metafor' package:

$g = \frac{\bar{y}_1 - \bar{y}_2}{s_p}$, $where\ s_p = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{(n_1-1)+(n_2-1)}}$ (Viechtbauer 2010; Hedges 1981).

That command also generated the associated sampling variances using the large-sample approximation method (Hedges 1982, l. 8). Where the SD was not available, it was back-calculated using the reported Standard Error (SE) and sample size (n). Where the mean and SD/SE were not available, raw data was used to calculate them (extracted from data tables or graphs using DataThief III software (Tummers 2016)).

Of the 24 eligible studies, it was not possible to calculate effect sizes for four studies. In two this was due to a lack of replication (n=1 in some groups) arising from either differing study objective (Kates and Turner 1953) or the host death mid-experiment (Kates and Turner 1960a), and in the other two this was because neither the raw data, nor the SD/SE were

reported, and no author contact details were available to trace the raw data (Blanchard and Wescott 1985; Dash 1981). Therefore, effect sizes were calculated from a total of 20 studies. As many studies contained multiple experimental groups (see below) a total of 69 effect sizes were obtained from 26 experiments within those 20 studies.

Experimental Design Metadata

During data extraction, the following methodological details were also recorded: the principal species; the co-infecting species; the breed of sheep (if stated); age at first infection (recorded in days, months assumed to contain 30 days, midpoint used if a range given); inter-infection period in days; and post-infection period (days from last infection until necropsy). In addition, for both the principal and the co-infecting species, the total infectious dose (total number of third stage larvae (L_3) administered - always identical in experimental and control groups) and the duration of the infection administration (number of days from first administration to final administration) were recorded. Experimental methods were then classified into four categories according to their design (Figure 5). As only one experiment utilized the 'anthelmintic attenuated' design, this category was collapsed with the 'anthelmintic terminated' design, giving a total of three experimental design categories: sequential, simultaneous and anthelmintic.

GIN species were then classified according to their predilection for either the abomasum (stomach) (*Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus axei)* or intestines (*Nematodirus battus, Nematodirus spathiger, Trichostrongylus colubriformis, Trichostrongylus vitrinus*). An additional variable 'Anatomic Direction' with three levels was then assigned: 'Within Organ' (both species infect the same site); 'Downstream' (co-infecting

species infects the abomasum and the principal species infects the intestines); and 'Upstream' (co-infecting species infects the intestines and the principal species infects the abomasum).

In some experiments, sheep were euthanized for necropsy at various time points after the last infection. These animals provided separate effect sizes, although they are clearly not entirely independent of each other. Similarly, some studies compared multiple experimental groups against a common control group (e.g. Species A co-infected with Species B vs Species A mono-infection; and Species A co-infected with Species C vs Species A mono-infection). Also, some studies compared a single experimental group against multiple control groups (Figure 5D). To account for this interdependence, individual studies were divided into separate experiments, with effect sizes considered to be derived from the same experiment if they fitted any of the examples given above. (More in-depth descriptions of the original authors' methodologies are included in the complete dataframe, accessible in the online supplementary materials.) Further interdependence may exist between studies due to the use of similar sheep breeds, parasite strains, necropsy protocols etc. by authors operating within wider research groups. Studies were therefore classified into 'research groups' if they shared any author with any other study. The following hierarchy of independence was therefore produced: Research Group (10) > Study (20) > Experiment (26) > Effect (69) (Table 2).

Meta-Analysis

All analysis was conducted in RStudio v2022.12.0 using R v4.2.2 (R Core Team 2023). Meta-analytic modelling was performed using the 'metafor' package (Viechtbauer 2010). Plots were created using 'orchaRd' (Nakagawa et al. 2021), 'tidyverse' (Wickham 2017), 'cowplot' (Wilke

2018) and 'tmap' (Tennekes 2018) packages. The dataset and R scripts can be accessed at DOI: 10.17632/275d8w3x3j.1

We conducted our meta-analysis using multi-level mixed-effects maximum likelihood models. A random-effects model was first constructed by fitting research group, study and experiment as a three-tier nested random effect, in order to account for interdependence between multiple effects. This random effects model was used to estimate the global effect and the 95% confidence interval (CI) and 95% prediction interval around the global effect. In addition, the proportion of heterogeneity explained by the random effects structure was estimated by calculating I^2 for the random-effects model using the 'i2_ml' command from the 'orchaRd' package (Nakagawa et al. 2021) and comparing it against I^2 for a null model with no random effects.

The effects of 'Experimental Design', 'Co-Infecting Parasite Dose', 'Co-infecting Species', 'Principal Species' and 'Anatomic Direction' were then tested by adding each to the random effects model as a single moderator. Each moderator was judged to be significant where the Wald-type χ^2 test for moderators (Q_M) provided a *P*-value <0.05. A more normal distribution for 'Co-Infecting Parasite Dose' was achieved using a natural logarithm (ln) transformation prior to fitting.

To assess the significance of potential confounding methodological variables on SMD, the following experimental design data were also tested as single moderators: 'Co-infection Duration' (as a continuous moderator in days); 'Co-Infection Duration' (discretised into 'single bolus' or 'multiple bolus/trickle infection'); and 'Inter-Infection Interval' (as a continuous moderator in days). The following methodological details were not tested as for each effect they were controlled for by identical conditions in the within-experiment control group: the

principal parasite dose; the principal parasite infection duration; the age at experiment onset; and the post-infection period.

All significant single moderators were then fitted together into a multiple moderator model. Moderators were considered significant after controlling for the other moderators if the Wald-type χ^2 test (Q_M) provided a *P*-value <0.05. To control for potential publication bias 'Publication Year' and $\sqrt{1/\tilde{n}_i}\ where\ \tilde{n}_i = \frac{n_{1i}n_{2i}}{n_{1i}+n_{2i}}$ were also fitted to the multiple moderator model (Nakagawa et al. 2022). As the $\sqrt{1/\tilde{n}_i}$ term was statistically significant it was replaced in the model by $1/\tilde{n}_i$ as recommended by Nakagawa et al. (2022). That replacement had no effect on the significance of any of the other moderators, therefore only the latter is reported in the results (both versions are present in the Supplementary Material).

Pairwise *post hoc* testing for all levels of categorical moderators that were significant in the multiple moderator model was performed with Benjamini-Hochberg correction using the 'multcomp' package (Hothorn, Bretz, and Westfall 2008).

One study (Herlich 1965) had very large SEs and was therefore considered a potential outlier. It also had a much longer co-infection duration and utilised a pair of parasites (*Cooperia oncophora* and *Cooperia pectinata*) not represented in any of the other studies, therefore its inclusion would have prevented fitting the multiple moderator meta-regression. Consequently, this study (two effects) was excluded from all the meta-analyses reported below. For completeness the single moderator meta-regressions were repeated without excluding that study and no change to the effects or conclusions were seen (Online Supplementary Materials).

Results

Multi-level meta-analysis indicates that sheep co-infected with two GIN species usually have significantly fewer worms of the principal species than sheep mono-infected with just the principal species (Figure 6). The global estimate for the standardized mean difference (SMD) in *post-mortem* worm counts of co-infected sheep compared to mono-infected control sheep (β_{global}) was -0.732 (95% confidence interval (CI) = -1.03 - -0.431, Z = -4.78, $P < 0.001$). Total heterogeneity was substantial (I^2 = 62.60%), whilst heterogeneity in the random-effects model was moderate (I^2 = 52.31%), indicating that the nested random effects structure accounted for 20.3% of the inter-effect heterogeneity.

Single moderator meta-regression analysis showed the anatomic direction of the co-infection had no significant effect on *post-mortem* worm counts (Q_M = 1.30, df = 2, P = 0.523) (:). Co-infection duration had no significant effect either as a continuous moderator (Q_M = 2.18, df = 1, P = 0.140) or as a two-level discrete moderator (bolus vs trickle) (Q_M = 1.22, df = 2, P = 0.269). Similarly, the inter-infection interval in days had no significant effect (Q_M = 1.64, df = 1, P = 0.201). Significant single moderator effects were identified for: experimental design (Q_M = 8.68, df = 2, P = 0.013); ln(co-infecting parasite dose) (Q_M = 5.18, df = 2, P = 0.023); co-infecting species (Q_M = 13.46, df = 5, P = 0.019); and principal species (Q_M = 13.27, df = 6, P = 0.039). Therefore these variables were carried forward into the multiple moderator meta-regression, where they all remained significant (Figure 8).

Sequential co-infection experiments without the use of anthelmintics had significantly more negative effects on worm count than sequential co-infection experiments where an anthelmintic was administered between infections (*post hoc* t = -3.28, $P_{(BH\ adjusted)}$ = 0.005). Simultaneously administered co-infections occupied an intermediate position relative to

those other experimental designs, although the 95% CI did not cross the zero, suggesting simultaneous GIN co-infections also interact negatively (Figure 8A). There was also a significant negative effect of ln(Co-Infecting Dose), indicating that higher doses of co-infecting worms exerted greater antagonism on the principal infection (Figure 8B).

GIN species differed significantly in both the degree to which they inhibited a co-infecting GIN (Figure 8C) and the degree to which they were inhibited by a co-infecting GIN (Figure 8D). No significant pairwise comparisons between GIN species were found on Benjamini-Hochberg adjusted *post hoc* testing; however visual examination of the 95% CIs suggests *T. vitrinus*, *T. axei* and *H. contortus* may have more negative effects on co-infecting GIN (Figure 8C), and at the same time *T. vitrinus* and *H. contortus* may be more greatly affected by a co-infecting GIN compared to the potentially more resilient *T. axei* (Figure 8D).

There is no evidence that these results were significantly affected by publication bias, given publication year and $1/\tilde{n}_i$were non-significant in the multiple moderator meta-regression (Table 1 and Figure 10).

The majority of inter-effect heterogeneity was explained together by experimental design, co-infecting species, principal species and co-infecting parasite dose, whilst controlling for publication year, effective sample size, research group and study. I^2 for the multiple moderator model was 13.18%, indicating a 74.81% reduction in the heterogeneity present in the random-effects model.

There were marked differences in the temporal and the geographic distributions of experimental studies identified during the literature search, compared to the excluded observational reports. The observational reports were globally distributed across 41 countries (Figure 9A), whereas the experimental infections were performed in only 5 countries (Figure

9B). The cumulative number of experimental studies increased approximately linearly through the 1960s to the 1990s but plateaued after the millennium; in contrast, the cumulative number of observational studies has expanded more exponentially with the greatest number of annual publications (7) in 2021 (Figure 9C).

Discussion

There is strong evidence from this meta-analysis that GIN species co-infecting sheep interact with each other, and although there is wide variation the general effect is predominately antagonistic, leading to a decrease in the number of worms of one species, relative to a mono-infection. This effect is also dose dependent, with greater antagonism seen at greater infectious doses. Further, GIN species vary in both their effect on co-infecting species and their susceptibility to such effects, but these interactions appear more complex than simply the relationship between their anatomic locations, given that factor was not significant in the single moderator analysis. This finding may simply reflect a lack of power given the small number of experiments examining upstream effects. However, it could also suggest that the mechanisms responsible for interactions among GIN (e.g. immunity, resource competition) are not local, but operate across the GI tract. This could have important implications for the development of immunity against natural mixed infections across multiple sites. Combinations of other life history factors may also be driving the observed variation between species, and specific parasite pair combinations may be important; however, unfortunately there were insufficient studies to fit an interaction between the co-infecting and principal species in the multiple moderator model.

Our results are unable to definitively state how individual species vary from each other in their co-infection interactions but the observed differences provide interesting points for

discussion. Lello et al. (2018) proposed that *H. contortus* has a facilitative effect on co-infecting *T. colubriformis* (mediated via immunosuppression); however, the multiple moderator model suggests *H. contortus* has antagonistic effects on co-infecting GIN in general, raising questions about how the host response against *T. colubriformis* may differ from other species. Lello et al. (2018) also suggested that blood-feeding by *H. contortus* may make it particularly vulnerable to serum antibodies raised against a co-infecting species. That idea is supported by previous vaccine trials against *H. contortus* using antigens from *Ostertagia ostertagi* (W. D. Smith, Smith, and Pettit 2000) and *T. circumcincta* (W. D. Smith, Pettit, and Smith 2001), and receives further support from the strongly negative estimate for *H. contortus* as the principal parasite in our meta-analysis (Figure 8D). *T. vitrinus* was negatively affected by co-infection with *T. circumcincta* (an abomasal species), and the authors of those experiments proposed that this was due to a vulnerability of this species to pH changes induced by co-infection with abomasal parasites (Coop et al. 1988; Jackson et al. 1992). This hypothesis is potentially supported by the fact that Roy, Host & Beveridge's (2004) experiment co-infecting *T. vitrinus* with *T. colubriformis* (another intestinal species) provided three effect sizes close to zero (Figure 8D). Our meta-analysis suggests that *T. axei* may be more resilient to the effects of a co-infecting GIN, albeit based on data from a single experiment. This species is arguably the most generalist of GIN species, capable of infecting many host species and potentially infecting the duodenum in addition to its abomasal predilection site. This generalist role may give it a wide ecological niche and the flexibility to potentially modify its site of infection and thereby mitigate the antagonism of a co-infecting species, as suggested by Pustovoi (1972).

A further striking finding of our meta-analysis was that the effects of a co-infection were lost following anthelmintic treatment. This suggests that either live parasites are necessary to

mediate their effects, or that any indirect mechanisms are quickly lost after the clearance of the first species. Experimental design was significant both as a single and as a multiple moderator, and the pairwise comparison between sequential and anthelmintic-treated designs was significant on *post hoc* testing. Simultaneous co-infections also had smaller effect sizes than sequential co-infections. Whilst *post hoc* testing of this pairwise comparison was not significant, it is intuitive that effects would be greater if one species has first either modified its environment to suit its own niche, or has pre-stimulated non-specific host immune or physiological responses. The indication that live parasites are necessary for antagonistic effects to occur raises important questions about the underlying mechanisms through which parasites may mediate antagonistic effects (e.g. via parasite excretory-secretory products) and why mucosal immune responses may be so short lived as to be lost quicky after anthelmintic treatment.

Although this meta-analysis has provided strong support for several important findings, the ability to investigate species-specific factors was limited due to the low number of reports relative to the number of potential pairwise combinations of parasites. The potential range of species interactions is even greater when the limited geographical distribution of the included studies is considered against the globally distributed observational reports. Although it would be interesting to expand the range of species studied, the number of pairwise comparisons would expand exponentially, and it would be unfeasible to test so many pairwise co-infections *in vivo*. Further, two-species co-infections are only a single step towards the biological reality of the multi-species co-infections that occur in the field. There is therefore value in studies utilising complex natural infections. The scope of this meta-analysis was also limited, in that it was only possible to assess the effect of co-infection on worm number, rather than fecundity or pathology. There is evidence that GIN co-infections may reduce

worm egg production (Dobson and Barnes 1995; Jackson et al. 1992; Mapes and Coop 1971) and that their pathologic impact on the host may be positive or negative (Coop et al. 1986; Steel, Jones, and Symons 1982; Sykes, Poppi, and Elliot 1988). There is hence also a need for further work on the impacts of co-infection interactions.

Two clear avenues for future research therefore open from this meta-analysis: firstly, studies of natural GIN co-infections able to identify multi-species interactions and quantify their impacts; and secondly, further controlled co-infection experiments aimed at identifying the mechanisms underlying them. The development of high throughput techniques for quantifying GIN species offers the potential for observational studies of natural co-infection dynamics (across the global breadth of host biomes). In contrast to the findings of this meta-analysis, observational studies of natural infections have generally reported positive correlations between co-infecting species (Barger 1984; Cabaret and Hoste 1998; Diez-Baños, Cabaret, and Diez-Baños 1992; Hoste and Cabaret 1992; Morales, Pino, and Sandoval 2006; Rehbein et al. 1997; Stear et al. 1998; J. P. A. Sweeny et al. 2012). However, those studies were all cross-sectional, a study design which Fenton et al. (2014) showed to have limited power to identify interspecies interactions in co-infections. To resolve this issue, future studies should consider longitudinally sampling a large number of individual hosts with high temporal granularity. Within such studies, the collection of good quality long-term measures of host fitness/production (e.g. lamb growth and survival, and ewe rearing success and longevity) would provide the most meaningful measure of the impact of co-infection dynamics, and the contextualisation alongside species-specific pasture larval counts would enable the greatest epidemiological inference. Analysing the effects of anthelmintic treatment or monovalent vaccines on GIN community composition would provide further insight and could be designed as deliberate perturbation experiments or could utilise clinical

samples from faecal egg count reduction tests or vaccine trials. Once these studies have identified significant interaction pathways, it would be valuable to return to controlled co-infection experiments targeting the causative mechanisms. Such studies could be *in vivo*, for example focussing on the influence of co-infections on mucosal immune responses along sites in the GI tract. Alternatively, they could be *in vitro*, perhaps looking for evidence of direct communication between nematode species, or using organoid models to examine whether changes in epithelial phenotypes (e.g. gastric remodelling (Faber et al. 2022)) are evidence of niche alterations that may affect the invasion success of a subsequent heterologous infection.

Acknowledgements and Interests

Funding Source: This work was primarily supported by ME's ECAT-V Fellowship, awarded by the Wellcome Trust and University of Edinburgh. AH is supported by a Moredun Foundation Research Fellowship; AH, FK and TNM receive funding from the Scottish Government Rural and Environment Science and Analytical Services (RESAS). YCM is supported by the large NERC grant (NE/R016801/1), awarded to DHN and TNM, which underpinned the conceptualisation of this study.

Competing Interests: None to declare

CRediT Authorship Statement: Conceptualization ME, YCM, AH, FK, TM, DN; Methodology ME, YCM, AH, FK, TM, DN; Formal analysis ME, AH, DN; Investigation ME; Data Curation ME; Writing - Original Draft ME; Writing - Review & Editing ME, YCM, AH, FK, TM, DN; Visualization ME, AH; Supervision YCM, AH, FK, TM, DN.

Figures

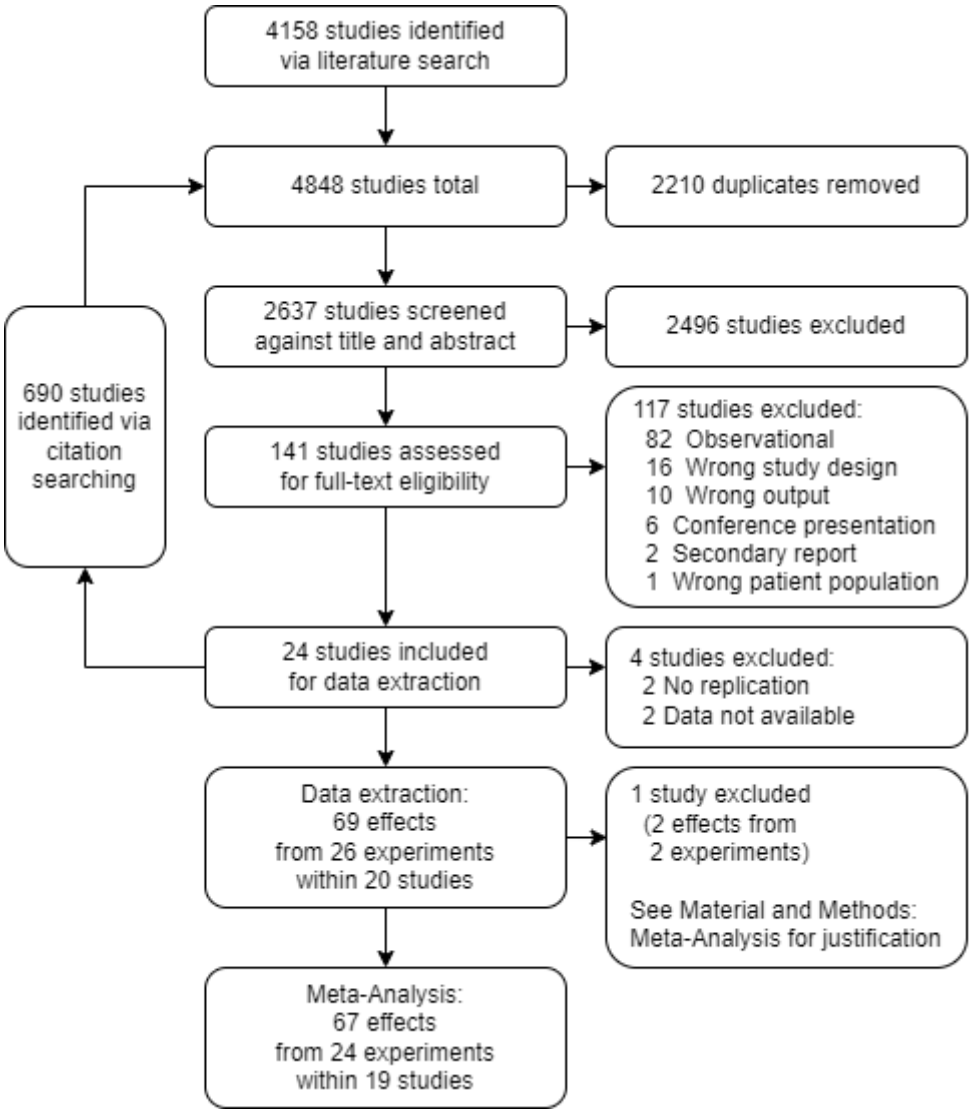


Figure 4 PRISMA diagram illustrating study selection. All screening and data extraction was performed by the lead author (ME)

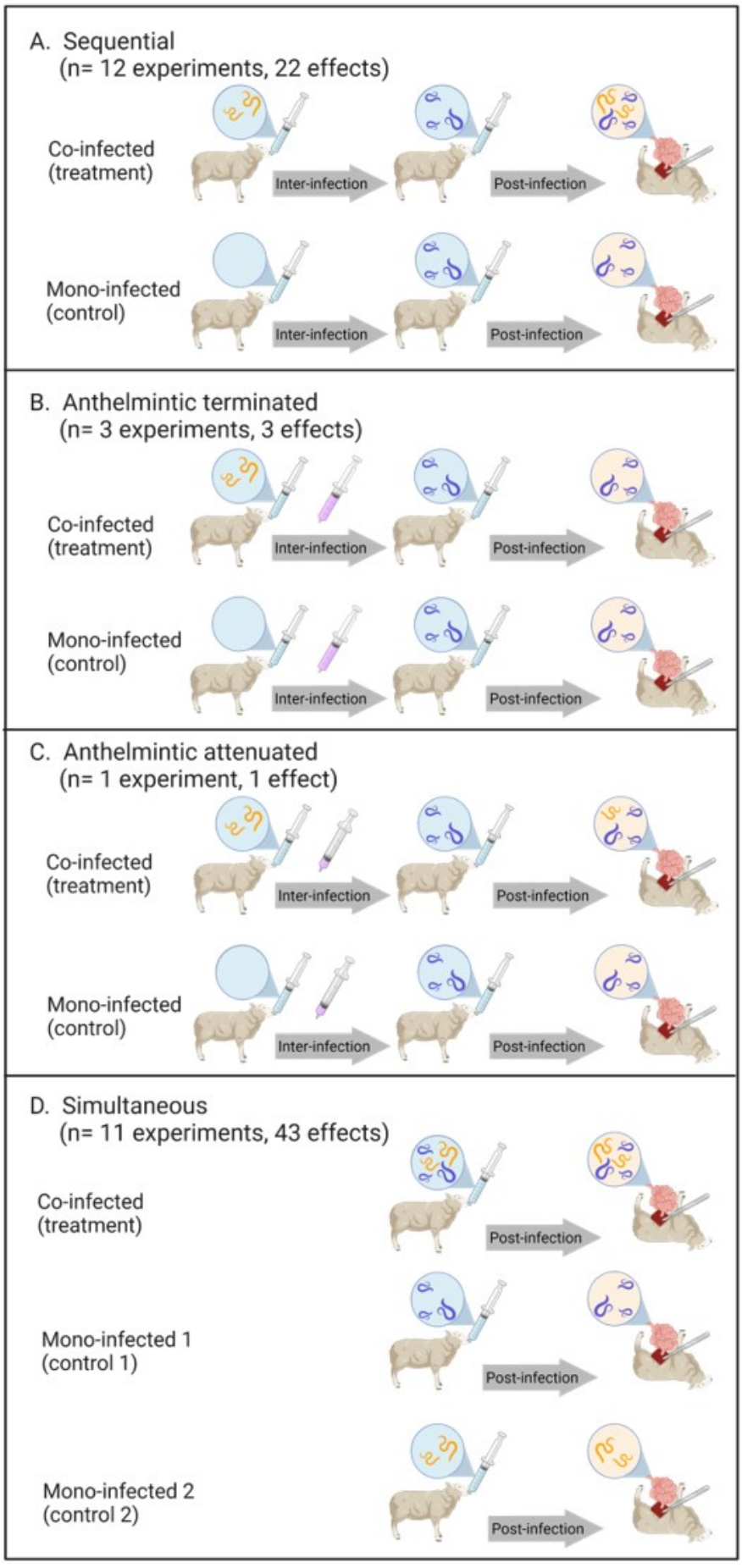

A. Sequential
(n= 12 experiments, 22 effects)
Co-infected
(treatment)
Inter-infection
Post-infection
Mono-infected
(control)
Inter-infection
Post-infection
B. Anthelmintic terminated
(n= 3 experiments, 3 effects)
Co-infected
(treatment)
Inter-infection
Post-infection
Mono-infected
(control)
Inter-infection
Post-infection
C. Anthelmintic attenuated
(n= 1 experiment, 1 effect)
Co-infected
(treatment)
Inter-infection
Post-infection
Mono-infected
(control)
Inter-infection
Post-infection
D. Simultaneous
(n= 11 experiments, 43 effects)
Co-infected
(treatment)
Post-infection
Mono-infected 1
(control 1)
Post-infection
Mono-infected 2
(control 2)
Post-infection

Figure 5: Experimental design classifications (created with BioRender.com).

A: Sequential. Co-infected sheep were infected with the co-infecting species (yellow) and subsequently infected with the principal species (blue); control sheep were mono-infected with the principal species (blue) only. Post-mortem worm counts of the principal species (blue) in the co-infected group were compared against those in the mono-infected group.

B: Anthelmintic terminated. Design as per A, except with a therapeutic dose of anthelmintic administered between the co-infecting species (yellow) and the principal species (blue) in order to eliminate the co-infecting species.

C: Anthelmintic attenuated. As per B, except using a subtherapeutic dose of anthelmintic intended to reduce the intensity of the co-infecting species without eliminating it. Note B & C were combined for analysis into a single 'Anthelmintic' group.

D: Simultaneous. Co-infected sheep were infected with the principal species and the co-infecting species simultaneously; control sheep were mono-infected with the principal species only. In some cases, mono-infections with both species were performed, therefore two effects could be obtained (co-infected vs mono-infected 1 - blue worms are the principal species; or co-infected vs mono-infected 2 - yellow worms are the principal species).

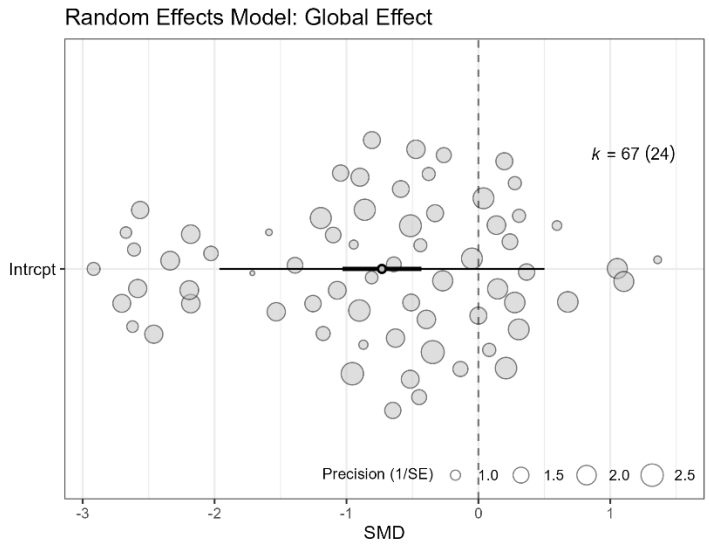


Figure 6: Orchard plot showing significant effect of GIN co-infection on the Standardised Mean Difference (SMD) in post-mortem worm count of the principal GIN. Circles show individual effects, with their diameter inversely proportional to the standard error (SE) of the SMD; central circle shows the global estimate from the random effects model, with the thick lines showing the 95% confidences intervals associated with that estimate; the thin lines show the 95% prediction interval for the model; k shows the number of effects, with the number of experiments in brackets.

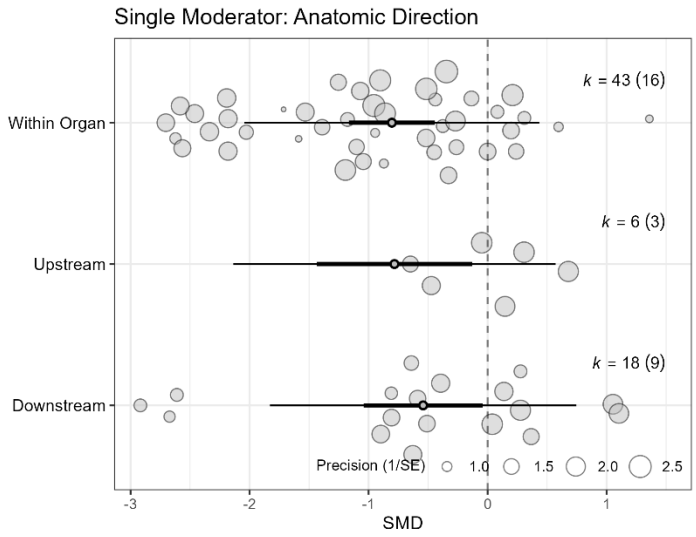


Figure 7: Orchard plot showing the lack of effect of anatomical direction on the Standardised Mean Difference (SMD) in post-mortem worm count of the principal GIN.

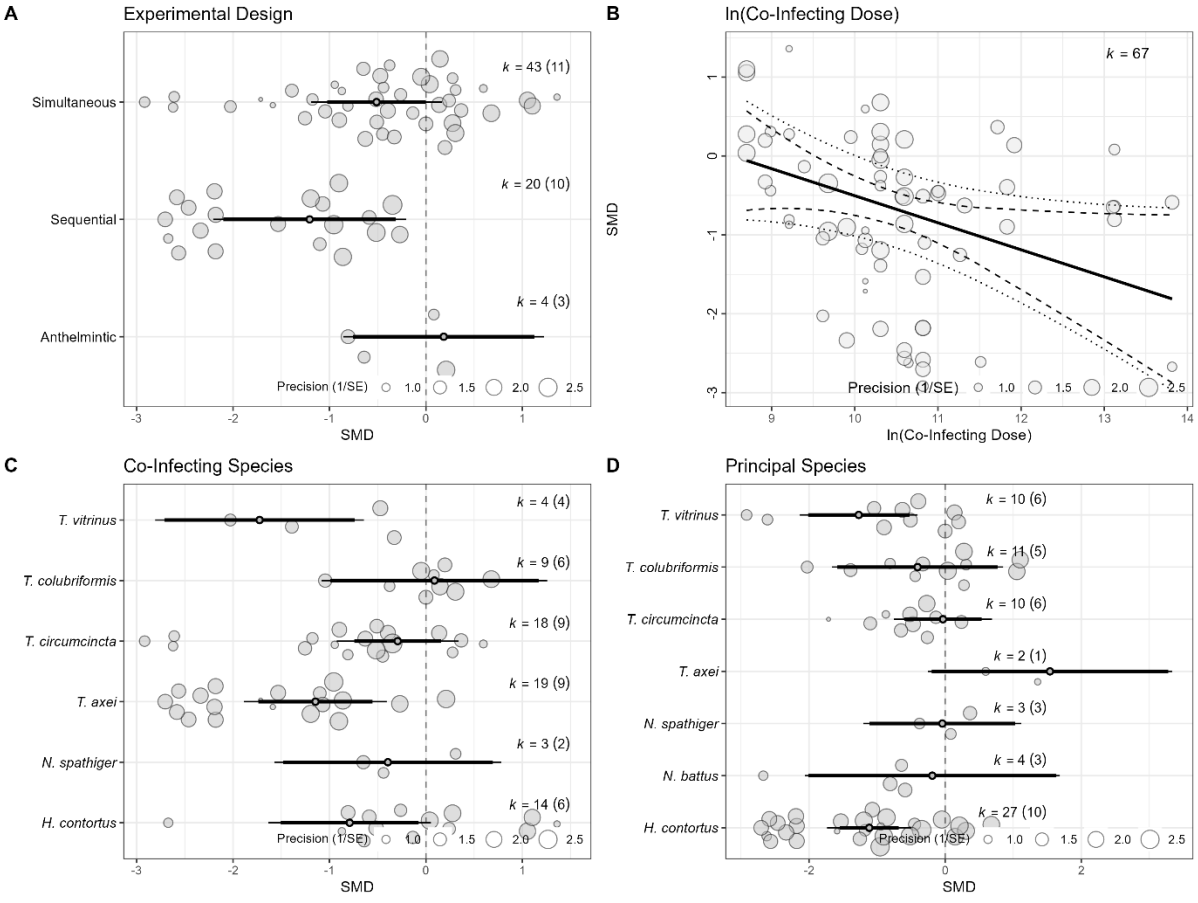


Figure 8: Outputs from the multiple moderator meta-regression describing effects of experimental design and GIN species on post-mortem worm count of the principal GIN. Orchard plots (A, C & D) as described for Figure 6. B: Bubble plot as per Orchard plots but with the solid line representing the estimated effect, the dashed lines representing the 95%CI around the effect, and the dotted lines representing the 95% prediction interval. SMD = Standardised Mean Difference in post-mortem worm count of the principal GIN.

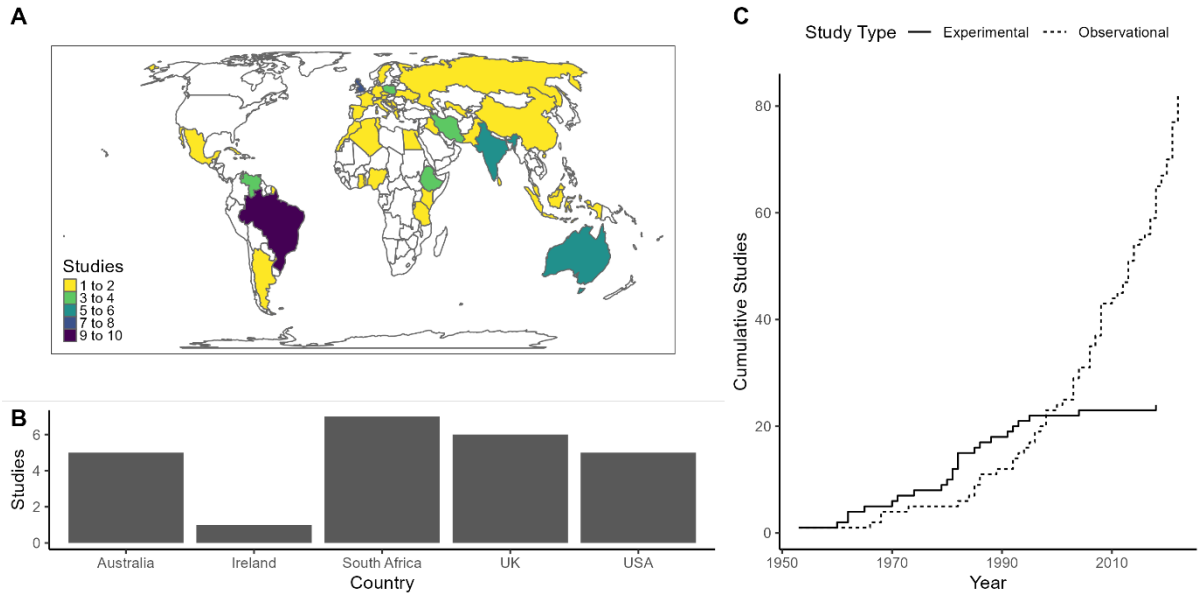


Figure 9: Choropleth map (A) and bar chart (B) showing total number of observational (A) and experimental (B) studies from each country identified during the systematic literature review. C: Cumulate frequencies of the studies in A and B against time.

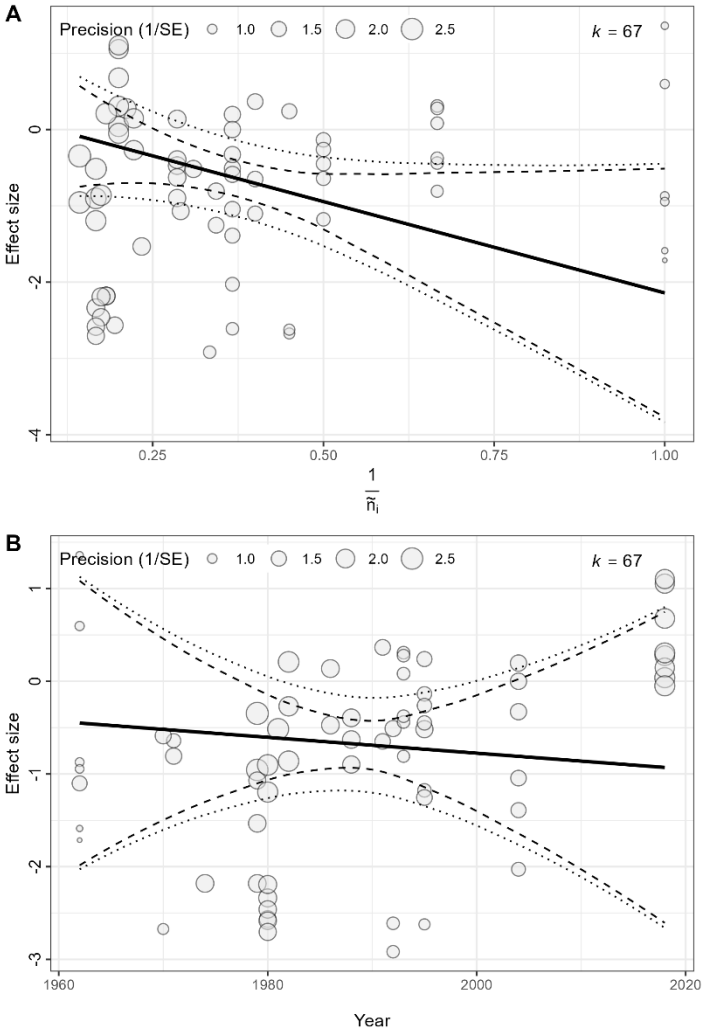


Figure 10: Bubble plots showing the non-significant effects of Year and $\frac{1}{\tilde{n}_i}$ *in the multiple moderator model*

Tables

Table 1 Results from the multiple-moderator meta-regression. QM, DF and P refer to the test for significant differences in effect size due to the moderator. Within moderators the estimates, 95% CIs, Z and P are relative

to the reference level. Significantly different groups of moderator levels according to post hoc pairwise testing are indicated by different letters.

Variable	Level	Q_M	DF	*P*	Estimate	95% CI	*Z*	*P*	*Post hoc*
Intercept	Intercept				21.17	-87.33 - 129.67	0.382	0.702	
Experimental design	Anthelmintic	10.91	2	0.004					a
	Sequential				-1.39	-2.27 - -0.565	-3.30	0.001	b
	Simultaneous				-0.700	-2.00 - 0.607	-1.05	0.295	ab
Co-infecting species	*H. contortus*	13.35	5	0.020					a
	N. spathiger				0.396	-0.857 - 1.65	0.619	0.536	a
	T. circumcincta				0.498	-0.487 - 1.48	0.991	0.322	a
	T. axei				-0.355	-1.46 - 0.754	-0.623	0.530	a
	T. colubriformis				0.879	-0.367 - 2.13	1.38	0.167	a
	T. vitrinus				-0.934	-1.95 - 0.085	-1.80	0.072	a
Principal species	*H. contortus*	15.19	6	0.019					a
	N. battus				0.926	-1.06 - 2.92	0.912	0.362	a
	N. spathiger				1.07	-0.082 - 2.23	1.82	0.069	a
	T. circumcincta				1.08	0.285 - 1.88	2.66	0.008	a
	T. axei				2.66	0.775 - 4.54	2.77	0.006	a
	T. colubriformis				0.709	-0.613 - 2.03	1.05	0.293	a
	T. vitrinus				-0.155	-0.904 - 0.594	-0.405	0.686	a
ln(Co-infecting dose)		4.62	1	0.032	-0.343	-0.655 - 0.030	-2.15	0.032	

Year	0.095	1	0.759	-0.009	-0.063 - 0.046	- 0.307	0.759
$1/\tilde{n}_i$	3.29	1	0.070	-2.39	-4.98 - 0.192	-1.81	0.070

Table 2: Hierarchical classification of effects within experiments, within studies, within research groups

Research Group	Study	Experiment	Effect ID	Within study description
Coop, Mapes, Jackson et al.	(Coop et al. 1986)	A	Coop et al., 1986_expA_eff1	T. vit. (principal), T. circ. (co-infecting)
			Coop et al., 1986_expA_eff2	T. circ (principal), T. vit. (co-infecting)
	(Coop et al. 1988)	A	Coop et al., 1988_expA_eff1	High dose T. circ. (principal), Low dose T. vit. (co-infecting)
			Coop et al., 1988_expA_eff2	Low dose T. vit. (principal), High dose T. circ. (co-infecting)
			Coop et al., 1988_expA_eff3	High dose T. vit. (principal), High dose T. circ. (co-infecting)
	(Jackson et al. 1992)	A	Jackson et al., 1992_expA_eff1	Short
			Jackson et al., 1992_expA_eff2	Medium
			Jackson et al., 1992_expA_eff3	Long
	(Mapes and Coop 1970)	A	Mapes and Coop, 1970_expA_eff1	Exsheathed Nematodirus

		B	Mapes and Coop, 1970_expB_eff1	Un-exsheathed Nematodirus
	(Mapes and Coop 1971)	A	Mapes and Coop, 1971_expA_eff1	Long
			Mapes and Coop, 1971_expA_eff2	Short
Dobson & Barnes	(Dobson and Barnes 1995)	A	Dobson and Barnes, 1995_expA_eff1	T. circ. (principal), Haemonchus (co-infecting), 4 weeks
			Dobson and Barnes, 1995_expA_eff2	T. circ. (principal), Haemonchus (co-infecting), 7 weeks
			Dobson and Barnes, 1995_expA_eff3	T. circ. (principal), Haemonchus (co-infecting), 10 weeks
			Dobson and Barnes, 1995_expA_eff4	T. circ. (principal), Haemonchus (co-infecting), 12 weeks
			Dobson and Barnes, 1995_expA_eff5	Haemonchus (principal), T. circ. (co-infecting), 4 weeks
			Dobson and Barnes, 1995_expA_eff6	Haemonchus (principal), T. circ. (co-infecting), 7 weeks
			Dobson and Barnes, 1995_expA_eff7	Haemonchus (principal), T. circ. (co-infecting), 10 weeks

			Dobson and Barnes, 1995_expA_eff8	Haemonchus (principal), T. circ. (co-infecting), 12 weeks
Downey	(Downey 1962)	A	Downey, 1962_expA_eff1	Single effect study
Emery, Wagland & McClure	(Emery, Wagland, and McClure 1993)	A	Emery et al., 1993_expA_eff1	N. spath. (principal), T. col. (co-infecting), anthelmintic
		B	Emery et al., 1993_expB_eff1	T. col. (principal), N. spath. (co-infecting), Short
			Emery et al., 1993_expB_eff2	T. col. (principal), N. spath. (co-infecting), Long
			Emery et al., 1993_expB_eff3	N. spath. (principal), T. col. (co-infecting), Long
			Emery et al., 1993_expB_eff4	T. circ. (principal), T. col. (co-infecting), Short
			Emery et al., 1993_expB_eff5	T. circ. (principal), T. col. (co-infecting), Long
Heath & Connan	(Heath and Connan 1991)	A	Heath and Connan, 1991_expA_eff1	T. circ. (principal), N. spath. (co-infecting)
			Heath and Connan, 1991_expA_eff2	N. spath. (principal), T. circ. (co-infecting)
Herlich	(Herlich 1965)	A	Herlich, 1965_expA_eff1	C. oncophora (co-infecting) then C. punctata (principal)
		B	Herlich, 1965_expB_eff1	C. punctata (co-infecting) then C. oncophora (principal)
Lello et al.	(Lello et al. 2018)	A	Lello et al., 2018_expA_eff1	T. col. (principal), Haemonchus (co-infecting), 6 weeks

			Lello et al., 2018_expA_eff2	T. col. (principal), Haemonchus (co-infecting), 10 weeks
			Lello et al., 2018_expA_eff3	T. col. (principal), Haemonchus (co-infecting), 14 weeks
			Lello et al., 2018_expA_eff4	T. col. (principal), Haemonchus (co-infecting), 18 weeks
			Lello et al., 2018_expA_eff5	Haemonchus (principal), T. col. (co-infecting), 6 weeks
			Lello et al., 2018_expA_eff6	Haemonchus (principal), T. col. (co-infecting), 10 weeks
			Lello et al., 2018_expA_eff7	Haemonchus (principal), T. col. (co-infecting), 14 weeks
			Lello et al., 2018_expA_eff8	Haemonchus (principal), T. col. (co-infecting), 18 weeks
Reinecke et al.	(Reinecke, Snyman, and Seaman 1979)	A	Reinecke et al., 1979_expA_eff1	Haemonchus (principal), T. circ. (co-infecting)
			Reinecke et al., 1979_expA_eff2	Haemonchus (principal), T. axei. (co-infecting)
		B	Reinecke et al., 1979_expB_eff1	High dose, Short
			Reinecke et al., 1979_expB_eff2	High dose, Long

			Reinecke et al., 1979_expB_eff3	Low dose
	(Reinecke, Bruckner, and De Villiers 1980)	A	Reinecke et al., 1980_expA_eff1	Low dose, divided
			Reinecke et al., 1980_expA_eff2	Low dose, single bolus
			Reinecke et al., 1980_expA_eff3	Medium dose, divided
			Reinecke et al., 1980_expA_eff4	Medium dose, single bolus
			Reinecke et al., 1980_expA_eff5	Higher dose, divided
			Reinecke et al., 1980_expA_eff6	Higher dose, single bolus
			Reinecke et al., 1980_expA_eff7	Highest dose, divided
			Reinecke et al., 1980_expA_eff8	Highest dose, single bolus
	(Reinecke, Bruckner, and De Villiers 1981)	A	Reinecke et al., 1981_expA_eff1	Single effect study
	(Reinecke, Brückner, and De Villiers 1982)	A	Reinecke et al., 1982a_expA_eff1	Single effect study
	(Reinecke, De Villiers, and Brückner 1982)	A	Reinecke et al., 1982b_expA_eff1	Single effect study
	(Reinecke, De Villiers, and Joubert 1982)	A	Reinecke et al., 1982c_expA_eff1	Single effect study

	(Reinecke 1974)	A	Reinecke, 1974_expA_eff1	Single effect study
Roy, Hoste & Beveridge	(Roy, Hoste, and Beveridge 2004)	A	Roy et al., 2004_expA_eff1	T. vit. (principal), T.col. (co-infecting), Low doses
			Roy et al., 2004_expA_eff2	T. col. (principal), T.vit. (co-infecting), Low doses
		B	Roy et al., 2004_expB_eff1	T. vit. (principal), T.col. (co-infecting), Medium doses
			Roy et al., 2004_expB_eff2	T. col. (principal), T.vit. (co-infecting), Medium doses
		C	Roy et al., 2004_expC_eff1	T. vit. (principal), T.col. (co-infecting), High Doses
			Roy et al., 2004_expC_eff2	T. col. (principal), T.vit. (co-infecting), High Doses
Turner, Wilson & Kates	(Turner, Kates, and Wilson 1962)	A	Turner et al., 1962_expA_eff1	T. circ. (principal), Haemonchus (co-infecting)
			Turner et al., 1962_expA_eff2	T. axei. (principal), Haemonchus (co-infecting)
			Turner et al., 1962_expA_eff3	Haemonchus (principal), T. circ. (co-infecting)
			Turner et al., 1962_expA_eff4	T. axei. (principal), T. circ. (co-infecting)
			Turner et al., 1962_expA_eff5	Haemonchus (principal), T. axei. (co-infecting)
			Turner et al., 1962_expA_eff6	T. circ. (principal), T. axei. (co-infecting)

Chapter 3 - Longitudinal dynamics of nematode co-infection in wild sheep

Abstract

Gastrointestinal nematode infections have significant impacts on people, livestock and wildlife globally. The majority of these infections are co-infections of multiple species which can interact with each other and the host in different ways. The natural dynamics of GIN communities in the absence of human management and drug treatment remain poorly understood, but are important both for our understanding of wildlife disease and our ability to design sustainable interventions. The development of ITS2-sequence-based nemabiome speciation allows nematode community structure and dynamics to be estimated from faecal samples at much higher throughput than before. In this chapter I applied this technique to over 3000 samples collected across four years from over 500 sheep living in an unmanaged population on St Kilda. The results show how parasite community diversity and the species-corrected faecal egg counts vary with season, reproductive status and age, in the absence of human intervention. The results indicate that *Teladorsagia circumcincta* and *Nematodirus* spp. conform to epidemiological patterns previously described in domestic sheep, whereas *Trichostrongylus vitrinus* showed an unexpected periparturient rise and higher than expected abundances in lambs in July. Our results also provide rare insight into the epidemiology of species which are highly sensitive to anthelmintic treatment and are therefore relatively poorly studied in domestic sheep (especially *Bunostomum trigonocephalum)*. The results also show that unlike in domestic sheep, but in common with other wild mammals, GIN alpha-

diversity is higher in young animals than mature animals, emphasising the value of fundamental research in unmanaged populations.

Introduction

Gastrointestinal nematodes (GIN) infect over half the world's human population (Chan 1997; Horton 2003) and are near ubiquitous parasites in wildlife and grazing livestock. Understanding GIN epidemiology and its effects on host health, as well as designing sustainable treatment strategies, are made particularly challenging as the vast majority of parasitic infections are multi-species co-infections (Bordes and Morand 2009; Pullan and Brooker 2008; Avramenko et al. 2015, 2018; Poulin 2001; Redman et al. 2019). Co-infecting GIN species vary in their pathogenicities, predilection sites and epidemiologies (M. A. Taylor, Coop, and Wall 2015), as well as their immunomodulatory capabilities (McNeilly and Nisbet 2014) and drug resistances (McIntyre et al. 2018; Zahid et al. 2023). Further, there is evidence that co-infecting GIN species interact both with each other and with other pathogens within the host environment (Christensen et al. 1987; Pedersen and Antonovics 2013; Midha, Schlosser, and Hartmann 2017; Evans et al. 2023). The recent development of next-generation sequencing technology to determine the species of GIN within faecal samples offers an exciting means of monitoring community structure and addressing how these different processes shape parasite community dynamics and epidemiology.

It is near impossible to recreate the complexities of natural GIN communities in controlled settings. Thus, the majority of experimental work has focussed on mono-infections, with a smaller body investigating pairwise co-infections indicating antagonistic interactions between co-infecting species (Chapter 2; Evans et al., 2023). A large proportion of that work has been conducted in domestic sheep, both because of the high translational value in this species (Charlier et al. 2020; Nieuwhof and Bishop 2005; Gilbert et al. 2018) and their tractability as a model organism. However, agricultural management practices – particular regular drug

treatment – profoundly impact parasite epidemiology (Morgan and van Dijk 2012). Therefore, research within specific agricultural systems may not generalise well when management practices vary, and hence have limited value for informing our knowledge of parasite community dynamics in wildlife and across livestock systems. Recently there has also been renewed interest in utilising knowledge of unmanaged ungulate populations to inform parasite evasion strategies in grazed livestock, often referred to as holistic or regenerative grazing (Hawkins 2017; Newton et al. 2020). Studies in unmanaged or wild host systems therefore provide the opportunity to address questions regarding the evolutionary ecology of parasite co-infections, whilst also providing a more fundamental archetype against which to plan and assess management interventions.

Observational field studies have been constrained in the past by the difficulty of differentiating eggs of different nematode species without laborious culture and morphological speciation of larvae (Van Wyk and Mayhew 2013). However, the recent application of deep-amplicon sequencing of the ITS-2 locus to identify nematode species from faecal samples ('nemabiome' sequencing) has opened up the possibility of large-scale non-invasive studies of GIN community dynamics (Avramenko et al. 2015). Cross-sectional studies using this approach in wild and managed ungulates have shown geographic and seasonal variation in the composition of GIN communities, often by pooling faecal samples from different individuals (Abbas et al. 2024; Bourgoin et al. 2021; Redman et al. 2015). Longitudinal studies, in which known individuals are repeatedly sampled over time, offer considerably greater insight into GIN community dynamics, allowing us to test how species abundance and diversity changes within-individuals over time and space and compare those dynamics among individuals of different age, sex or breed. They are also a more robust

approach to identifying and measuring parasite interactions within the host (Fenton et al. 2014).

In this chapter I used over 3000 faecal samples that were collected from over 500 individual sheep across four years in an unmanaged Soay sheep population. As part of a larger project (see declarations), I applied ITS2-sequencing to those samples generate individual-level nemabiome data. I then used these nemabiome data to describe the seasonal epidemiology of six GIN species and compared our observations against those observed in previous studies of domestic sheep (Figure 11). Firstly, I investigated the GIN co-infection of lambs from July to November of their first year of life. Studies in domestic sheep predict these would be characterised by a decline in *Nematodirus spp.* and *Te. circumcincta*, and an increase in *Tr. vitrinus* from July to November. Are those epidemiological patterns maintained in an unmanaged population with free co-grazing by all age/sex classes and no anthelmintic intervention? Secondly, I investigated how the nemabiome changes through the first two years of life into mature adulthood, for which we had no prior expectations given the lack of research in this area, especially for GIN species that are relatively uncommon on farms with regular anthelmintic treatment (*Chabertia ovina* and *Bunostomum trigonocephalum*). Thirdly, I investigated the interaction between seasonal and sexual/reproductive effects. Here I expected to see a rise in egg counts in reproductive females in spring, principally driven by *Te. circumcincta*, as a consequence of the periparturient relaxation in immunity (PPRI). Under unmanaged grazing conditions and no anthelmintic treatment, does the PPRI affect GIN species differently? And how does the nemabiome of rams vary given the strong inter-male competition present in this system in the autumn?

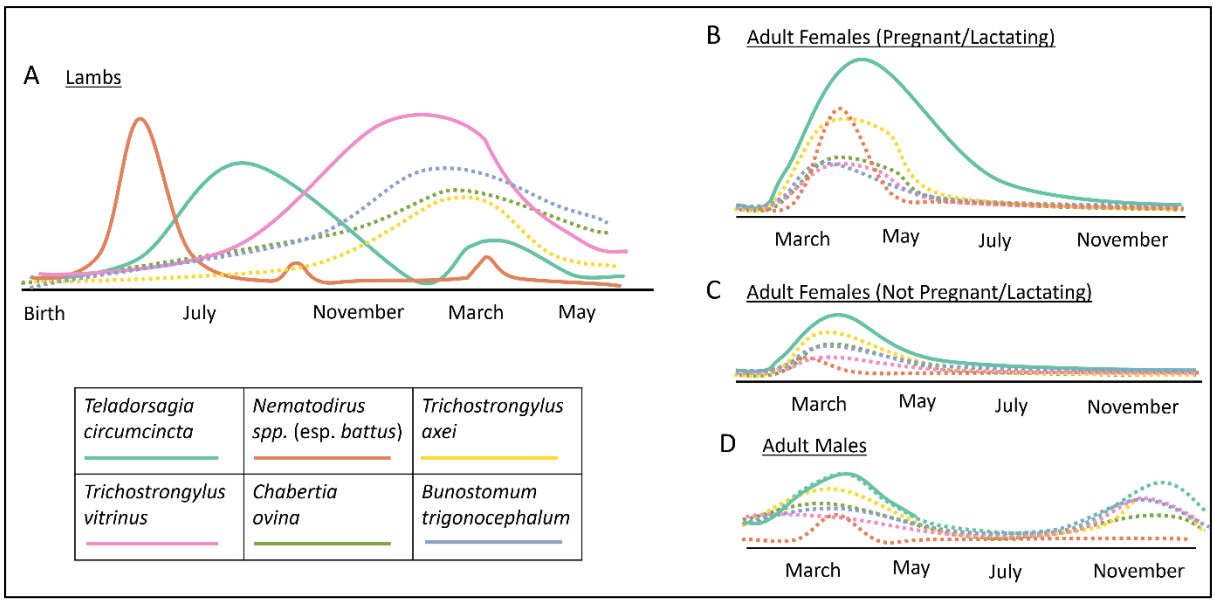


Figure 11 Hypothesised species-specific faecal egg counts in lambs and ewes over the course of a year based on previous studies of the same parasites in domestic sheep (Boag and Thomas 1977, 1975; H. D. Crofton 1957; D. J. Wilson et al. 2008; Evans et al. 2021; Jackson, Jackson, and Williams 1988; Hamer et al. 2019; Brunsdon and Vlassoff 1971; Kidane et al. 2009; Parnell et al. 1954; E. G. Williams et al. 2024; Geddes et al. 2024). *Dotted lines indicated species with more limited descriptions in those studies and hence lower prior confidence.*

Methods

Study System

This study was conducted in the wild population of Soay sheep (*Ovis aries*) on the island of Hirta in the St Kilda archipelago (65 km northwest of the Outer Hebrides, Scotland). Soay sheep are thought to have lived wild on the island of Soay since the Bronze Age, with 107 sheep transferred from Soay to the depopulated island of Hirta in 1934, resulting in the present unmanaged population. Since 1985 the sheep living in Village Bay area of Hirta (around a third of island), have been part of a long-term individual-based study in which neonates are captured and ear-tagged within a few days of birth allowing them to be identified and monitored throughout life (Clutton-Brock and Pemberton 2004).

The sheep in this system exhibit seasonal reproduction with mating occurring in November, with intense inter-male conflict and competition (butting and horn-clashing) to mate with oestrous females. Lambing occurs in April, with most adult females producing at least one lamb each year. The majority of females give birth for the first time at two years of age but a smaller proportion of females give birth at one year of age. Twinning rates are usually below 20% and triplets are extremely rare. There is no adult predation on the island (great skuas occasionally attack and kill weak neonate lambs) and mortality occurs mostly over-winter among lambs/yearlings, mediated by limited nutritional resources and parasitism.

Sample Collection

Four two-week sampling periods were planned for each of the four years of the study (2019-2022). Samples taken in early March (henceforth referred to as 'March') coincided with late-pregnancy in females. Samples taken in mid-May ('May') coincided approximately with peak lactation in reproductive females. Samples taken in late-July ('July') coincided approximately with natural weaning of lambs. Samples taken in late October to early November ('November') were just prior to the mating season. Due to restrictions during the COVID-19 pandemic no sampling was performed in May 2020, and sampling for March 2021 was delayed until early April.

Sampling was carried out in targeted individuals in our core study area, chosen to cover an even range of ages and sex, starting with a group of 121 adults (ranging from 2 to 10 years old) during March and May 2019, and incorporating a cohort of 120 lambs from July onwards. The faecal collection from these animals continued during the 4 years, incorporating new adults if study animals died, plus a new cohort of lambs each year.

Faecal samples were collected by following the target individuals and collecting faecal samples from the ground within 1-2min of defecation. Samples were immediately placed into plastic bags and the air extruded to provide anaerobic conditions and maintain humidity. Samples were divided into two subsamples: one for faecal egg counts (of 2g for adults and 1g for lambs) which were stored in anaerobic conditions at 4°C; and the second was the remainder which was used for coprocultures and therefore stored at room temperature in plastic bottles with a loose lid to allow air to enter. Samples were dispatched to the mainland for processing within 20 days (all except 1 sampling season were within 15 days) of collection. Samples were not collected from lambs in the May of their first year of life (aged c. 4 weeks) due to low faecal volumes. Across the four years a total of 3208 faecal samples were collected from 563 individual sheep.

Faecal Parasitology

Faecal egg counts (FEC) were conducted on the anaerobically stored subsample using a modified salt-flotation technique with a sensitivity of 1 egg per gram (epg), first described by Jackson (1974) and detailed within this study system by Hayward et al. (2019) and Sweeny et al. (2022). For this study, FEC were morphologically identified as either *Nematodirus* spp. (principally *N. battus*) or strongyle-type eggs (which include *Bunostomum trigonocephalum, Chabertia ovina, Teladorsagia circumcincta, Trichostrongylus axei* and *Trichostrongylus vitrinus*). Other nematode eggs were not enumerated due to their extremely low prevalence (*Trichuris ovis* and *Capillaria longipes*) or their low clinical significance (*Strongyloides papillosus*) (Gulland and Fox 1992; Craig 2005; Chambers 2020).

After arrival at the laboratory, the coproculture samples were cultured to allow nematode eggs to develop to the third larval stage (L_3) by storing at 25°C for a further 10 days in the

plastic pots with loose-fitted lids (to allow gas transfer, whilst maintaining humidity). After culturing, samples were flooded with tepid water and left overnight to encourage L_3 to migrate out of the faecal mass, before isolating the larvae by overnight by Baermannisation through filter paper (MAFF 1986). L_3 were concentrated by centrifugation at 200×*g* for 5 minutes, followed by incubation on the bench at room temperature for 2 hours to allow any larvae still floating to settle. The water volume for each sample was then reduced to 1ml by vacuum line.

Numbers of isolated L_3 were estimated by counting all ensheathed larvae within three 10µl aliquots under light microscopy at 100x magnification. Any free-living larvae (i.e., those without a sheath) were not counted. If no L_3 were seen, a further 3 aliquots were investigated. Where no larvae were counted in all six 10µl aliquots, the sample was discarded as the larval yield was assumed to be too low for useful sequence data. Where larval counting showed there to be more than 1000 L_3 per ml, an aliquot equivalent of 1000 L_3 was taken for DNA extraction was prepared by dilution and taken forward for DNA extraction. For the samples with <1000 L_3, the entire sample was taken forward for DNA extraction. 1986 of the 3208 faecal samples yielded sufficient L_3 to proceed to DNA extraction.

DNA extraction, PCR and Sequencing

Genomic DNA was extracted, the ITS-2 region amplified and barcodes added using previously described techniques (Avramenko et al. 2015; Redman et al. 2019), briefly explained below.

The L_3 sheaths were first degraded by incubating at 95°C for 15 minutes whilst shaking at 1000rpm and then freezing at -80°C for a minimum of 1 hour. Following exsheathment, genomic DNA (gDNA) was extracted from the L_3 by incubation in a buffered proteinase K solution (120µg/ml) at 60°C for 120 minutes whilst shaking at 700rpm, followed by incubation

at 95°C for 20 minutes to inactivate the proteinase K. Extracted DNA was then allocated to one of ten libraries, each composed of four 96-well plates. Samples within a single season were divided across two of these libraries, to account for any potential technical variation between libraries.

The nematode ITS-2 region was amplified using equal proportions of four forward and four reverse universal adapter primers (Avramenko et al. 2015). Each 25µl PCR reaction contained the following components: 0.75µl 10µM forward adapter primers, 0.75µl 10µM reverse adapter primers, 0.75µl 10mM dNTPs, 0.5µl KAPA HIFI polymerase enzyme (KAPA Biosystems, USA), 5µl 5×buffer (KAPA Biosystems, USA), 0.4µl gDNA (diluted 1:10 in nuclease-free water), and 13.25µl nuclease-free water. Thermocycling conditions were 95°C for 2 min, followed by 35 cycles of 98°C for 20s, 60°C for 15s, 72°C for 15s. PCR products were then purified with AMPure XP Magnetic Beads according to the manufacturer's instructions (Beckman Coulter, USA) and eluted in a final volume of 32µl.

A second round of PCR was performed using combinations of 16 forward and 24 reverse barcoded primers (Illumina, USA), obtaining a unique combination of barcoded primers for each sample within a library. Each PCR reaction contained 2µl of the first round PCR purified product as a template, 0.5µl KAPA HiFi polymerase (KAPA Biosystems, USA), 0.75µl dNTPs (10 mM), 5µl 5×KAPA HiFi Fidelity buffer (KAPA Biosystems, USA), 1.25µl of each corresponding reverse and forward combination primer (10µM), and 13.25µl of nuclease-free water. PCR conditions were 98 °C for 45s, followed by seven cycles of 98°C for 20s, 63°C for 20s, and 72°C for 2 min. PCR products were again purified with AMPure XP Magnetic Beads according to the manufacturer's instructions (Beckman Coulter, USA) and eluted in a final volume of 32µl.

The DNA concentrations of the second round PCR products were then quantified using Quantifluor dsDNA kits according to the manufacturer's instructions (Promega, USA). Where samples yielded a second-round product with less than 20ng/µl dsDNA, the PCR was repeated, using a greater quantity of extracted DNA (iteratively from 1µl up to 10µl). Second round products were then pooled for each library, such that the pool contained 200ng dsDNA from each sample. Any samples that yielded less than 10ng/µl after repeating were discarded. Libraries were then sent to Edinburgh Genomics, Edinburgh, where they were run on an Illumina MiSeq sequencer to yield at least 11M reads. During the post-run processing, all sequences were split by sample identity using the barcoded indices to produce FASTQ files with primer sequences removed.

Bioinformatics and Data processing

Raw sequences were processed in RStudio v2024.09.1 using R v4.3.1 (R Core Team 2023) using a 'dada2' bioinformatic workflow (Callahan et al. 2016) adapted from the workflow provided at www.Nemabiome.ca and briefly outlined here. Sequences were first filtered for quality with maximum expected errors set at 2 for the forward sequence and 5 for the reverse sequences, and truncation after a quality score of 2 or lower. Sequences were then denoised, paired ends were merged and chimeras removed. Taxonomies were then assigned using an RDP Naive Bayesian Classifier algorithm with kmer size of 8 and 100 bootstrap replicates (Q. Wang et al. 2007) using the curated Fasta reference file provided at (www.Nemabiome.ca downloaded 22 November 2024). Reads assigned to spurious taxa were then removed (taxa were retained if they had more than 2000 reads in more than 1% of samples) and individual samples with fewer than 5000 total reads were then also removed. Of the 1986 samples that underwent PCR and sequencing, 20 did not yield sufficient quality DNA to pass these

thresholds and were therefore rejected, leaving 1966 samples with high quality ITS-2 read counts.

These read counts were then adjusted using established correction factors to correct for L_3 size and PCR biases (Redman et al. 2019; Chambers 2020), before being converted into a proportion of the total reads in each sample. These species proportions were then multiplied by the strongyle-type faecal egg count for that sample and rounded to the nearest integer to give species-corrected egg counts for the five strongyle-type species. These data were then combined with the morphologically identified *Nematodirus spp*. egg counts, to give six species-corrected FECs (sFECs) for each sample.

192 of the initial 3208 samples had a strongyle-type FEC of <1epg. These were assigned 0epg for all five strongyle-type species, giving a total dataset of 2158 samples (1966 plus 192). Sub-sampling of this dataset, revealed that the drop-out of samples during processing and the inclusion of the zero FECs did not introduce species-level bias into the dataset (Figure 15, supplementary materials).

Alpha- and Beta-Diversity

Alpha-diversity was assessed using the Shannon Index and beta-diversity using the Bray-Curtis Dissimilarity Index, both calculated using the 'vegan' package (Oksanen et al. 2022). Samples with zero FEC were excluded from both alpha- and beta-diversity analyses (diversity metrics for a zero-count community are either zero or infinity and somewhat meaningless either way).

The first grazing season in lambs

The data were restricted to only lambs in July and November of their first year of life. For sFECs, six generalised mixed models (GLMMs) were constructed using the 'glmmTMB'

package (Brooks et al. 2017), one for each GIN species. For each of these models, sFEC was the response variable, and Season was included as the fixed-effect of interest. Year, Sex and Sibling Status were also included as fixed-effects and individual ID was included as a random effect, to control for variation associated with them. Poisson models (with observation-level random effects), linearly parametrised negative binomial models ($V=\mu(1+\phi)$) and quadratically parameterised negative binomial models ($V=\mu(1+\mu/\phi)$) were all considered, both with and without zero-inflation. On the basis of visual assessment of plots of simulated residuals in the package 'DHARMa' (Hartig 2022), quadratically parameterised negative binomial models (nbinom2: $V=\mu(1+\mu/\phi)$) were chosen for all analyses. Alpha-diversity was analysed using a GLMM with identical fixed-and random effects, but with rank-transformed Shannon Index as the response variable.

Fixed effect significance was assessed by Type-II Wald χ^2 tests. Predicted effects were visualized by plotting estimated average marginal means. Pairwise comparisons were then made by Benjamini-Hochberg (BH)-corrected *post hoc* tests for differences in marginal means.

Beta-diversity was analysed using a PERMANOVA (1000 permutations) of the Bray-Curtis Dissimilarity Index, using the same fixed effects but without the random effect (Oksanen et al. 2022). Differences in beta-diversity were visualised using the first two axes of a principal components analysis of the six sFECs, log-transformed prior to centring and scaling.

Effects of age

The data were restricted to include all ages, but only samples collected in July and November. This allowed direct comparison with patterns in lambs, where samples from March (before they were born) or in May (faecal samples too small) were not available. Six GLMMs were constructed for each species sFEC, this time with Age Class as the fixed-effect of interest,

categorised as: Lambs (born that year), Yearlings (born the previous year), Adults (aged 2-6 years), and Geriatrics (aged 7 years or over). Season and year were included as fixed effects, and ID was again included as a random effect. Negative binomial GLMMS were again preferred after inspection of simulated residuals. Model terms were similarly assessed by Type-II Wald χ^2 tests and *post hoc* BH tests. The same approach was then followed for alpha- and beta-diversity as for the analysis of lambs in the first grazing season diversity (i.e. one GLMM for alpha-diversity; and one PERMANOVA and PCA plots for beta-diversity).

Effects of sex and reproduction

The data were restricted to include all seasons, but excluding samples collected from lambs in July and November. Six GLMMs were constructed for each species sFEC, with Age Class, Season, Year and Sex/Reproductive Class as fixed effects, and ID as a random effect. An interaction between Season and Sex/Reproductive Class was added to test whether seasonal effects depended on sex or reproductive status. Sex/Reproductive Class was categorised as: Male; Reproductive Female, or Non-Reproductive Female. Females were assigned as reproductive if they gave birth in the April of the calendar year of sampling. Females sampled in March that then died between March and April were assigned as reproductive if foetuses were present on post-mortem examination. Negative binomial GLMMs were again preferred, fixed effect significance was assessed by Type-II Wald χ^2 tests and *post hoc* BH tests. The same approach was followed for alpha- and beta-diversity as for previous analyses (i.e. one GLMM for alpha-diversity; and one PERMANOVA and PCA plots for beta-diversity).

Results

The first grazing season in lambs

The nemabiome of lambs in July (aged c.3 months) was predominantly composed of *Nematodirus spp.*, *Te. circumcincta*, and *Tr. vitrinus* (Figure 12A and B). By November, levels of *Nematodirus spp.* had declined to almost zero; levels of *Te. circumcincta* sFECs also exhibited a significant, but much smaller, decrease (Figure 12A). During the same interval, sFECs for *Tr. axei*, *C. ovina*, and *B. trigonocephalum* all increased significantly, whilst *Tr. vitrinus* sFECs were unchanged (Figure 12A). This seasonal progression resulted in a significant difference in beta-diversity (Figure 12B; Table 4, supplementary) and a significant increase in alpha-diversity from July to November (Figure 12C).

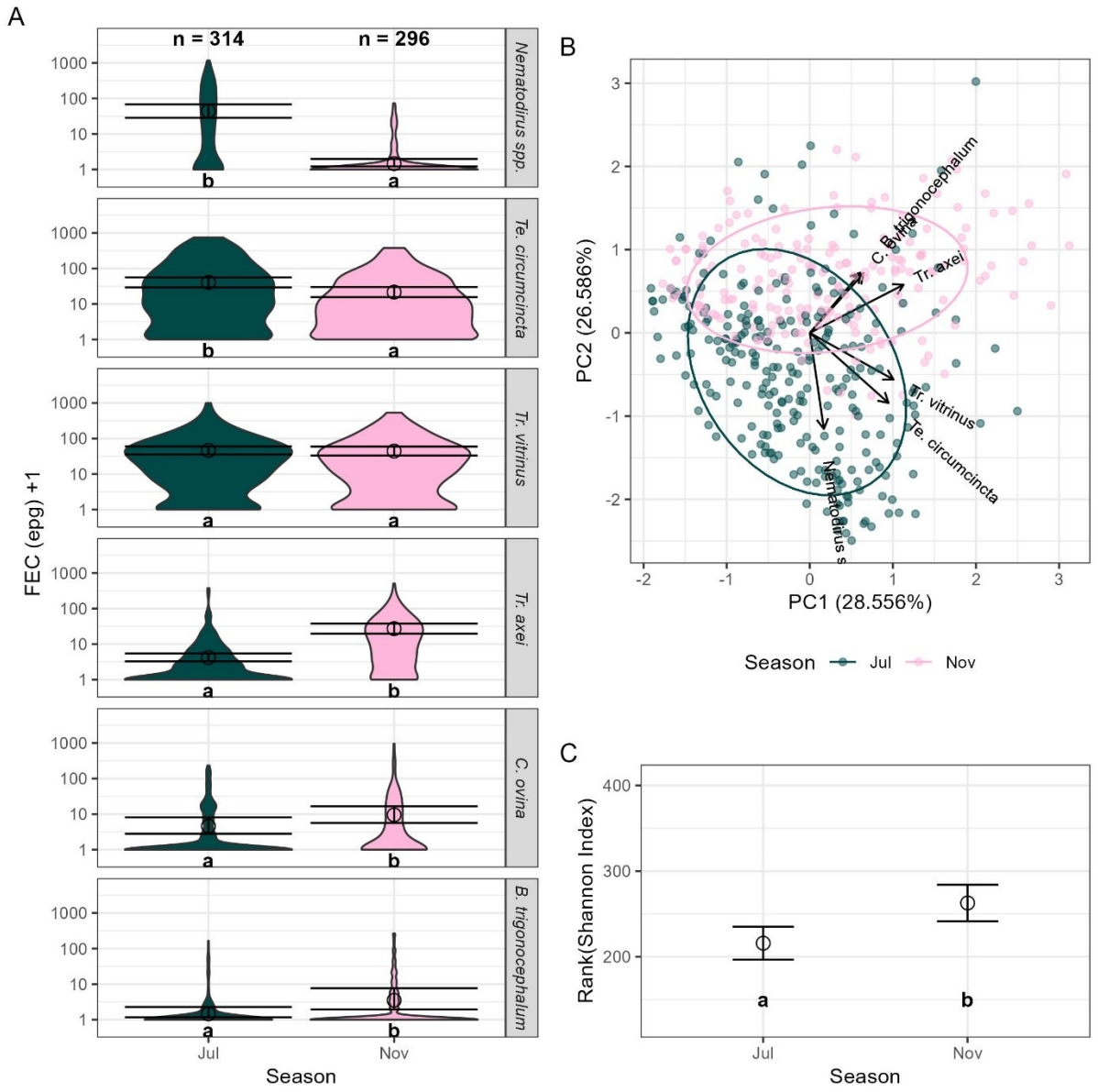


Figure 12 A: sFEC results for lambs in July and November. Points and bars show estimated marginal means with 95% confidence intervals from each model. Significantly different marginal means between sampling points for each species (according to BH-corrected post hoc testing) are indicated by non-shared letters printed below. Violin plots show the raw data distributions. B: First two axes of the principal components analysis for sFEC (log transformed prior to centring and scaling), coloured according to season. Ellipses represent 95% confidence intervals. Arrows indicate loadings towards each of the six species. C: Estimated marginal mean Shannon index ranks (with 95% confidence intervals) obtained from the non-parametric model of alpha-diversity in lambs.

Effects of age

As lambs matured through their first two years of life, there was a progressive decline in sFECs for *Nematodirus spp.*, *Te. circumcincta*, and *Tr. vitrinus* (Figure 13A). This resulted in a significant effect of age class on beta-diversity (Figure 13B; Table 4, supplementary) and a progressive decrease in alpha-diversity (Figure 13C). *Tr. axei and C. ovina* sFECs were also lowest in adult and geriatric animals, although there was no significant difference in sFEC between lambs and yearlings (Fig. 3A). The age-related changes in *B. trigonocephalum* sFECs were markedly different however, with the estimated marginal mean sFEC increasing from lambs to yearlings, before decreasing to an intermediate level in adults and geriatrics (Figure 13A). There were no significant differences in alpha- or beta-diversity between adults and geriatrics (Figure 13B&C; Table 4, supplementary) and *B. trigonocephalum* was the only species for which there was a significant difference in sFECs between adults and geriatrics (Figure 13A).

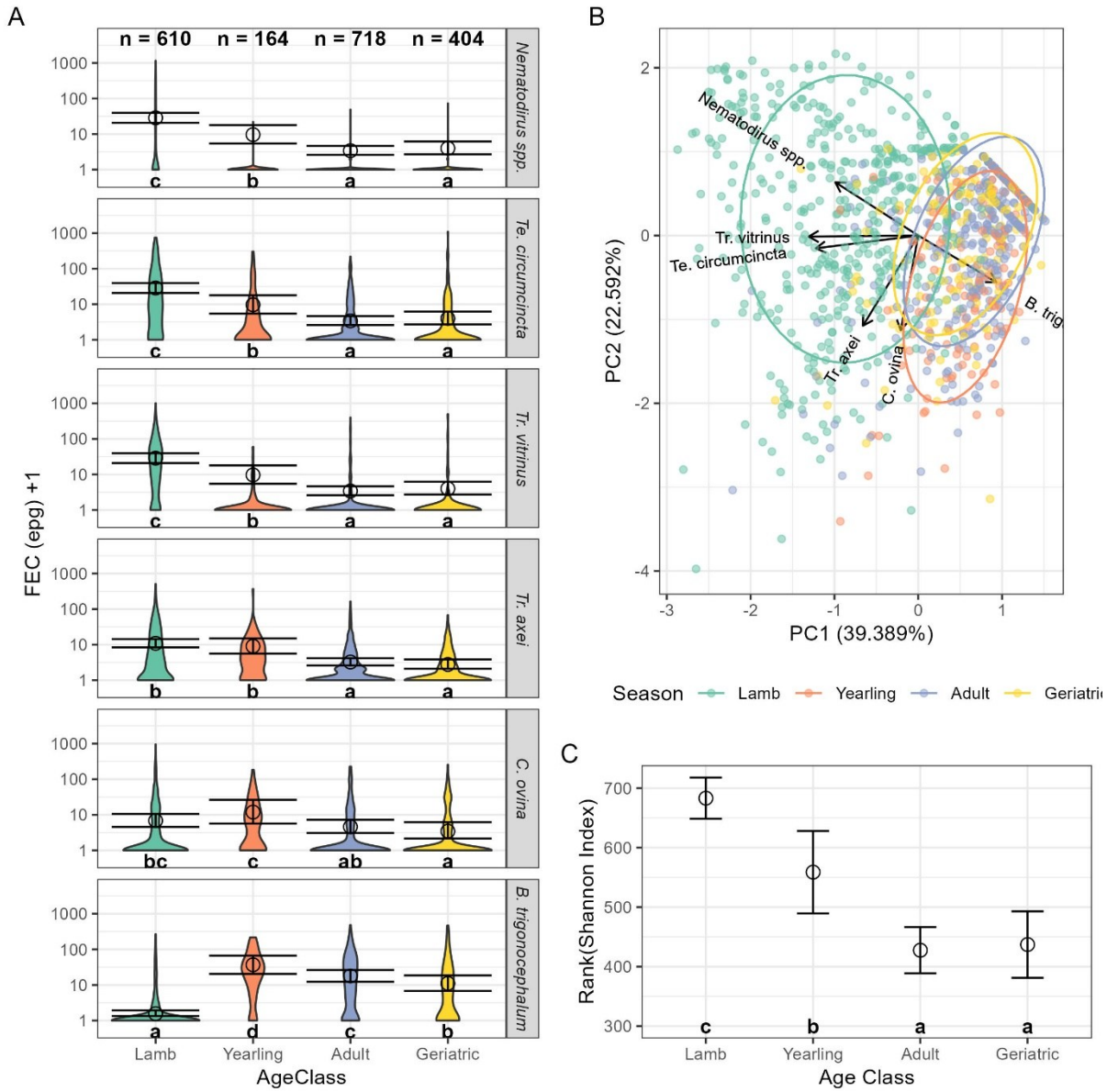


Figure 13 A: sFEC results for all age classes in July and November. Points and bars show estimated marginal means with 95% confidence intervals from each model. Significantly different marginal means (according to BH-corrected post hoc testing) are indicated by non-shared letters printed below. Violin plots show the raw data distributions. B: First two axes of the principal components analysis for sFEC (log transformed prior to centring and scaling), coloured according to age class. Ellipses represent 95% confidence intervals. Arrows indicate loadings towards each of the six species. C: Estimated marginal mean Shannon index ranks (with 95% confidence intervals) obtained from the non-parametric model of alpha-diversity for all age classes in July and November.

Effects of sex and reproduction

There was a significant interaction between season and sex/reproductive status on sFEC for all six GIN species (Table 3, supplementary), as well as alpha-diversity (Figure 14; Table 3, supplementary) and beta-diversity (Figure 16B, supplementary; Table 4, supplementary). Alpha-diversity was comparable between all sex/reproductive classes in March. In non-reproductive females it then decreased progressively until November, whilst it peaked in May among reproductive females, and among males it was higher in November than May or July (Figure 14B).

Reproductive females showed broadly the same patterns for all species sFECs (Figure 14A), being generally high early in the year before declining. For all species, the peak sFEC among reproductive females was in May, with the highest marginal mean sFECs for *Te. circumcincta*, *Tr. vitrinus* and *B. trigonocephalum*. This spring increase began earlier in the year for *Te. circumcincta* and *Tr. axei* than for the other species and whilst the fall from the peak in May to July was large for *Nematodirus* spp., *Te. circumcincta* and *Tr. vitrinus,* it was less extreme for *Tr. axei*, *C. ovina* and *B. trigonocephalum*.

In contrast, non-reproductive females generally showed a decline through the year for most species (Figure 14A). This was not the case for *Nematodirus* spp. where marginal mean sFECs were close to zero year-round, nor for *B. trigonocephalum* which showed no discernible seasonal pattern.

Males showed three different trends in sFEC divided between the different GIN species. For *Nematodirus* spp., *Tr. vitrinus* and *Tr. axei* there steady seasonal declines from March onwards. *Te. circumcincta* and *C. ovina* showed a U-shaped pattern, with peaks in March and November, but lower marginal mean sFECs in May and July. *B. trigonocephalum* again showed

a unique seasonal pattern being lowest among males in March and high at all other time points

Nematodirus sFECs were very low in adults across most seasons, with the significant interaction between season and reproductive status appearing to be driven by a mild increase in pregnant females in May and very minor increases across all reproductive classes in March (Fig. 4A).

The raw data distributions showed that the sFECs were highly over-dispersed with strong right-skews visible even on a logarithmic scale for all species except *B. trigonocephalum*, which showed left-skews on the logarithmic scale particularly among males (Figure 16, supplementary).

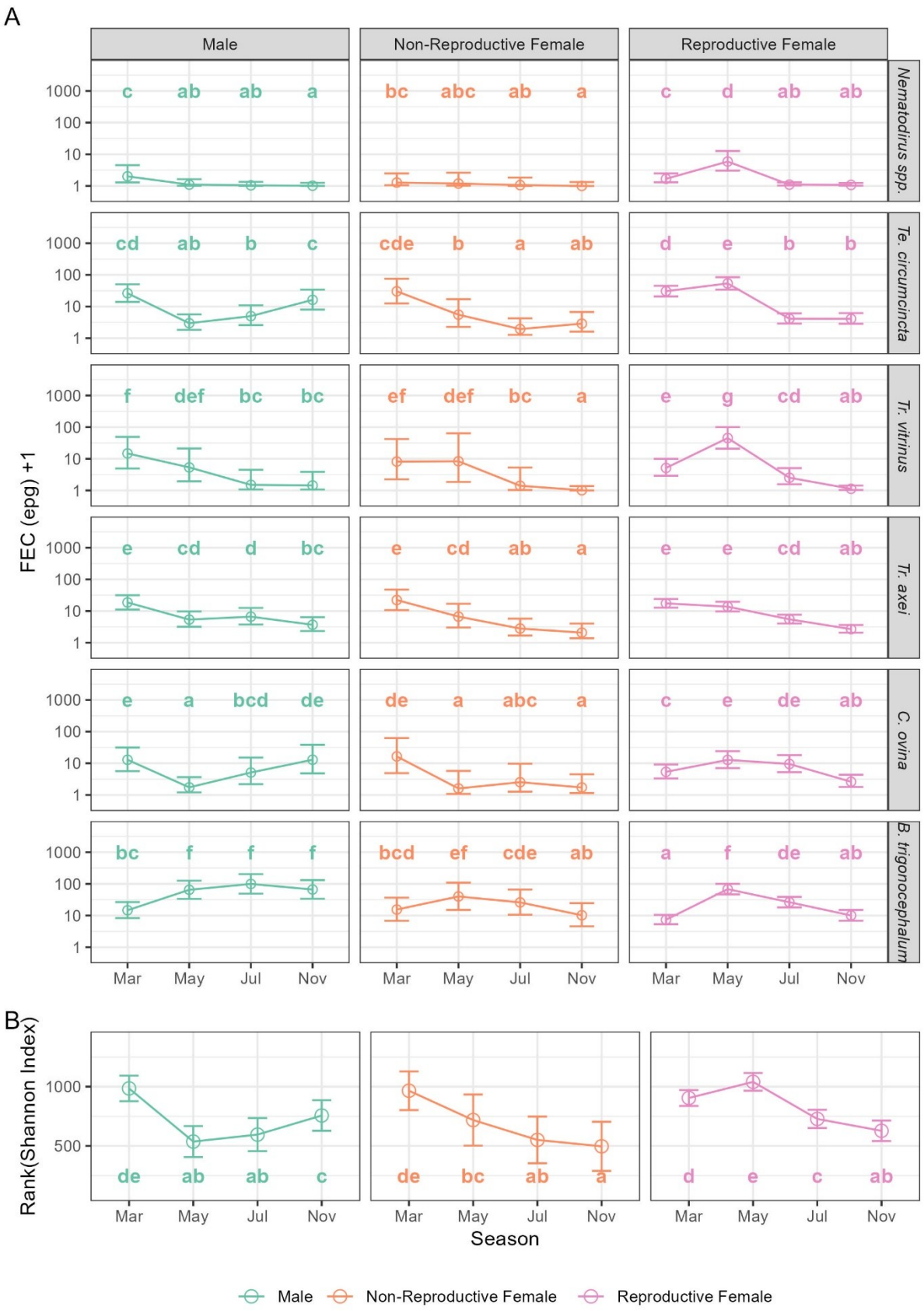

A
Male
Non-Reproductive Female
Reproductive Female
Nematodirus spp.
Te. circumcincta
Tr. vitrinus
Tr. axei
C. ovina
B. trigonocephalum
FEC (epg) +1
1000
100
10
1
c ab ab a
bc abc ab a
c d ab ab
cd ab b c
cde b a ab
d e b b
f def bc bc
ef def bc a
e g cd ab
e cd d bc
e cd ab a
e e cd ab
e a bcd de
de a abc a
c e de ab
bc f f f
bcd ef cde ab
a f de ab
Mar May Jul Nov
B
Rank(Shannon Index)
1000
500
de ab ab c
de bc ab a
d e c ab
Season
Male
Non-Reproductive Female
Reproductive Female

Figure 14: The interaction between season and reproductive status for all animals except lambs for sFEC models (A) and for alpha-diversity (B). Points and bars show estimated marginal means with 95% confidence intervals. Significantly different marginal means between sampling points for each species (according to BH-corrected post hoc testing) are indicated by non-shared letters. Estimated marginal means have been linked by lines to aid interpretation. The associated violin plots (including the number of individuals in each group) and PCA plots are in Figure 16 (supplementary).

Discussion

These results provide strong evidence that different GIN species respond differently to the age, sex and reproductive status of their hosts, and to season. The seasonal dynamics for *Te. circumcincta* and *Nematodirus* spp. were remarkably similar in this unmanaged system to descriptions in domestic systems, but the dynamics of *Tr. vitrinus* were different from domestic sheep. Ageing from lamb through yearling to adult was generally associated with a decline in alpha-diversity and a shift towards *B. trigonocephalum*, suggesting immunity against that species may be less effective. The complexity and variability in GIN community dynamics would not be observable without employing species-specific methods, emphasising the power of nemabiome approaches to aid understanding of the distinct epidemiologies of multispecies GIN coinfections.

As stated above, the seasonal epidemiological patterns of *Te. circumcincta* and *Nematodirus* spp. in unmanaged Soay lambs during their first grazing season were in line with expectations derived from studies in domestic sheep (Boag and Thomas 1977; H. D. Crofton 1957; Evans et al. 2021; Brunsdon 1970; D. J. Wilson et al. 2008; Reid and Armour 1975). The large decline in *Nematodirus* spp. sFECs in lambs between July and November, is consistent with the expected seasonal mass hatching of *Nematodirus* spp. eggs in spring, followed by robust immune

clearance at a similar time to the rapid decline in L_3 challenge from pasture (D. M. Taylor and Thomas 1986; van Dijk and Morgan 2008; Gibson and Everett 1963). In contrast *Te. circumcincta* counts in lambs declined more gradually, with a less dramatic change in the shape of the data distribution. This is most likely a result of the more gradual development of anti-*Te. circumcincta* immunity, alongside a more sustained intake of *Te. circumcincta* L_3 from pasture (McRae et al. 2015; O'Connor, Walkden-Brown, and Kahn 2006).

The seasonal epidemiology of these two species in adults was also consistent with patterns described in domestic sheep. Seasonal mass hatching was likely responsible for the increase in *Nematodirus* spp. sFECs adults of all sex/reproductive classes in March. The rise in *Te. circumcincta* across all sex/reproductive classes at the same time was presumably caused by the emergence of overwintered L_4 in the abomasum. The emergence of hypobiotic/inhibited L_4 is at least partly due to a loss of immunity (W. D. Smith 2007), and it is not surprising that this coincides with the nutritional/conditional nadir in this population (Craig, Pilkington, and Pemberton 2006; Gulland 1992). Estimated marginal mean *Te. circumcincta* sFECs then dropped in May for non-lactating females and males but was maintained in lactating females, presumably due to the PPRI.

In addition, it is interesting to note that a small proportion of lambs still had positive *Nematodirus* counts in November. Autumn peaks in *Nematodirus battus* associated with the hatching of non-chilled eggs have been observed in domestic sheep and this may be responsible for the positive counts among some lambs in November (van Dijk and Morgan 2008, 2010; Kenyon et al. 2009; Sargison, Wilson, and Scott 2012). Alternatively, these counts may be the result of a failure to develop immunity among a small proportion of lambs – a phenomenon previously observed in domestic sheep (D. M. Taylor and Thomas 1986) that

appears to be genetically- rather than nutritionally-mediated (Israf, Coop, Stevenson, et al. 1996; Israf, Coop, Jackson, et al. 1996). Interestingly, the raw data distributions suggest that the increase in estimated marginal mean *Nematodirus* spp. sFECs in reproductive ewes in May was driven by a very small proportion of individuals. This suggests that there is inter-individual variation in both *Nematodirus* clearance in lambs over summer and susceptibility in lactating ewes in May, the precise causes of which bear further investigation. It is additionally worth noting that this variation would likely have been missed with smaller sample sizes or analysis of pooled faecal samples.

Whilst the seasonal epidemiologies of *Te. circumcincta* and *Nematodirus* spp. conformed to our prior expectations, the seasonal epidemiology of *Tr. vitrinus* did not. Previous work in domestic sheep suggests that abundances for *Tr. vitrinus* are low in young lambs, with FECs rising from September onwards (Boag and Thomas 1977; H. D. Crofton 1957; D. J. Wilson et al. 2008; Evans et al. 2021; Geddes et al. 2024), whereas our results show comparable abundances in lambs in July and November. Similarly, work in domestic sheep suggests the immunity of ewes against *Tr. vitrinus* is maintained during the periparturient period (Jackson, Jackson, and Williams 1988), whereas our results show a clear periparturient rise in ewes.

The higher than anticipated *Tr. vitrinus* abundances in lambs in July could theoretically have arisen due to differences in exposure or susceptibility. Increased exposure of lambs on St Kilda to infectious *Tr. vitrinus* larvae on pasture could arise from any climatic or microclimatic differences that favour increased overwinter survival of *Tr. vitrinus* larvae on pasture (O'Connor, Walkden-Brown, and Kahn 2006). Alternatively, increased exposure could have arisen from spatial overlap between lambs/yearlings in winter and young lambs in spring. (This spatial overlap is uncommon in domestic systems as older lambs are slaughtered or

moved away from lambing pastures.) However, these hypotheses seem unlikely, given the same pattern was not seen for *Tr. axei*, despite similar larval ecologies for these two closely-related species (Callinan 1979, 1978b), and the sustained presence of *Tr. axei* in yearlings in this population.

The remaining hypothesis for earlier *Tr. vitrinus* exposure in this system is that the pasture is more heavily contaminated with *Tr. vitrinus* early in the year by adult sheep. The *Tr. vitrinus* counts in adults in July and November were generally low, but there was then a significant rise in the marginal means for *Tr. vitrinus* FECs in reproductive ewes in May, driven by a relatively small number of individuals with very high counts (seen in raw data distributions in Figure 16, supplementary). This could suggest that the factors driving the rise are less evenly distributed among sheep within that reproductive class. A similar rise in marginal means accompanied by more skewed raw data was also seen in adult males and non-pregnant ewes in March. As previously discussed, body condition and protein nutrition at this point in the late winter would be an obvious candidate factor that could be driving this variation in *Tr. vitrinus* counts in adults. Further work expanding mechanistic models of nematode epidemiology (Rose Vineer, Verschave, et al. 2020) to include *Tr. vitrinus* could be a valuable way to establish the relative epidemiological contributions of different age and reproductive classes.

At the same time, differences in susceptibility could account for the higher-than-expected levels of *Tr. vitrinus* in young lambs. Susceptibility may also be impacted by interactions between co-infecting GIN species, with a meta-analysis of pairwise experimental co-infections (Evans et al. 2023) and a community perturbation experiment (Craig et al. 2009) both suggesting *Tr. axei* and *Tr. vitrinus* are particularly susceptible to antagonistic effects of pre-

established co-infections. It is easy to envisage how these effects could be compounded with variation in exposure and it may be the case that in systems where lambs are initially exposed to mostly *Te. circumcincta*, *Tr. vitrinus* is excluded until *Te. circumcincta* levels wane (as hypothesised by Jackson et al. (1992)). In contrast, in systems where initial exposure to *Tr. vitrinus* is higher, both *Te. circumcincta* and *Tr. vitrinus* may simultaneously establish in young lambs. Further work investigating the impact that these co-infection dynamics have upon the health and fitness of the lambs would therefore be of great relevance both to this wild population and to domestic sheep.

The results of this study have also shown that alpha-diversity decreases progressively from lambs, to yearlings, to adults. This is at odds with studies in domestic sheep (Evans et al. 2021; Jouffroy et al. 2025) and consistent with studies in other wild mammals (Beaumelle et al. 2021; Ahn et al. 2024). This emphasises the impact that regular anthelmintic treatment of youngstock has on GIN epidemiology and the value of performing fundamental research in unmanaged populations. This decline in alpha-diversity is associated with a shift in beta-diversity away from *Nematodirus spp., Te. circumcincta* and *Tr. vitrinus* after the first year of life, to *Tr axei and C. ovina* in the second year, before being dominated by *B. trigonocephalum* in adults.

The high *B. trigonocephalum* sFECs among adults may have been mediated to some extent by high fecundity in this species, but that cannot be the only explanatory factor given its relative fecundity is comparable to *C. ovis* and only 1.5 times that of *Te. circumcincta* (H. Crofton 1963). The raw data distributions for *B. trigonocephalum* were also remarkably different from the other species, showing a more left-ward skew in adults, especially males, that may suggest less extreme inter-individual variation in this species. The high egg *B. trigonocephalum* sFECs

in adults may alternatively suggest that immunity against this species is comparatively limited, and there is limited experimental evidence that the development of immunity against *B. trigonocephalum* may be dependent on plane of nutrition (Neumayer and Lucker 1947).

Despite these clear differences in sFECs between age classes however, the age effect accounted for only 13.2% of the variation in beta-diversity with 79.5% of the variation unexplained in that PERMANOVA analysis (Table 4, supplementary). There are therefore unidentified factors that drive inter-individual variation in GIN community composition that warrant further investigation. Clearly the interaction between season and sex/reproductive status is one such driver, with clear periparturient effects in females for all species bar *B. trigonocephalum*. There is a lack of evidence regarding seasonal patterns in domestic rams but the additional rise in *Te. circumcincta* FECs may potentially be associated with the metabolic demands of the rut. This supports the theory that in addition to periparturient effects, relaxations in immunity against parasites may be driven by resource partitioning at other times of physiological stress (Coop and Kyriazakis 1999).

As mentioned above, the seasonal effects between reproductive classes were markedly different for *B. trigonocephlaum* compared to the other species, with decreased FECs in females in March that were most pronounced in reproductive females, and high egg counts and prevalences in males in May, July and November. These higher counts in male animals are consistent with increased levels of *B. trigonocephalum* observed in post mortem examinations of male Soay sheep (Craig 2005). *B. trigonocephalum* is relatively understudied in domestic sheep as it is now uncommonly seen in commercial farming systems in temperate regions, although it remains a clinical problem in a global context (Khare et al. 2018; Naem and Gorgani 2011; Aragaw and Gebreegziabher 2014; Atayev et al. 2022; Ma et al. 2014). It is

therefore challenging to derive specific hypotheses for the unusual patterns seen in *B. trigonocephalum* but they could represent: the influence of the rut in males with a time-lag due to the long prepatent period, differences in mechanisms of host immunity given its tissue-migration phase and blood-feeding, or temporospatial differences in exposure given its transcutaneous route of infection (Kamenov and Kanchev 2022; Westen 1967; Bhatt, Srivastava, and Subramanian 1969). The life history of this parasite is also very similar to the human hookworms *Necator americanus* and *Ancylostoma duodenale* (Hoagland and Schad 1978). These human hookworms are noted for their immunosuppressive and microbiome-modulatory activities (Abuzeid et al. 2020; Jenkins et al. 2021; Geiger et al. 2007), therefore further work on immunity against this *B. trigonocephalum* in the context of the wider GIN community and ovine microbiome could provide one-health insights.

Again, the effect of season and sex/reproductive status and the interaction between them together explained only 14.0% of the variation in adult beta-diversity (Table 4, supplementary), emphasising the need for further investigation of the drivers of inter-individual variation. Possible avenues for further research of this residual variation could include multivariate modelling of these GIN data with measures of apicomplexan burden (A. R. Sweeny et al. 2022), combining this dataset with estimates for host spatial density (Albery et al. 2024), incorporating measures of anti-parasite immunity (Corripio-Miyar et al. 2022) or comparing against genomic predictors of anti-parasite resistance (Sparks et al. 2019).

Conclusions

- GIN species respond differently to age, sex and reproductive status of their hosts, and to season.

- The seasonal dynamics for *Te. circumcincta* and *Nematodirus* spp. in this unmanaged population are comparable to descriptions in domestic systems, suggesting the drivers are not confined to agricultural systems
- The dynamics of *Tr. vitrinus* were different from domestic sheep with higher-than-expected abundances in lambs in July, possibly driven by an unexpected periparturient rise that may be a consequence of over-winter nutrition.
- Unlike in domestic sheep but in common with other wild mammals, ageing from lamb through yearling to adult was generally associated with a decline in alpha diversity, likely due to the absence of anthelmintic treatment in lambs
- Adult nemabiomes were shifted towards *B. trigonocephalum*, suggesting immunity against that species may be less complete, or that it is a more effective immunosuppressor.
- The complexity and variability in GIN community dynamics would not be observable without employing species-specific methods, emphasising the power of nemabiome approaches to aid understanding of the distinct epidemiologies of multispecies GIN coinfections.

Supplementary Materials

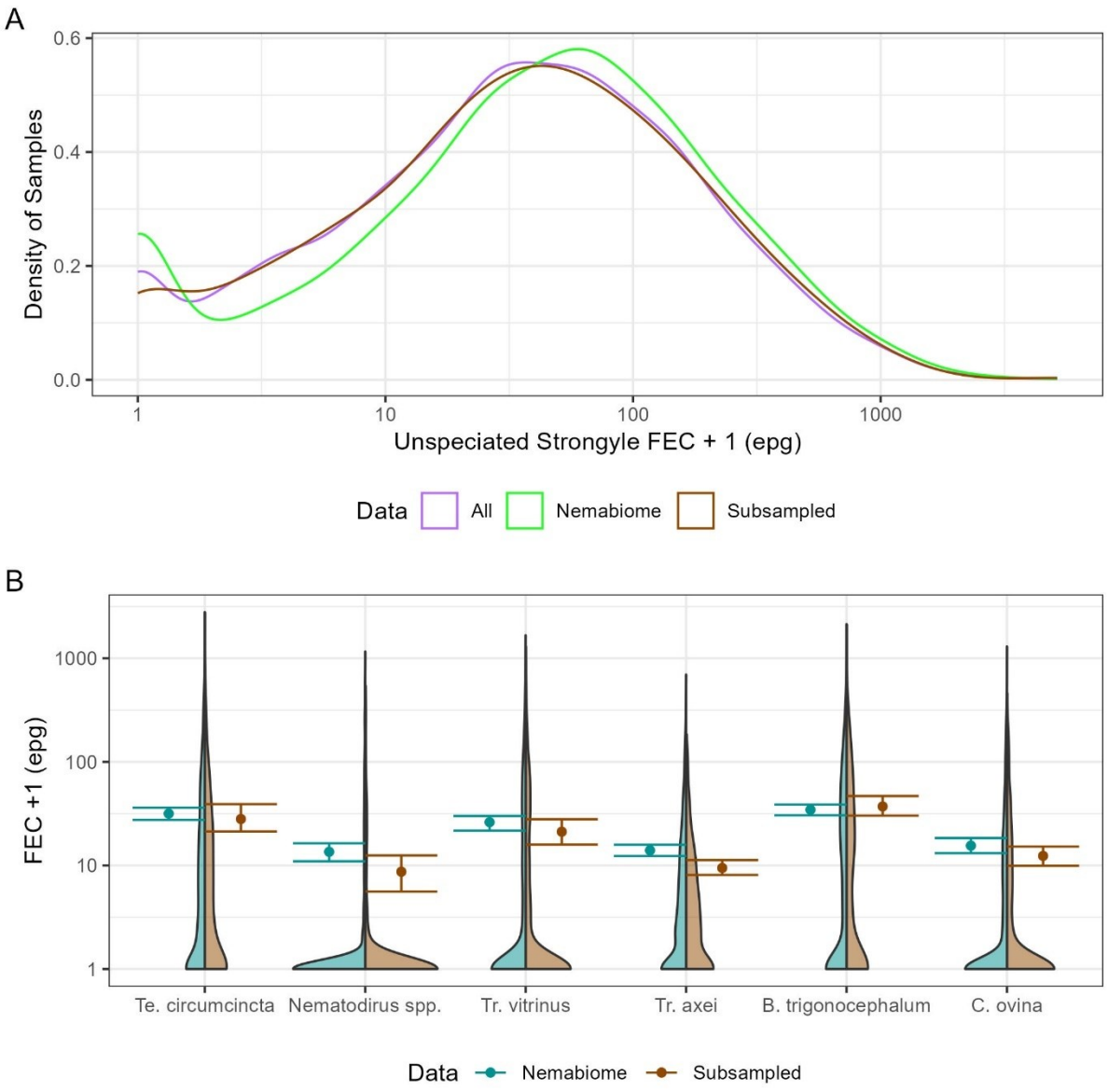


Figure 15 Assessing for species-level bias introduced by the higher rate of coproculture failure of faecal samples with low FEC. The Nemabiome data were divided into bins by total FEC (10 bins), sample timepoint and age class. A randomly subsampled dataset was then produced, subsampling proportionally more samples from bins that were under-represented in the Nemabiome dataset. A: Density plot showing the difference in the distribution of samples with Nemabiome data (green) versus all samples (purple). The subsampling procedure accurately reproduced the distribution of FECs seen in all samples (brown). B: The means (with 95% confidence

intervals generated by 500 non-parametric bootstraps) and distributions of sFECs from the Nemabiome dataset and the subsampled dataset. The means and distributions are comparable between both datasets, indicating that the loss of samples due to low coproculture yield did not introduce species-level bias.

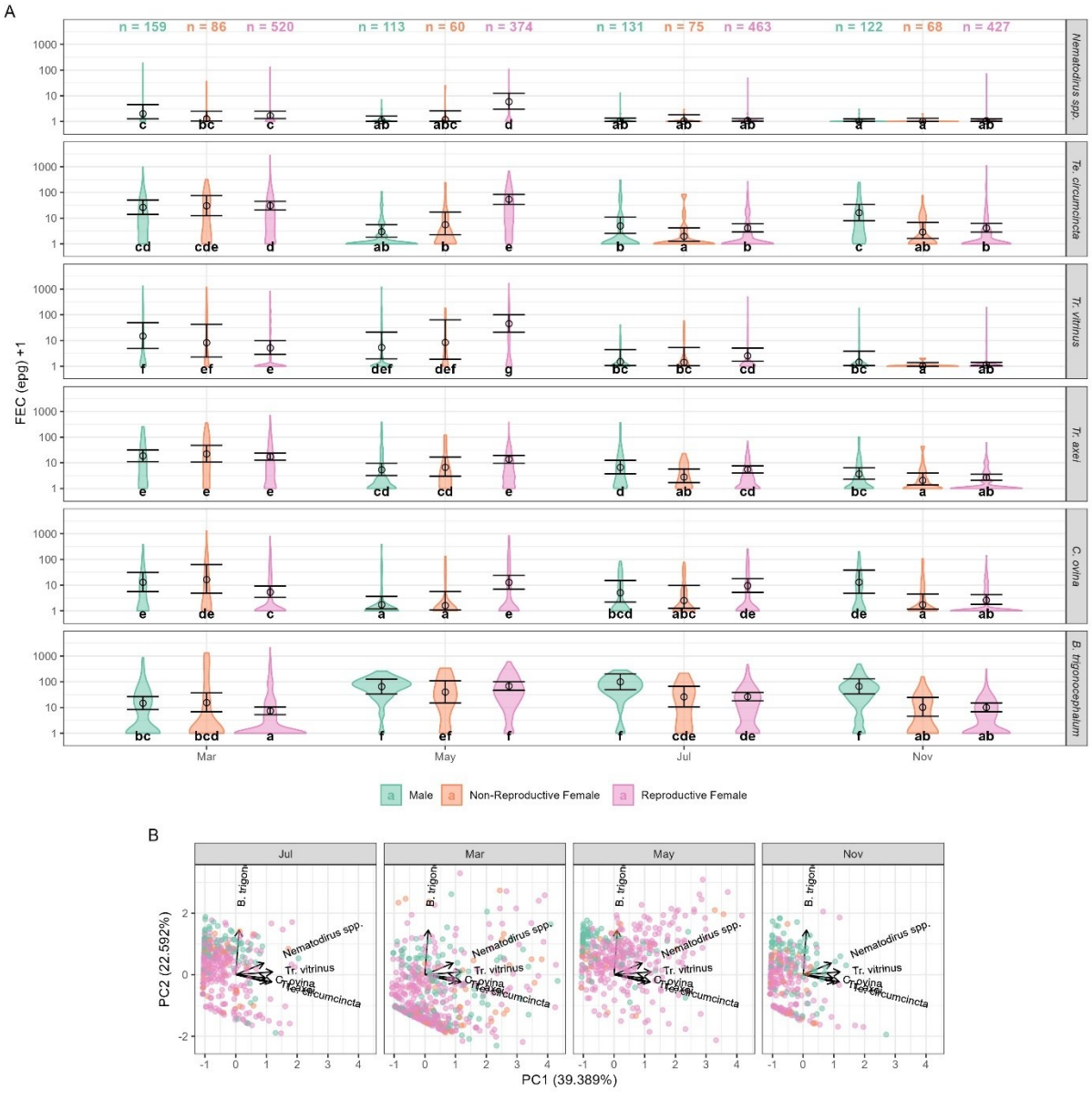


Figure 16: The violin plots (A) and ordination plots (B) not shown in Figure 14. A: sFEC results for all age classes except lambs, grouped both by season and by sex and reproductive status. Points and bars show estimated marginal means with 95% confidence intervals from each model. Significantly different marginal means

(according to BH-corrected post hoc testing) are indicated by non-shared letters printed below. Violin plots show the raw data distributions. B: First two axes of the principal components analysis for sFEC (log transformed prior to centring and scaling), coloured according to sex and reproductive status. Ellipses represent 95% confidence intervals. Arrows indicate loadings towards each of the six species.

Table 3: Wald Type-II Chi Square test results

model term	Chisq	Df	Pr(>Chisq)	Response Variable
First year				
Year	37.556	3	<0.001	C. ovina
Sex	0.033	1	0.856	C. ovina
SiblingStatus	0.134	1	0.714	C. ovina
Season	6.843	1	0.009	C. ovina
Year	15.789	3	0.001	B. trigonocephalum
Sex	7.302	1	0.007	B. trigonocephalum
SiblingStatus	0.059	1	0.809	B. trigonocephalum
Season	9.335	1	0.002	B. trigonocephalum
Year	37.865	3	<0.001	Tr. axei
Sex	4.957	1	0.026	Tr. axei
SiblingStatus	4.335	1	0.037	Tr. axei

Season	138.945	1	<0.001	Tr. axei
Year	24.008	3	<0.001	Tr. vitrinus
Sex	17.689	1	<0.001	Tr. vitrinus
SiblingStatus	32.503	1	<0.001	Tr. vitrinus
Season	0.091	1	0.763	Tr. vitrinus
Year	45.724	3	<0.001	Nematodirus spp.
Sex	12.342	1	<0.001	Nematodirus spp.
SiblingStatus	2.228	1	0.136	Nematodirus spp.
Season	202.275	1	<0.001	Nematodirus spp.
Year	70.618	3	<0.001	Te. circumcincta
Sex	10.768	1	0.001	Te. circumcincta
SiblingStatus	10.943	1	0.001	Te. circumcincta
Season	14.497	1	<0.001	Te. circumcincta
Year	37.039	3	<0.001	Rank(Shannon Index)
Sex	2.252	1	0.133	Rank(Shannon Index)
SiblingStatus	0.111	1	0.739	Rank(Shannon Index)
Season	15.908	1	<0.001	Rank(Shannon Index)
Age				

Year	36.684	3	<0.001	Te. circumcincta
Season	0.975	1	0.323	Te. circumcincta
AgeClass	13.931	3	0.003	Te. circumcincta
Year	107.777	3	<0.001	B. trigonocephalum
Season	0.157	1	0.692	B. trigonocephalum
AgeClass	159.354	3	<0.001	B. trigonocephalum
Year	25.3	3	<0.001	Te. circumcincta
Season	3.649	1	0.056	Te. circumcincta
AgeClass	104.326	3	<0.001	Te. circumcincta
Year	107.777	3	<0.001	Tr. vitrinus
Season	0.157	1	0.692	Tr. vitrinus
AgeClass	159.354	3	<0.001	Tr. vitrinus
Year	107.777	3	<0.001	Nematodirus spp.
Season	0.157	1	0.692	Nematodirus spp.
AgeClass	159.354	3	<0.001	Nematodirus spp.
Year	107.777	3	<0.001	Te. circumcincta
Season	0.157	1	0.692	Te. circumcincta
AgeClass	159.354	3	<0.001	Te. circumcincta

Year	28.451	3	<0.001	Rank(Shannon Index)
Season	0.503	1	0.4478	Rank(Shannon Index)
AgeClass	170.186	3	<0.001	Rank(Shannon Index)
Seasonal reproduction				
Year	52.787	3	<0.001	C. ovina
SexRepro	2.927	2	0.231	C. ovina
Season	26.683	3	<0.001	C. ovina
AgeClass	77.334	2	<0.001	C. ovina
SexRepro:Season	92.048	6	<0.001	C. ovina
Year	117.56	3	<0.001	B. trigonocephalum
SexRepro	28.02	2	<0.001	B. trigonocephalum
Season	255.925	3	<0.001	B. trigonocephalum
AgeClass	30.217	2	<0.001	B. trigonocephalum
SexRepro:Season	53.003	6	<0.001	B. trigonocephalum
Year	32.497	3	<0.001	Tr. axei
SexRepro	3.573	2	0.168	Tr. axei
Season	304.151	3	<0.001	Tr. axei
AgeClass	109.998	2	<0.001	Tr. axei

SexRepro:Season	33.94	6	<0.001	Tr. axei
Year	60.198	3	<0.001	Tr. vitrinus
SexRepro	3.137	2	0.208	Tr. vitrinus
Season	185.153	3	<0.001	Tr. vitrinus
AgeClass	60.751	2	<0.001	Tr. vitrinus
SexRepro:Season	38.212	6	<0.001	Tr. vitrinus
Year	3.516	3	0.319	Nematodirus spp.
SexRepro	14.811	2	0.001	Nematodirus spp.
Season	112.576	3	<0.001	Nematodirus spp.
AgeClass	84.06	2	<0.001	Nematodirus spp.
SexRepro:Season	33.485	6	<0.001	Nematodirus spp.
Year	81.126	3	<0.001	Te. circumcincta
SexRepro	13.636	2	0.001	Te. circumcincta
Season	286.857	3	<0.001	Te. circumcincta
AgeClass	22.234	2	<0.001	Te. circumcincta
SexRepro:Season	169.227	6	<0.001	Te. circumcincta
Year	13.558	3	0.004	Rank(Shannon Index)
SexRepro	16.371	2	<0.001	Rank(Shannon Index)

Season	164.388	3	<0.001	Rank(Shannon Index)
AgeClass	93.723	2	<0.001	Rank(Shannon Index)
SexRepro:Season	125.791	6	<0.001	Rank(Shannon Index)

Table 4: PERMANOVA results

	Df	SumOfSqs	R2	F	Pr(>F)
First year					
Season	1	9.263	0.064	32.990	0.001
Sex	1	1.192	0.008	4.245	0.001
Year	3	6.677	0.046	7.927	0.001
SiblingStatus	1	1.588	0.011	5.656	0.001
Residual	445	124.945	0.869		
Total	451	143.843	1.000		
Age					
Season	1	4.253	0.011	14.658	0.001
Year	3	11.432	0.029	13.134	0.001
AgeClass	3	51.989	0.132	59.727	0.001

	Df	SumOfSqs	R2	F	Pr(>F)
Residual	1079	313.070	0.795		
Total	1086	393.665	1.000		
Seasonal Reproduction					
Year	3	8.142	0.016	9.889	0.001
AgeClass	2	7.106	0.014	12.946	0.001
Season:SexRepro	6	8.939	0.018	5.428	0.001
Residual	1501	411.970	0.825		
Total	1517	499.147	1.000		

Chapter 4 - The impact of the nemabiome on lamb bodyweight and survival

Abstract

Co-infections with multiple gastrointestinal nematodes (GIN) impact the health and wellbeing of humans, domestic animals and wildlife. These co-infecting parasites are likely to interact with each other but there is limited evidence regarding the impact co-infections have upon host condition and fitness, and whether there is value to the host in being infected by a more or less diverse community of parasites. This chapter combines individual-level nemabiome data from lambs in the unmanaged population of Soay sheep resident on the remote St Kilda archipelago (presented in Chapter 3) with their future bodyweight and overwinter survival, directly linking meta-barcoded parasite community data to individual host condition and fitness outcomes for the first time. This approach reveals that species-specific data explain more variation in early life host health/fitness outcomes than more widely applied total GIN egg counts. This seems driven by specific GIN species, with *Trichostrongylus vitrinus* having specific negative effects upon both bodyweight and survival, whilst the more intensively studied species *Teladorsagia circumcincta* has no specific association with either. These species-specific measures explained more variation in bodyweight and survival than summary measures of parasite alpha- and beta-diversity. However, the approach also identified a robust, positive association between *Tr. axei* and August bodyweight, that suggests the impacts of parasites in natural co-infections may be more complex than simply the addition of their individual pathological effects.

Introduction

Gastrointestinal nematodes (GIN) infect the majority of the human population worldwide (Stepek et al. 2006) and are near ubiquitous in grazed livestock, where they impact animal welfare, farm productivity and carbon emissions (Charlier et al. 2020; Mavrot, Hertzberg, and Torgerson 2015; Kenyon et al. 2013). GIN are similarly common in wildlife, with significant effects on host fitness and demography (Tompkins and Begon 1999; Coulson et al. 2018). The majority of these infections are co-infections of multiple species that may interact with each other both via the host immune system and via the alteration of the environment and resources available within the host (Cox 2001; Pedersen and Fenton 2007). Widespread anthelmintic drug treatment in humans and domestic animals reduces parasite burden, but may also change GIN diversity and community structure, as the prevalence of anthelmintic resistance varies among GIN species (Cai et al. 2024; Rose Vineer, Morgan, et al. 2020; McIntyre et al. 2018). Understanding naturally-occurring variation in GIN community structure and its association with host health is therefore important to gain broader understanding of the epidemiology of these parasites. Here, I examine the relationship between naturally-varying GIN community structure and its relationship with host growth and survival during early life in an unmanaged population of Soay sheep.

Cross-sectional observations (across host and parasite taxa) mostly report negative impacts of parasite co-infection on host condition, but typically do not control for the likelihood that hosts in poor condition may be more susceptible to infection (Donohue, Cross, and Michael 2019; Lello, Boag, and Hudson 2005; Holmstad, Hudson, and Skorping 2005; Bordes and Morand 2011). Controlled coinfection experiments have more power to identify the impacts of coinfections on host condition but are extremely limited in number. Two-species GIN co-

infection studies examining the impact on host condition have been mostly performed in sheep, likely as a result of the large economic impact of GIN infections in this species (Charlier et al. 2020; Nieuwhof and Bishop 2005; Brunsdon 1988), These studies have provided mixed evidence regarding the impact on host condition of GIN co-infections versus mono-infections of equivalent intensity (Coop et al. 1986; Sykes, Poppi, and Elliot 1988; Steel, Jones, and Symons 1982; A. R. Williams et al. 2010). However, two-species co-infections are still a limited representation of the complex multispecies communities found in natural infections.

Community perturbation experiments are another powerful means of infering causal effects of complex natural co-infections on host fitness, and have indicated benefical impacts on host health of co-infections between helminths and other pathogens. For example between GIN apicomplexans in buffalo (Seguel et al. 2023), between GIN and tuberculosis in buffalo (Ezenwa and Jolles 2015), and between nematodes and a viral pathogen in mice (A. R. Sweeny et al. 2020). However, the broad-spectrum activity of most anthelmintics makes it extremely challenging to target perturbation at a specific GIN species within a GIN community. Further, these experiments may be unsuited to examine longer-term impacts of coinfection that may persist after the perturbation (for example host condition and cross-protective immune responses). Observational studies are therefore necessary to address these questions, with longitudinal studies better able to identify effects than cross-sectional studies, as repeated measures from the same individuals can overcome the challenge of colinearity between co-infection status and host fitness (A. R. Sweeny et al. 2022; Thumbi et al. 2013).

The most accurate way of assessing GIN burdens is via post-mortem worm counts, however this naturally precludes longitudinal study designs. Therefore minimally-invasive faecal egg counts (FECs) have commonly been used to provide esimates of GIN burden. Unfortunately

the eggs of many GIN species are morphologically indistinguishable and the morphological identification of larvae is highly-skilled and time-consuming (Van Wyk and Mayhew 2013).The recent development of high-throughput ITS-2 sequence-based speciation (nemabiome sequencing) (Avramenko et al. 2015) has opened the possibility of large-scale observational studies of GIN co-infections, providing insights into seasonal, geographical and multi-host-species epidemiology (Evans et al. 2021; Sargison et al. 2022; Redman et al. 2019; Beaumelle et al. 2022; Abbas et al. 2024), as well as changes associated with host age, sex and reproduction (Beaumelle et al. 2021; Ahn et al. 2024; E. G. Williams et al. 2024). However, no prior studies have been able to link individual-level nemabiome data to individual host outcomes.

Chapter 3 demonstrated that the nemabiome of lambs in July is dominated by *Te. circumcincta*, *Nematodirus* spp. and, more surprisingly*, Tr. vitrinus.* Then by November the community is more diverse, with higher abundances of *Tr. axei*, *C. ovina* and *B. trigonocephalum*. Previous work in the same population of Soay sheep on St Kilda has provided strong evidence linking nematode FECs in August with both bodyweight, an important predictor of future survival and fitness in this system, and with overwinter lamb mortality directly (K. Wilson et al. 2004; Clutton-Brock et al. 1992; Clutton-Brock and Pemberton 2004; Craig et al. 2008). However, those studies utilised unspeciated FECs, therefore the impacts of individual GIN species and species diversity upon the host remain unknown. In this chapter I take the nemabiome data from lambs presented in Chapter 3 and combine it with bodyweight and survival data collected as part of the long-running St Kilda Soay Sheep Project. Through this I examine how GIN community composition shapes early life health and fitness in a wild mammal, specifically asking whether total FEC, sFECs, alpha-diversity or beta-diversity best predict early life bodyweight and mortality.

Methods

Study system and sample collection

Soay sheep are thought to have lived wild on the island of Soay in the remote St Kilda archipelago since the Bronze Age. In 1934, 107 sheep were transferred from Soay to the depopulated island of Hirta, this population have since expanded across the island and remained unmanaged. Since 1985, the sheep living in Village Bay area of Hirta (around a third of island's 628ha area), have been part of a long-term individual-based study in which neonates are captured and ear-tagged within a few days of birth allowing them to be identified and monitored throughout life (Clutton-Brock and Pemberton 2004).

The sheep exhibit seasonal reproduction with lambing occurring in spring (late March–early May). Most (c.80%) adult females produce a lamb in each year, and a smaller proportion of females lambing at one year of age (c.15-40%). Twinning rates are usually below 20% and triplets are extremely rare. Lambs are captured within two weeks of birth (c.90% within the first week), weighed, blood sampled, and ear tagged before being returned to their mother. Each August corral traps are constructed by a team of project fieldworkers and approximately 50-60% of the Village Bay population are caught in them and their bodyweights recorded. (Clutton-Brock and Pemberton 2004).

There is minimal predation on the island (skuas may occasionally take small lambs) and mortality occurs mostly over-winter among lambs/yearlings, mediated by limited nutritional resources and parasitism (Craig, Pilkington, and Pemberton 2006; Gulland 1992). Ten censuses of the study area are performed each April, August and November during which time the identities and locations of any animals seen are recorded. The identity of any carcasses identified during these census periods are recorded. Lambs were judged to have

survived over winter if they were seen alive after the first of May in the calendar year after their birth (26% of the animals in this study). Lambs were judged to have not survived over winter if they were found dead prior to the first of May in the year after their birth (62% of animals in this study). Lambs not that were not observed again, or not found dead until a later date were excluded from mortality analyses (12% of the animals in this study).

In each of the years 2019-2022, faecal samples were collected from individual lambs during two weeks in July and two weeks in late-October – early-November (henceforth referred to as November for simplicity). These samples were collected from the ground immediately after observed voiding (including observation of the animal's uniquely identifying ear tag) and stored at 4°C in polythene bags with the air extruded until parasitology was performed. The timing of these faecal samples is illustrated in Figure 17A.

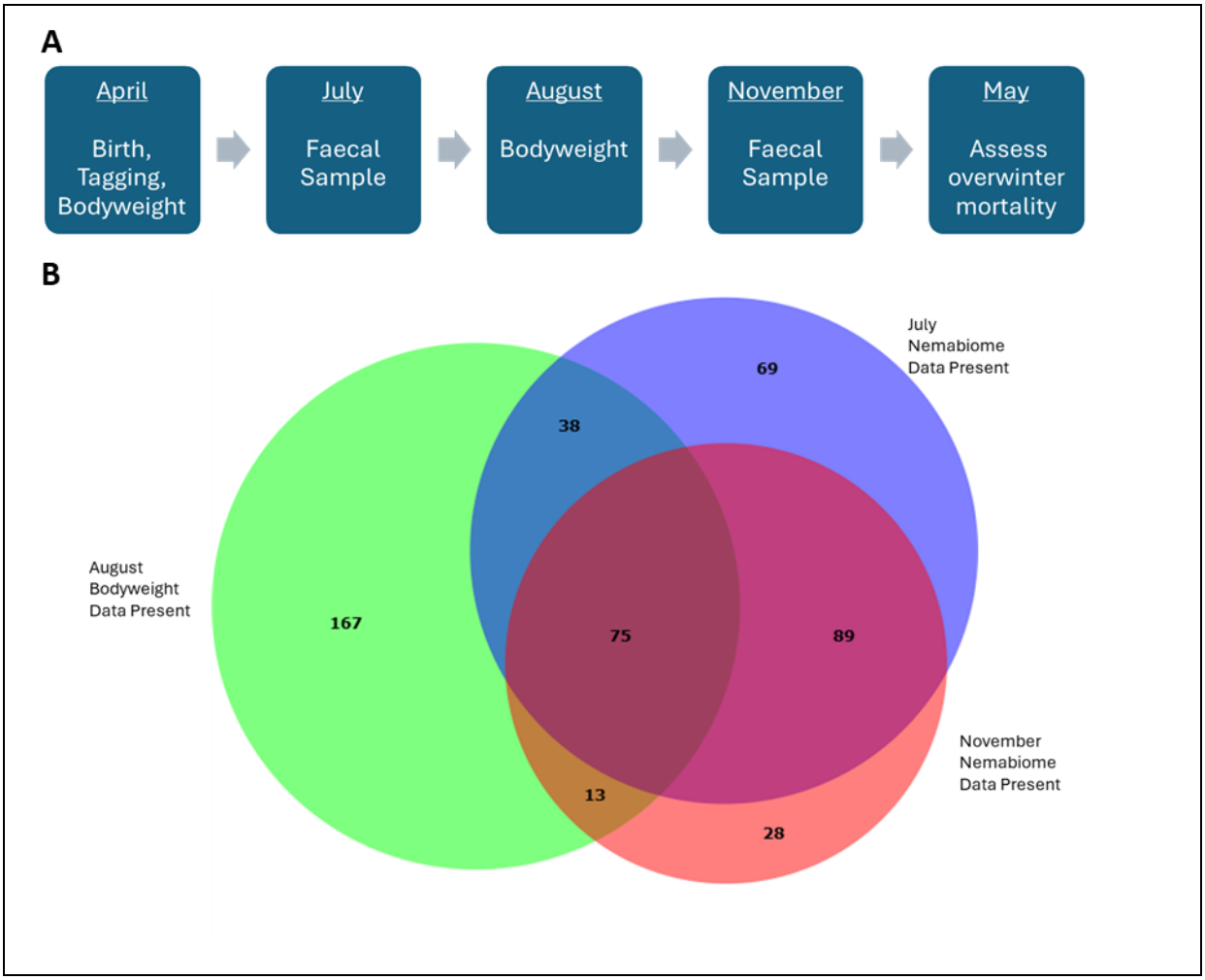


Figure 17 A: Study timeline. This was performed in each of the four years 2019-2022. B: Venn diagram illustrating the degree of overlap between the three datasets. For example, a model using both August bodyweight data and July Nemabiome data, the total number of individuals would equal 113 (38+75). Similarly, for a model using both August bodyweight data and November Nemabiome data, the total number of individuals would equal 88 (13+75), whereas for a model using just November Nemabiome data the total number of individuals would equal 205 (75+89+13+28). Venn diagram produced using Deep Venn (Hulsen 2022).

Parasitology

Parasitological methods are given in full in Chapter 3. Briefly, strongyle-type and *Nematodirus* spp. (chiefly *N. battus*) eggs were enumerated by a salt-flotation technique with a sensitivity of 1 egg per gram (epg) (Jackson 1974; A. R. Sweeny et al. 2022). Samples were then cultured

to isolate third stage nematode larvae, from which DNA was extracted and species proportions calculated by ITS-2 amplicon sequencing (nemabiome) (Avramenko et al. 2015). Species proportions were then multiplied by the strongyle-type FEC to give six species-corrected faecal egg counts (sFECs) (*Teladorsagia circumcincta, Trichostrongylus vitrinus, Trichostrongylus axei, Chabertia ovina* and *Bunostomum trigonocephalum* from the strongyle-type eggs, plus the morphologically identified *Nematodirus* spp. eggs). Shannon index was calculated from these sFECs using the 'vegan' R package (Oksanen et al. 2022). The first two axes of a principal components analysis of the log-transformed sFECs (together explaining 55% of the variation) was used as measure of beta-diversity.

Across the four years, nemabiome data were present for 271 lambs in July and 205 lambs in November, with 126 lambs of these being caught during the August catches (in addition to the capture of 167 lambs without July or November nemabiome data). Known individuals were followed until defaecation was observed (without the need for capture), therefore there was a high degree of overlap between the individuals present in the nemabiome datasets. In contrast, bodyweight measurement was dependent upon catching individuals, therefore there was less overlap between the bodyweight dataset and the nemabiome datasets (Figure 17B).

Statistical analysis

A linear regression between first capture weight and capture age (in days) was first performed using data from all years of the Soay Sheep Project (1985 onwards). The residuals from this model (R^2=0.19) were then calculated and these residuals were used in subsequent models as an estimate for birthweight corrected for age at first capture (henceforth referred to as

"Birthweight Residual") (Overall et al. 2005). Four animals missing birth weight data were assigned a Birthweight Residual of 0 (i.e. imputing that they were of mean birthweight).

In order to test the relationship between measures of parasite community composition in the preceding season, and both bodyweight in August and over-winter survival, base generalised linear mixed-effects models (GLMMs) were first constructed using the glmmTMB package (Brooks et al. 2017). A base model was first built with August bodyweight as the response variable, assuming a Gaussian distribution. This model included birth year, sex and sibling status (singleton or twin) as fixed effects, birthweight residual and age (days between date of birth and date of August bodyweight) as covariates; and maternal identity as a random effect. Next, a base GLMM of over-winter survival as a binomial response variable (1 = survived, 0 = died) was fitted with a logit link. This included birth year, sex and sibling status as fixed effects; and maternal identity as a random effect.

These weight and survival models were then extended by including additional covariates reflecting different measures of the GIN community structure in the preceding sampling season. For weight, this was July samples (N = 113) and for survival it was November (N = 180 as 25 of the 205 lambs with November Nemabiomes did not have a known overwinter survival status, see Figure 1B for details of sample sizes available across all key variables). The competing parasitological covariates were fitted in separate models, to test different ways in which the GIN community might predict weight and survival:

Morphological FEC: Strongyle-type FEC and *Nematodirus* spp. FEC, each transformed by the natural logarithm (plus one).

Alpha diversity: Shannon Index

Beta diversity: The first two axes of a principal components analysis

Complete nemabiome: All six sFECs (log-transformed) fitted together in a single model

Individual sFEC: Each sFEC (log-transformed) included separately (i.e. 6 models)

These competing models were then assessed by AICc calculated in the 'performance' package (Lüdecke et al. 2021) with the model with the lowest AICc considered the best, provided the AICc was at least 2 units lower than that of the base model, as this is widely considered to represent a clearly improved model (Burnham and Anderson 2002). The significance of the model terms in the best model were assessed using Wald Type-II χ^2 tests using the 'car' package, with significance judged as $P<0.05$ (Fox and Weisberg 2019). Model fit and the violation of model assumptions for the best model were assessed by visual inspection of simulated residual plots using the 'DHARMa' package (Hartig 2022) and assessment of the Variance Inflation Factor (Lüdecke et al. 2021).

FECs and sFECs have highly skewed distributions, even after log transformation, therefore models incorporating them as covariates are potentially vulnerable to a small number of outlying data points with high statistical leverage. Removing outlying datapoints can introduce bias and seems particularly unsound when there is a large body of evidence demonstrating that parasite counts are usually highly over-dispersed (D. J. Shaw and Dobson 1995). Instead of removing outlying datapoints, the robustness of the best model to their influence was assessed by 90% winsorization (Ruppert 2014), wherein the best model for each response variable was refit after setting observations greater than the 95th percentile equal to the value of the 95th percentile and all observations less than the 5th percentile equal to the value of the 5th percentile, using the 'DescTools' R package (Signorell 2025). A result was

deemed robust to outlying values if the model term remained significant on Wald Type-II χ^2 testing after 90% winsorization.

The models of August weight and overwinter survival provided evidence of a negative effect of July and November *Tr. vitrinus* sFEC on these variables, respectively (see Results). We sought to test *post hoc* whether the association between November *Tr. vitrinus* sFEC and survival was due carry over effects of July *Tr. vitrinus* (i.e. counts in July predict counts in November which predicts survival). We did this by adding July *Tr. vitrinus* sFEC as a response variable to our best fitting survival model in place of November *Tr. vitrinus* sFEC. We also sought to test *post hoc* whether variation in body weight in August might mediate the association between November *Tr. vitrinus* sFEC and survival by similarly re-running our best survival model including August bodyweight.

Results

All terms in the base model of August bodyweight were significant, with weight increasing with lamb age, and varying with sibling status (singletons heavier) and sex (males heavier) (Table S1). When comparing model fits with different GIN parasite community measures in July, the best model was the complete nemabiome model, including separate terms for each of the six species-specific sFECs (Table 1). Within that model the only two species significantly associated with body weight were *Tr. axei* (P=<0.001) and *Tr. vitrinus* (P=0.003) (Table 2). The effect of *Tr. vitrinus* was negative with higher sFECs in July being associated with lower bodyweight in August, whereas the effect of *Tr. axei* was positive (Fig. 3A). Both these effects were robust to Winsorization (Figure 18B, Table 2) and the variance inflation factors for all model terms in the complete nemabiome model (with and without Winsorization) were less than 5, suggesting there was minimal influence of any collinearity on parameter estimation

(Lüdecke et al. 2021). The model incorporating unspeciated FEC had a worse fit than the base model (Table 5) and showed no evidence of any association between GIN infection intensity in July and August bodyweight (Figure 18C).

Table 5: AICc values for each of the competing models. The AICc of the best model is in bold and underlined

Model	**AICc – August bodyweight**	**AICc – Overwinter survival**
Base	398.805	215.900
Morphological	401.642	218.398
Alpha-Diversity	400.345	216.601
Beta-Diversity	403.710	218.013
Nemabiome (All six)	**393.537**	219.918
Nematodirus spp.	399.591	217.839
Tr. vitrinus	398.062	**210.871**
Tr. axei	395.080	216.831
Te. circumcincta	400.906	218.003
B. trigonocephalum	400.895	217.756
C. ovina	400.071	216.279

Table 6: Wald Type-II χ^2 test results for the best bodyweight model with and without 90% Winsorization.

	Without Winsorization			**With 90% Winsorization**		
Model term	**χ^2**	**Df**	**P(>χ^2)**	**χ^2**	**Df**	**P(>χ^2)**
Birthweight Residual	82.951	1	<0.001	82.191	1	<0.001
Birth Year	15.239	3	0.002	13.910	3	0.003

Sex	37.088	1	<0.001	35.436	1	<0.001
Sibling Status	6.735	1	0.009	5.520	1	0.019
Age	17.098	1	<0.001	15.539	1	<0.001
Nematodirus spp.	0.679	1	0.410	0.716	1	0.397
B. trigonocephalum	1.600	1	0.206	1.034	1	0.309
C. ovina	0.666	1	0.414	0.651	1	0.420
Te. circumcincta	0.505	1	0.477	0.619	1	0.431
Tr. axei	10.966	1	0.001	6.978	1	0.008
Tr. vitrinus	8.635	1	0.003	6.267	1	0.012

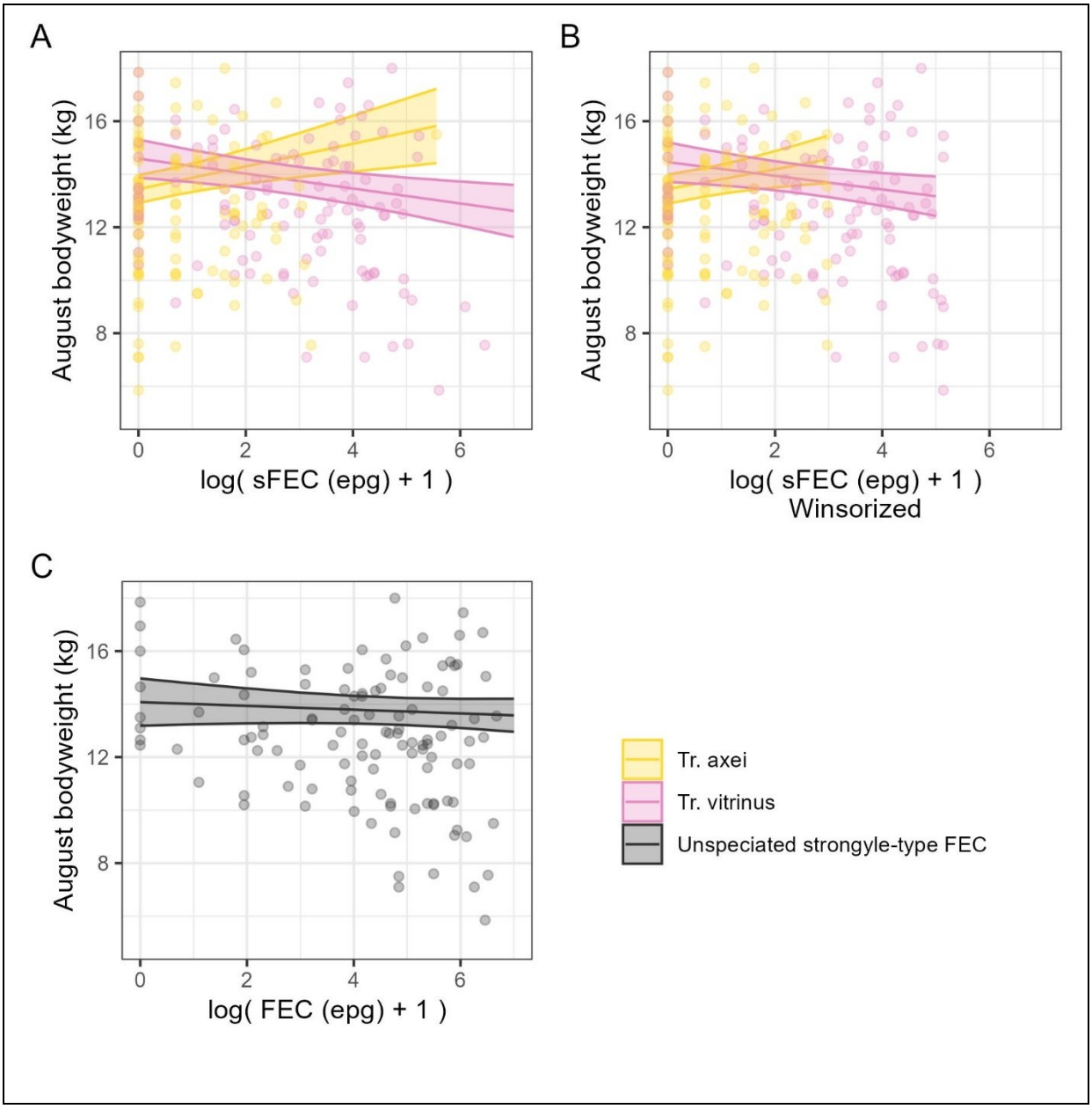


Figure 18 A: Predicted association between July Tr. vitrinus and July Tr. axei and August bodyweight, from the nemabiome model. B: Predicted association between July Tr. vitrinus and July Tr. axei and August bodyweight, from the nemabiome model, after winsorization of the sFECs. C: Predicted (non-significant) association between unspeciated strongyle-type FEC and August bodyweight, from the morphological model. N.B. The x-axis is on the natural log scale, for reference $e^2 \simeq 7$, $e^4 \simeq 50$, $e^6 \simeq 400$, $e^8 \simeq 3000$. Shaded ribbons indicated 95% confidence intervals. Translucent points represent individual data values (n=113).

There were significant associations in the base survival model between survival and sex (females more likely to survive) (Table 9, supplementary) and between survival and sibling status (singletons more likely to survive) (Table 9, supplementary). When comparing model fits with different GIN parasite community measures in November, the best model for overwinter survival was that which included *Tr. vitrinus* on its own (Table 5). Within that model the *Tr. vitrinus* term was significant (P=0.010, Table 7) and negative, with higher *Tr. vitrinus* sFECs in November being associated with a lower probability of lamb overwinter survival (Figure 19A). The effect was also robust to winsorization (Table 7, Figure 19B). The model incorporating unspeciated FEC had a worse fit than the base model (Table 5) and showed no evidence of an association between unspeciated FEC in November and overwinter survival (Figure 19C). When July *Tr. vitrinus* sFEC was added to the best fitting survival model in place of November *Tr. vitrinus* sFEC, it was not significant (χ^2_1=0.195, P=0.659). When August bodyweight and November *Tr vitrinus* sFEC were included together in a model of overwinter survival neither the *Tr. vitrinus* nor the August bodyweight term were significant (χ^2_1=1.479, P=0.224 and χ^2_1=1.369, P=0.242, respectively). Not all lambs with November parasitology data were caught during August, therefore that model was fit with a smaller subset of data (N=88, Figure 17B).

Table 7: Wald Type-II χ^2 test results for the best survival model with and without 90% Winsorization.

	Without Winsorization			**With 90% Winsorization**		
Model term	**χ^2**	**Df**	**P(>χ^2)**	**χ^2**	**Df**	**P(>χ^2)**
Birth Year	2.113	3	0.549	2.115	3	0.549
Sex	3.205	1	0.073	3.238	1	0.072
Sibling Status	4.220	1	0.040	4.265	1	0.039

Tr. vitrinus	6.703	1	0.010	6.537	1	0.011

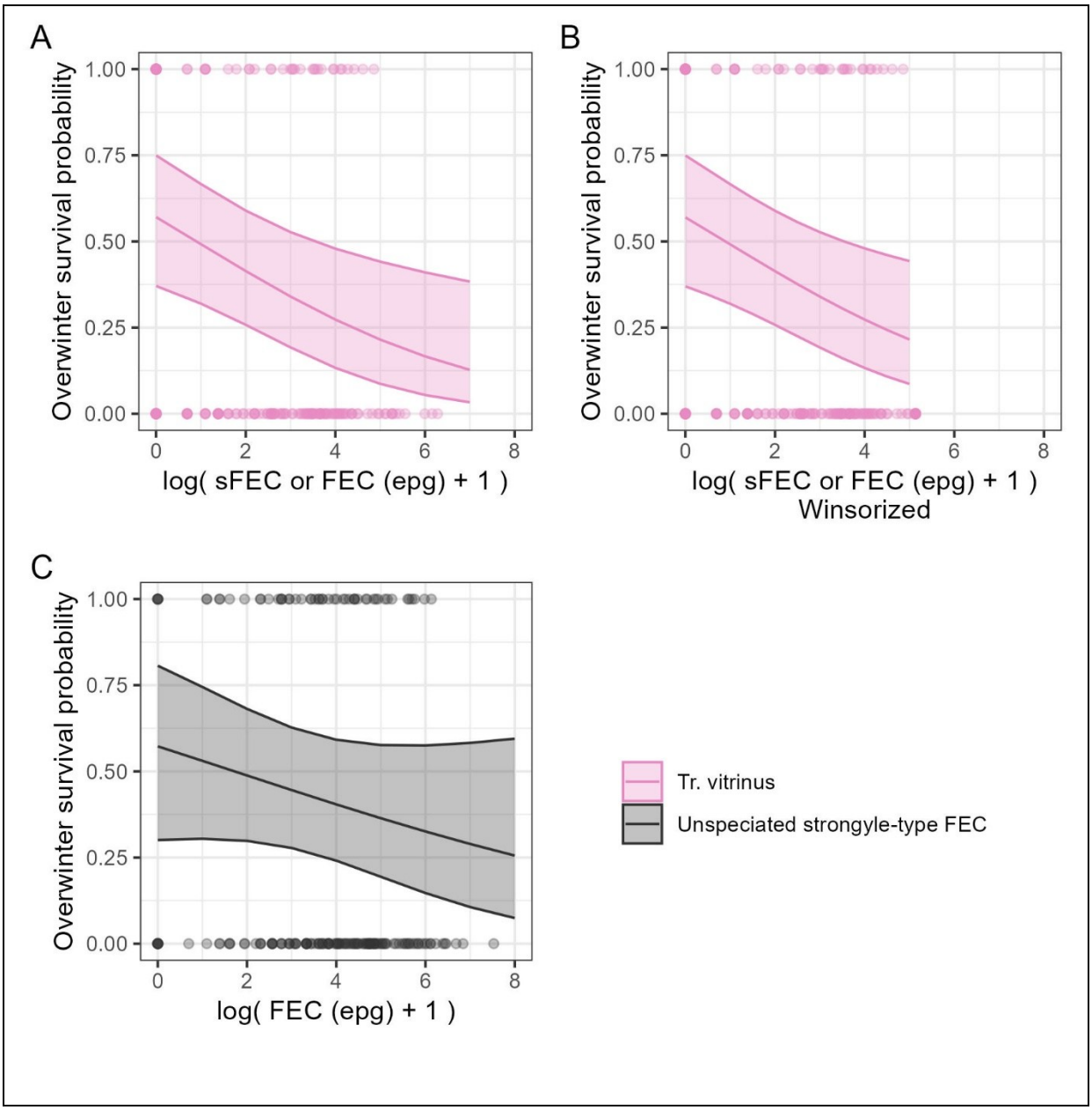


Figure 19 A: Predicted association between November Tr. vitrinus and overwinter survival from the best model. B: Predicted association between November Tr. vitrinus and overwinter survival from the best model, after winsorization of the sFECs. C: Predicted (non-significant) association between unspeciated strongyle-type FEC

and August bodyweight, from the morphological model. N.B. The x-axis is on the natural log scale, for reference $e^2 \simeq 7$, $e^4 \simeq 50$, $e^6 \simeq 400$, $e^8 \simeq 3000$. Shaded ribbons indicated 95% confidence intervals. Translucent points represent individual data values (n=205).

Discussion

These results provide strong evidence that a 'nemabiome' approach offers far greater insight into the role GIN infections play in host health and fitness of animal populations than combined, unspeciated egg counts. They show that single-species FECs derived from meta-barcoding data explain more variation in growth and survival than either unspeciated FECs and measures of GIN community diversity or structure as predictors of growth and survival during early life in wild Soay sheep. This appears to be because more of the variation in growth and survival is explained by one or two individual species counts, with the overall signal muted or lost in the total unspeciated FECs. Previous nemabiome studies have provided notable insights into temporal, geographic and host-species differences in GIN community composition (Evans et al. 2021; Redman et al. 2019; Beaumelle et al. 2022; Avramenko et al. 2018; Ahn et al. 2024; Abbas et al. 2024; Beaumelle et al. 2021); however, this is the first study to use individual-level nemabiome data to illuminate the role of specific GIN parasites in host health and fitness. This demonstrates the power of next-generation sequencing approaches when applied to closely monitored individuals in an unmanaged population.

The results specifically highlight the importance of early-life *Tr. vitrinus* infections in this study system, with sFECs in July being negatively associated with August bodyweight and sFECs in November being negatively associated with overwinter survival. Experimental work in domestic sheep has demonstrated *Tr. vitrinus* infection can have significant negative impacts on weight gain in lambs (Blackburn, Carmichael, and Walkden-Brown 2015) and *Tr. vitrinus*

was the second most numerous GIN identified in lambs that died on St Kilda over the winter of 2001-2002, and the best predictor of overwinter weight loss in those animals that had died (Craig, Pilkington, and Pemberton 2006). Further, previous work in this study system has shown unspeciated FECs taken from lambs in August to be negatively associated with bodyweight (Coltman et al. 2001) and overwinter survival (Hayward et al. 2011) and that anthelmintic treatment decreases the probability of overwinter mortality (Gulland 1992). However, until now, *Te. circumcincta* and not *Tr. vitrinus* was assumed to be a major contributor to the impacts of early life parasitism on young Soay lambs. This owed to the classic epidemiological descriptions from domestic sheep of a seasonal epidemiological progression from *Te. circumcincta* infections in young lambs through to *Tr.* spp. infections in older lambs in late winter (Boag and Thomas 1977; Coop et al. 1986; H. D. Crofton 1957; Parnell et al. 1954; Reid and Armour 1972; S. M. Taylor and Pearson 1979), plus the much larger veterinary research focus on *Te. circumcincta* than *Tr. vitrinus* (11,800 vs 1,780 google scholar results [accessed 17th September 2025]).

Chapter 3 found that *Tr. vitrinus* counts in lambs on St Kilda rose much earlier in these seasons than classical epidemiological descriptions in domestic sheep would predict. However, those descriptions are either close to 70 years old (Parnell et al. 1954; H. D. Crofton 1957), confined to a single experimental farm (Boag and Thomas 1977), or from farms with monthly anthelmintic treatment of all lambs (Reid and Armour 1975; D. J. Wilson et al. 2008). Whilst they were replicated using a nemabiome approach by Evans *et al.* (2021) and Geddes *et al.* (2024), they were in again in managed flocks using regular anthelmintic treatments that could have favoured the establishment of anthelmintic resistant *Te. circumcincta* populations. In contrast, Redman *et al.* (2019) showed greater intensities of *Tr.* vitrinus than *Te. circumcincta* in pooled post-weaning samples taken from 61 groups of lambs from farms across the UK.

This demonstrates the need to review the epidemiology of the major GIN infecting domestic sheep in the context of varied farming systems, and recent reductions in the reliance upon anthelmintic treatments. My results from an unmanaged, wild sheep population may support development of epidemiological models of the role of GIN in sheep farming in the absence of anthelmintics. They also demonstrate the value of the nemabiome approach to understanding the epidemiological drivers of parasite-induced impacts upon the fitness of wild hosts.

In Chapter 3 I hypothesised that the high *Tr. vitrinus* sFECs in lambs in July may be a result of a periparturient rise in FEC in this species, or the presence of lambs/yearlings overwinter on the same pastures that will go on to be home to the next generation of lambs in the following spring. The combination of this epidemiology and the specific impact of this parasite could have interesting implications for the evolutionary ecology of these wild sheep. Namely, if the ewes are the primary source of *Tr. vitrinus* infectious pressure, harsh winters would likely lead to greater burdens in lambs the following spring, as a consequence of a larger periparturient rise (Houdijk 2008). In contrast, if yearlings are the primary source of *Tr. vitrinus* infectious pressure, harsh winters may lead to lower burdens in lambs the following spring due to increased mortality in the previous generation. Both these scenarios would then have follow-on consequences for mortality of the next cohort in the subsequent winter. This interesting epidemiological dynamic could influence the demographics in this wild system, with parasites transferring impacts of the environmental conditions faced by one generation onto the fitness of a subsequent generation.

In this study system, August bodyweight has been shown to negatively impact survival probability (Clutton-Brock et al. 1992). In our study, it was not possible to separate the effect

of November *Tr. vitrinus* sFEC on overwinter survival from the effect of August bodyweight on survival. Owing to incomplete overlap between the Nemabiome datasets and the bodyweight datasets, sample size for that model was reduced. As a *post hoc* check, the best survival model was refit with that smaller dataset and the *Tr. vitrinus* effect ceased to be significant (χ^2_1= 1.477, P=0.224). Also, there was no significant effect of weight if that model was refit using weight instead of *Tr. vitrinus* sFEC (χ^2_1= 1.382, P=0.240). This suggests that the inability to separate the effects of weight and November *Tr. vitrinus* sFEC on survival was due to inadequate power in that smaller data subset. However, the absence of a significant effect of July *Tr. vitrinus* on overwinter survival indicates that the association between November *Tr. vitrinus* and survival was not mediated by the association between July *Tr. vitrinus* and August bodyweight.

It is surprising that there was no evidence for an association between *Te. circumcincta* sFECs and either bodyweight or survival given sFECs for *Te. circumcincta* and for *Tr. vitrinus* were comparable in July (*Te. circumcincta:* mean = 13.8, range = 0-745; *Tr. vitrinus*: mean = 14.0, range = 0-998) and were moderately well correlated with each other (r=0.510). *Te. circumcincta* is known to cause major production losses in young domestic lambs (Brunsdon 1988; Vlassoff and McKenna 1994) but it is possible that any impact of *Te. circumcincta* may have been more difficult to identify because of density dependent fecundity. Density-dependent fecundity is well described in *Te. circumcincta* (Bishop and Stear 2000) and its close relatives found in other hosts (Stien et al. 2002; G. Smith, Grenfell, and Anderson 1987), but this effect has not been observed for the majority of other ruminant GIN (Coyne, Smith, and Johnstone 1991; Barger 1987). This could lead to a weaker association between sFEC and worm burden, obscuring the impacts. This relationship may suggest that nemabiome-derived

egg counts may be more useful measures of productivity/health in GIN species without density-dependent fecundity.

Heterogeneity in host anti-parasite strategies could have also masked the costs of *Te. circumcincta* parasitism. Theoretical models suggest that in the presence of parasite-associated immunopathology, the fitness benefits of tolerance versus resistance (as anti-parasite strategies) are dependent upon resource availability (Dean et al. 2024). Therefore in a natural environment with fluctuating resources, heterogeneity in host strategy ought to be high (a theory empirically supported within the St Kilda system (Hayward et al. 2011)). Immunosuppression experiments indicate there are higher immunopathological costs of resistance against *Te. circumcincta* than against *Tr. vitrinus* (Greer et al. 2008; Blackburn, Carmichael, and Walkden-Brown 2015). In addition, anti-*Te. circumcincta* resistance has a specific impact on female worm fecundity (Stear et al. 1995). Therefore, for a given *Te. circumcincta* burden, more resistant hosts would be expected to have low sFEC but high immunopathological costs, whilst more tolerant hosts would have high sFEC and low immunopathological costs. This would potentially lead to the costs of *Te. circumcincta* parasitism being obscured when using sFEC as an estimate of burden.

Alternatively, the costs of *Te. circumcincta* infection may have been limited by the other parasite species present in the July samples. The results in this chapter don't provide direct evidence for this, but they do show a robust, significant, positive association between July *Tr. axei* sFEC and August bodyweight that raises questions about the role of less pathogenic species in the net impact of parasite co-infections. *Tr. axei* is often considered less pathogenic than the other *Tr.* spp. and *Te. circumcincta* (Stubbings et al. 2022); however, a positive causal effect of this parasite on bodyweight is extremely unlikely, given controlled infections have

shown *Tr. axei* to suppress appetite and reduce digestive efficiency (Spedding 1954) and it has been associated with mortality in natural clinical cases (Abbott and McFarland 1991). An interaction between *Tr. axei* and *Tr. vitrinus* was added to the bodyweight model to test *post hoc* whether it could explain the positive effect of *Tr. axei*; however, that interaction was not significant (χ^2_1=3.799, P=0.051) and did not alter than direction of the gradient of the *Tr. axei* main effect. Chapter 2 showed that interactions between co-infecting GIN are more antagonistic when sequential rather than concurrent, therefore an inter-species interaction at an unmeasured timepoint could nonetheless explain the positive effect of July *Tr. axei*. As discussed in Chapter 2, cross-species immunity could be responsible for the antagonistic interactions that occur between co-infecting GIN, and this could explain how the early presence of a less pathogenic GIN species such as *Tr. axei* was associated with increased bodyweight. It is therefore conceivable that farm management practices and species-level pasture larvae monitoring/forecasting could be used to push L_3 exposure towards less-pathogenic species and thereby induce anti-GIN resistance whilst minimizing production impacts. This positive association between one parasite species and host condition also raises questions about how co-infection dynamics feedback on host demography, and further, how interactions could influence the fitness of the parasites themselves in species that share hosts and hence have evolutionarily entwined fates.

Conclusions

- Species-specific FECs explain more variation in early life host health/fitness outcomes than more widely applied total GIN egg counts or summary measures of parasite alpha- and beta-diversity.

- In the absence of human intervention, *Trichostrongylus vitrinus* has specific negative effects upon both bodyweight and survival, whilst the more intensively studied species *Teladorsagia circumcincta* has no specific association with either.
- In this population of wild sheep there is a robust, positive association between *Tr. axei* and August bodyweight, that suggests the impacts of parasites in natural co-infections may be more complex than simply the addition of their individual pathological contributions.

Supplementary Materials

Table 8: Wald Type-II χ^2 test results for the base bodyweight model

Model term	χ^2	Df	P(>χ^2)
Birthweight Residual	88.796	1	<0.001
Birth Year	19.234	3	<0.001
Sex	28.562	1	<0.001
Sibling Status	5.109	1	0.009
Age	17.040	1	<0.001

Table 9: Wald Type-II χ^2 test results for the base survival model

Model term	χ^2	Df	P(>χ^2)
Birth Year	1.264	3	0.738
Sex	4.549	1	0.033
Sibling Status	6.327	1	0.012

Chapter 5 - Abomasal immune responses triggered by infection with an intestinal parasite

Abstract

Gastrointestinal nematodes (GIN) place major constraints on grazing livestock production, particularly in sheep. Widespread anthelmintic resistance necessitates alternative management options including the development of immune protection. However, the majority of GIN infections in temperate climates are multispecies co-infections with sequential seasonal epidemiologies and antagonistic interactions between species. Cross-protective immunity is likely to play a role in these interactions, yet there is limited direct evidence that this occurs, nor the effector mechanisms which may be involved.

Investigating cross-protective immunity between sites within the gastrointestinal tract can be challenging due to the aboral flow of immune effectors in the mucus and digesta. Here we instead look 'upstream', using sheep infected with the intestinal nematode *Trichostrongylus colubriformis* to investigate immune responses generated in the abomasum which may be effective against abomasal GIN.

This study demonstrates that *Tr. colubriformis* infection induces IgG within intestinal and abomasal mucus which cross-reacts with somatic and excretory-secretory antigens of third and fourth-stage larvae of the abomasal-dwelling parasite *Teladorsagia circumcincta*. In contrast, cross-reactive IgA was only found in intestinal mucus. The somatic-antigen targets of these cross-reactive antibodies were unaffected by periodate treatment suggesting they do not recognise glycan moieties. Furthermore, proteomic analysis indicated that cross-reactive IgG recognises conserved structural and metabolic proteins unlikely to be exposed in

live nematodes and hence unlikely to be effective targets of cross-protective immunity. Instead, we show that *Tr. colubriformis* infection results in upregulation of other immune pathways within the abomasal fundus and pylorus associated with anti-GIN immunity, including mast cell degranulation; immunoglobulin binding; mucus secretion; Interleukin (IL)-13 production; IL-5, IL-13 and IL-17 receptor expression; and tuft cell differentiation.

Together, these findings provide potential mechanisms for antagonistic interactions between co-infecting nematodes in different regions of the gastrointestinal tract which are mediated by immunological changes within the mucosa rather than cross-reactive antibodies. This highlights the need for work on immunological control of GIN which considers the potential benefits of immune responses common across mucosal sites.

Introduction

Gastrointestinal nematode (GIN) parasites impact the productivity, welfare and environmental sustainability of grazed livestock agriculture, especially sheep farming (Charlier et al. 2020; Mavrot, Hertzberg, and Torgerson 2015; Kenyon et al. 2013). Widespread anthelmintic resistance necessitates alternative management options including the development of immune protection via selective breeding, managed parasite exposure or vaccination (Hayward 2022; Abbott, Taylor, and Stubbings 2012; Nisbet et al. 2013; Kebeta et al. 2021). However, the vast majority of GIN infections in temperate climates are multispecies co-infections with sequential seasonal epidemiologies (Boag and Thomas 1977) and antagonistic interactions between these sequentially infecting parasite species (Evans et al. 2023). Cross-protective immunity is a likely mechanism for these interactions, yet there is little evidence that vaccines effective against single species provide cross-species protection (Andronicos et al. 2025; Nisbet et al. 2024), or that targets for selective breeding indices provide direct multi-species immunity (Harrison et al. 2008).

The protective immune response against many helminth parasites is largely driven by T helper type 2 (Th2) responses characterized by increases in the levels of interleukins (IL) 4, 5, 9, 13, 21 and 25, which trigger activation and expansion of $CD4^+$ Th2 cells, plasma cells secreting IgE, eosinophils, mast cells, basophils and alternatively activated macrophages (Anthony et al. 2007). These Th2-type cytokines, particularly IL-13, also stimulate increased mucus production (Gossner et al. 2013; McKenzie et al. 1998), which provides a physical barrier against GIN as well as being the carrier medium for inhibitory/immune mediators (Douch et al. 1984; Harrison et al. 1999). In temperate climates, sheep face the challenge of multiple GIN species in sites across the GI tract, including *Teladorsagia circumcincta* in the abomasum,

and *Trichostrongylus colubriformis* and *Tr. vitrinus* in the small intestines (O'Connor, Walkden-Brown, and Kahn 2006). Immunity against the intestinal GINs *Tr. colubriformis* and *Tr. vitrinus* is characterised by an initial reduction in the number of incoming L_3 that become established (Seaton et al. 1989a; Dobson, Waller, and Donald 1990a). This is mediated by IgG and IgA responses against L_3 somatic antigens (Harrison et al. 2008) which can be detected in serum from 4 weeks post-infection (Cardia et al. 2011). This is followed by arrested worm development, reduced worm fecundity and the eventual expulsion of the established worm population (Dobson, Waller, and Donald 1990b, 1990c; Eysker 1987; Sykes, Coop, and Angus 1979). In contrast, the initial response against the abomasal GIN *Te. circumcincta* is an IgA-mediated reduction in worm length and via that fecundity (Stear, Strain, and Bishop 1999). This is followed by increases in mast cells, eosinophils, globule leukocytes and IgA plasma cells in the both the pylorus and fundus with an immediate-type hypersensitivity reaction against incoming L_3 reducing further parasite establishment (W. D. Smith et al. 1984; Seaton et al. 1989b; Stear et al. 1995).

Potential mechanisms for cross-protection include cross-reactive antibodies, which due to the common mucosal immune response, may be induced at one site within the gastrointestinal tract (GIT) and be produced at other sites within the GIT. The glycolipid known as CarLA (carbohydrate larval antigen) has been a focus of much research, and animals with higher salivary IgA against CarLA have lower parasite faecal egg count (FEC), lower breech soiling and improved weight gain in field grazed sheep (presumably facing a multi-species challenge) (R. J. Shaw, Morris, and Wheeler 2013; R. J. Shaw et al. 2012). Further, anti-CarLA antibodies produced during experimental *Tr. colubriformis* infections cross-react with similarly sized antigens in somatic extracts of multiple species of ovine GIN (Harrison, Pulford, Hein, Severn, et al. 2003a). Harrison et al. (2008) found that preincubating *Tr. colubriformis* L_3 with these

antibodies then provided passive protection against infection by surgical implantation, but cross-species passive protection was not provided when the antibodies were incubated with *Te. circumcincta* or *Haemonchus contortus* L_3. Similarly, serum levels of *Te. circumcincta* L_3 somatic antigen (*Te.c*L3)-specific IgG are associated with protection against GIN, being negatively associated with FEC and positively associated with both adult and lamb survival in wild sheep (Sparks et al. 2020, 2018; Froy et al. 2019). Interestingly, in these studies, serum IgG responses to *Te. circumcincta* L_3 were highly correlated with responses against L_3 antigen prepared from other sheep and mouse GIN (and even non-parasitic soil-dwelling nematodes), suggesting the antibodies largely targeted conserved pan-nematode antigens (Froy et al. 2019). However, it is currently unclear what antigen targets these cross-reactive antibodies recognised, and whether they contribute to immune protection.

In order for an immune response to be triggered, the pathogen must first be detected. Mouse models indicate that intestinal tuft cells sense and initiate the Th2-type response against GIN infection (Gerbe et al. 2016; Howitt et al. 2016). These cells increase in number in the ovine abomasum in response to *Te. circumcincta* infection and possess conserved signalling pathways with murine tuft cells, suggesting they play a similar function in parasite sensing (Hildersley et al. 2021). Subepithelial dendritic cells may also play an important role in the recognition of GIN infection within the abomasum, with glycan moieties potentially influencing T helper cell polarization (McNeilly, Devaney, and Matthews 2009). Communication between immune tissues across mucosal sites is well described (Iijima, Takahashi, and Kiyono 2001), however there is a degree of compartmentalization in immunity within the gastrointestinal (GI) tract (Brown and Esterházy 2021; Mowat and Agace 2014; Agace and McCoy 2017) and it is unclear to what extent the ability to detect and generate responses against co-infecting GIN are compartmentalized within the GI tract.

Investigating this cross-protective immunity between sites within the GIT can be challenging due to the aboral flow of immune effectors in the mucus and digesta. In this study we utilised the unidirectional flow of GI contents to test for the presence of cross-reactive antibodies 'upstream' in the abomasum in response to *Tr. colubriformis* infection. We then evaluated the influence of glycan moieties on their antigenicity and performed proteomics on purified antigens to identify the cross-reactive protein targets. Finally, we used RNA sequencing to investigate whether *Tr. colubriformis* infection induces a cellular response in the abomasum that could mediate antagonistic interactions between these species.

Methods

Animal trials

This study utilised samples obtained from two experimental infections, A & B (Figure 20). In A, three groups of female Texel-cross (TX) lambs (aged c. 7 months at day 0) were used. The first group (Group A1 – Control) consisted of 5 lambs raised in conditions to avoid pre-exposure to helminth parasites (helminth-naïve). In the second group (Group A2 – *Tr. colubriformis* Infected) 8 helminth-naïve lambs were trickle infected with 2,000 *Tr. colubriformis* third stage larvae (L_3) suspended in water, administered orally three times weekly (Mon, Weds, Fri) for 16 consecutive weeks. Anthelmintic treatment (7.5mg/kg levamisole *per os*) was then administered on day 110, followed by a larger bolus challenge of 10,000 *Tr. colubriformis* L_3 on day 117. The third group (Group A3 – *Te. circumcincta* Infected) comprised 8 helminth naïve lambs infected exactly as Group A2, only with *Te. circumcincta* larvae instead of *Tr. colubriformis* larvae. All animals were then killed on day 132 by intravenous overdose of barbiturate (Pentoject, Animalcare).

Experient B also used two groups of helminth-naïve female lambs (aged c. 8mo at day 0). In the first group (Group B1 – Control) 6 helminth naïve TX lambs were used as uninfected control animals. The second group (Group B2 – Infected) was a group of 6 helminth naïve TX lambs that were trickle infected with 2000 *Tr. colubriformis* third stage larvae (L_3) three times weekly (Mon, Weds, Fri) for 12 consecutive weeks and then all animals were killed by intravenous overdose of barbiturate (Pentoject, Animalcare).

In addition, venous blood samples and faecal samples were taken regularly throughout both trials (Figure 20).

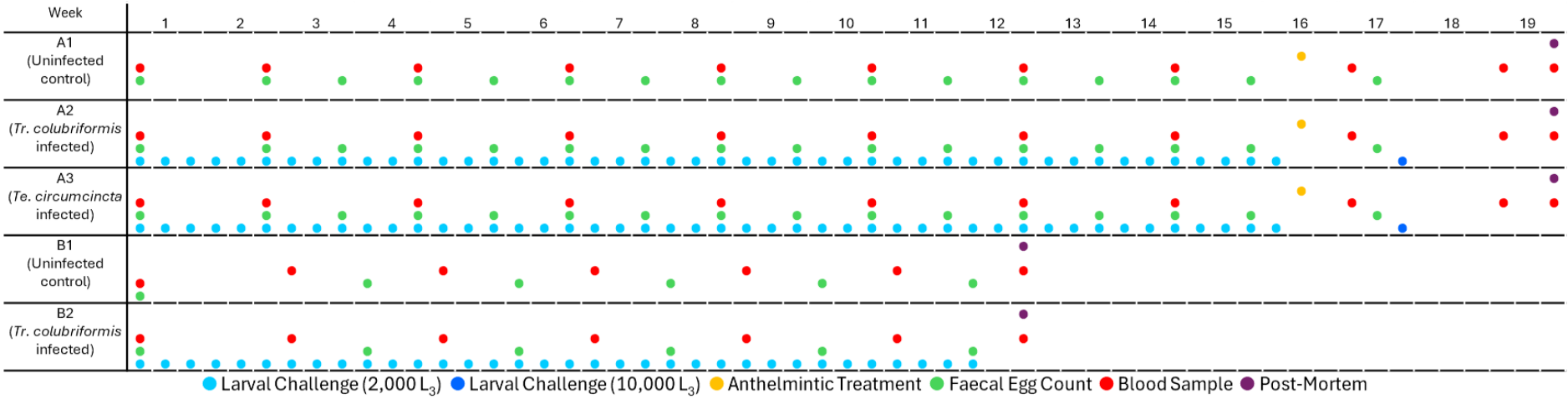


Figure 20: Experimental timetable for animal trials A and B.

*In **Group A1**, 5 helminth-naïve lambs were maintained in conditions to avoid exposure to helminths for 19 weeks. During that time, they were subject to regular Faecal Egg Counts (FECs) and venous blood-sampling, and an anthelmintic treatment (7.5mg/kg levamisole per os) on day 110. All lambs were then killed at the end of week 19. In **Group A2**, 8 helminth-naïve lambs were subject to the same procedures as Group A1, with the addition of a trickle infection of 2,000 Trichostrongylus colubriformis third stage larvae (L3) suspended in water, administered orally three times weekly for the first 16 weeks, followed by larger bolus challenge of 10,000 Tr. colubriformis L3 on day 117. In **Group A3**, 8 helminth-naïve lambs were subject to the same procedures as Group A2, except using Teladorsagia circumcincta L3 rather than Trichostrongylus colubriformis L3.*

*In **Group B1**, 6 helminth-naïve lambs were maintained in conditions to avoid exposure to helminths for 12 weeks. During that time, they were subject to regular Faecal Egg Counts (FECs) and venous blood-sampling. All lambs were killed at the end of week 12. In **Group B2**, 6 helminth-naïve lambs were subject to the same procedures as Group B1, with the addition of a trickle infection of 2,000 Trichostrongylus colubriformis third stage larvae (L3) suspended in water, administered orally three times weekly.*

Faecal Egg Counts

Strongyle-type faecal eggs in each faecal sample were counted using a modified salt flotation technique with a sensitivity of 1 egg per gram (epg) (Jackson 1974; Hayward et al. 2019).

Mucus sampling

In experiment A, the abomasum and first three metres of small intestines were separately removed from each carcase immediately after killing. The abomasal and small intestinal mucosae were rinsed using 0.9% w/v saline at 37°C and the surfaces swabbed with gentle pressure using 10mm diameter cotton dental rolls (one roll for the abomasum and three for the intestines). The cotton rolls were then each placed into ice-cold PBS + protease inhibitor (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail, Roche), vortexed and placed on ice. The contents mucus and PBS+ protease inhibitor mix were expressed from the dental rolls, filtered (0.2µm), centrifuged (10,000 x *g* for 3 min) and the supernatant retained. Henceforth these supernatants will be referred to as abomasal and small intestinal 'mucus' for simplicity, although they are more precisely a PBS dilution of the water-soluble elements contained within the mucus.

RNA Extraction

For each animal in experiment B, post-mortem tissue samples were taken from the abomasal fundus, the pylorus and the proximal first three metres of the small intestine. Three spatially distributed samples ($1cm^2$) were taken from each site and stored in 5ml RNAlater (ThermoFisher) prior to RNA extraction, resulting in a total of 108 samples.

During the necropsy of one of the infected sheep an encapsulated abscess adhered to the body wall adjacent to the abomasum and with associated omental thickening was identified. This was presumed to be the result of a penetrating injury unrelated to the challenge infection

as a skin lesion was present on the exterior surface. Data from this animal were subsequently excluded (see RNA sequencing results).

Total RNA was extracted from 30mg tissue samples by homogenisation in RLT buffer (Qiagen) using a Precellys bead basher (Bertin Instruments) with Precellys CK28 bead tubes (Bertin Technologies) at 5000 oscillations per minute for 15 seconds. Samples were centrifuged at 14,000 × *g* at 4°C for 10min and RNA extracted from the supernatants using RNeasy Mini Kits (Qiagen) according to the manufacturer's instructions, including on-column DNAse I digestion. RNA yield was estimated using a NanoDrop spectrophotometer (ThermoFisher), and RNA integrity was assessed using RNA 6000 Nano Chips (Agilent) and an Agilent 2100 Bioanalyzer (Agilent). RNA from triplicate samples from each site were then pooled at a 1:1:1 ratio such that each contributed an equal quantity of Total RNA to each pooled sample.

Te. circumcincta antigen preparation

Third and fourth stage larval somatic antigens (L_3SA and L_4SA) were prepared by homogenising L_3 or L_4 larvae in ice-cold PBS, centrifuging and extracting the supernatant that contains the PBS-soluble somatic antigens, as previously described (Nisbet et al. 2009). These solutions were filtered through a 0.45µm filter and then concentrated using Amicon® Ultra 10 kDa MWCO centrifugal filters (Merck). Fourth stage larval excretory-secretory products (L_4ES) were produced as previously described (McNeilly et al. 2013) and third stage larval excretory-secretory products (L_3ES) were produced as previously described for *Ostertagia ostertagi* (Geldhof et al. 2000; Faber et al. 2022).

Measurement of *Te. circumcincta*-specific antibody responses

Levels of *Te. circumcincta* antigen-specific IgG, IgA and IgE in the small intestinal and abomasal mucus samples were assessed by indirect ELISA as previously described (Nussey et al. 2014).

All washes were carried out five times with Tris-buffered saline + 0.5% of Tween 20 (TBST) and incubations at 37°C for 1 h. Briefly, Immulon® 2 HB Flat Bottom MicroTiter® Plates (Thermo Scientific) were coated overnight at 4°C with 2µg/ml of antigen in 0.05M carbonate buffer for each of four *Te. circumcincta* antigens (L_3SA, L_3ES, L_4SA and L_4ES). These were incubated with abomasal mucus and intestinal mucus from *Tr. colubriformis* infected animals (group A2) and helminth-naïve controls (group A1) diluted 1:1 with TBST. For all ELISAs three blank controls (TBST only) were included on each plate. Bound antibodies were detected using: HRP-conjugated polyclonal rabbit anti-sheep IgG (H+L) antibody (BioRad); HRP-conjugated polyclonal rabbit anti-sheep IgA antibody (BioRad); or unconjugated monoclonal anti-sheep IgE antibody (clone 2F1, Moredun Research Institute) followed by HRP-conjugated monoclonal goat anti-mouse IgG1 antibody (clone GT34, BioRad). Following the final wash, SureBlue TMB substrate was added to all wells and incubated for 5min. The reaction was stopped by the addition of an equal volume of 1M HCl. Absorbance values were read using a spectrophotometer (Tecan) at an optical density (OD) of 450nm.

ELISAs were also performed for all four *Te. circumcincta* antigens as above using serum from the same animals (diluted 1:12,800 in TBST), except for the following variation in the method. After the plates were coated and washed, half of the wells on each plate were then incubated with 50µl 0.02M sodium periodate in sodium acetate buffer at 37°C for 1h (to oxidise any glycan moieties), followed by 50µl 0.05M sodium borohydrate for 30mins (Longhi-Browne 2014). The other half of the wells were treated identically, only using sodium acetate buffer without the sodium periodate. Serum from each lamb was used in triplicate for both periodate-treated and non-periodate-treated wells. A pool of serum from *Te. circumcincta* infected sheep (Group A3) was used as a positive control for the effect of the periodate

treatment (the anti-*Te. circumcincta* L_4ES response is known to target the glycosylated cathepsin-F (Longhi-Browne 2014). Detection for these ELISAs was performed for IgG only.

ODs were corrected for each plate by subtracting the mean OD of the blank controls. For all ELISAs each set of replicates was contained within a single microtiter plate, therefore positive controls for inter-plate variation were not used. The means of the blank-corrected replicates were then taken and visualised in RStudio (version 2024.12.0) using the 'ggplot2' (Wickham 2009), 'cowplot' (Wilke 2018) and 'ggh4x' (van den Brand 2025) packages.

Western blotting of *Te. circumcincta* antigens

Each of the four *Te. circumcincta* antigens (L_3SA, L_3ES, L_4SA and L_4ES) were first denatured in LDS-buffer (Invitrogen) with 50mM DTT (Invitrogen) at 70°C for 10mins. They were then electrophoresed on NuPAGE® Bis-Tris 4–12% gels (loaded with 5µg protein per lane) under reducing conditions employing NuPAGE® MES SDS running buffer (Invitrogen). Antigens were transferred to nitrocellulose membranes according to the manufacturer's instructions (Invitrogen), with Ponceau-S solution (Thermo Scientific) staining to ensure successful transfer. After transfer membranes were washed in tris buffered saline with 0.5% Tween (TBST) and incubated for 1 hour with 5% (w/v) soy bean powder (SMA Nutrition) in TBST to block non-specific protein adsorption. Membranes were incubated overnight with abomasal mucus (diluted 1:20 in TBST), intestinal mucus (diluted 1:20) or serum (diluted 1:20 for IgA detection and 1:2000 for IgG detection), pooled from either *Te. colubriformis* infected animals (Group A2) or control animals (Group A1). Blots were washed and probed with either: HRP-conjugated monoclonal mouse anti-ovine IgG (clone GT-34, Sigma Aldrich, diluted 1:1000 in blocking buffer); or unconjugated mouse anti-ovine IgA (BioRad, diluted 1:5000) followed by HRP-conjugated polyclonal rabbit anti-mouse Ig (Dako, diluted 1:1000). Blots were then

washed again and peroxidase activity detected using Clarity Western ECL Substrate according to the manufacturer's instructions (BioRad).

Isolation of cross-reactive *Te. circumcincta* antigens by affinity chromatography and immunoprecipitation

IgG was first purified by affinity chromatography (HiTrap™ Protein G HP 1ml resin columns, Cytiva) on an ÄKTA go™ liquid chromatography system - Cytiva) from 1ml pooled serum, taken at post-mortem from either *Tr. colubriformis* lambs (Group A2), uninfected control lambs or *Te. circumcincta* infected lambs (Group A3). Each purified IgG was buffer exchanged to binding buffer (0.02M sodium phosphate, 0.5M NaCl solution pH7.4) using a HiTrap™ Desalting 5ml column (Cytiva). This yielded 4.8mg purified IgG from control animals, 6.68mg purified IgG from *Tr. colubriformis* infected animals and 6.80 mg purified IgG from *Te. circumcincta* infected animals as estimated using a Pierce BCA Protein Assay kit according to the manufacturer's instructions (Invitrogen).

An immunoaffinity column was prepared by binding 400mg *Te. circumcincta* L_3SA to a 1ml HiTrap™ NHS-Activated HP affinity column according to the manufacturer's instructions (Cytiva). L_3SA reactive IgG was then purified from control (Group A1), *Tr. colubriformis* infected (Group A2), and *Te. circumcincta* infected (Group A3) animals by passing 1.2mg of each purified IgG through this affinity column using an ÄKTA go™ FPLC (Cytiva). Following washing of columns with binding buffer, bound IgG was eluted in 0.1M glycine, 0.15M NaCl solution (elution buffer, pH2.7) and neutralised using 1M Tris-HCl (pH 9). The protein concentration of the eluates was quantified using a NanoDrop spectrophotometer (ThermoFisher). IgG from control animals was processed first, and the column washed

thoroughly between samples using 3 column volumes (cv) binding buffer, then 3cv elution buffer, then a further 10cv of binding buffer.

Isolated *Te. circumcincta* L_3SA specific antibodies were then incubated with 400mg *Te. circumcincta* L_3SA at RT for 2h with continuous gentle mixing. The antibody/antigen mixtures were then added to Pierce™ Protein G Magnetic Beads (Thermo Scientific) and incubated for 1h at RT. IgG and bound antigen were eluted from the beads using elution buffer (0.1M glycine, pH2.0).

Proteomic identification of cross-reactive *Te. circumcincta* antigens

The three bound fractions were then submitted to the Moredun Proteomics for proteomic identification. Samples were there first trypsin digested using an S-Trap micro column (Protifi) kit, following the manufacturer's instructions. Briefly, 1.5-3 µg of sample was reductively alkylated and bound to the S-Trap column. Contaminants and interfering compounds were removed with 4 washes S-Trap binding/washing buffer (100mM triethylammonium bicarbonate (TEAB) in 90% Methanol). Proteins were digested using sequencing grade trypsin (in 50 mM TEAB) in a 1:10 ratio to trapped protein for 16 hrs at 37°C. Tryptic peptides were eluted in 40µl 50mM TEAB, followed by 40µl 0.2% formic acid (FA), and finally 40µl 50 % acetonitrile (ACN) in water and 0.2% FA. Elutions were pooled and dried before being reconstituted in 20 µl of 0.1% FA.

Liquid chromatography−tandem mass spectrometry (LC−MS/MS) analysis was then performed using a Vanquish nano-LC system (Thermo Fisher Scientific) coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific). The LC system was equipped with an Acclaim Pepmap nano-trap column and an Acclaim Pepmap RSLC C18 EasySpray analytical column (both Thermo Fisher Scientific). The tryptic peptides were first loaded onto the trap

column then separated on the analytical column. The eluents used were 0.1% v/v formic acid (solvent A) in H_2O and 80% v/v CH_3CN in 0.1% v/v formic acid (solvent B). A 25 min gradient was used at 350 nl/ min from (i) 0–5 min, 4-25% B; (ii) 5-20 min, 25- 50% B (iii) 20-30 99% B. Data were acquired in positive mode using a data dependent approach, MS1 scans were acquired at 70,000 resolution over a mass range of m/z 380-1500. In each cycle, the 15 most intense ions were selected for MS/MS and subjected to high-energy collision dissociation (HCD) The dynamic exclusion time was set to 30s.

The MS raw data were processed using the Proteome Discoverer platform (version 2.4, Thermo Fisher Scientific) and Sequest HT algorithm. The MS data were searched against a combined database comprising annotations for *Te. circumcincta* (tci2_wsi3.0, n= 36,038 transcripts, ERZ24880405), *Tr. colubriformis* (nxTriColu_2.1, n=51,954 transcripts, GCA_963978875.1) and *Ovis aries* (ARS-UI_Ramb_v3.0, GCF_016772045.2), plus the Swiss-Prot database (SwissProt_2023_02). A maximum of two missed tryptic cleavages were allowed. The oxidation of Methionine and protein N-terminal acetylation were set as variable modifications, as was the Methylation addition to Cysteines. MS and MS/MS ion tolerances were set at 10ppm and 0.02Da, respectively. A maximum false discovery rate (FDR) of 1% was used at both the peptide and the protein levels. Two protein lists were then produced for each of the three immunoprecipitation products, the first list accepting only proteins identified with ≥2 peptides and the second list including single peptide matches (Gupta and Pevzner 2009). Venn diagrams were then produced illustrating the overlap between the protein lists for the different immunoprecipitations. The protein lists were then filtered to retain only proteins that were precipitated by IgG from *Tr. colubriformis* infected animals and not precipitated by IgG from the uninfected animals. The sequences of the proteins in these lists were then converted to FASTA files and exported into GenomicsBox (BioBam). A BLAST

protein search was performed and the resultant hits searched for in the Gene Ontology (GO) database and annotated with the matching GO terms.

RNA sequencing

Extracted RNA pools were sent to Novogene UK (Cambridge) for further library preparation and sequencing. There, the RNA was poly-A enriched, fragmented (150 bp – 500 bp) and used to produce cDNA libraries. They were then sequenced on an Illumina NovaSeq (150 bp paired end reads) to a minimum depth of 30 million paired end reads per sample (mean depth 34.3million). Raw reads were then filtered to remove reads containing adapters, reads containing more than 10% undetermined bases, and reads containing >50% low quality base (Qscore<= 5). The percentage of clean reads retained after filtering ranged from 98.73% to 99.37%. These clean reads were then returned as fastq files.

The cleaned sequences were the assessed for quality using fastQC (Andrews 2010). An index file was generated in Kallisto (v0.46.2) (Bray et al. 2016) using the annotated *Ovis aries* Rambouillet v2.0 cDNA assembly published at Ensembl. The reads were then then pseudo-aligned against that index (again with Kallisto) and read abundances estimated calculated as transcripts per million. These estimated read counts were then imported into R using the 'tximport' package (Soneson, Love, and Robinson 2015) and filtered to remove genes with fewer than 10 counts in total. The counts of the top 500 most variable genes were visualised by PCA plots after variance stabilising transformation with the 'DESeq2' package (Love, Huber, and Anders 2014).

Statistical analyses

Antibody data were analysed in R using Mann-Whitney tests, with the resultant P-values corrected for false discovery using the Benjamini-Hochberg (BH) algorithm. The differences

in antibody responses between infected and control animals were assessed for each combination of the four antigens and two periodate treatment levels (treated vs not treated) by one-tailed tests (alternative hypothesis: infected animals have higher antibody levels than controls). The effect of periodate treatment was assessed using paired one-tailed Mann-Whitney tests (alternative hypothesis: periodate treatment decreases affinity of IgG in serum from infected animals against all four antigens), again BH-corrected.

For RNAseq analysis, differential expression was assessed using a negative binomial glm in the 'DESeq2' package (Love, Huber, and Anders 2014) with a single six-level factor of all combinations of organ location and animal infection group as the only fixed effect. Significant genes were identified by Wald testing with BH-adjustment. Genes with adjust P-values <0.05 and with log2 fold change >1 and <-1 were considered significant. Functional enrichment analysis was then performed using gene ontology (GO) terms in g:Profiler (Kolberg et al. 2023) with FDR set at 0.05.

Results

Faecal Egg Counts

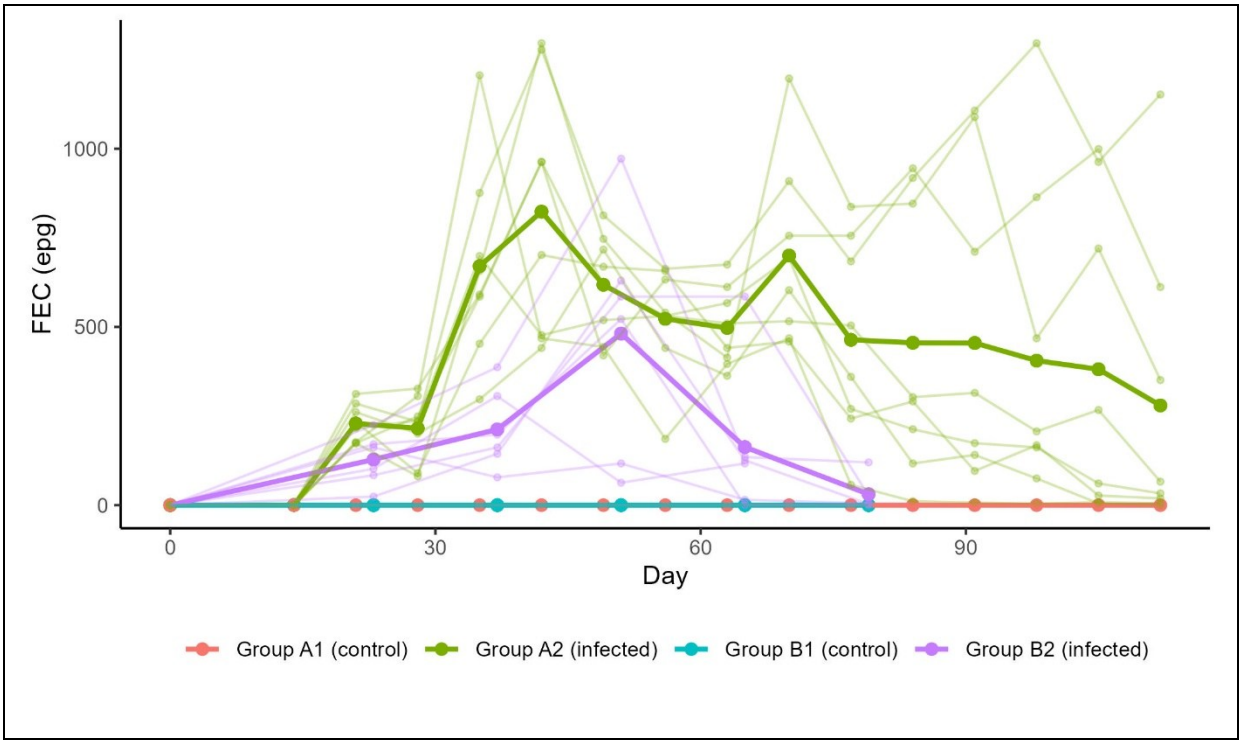


Figure 21: Faecal Egg counts (FEC) against time for the Tr. colubriformis infected sheep and helminth naïve controls in trial A (up to the day of anthelmintic administration) and Trial B (entire trial). The lighter points and lines show raw data, whilst the heavier points and lines show the group means. Group A1 (n=5) and Group B1 (n=6) are uninfected control lambs. The trickle infections methods are explained in the methods and illustrated in Figure 20, but briefly, all lambs in Group A2 (n=8) and Group B2 (n=6) were trickle infected with 2000 Trichostrongylus colubriformis third stage larvae (L3) suspended in water, administered orally three times weekly for either 16 weeks (Group A2) or 12 weeks (Group B2).

All animals were confirmed to be helminth free at the start of the trial (FEC = 0epg). All animals in the infected group had patent infections three weeks after the start of the trickle infection. In experiment B, FECs peaked at week 7 of the trickle infection and by week 11 had declined, suggesting development of an effective immune response (Figure 21). FEC results from

experiment A were similar, although FECs remained high beyond week 11 for three out of the eight infected animals.

Tr. colubriformis infection induces mucosal antibodies which cross-react with *Te. circumcincta* antigens.

Infection with *Tr. colubriformis* induced the production of mucosal IgG and IgA that cross-reacted against all four *Te. circumcincta* antigens. Cross-reactive IgA responses were confined to the site of the *Tr. colubriformis* infection (the small intestine) but the IgG responses were present both in the small intestinal mucus and also upstream in the abomasal mucus (Figure 22A). These cross-reactive IgG responses were also detectable in the serum of *Tr. colubriformis* infected animals (Figure 22B). Both IgG locations and the intestinal IgA showed the same pattern of effect sizes across antigens with the highest ODs for L_3-SA, followed by L_3ES, then L_4SA and L_4ES. There was no evidence of any cross-reactive IgE present in either the abomasal mucus or the intestinal mucus. Periodate treatment of the four antigens only reduced anti-L_4-SA reactivity, suggesting that cross-reactive IgG was largely directed against non-glycan targets (Figure 22C).

Western blotting was subsequently performed to investigate which *Te. circumcincta* antigens were recognised by the cross-reactive antibodies. The pattern of immunoreactive bands were comparable among the three sample types (abomasal mucus, small intestinal mucus and serum), within each antigen and isotype combination (Figure 23). Although the molecular weights of immunoreactive bands were comparable between immunoglobulin types, the intensities of each band did vary (in line with the estimated antibody activity seen in the mucus ELISAs). The comparable banding patterns and ELISA results suggested serum and

mucosal IgG responses were directed to similar antigens and permitted circulating serum IgG to be used as a proxy for abomasal mucosal IgG in the subsequent immunoprecipitations.

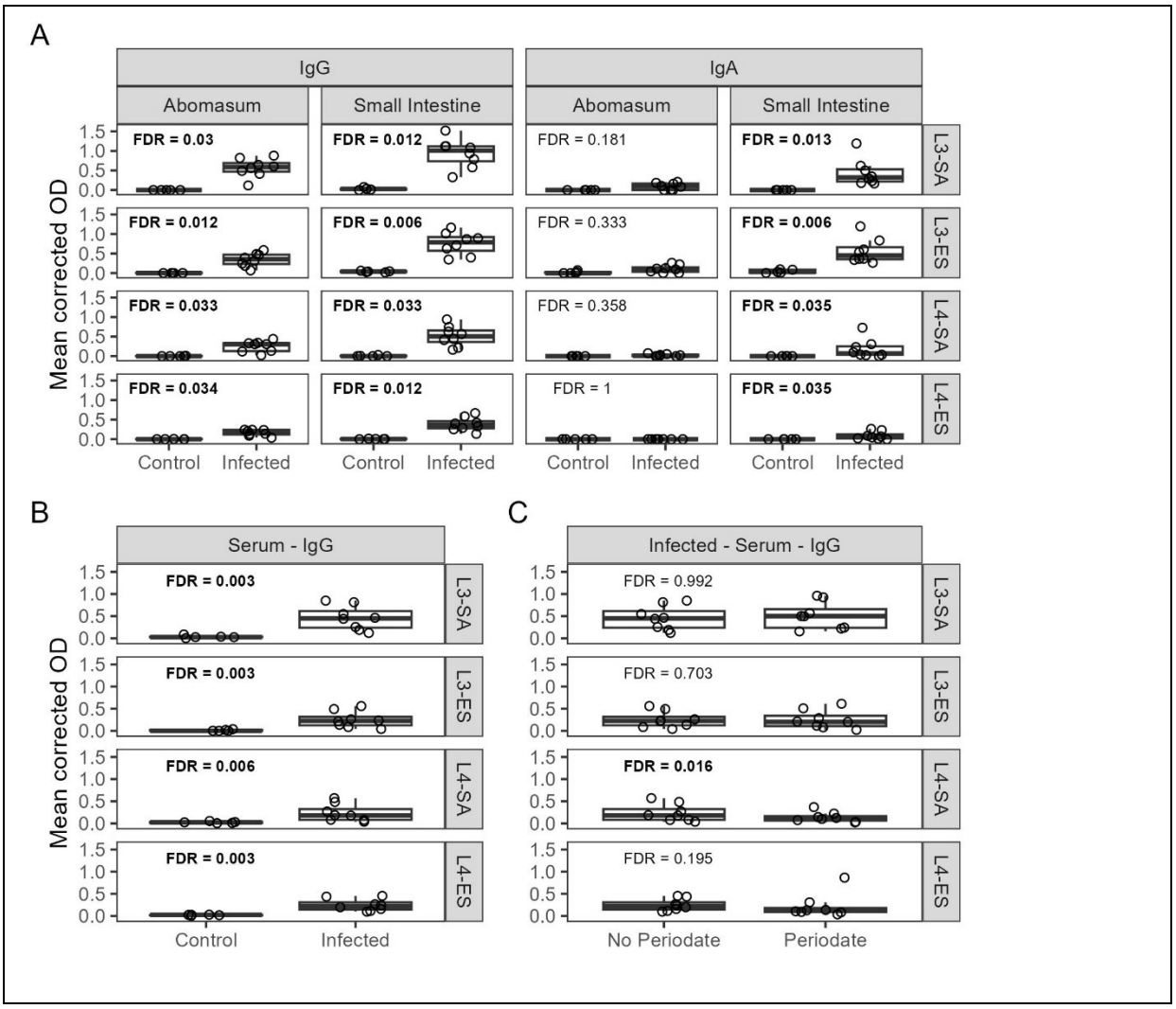


Figure 22: Box and whisker plots showing the ELISA results, with BH-corrected results of the Mann-Whitney tests are shown within each panel. A: Levels of mucosal IgG and IgA that bind each of four Te. circumcincta antigens from uninfected lambs (Group A1, n=5) versus Tr. colubriformis infected lambs (Group A2, n=8) (IgE data not shown.). B: Levels of serum IgG that bind each of four Te. circumcincta antigens from uninfected lambs (Group A1, n=5) versus Tr. colubriformis infected lambs (Group A2, n=8). C: The effect of periodate treatment on serum IgG responses against each of the four Te. circumcincta antigens from Tr. colubriformis infected lambs (Group A2, n=8).

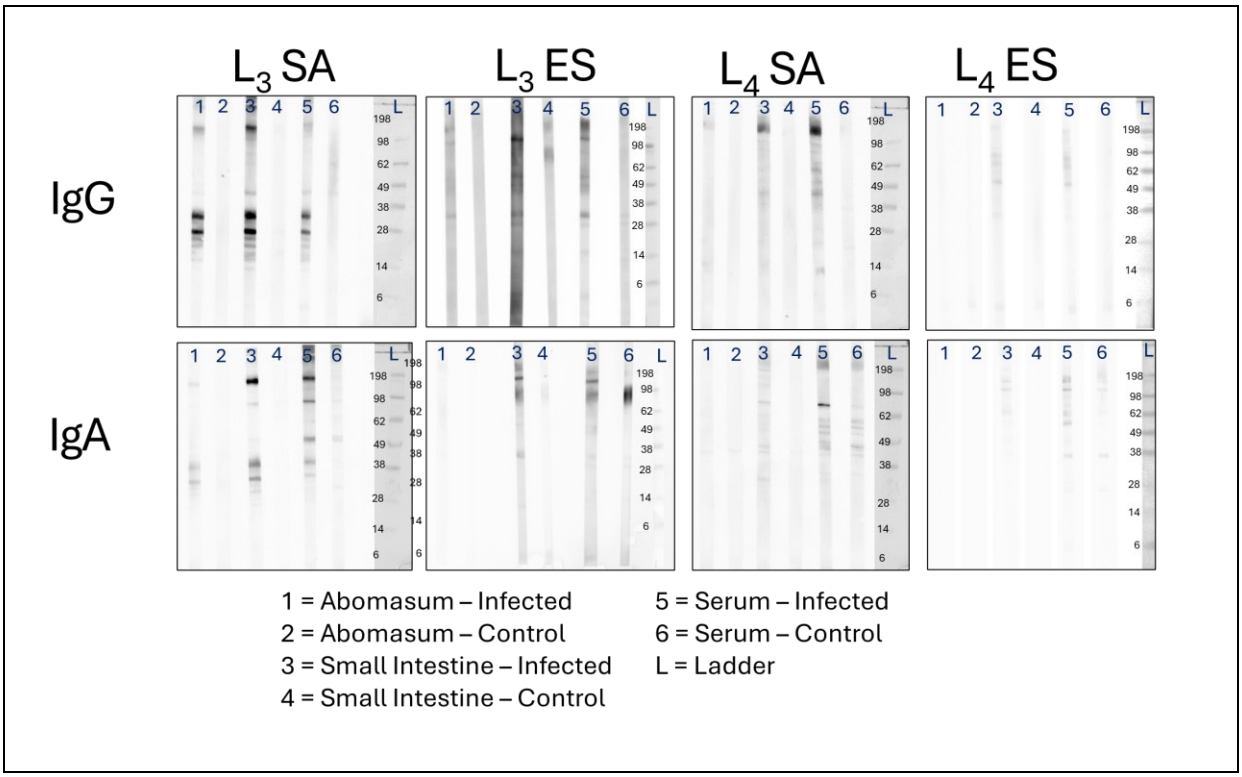


Figure 23: Western blots against each of four Te. circumcincta antigens after LDS+DTT denaturing (horizontal panels). Membrane strips were probed with abomasal mucus, small intestinal mucus or serum (taken at the point of killing), pooled from all individual animals in the uninfected control group or the Tr. colubriformis infected group. Chemiluminescent detection used either anti-IgG (top panel) or anti-IgA (bottom panel) antibodies. Approximate ladder weights (kDa) shown in the alongside the ladder.

Identification of *Te. circumcincta* antigens recognised by cross-reactive IgG

Proteomic analysis identified six *Te. circumcincta* proteins (with ≥ 2 peptide matches) that were precipitated by the IgG from the *Tr. colubriformis* infected animals but not by the IgG from the uninfected control animals. A total of 17 proteins were identified when this list was expanded to include proteins with single peptide matches (Table 10). All 17 of these proteins

were also precipitated by the IgG from the *Te. circumcincta* infected animals (Figure 24A). The molecular weights of the proteins identified by proteomics closely matched the approximate molecular weights of the immunoreactive bands identified by Western blot (Figure 24B).

Of the proteins identified, all but two were able to be linked to GO terms (Table 10). These GO terms indicated internal metabolic and structural proteins that would be unlikely to be expressed on the exterior surface of the L_3.

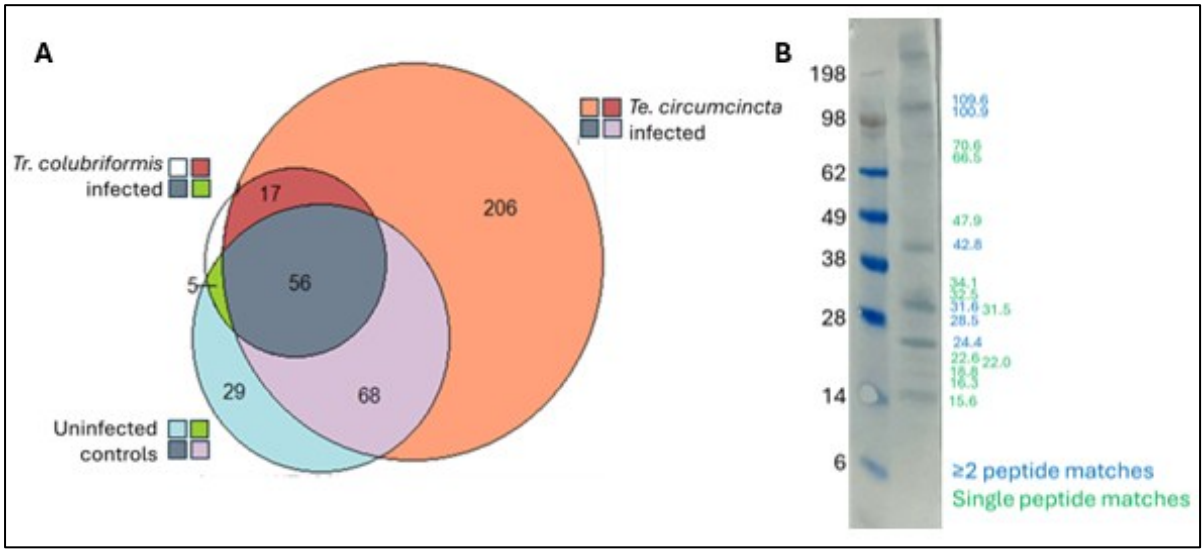


Figure 24 A: Venn Diagram illustrating the overlap in the list of proteins (including single peptide matches) precipitated from Te. circumcincta L_3SA by IgG purified from uninfected control animals, Te. circumcincta infected animals and Tr. colubriformis infected animals. B: A western blot of Te. circumcincta L_3-SA probed with serum from Tr. colubriformis infected animals and detected with anti-IgG antibodies (NB, this blot was developed using Diaminobenzidine and $CoCl_2$ (SIGMAFAST™, Sigma-Aldrich) instead of ECL). The molecular weights (kDa) of the proteins identified by proteomics are shown on the right-hand side of the western blot in their approximate positions relative to the ladder (on the left-hand side).

Table 10: Gene Ontology (GO) terms associated with the proteins identified by mass spectrometry that were present in the immunoprecipitation of Te. circumcincta L_3-SA using antigen-specific IgG derived from Tr. colubriformis infected animals and not from helminth-naïve animals

Protein	Associated Gene Ontology (GO) terms	Peptide Matches
Calponin family repeat-containing domain protein	P:actin filament organization; F:actin filament binding; C:actin cytoskeleton	≥2
Hypothetical protein	None identified	≥2
Hypothetical protein	C:mitochondrion; C:mitochondrial inner membrane	≥2
MFS transporter, SP family	P:hexose transmembrane transport; P:monosaccharide transmembrane transport; P:transmembrane transport; F:hexose transmembrane transporter activity; F:transmembrane transporter activity; C:membrane	≥2
Myosin tail	P:muscle contraction; P:actin filament organization; P:sarcomere organization; F:microfilament motor activity; F:cytoskeletal motor activity; F:actin binding; F:ATP binding; F:actin filament binding; C:cytoplasm; C:bicellular	≥2

	tight junction; C:actin cytoskeleton; C:membrane; C:myosin complex; C:myosin II complex; C:myofibril; C:sarcomere; C:myosin filament	
Ribosomal protein L6e	P:ribosomal large subunit assembly; P:cytoplasmic translation; P:translation; F:RNA binding; F:structural constituent of ribosome; C:ribosome; C:cytosolic large ribosomal subunit	≥2
ATP synthase	P:proton motive force-driven ATP synthesis; P:proton transmembrane transport; F:ATP binding; F:ADP binding; F:proton-transporting ATP synthase activity, rotational mechanism; C:proton-transporting ATP synthase complex	1
Carboxyl transferase domain protein	F:propionyl-CoA carboxylase activity; F:transferase activity; C:mitochondrion	1
EF hand	F:calcium ion binding	1
EF hand	F:calcium ion binding; C:muscle myosin complex	1
Galactoside-binding lectin	F:galactoside binding; F:carbohydrate binding	1
Hsp20/alpha crystallin family protein	P:response to heat; P:protein refolding; F:unfolded protein binding; C:nucleus; C:cytoplasm	1

Hypothetical protein	P:proton motive force-driven ATP synthesis; P:proton transmembrane transport; F:proton transmembrane transporter activity; C:mitochondrial inner membrane; C:proton-transporting ATP synthase complex	1
Hypothetical protein	P:regulation of muscle contraction; P:sarcomere organization; F:tropomyosin binding; C:troponin complex	1
Hypothetical protein	C:mitochondrion	1
Hypothetical protein	None identified	1
Intermediate filament tail domain protein	P:nuclear envelope organization; P:cytoskeleton organization; P:nuclear migration; P:heterochromatin formation; P:nuclear pore localization; P:protein localization to nuclear envelope; F:structural constituent of cytoskeleton; C:nuclear lamina; C:intermediate filament	1
Ribosomal l18p/L5e family protein	P:ribosomal large subunit assembly; P:translation; F:structural constituent of ribosome; F:5S rRNA binding; C:cytosolic large ribosomal subunit	1

Ribosomal protein S11	P:translation; F:structural constituent of ribosome; C:ribosome; C:ribonucleoprotein complex	1
Succinate dehydrogenase, flavoprotein subunit	P:tricarboxylic acid cycle; P:mitochondrial electron transport, succinate to ubiquinone; F:succinate dehydrogenase (quinone) activity; F:electron transfer activity; F:flavin adenine dinucleotide binding; C:mitochondrial inner membrane	1

Tr. colubriformis induces differential gene expression within the abomasal mucosa

All 36 pooled samples (one for each of the three locations from each of the animals in groups B1 and B2) yielded high-quality sequence data with at least 30 million reads per sample, of which at least 5 million were unique per sample. Phred score was high (>37) across all base positions of all samples (Phred score of 37 corresponds to a base-call error probability of 0.0002). GC content per sample ranged from 47% to 54% with approximately normal distributions of GC content across all sequences in each sample. The most common over-represented sequence was a 50-bp poly-G sequence that comprised 0.1% of all reads. There were no identifiable (e.g. ribosomal) over-represented sequences in any samples.

PCA analysis of the variance stabilising transformed gene counts for the top 500 most variable sequences showed the samples to cluster principally by tissue type, followed by infection

status, with the separation between infected and control samples more pronounced for small intestinal samples (Figure 25). The pyloric sample from animal the animal with the peritoneal abscess was separated from the other pyloric samples along PC2, therefore all samples from that animal were excluded from further analyses.

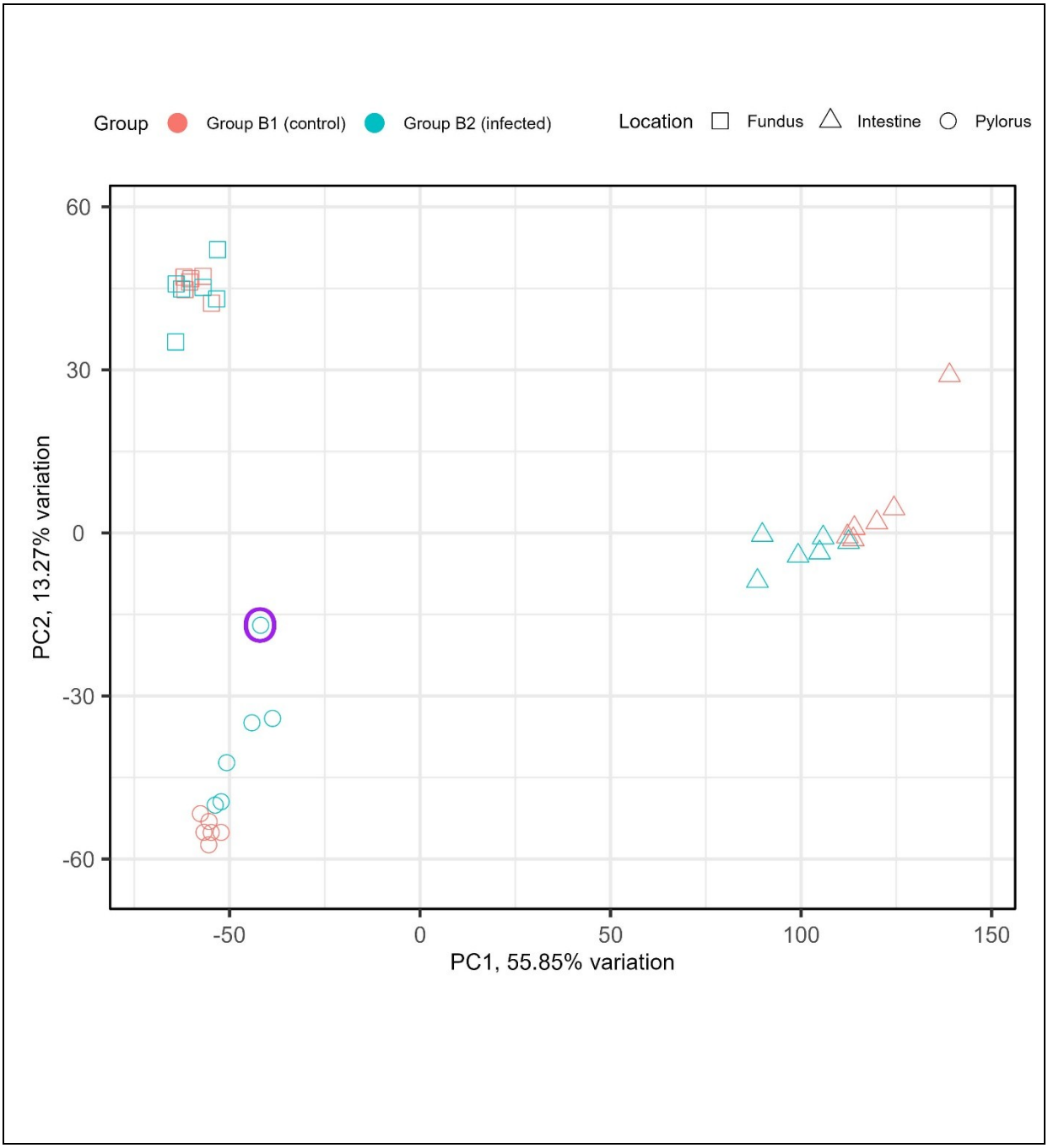


Figure 25: PCA plot for all top 500 most variable RNA sequences, after normalisation by variance stabilising transformation. Points are coloured according to infection status (Group B1 = uninfected, Group B2 = Tr. colubriformis infected) and shaped according to the anatomical location of the tissue sample. The outlying pyloric sample from the animal with the abscess is indicated by the purple ring.

Infection with *Tr. colubriformis* induced significant upregulation of 9 genes in the abomasal fundus, 157 genes in the pylorus and 392 genes in the small intestine, alongside downregulation of 44 genes in the pylorus and 391 genes in the small intestine (Figure 26). Within the site of infection (the small intestine), functional enrichment analysis revealed significant upregulation of many wound-healing and collagen binding pathways. Functional enrichment analysis of abomasal samples indicated significant upregulation IL-5 receptor activity in the fundus and upregulation of several immune processes in the pylorus (Table 11). Specific genes of interest that were significantly upregulated upstream of the site of infection (pylorus or fundus) but not specifically highlighted by the gene ontology analysis include: *clca1* (mucus production) in both the fundus and pylorus; IL-1 and IL-17 receptors and IL-13 (immune response) in the pylorus; and *trpm5* and *pou2af2* (tuft cell markers) in the pylorus (see Appendix II for complete lists of differentially expressed genes).

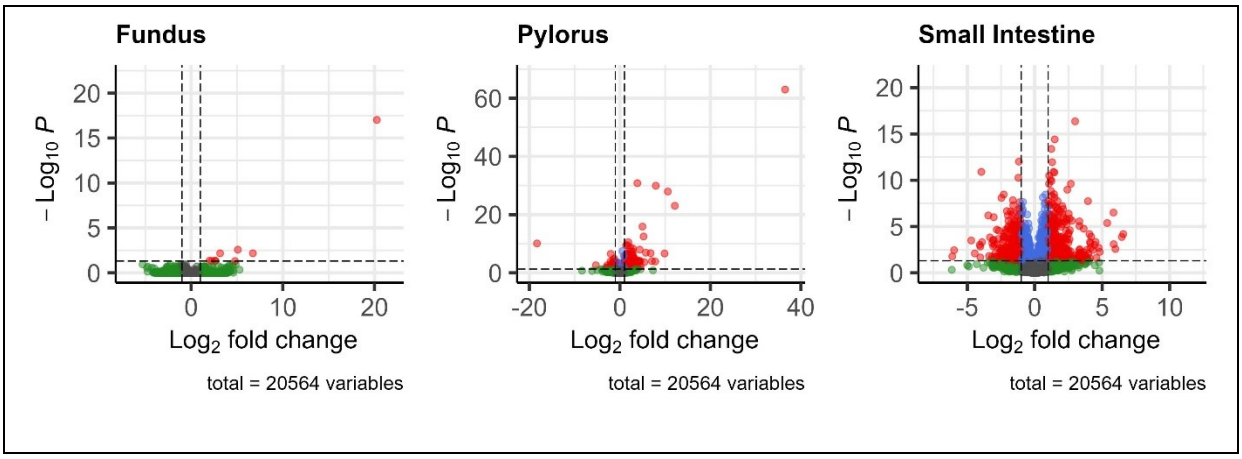


Figure 26: Volcano plots showing genes that were up- and downregulated in Tr. colubriformis infected animals (Group B2) compared to uninfected helminth-naïve animals (Group B1) for each tissue location. (Fundus: 9 differentially expressed genes (DEGs); Pylorus 201 DEGs; Small intestine 783 DEGs.) FDR was set at 0.05 and the absolute log_2 fold change threshold set at 1. Red points represent genes with FDR≤0.05 and absolute log_2-fold change ≥1. Blue points represent genes with FDR≤0.05 but absolute log_2-fold change <1. Green points represent

genes with FDR>0.05 but absolute log_2-fold change ≥1. Grey points represent genes with FDR>0.05 and absolute log_2-fold change <1.

Table 11: Gene ontology (GO) terms highlighted by functional expression analysis of the genes differentially expressed in each location in response to Tr. colubriformis infection. Table shows 'driver' terms selected by g:Profiler (Kolberg et al. 2023).

Fundus - upregulated	**Fundus - downregulated**
Interleukin-5 receptor activity	None
Pylorus - upregulated	**Pylorus - downregulated**
Immune receptor activity	Neuropeptide y receptor activity
Symporter activity	Multicellular organismal process
Solute:sodium symporter activity	Extracellular region
Immunoglobulin binding	
Immune system process	
Mast cell degranulation	
Leukotriene biosynthetic process	
Bone mineralization	
Cellular response to cytokine stimulus	
Side of membrane	
Extracellular region	
Small intestine - upregulated	**Small intestine - downregulated**
Collagen binding	Lipid transporter activity

Glycosaminoglycan binding	Oxidoreductase activity
Integrin binding	Solute:sodium symporter activity
Proteoglycan binding	Small molecule metabolic process
Protease binding	Plasma lipoprotein particle assembly
Multicellular organismal process	Sterol homeostasis
Wound healing	Lipid storage
Cell-substrate adhesion	Olefinic compound metabolic process
Extracellular matrix organization	Lipid localization
Actin cytoskeleton organization	Membrane
L-amino acid metabolic process	Brush border
Proteinogenic amino acid metabolic process	Extracellular region
	Peroxisome
Regulation of actin filament organization	
Synapse organization	
Extracellular region	
Cell periphery	
Cell surface	
Sarcoplasm	
Collagen trimer	
Cell junction	
Excitatory synapse	
Spectrin-associated cytoskeleton	
Axon	

Discussion

These results confirm previous findings that infection with *Tr. colubriformis* induces secretion into the intestinal mucus of both IgG and IgA that cross react with *Te. circumcincta* L_3SA (Harrison et al. 2008; A. R. Williams et al. 2010). In addition, there were also IgG and IgA responses against L_3ES, L_4SA and L_4ES, with lower magnitude responses against the L_4 antigens. This is consistent with the idea that the earlier larval stages are relatively conserved (given their similar life histories) but diverge once they infect the host and grow and adapt to their specific host niches (Lu et al. 2020).

The molecular weights of immunoreactive bands were comparable between mucus and serum for all antigens and for both IgG and IgA. These findings are consistent with work reporting that the majority of mucusal IgG in ruminants is serum-derived (Cripps, Husband, and Lascelles 1974; McNeilly et al. 2007) and the majority of serum anti-nematode IgA is mucus derived (De Cisneros et al. 2014) and supports the use of serum antibody titres for monitoring *in vivo*, and the use of serum antibodies as a proxy for mucous antibodies in *ex vivo* work.

However, IgA responses against all four antigens were restricted to the small intestinal mucus and absent from the upstream abomasal mucus. Whilst this does not preclude an IgA-mediated mechanism for antagonistic interactions between co-infecting GIN, the absence of cross-reactive IgA at the site of infection would at the least lead to a delay whilst plasma cells were recruited/stimulated. However, even then, Harrison *et al.* (2008) showed that the cross-reactive IgA components induced by *Tr. colubriformis* infection do not provide passive protection against *Te. circumcincta* infection.

In contrast, cross-reactive IgG was present upstream in the abomasal mucus, where it is feasible that it could exert an effect on *Te. circumcincta* in a co-infection. The periodate ELISAs indicated that this response was not targeted at glycan moieties. This is consistent with the weakness of the immunoreactive band at approximately 35kDa in the IgG-L_3SA and IgA-L_3SA western blots, which may correspond to low concentrations of anti-CarLA antibodies (Harrison, Pulford, Hein, Severn, et al. 2003b). The reason for this difference is unclear but perhaps indicates the influence on the immune response of the host genotype (Harrison et al. used Romney sheep rather than Texel-cross), the age of sheep (4-month-old lambs rather than 7-month-old lambs) or the experimental design (several truncated bolus infections rather than a truncated trickle followed by a bolus).

The lack of glycan moieties was helpful in our study, as it facilitated the use of proteomics to identify the antigens targeted by cross-reactive antibodies. Antigen yields from the immunoaffinity chromatography were relatively low, which may suggest that the cross-reactive antibodies are low affinity, and affinity testing would be a worthwhile future direction. Proteomic analysis of proteins bound by cross-reactive antibodies identified proteins with predicted molecular weights closely correlated with the molecular weights of immunoreactive bands identified by western blotting, validating the proteomic data. Those results revealed the *Te. circumcincta* L_3SA components antigens bound by IgG from *Tr. colubriformis* infected animals and absent in helminth-naïve control animals were dominated by internal structural or metabolic proteins (irrespective of the number of peptide matches used). These proteins would likely be inaccessible to antibodies during infection and consequently, cross-reactive IgG identified in this study are unlikely to play an active role in cross-parasite species resistance. Instead, it is possible that these cross-reactive IgG represent the ability of the host to kill the infecting parasites (via another mechanism) and expose

internal antigens to the host immune system. This would partly explain the negative correlation between L3-specific IgG and FEC in other studies (Sparks et al. 2018; Froy et al. 2019). Also, given their structural and metabolic functions, these proteins may be highly evolutionarily conserved, hence the antigenic cross-reactivity identified in this and other studies (Cuquerella et al. 1994; Froy et al. 2019). Alternatively, the proteins may simply be more antigenic, given several of them contain repetitive structures (e.g. myosin, intermediate filament tail, and calponin family repeat), and repetitive structures are commonly highly immunogenic (Sauerborn et al. 2010). Phylogenetic orthology inference and linear epitope prediction software could be used to assess the degree of conservation and the predicted antigenicity of these proteins.

RNA sequencing of the intestinal samples showed that wound-healing processes dominated the transcriptomic changes in the site of *Tr. colubriformis*. However, the RNA sequencing of the abomasal samples revealed that there is also differential expression of immune pathways upstream in both the fundus and pylorus. The differential expression of these immune pathways in the abomasum could provide mechanisms for co-infection interactions between *Tr. colubriformis* and abomasal GIN. Upregulation of mucus secretion via *clca*-1, could facilitate the 'weep-and-sweep' response (Nyström et al. 2019). Upregulation of mast cell degranulation pathways would release the multiple bioactive components within mast cell granules associated with inflammatory anti-parasite responses (Wernersson and Pejler 2014), whilst upregulated immunoglobulin binding pathways could trigger a variety of cellular responses including eosinophil and macrophage activation (Hirano et al. 2007; Rainbird, Macmillan, and Meeusen 1998). In mice IL-13 is associated with helminth expulsion (McKenzie et al. 1998) and IL-17 is associated with generating the Th2 response in helminth infection (Allen, Sutherland, and Rückerl 2015). In addition to these effects against

established parasites, tuft cells are involved in sensing helminth infection and initiating the mucosal Th2 response (Gerbe et al. 2016), whilst IL-5 has been associated with preventing establishment of incoming parasite larvae. Together these pathways could increase barriers to larval establishment and speed the initiation of immune responses by placing the upstream mucosal immune system on 'high alert' to a subsequently co-infecting abomasal nematode. Further, these results are consistent with findings from a previous *Te. circumcincta* mono-infection experiment that showed greater cellular responses in the pylorus than the fundus (Stear et al. 1995) and a *Te. circumcincta*/*Tr. colubriformis* co-infection experiment that showed an increase in circulating IL-5 in co-infected animals compared to mono-infected (A. R. Williams et al. 2010). The latter study also found statistically non-significant increases in abomasal eosinophils and globule leukocytes (degranulated mast cells (Huntley, Newlands, and Miller 1984)) in response to *Tr. colubriformis* mono-infection. Therefore, future work should include the quantification of these cell types, alongside tuft cells within the abomasum using histopathological samples also taken during our study but not yet analysed.

Conclusions

This chapter demonstrates that *Tr. colubriformis* infection induces the production of mucosal IgG and IgA which cross-reacts with somatic and excretory-secretory antigens of third and fourth-stage larvae of the abomasal-dwelling parasite *Te. circumcincta*. The cross-reactive IgA was confined to the site of *Tr. colubriformis* infection, but the IgG was also produced upstream in the abomasum, where it could have a biological effect. However, the L_3SA-targetted cross-reactive antibodies recognised conserved structural and metabolic proteins that are unlikely to be exposed in live nematodes and hence unlikely to be effective targets of cross-protective immunity. However, the expression of pathways associated with anti-GIN immunity were also upregulated in both the abomasal fundus and pylorus. These transcriptomic changes could

mediate antagonistic interactions between co-infecting nematodes in different regions of the gastrointestinal tract.

Chapter 6 - General Discussion

"The beginning of wisdom is to call things by their proper name."

Chinese proverb

Through this thesis I have combined scientific approaches across scales - from the population level down to the individual host, then within the host to the interactions between coinfecting parasites, before focusing in yet further on the transcriptomic responses that may drive these interactions. These multiple approaches were guided by each other and addressed specific questions, supported by both theory and prior evidence. This array of techniques has provided complementary insights into the interactions between coinfecting GIN and immunity. These key findings have important implications for both the health of domestic animals and the evolutionary ecology of wild animals, and provide clear directions for future research, which I will discuss below.

Key Findings

In Chapter 2 I demonstrated that interactions between coinfecting GIN in sheep are mostly antagonistic and that they are more so if they occur sequentially (i.e., the presence of an established infection reduces the establishment of a second species). This effect is dose-dependent and varies between GIN species, both in how strong an antagonistic effect a species exerts and how vulnerable a species is to such antagonism. Further, this antagonistic

effect occurs in coinfections within a GI compartment and coinfections across two GI compartments.

In Chapter 3 I observed how in a population of unmanaged sheep, the seasonal epidemiology of *Nematodirus* spp. and *Te. circumcincta* are comparable to those seen in domestic sheep (in Northern Hemisphere cool-temperate climates), but that *Tr. vitrinus* is more abundant in lambs in July than described in domestic sheep flocks. This difference in epidemiology is potentially due to increases in egg counts among reproductive females in May and spatial overlap between age groups that does not occur on many farms, and the resultant earlier exposure may allow *Tr. vitrinus* to avoid antagonistic exclusion by *Te. circumcincta*. I also observed that abundances for all species except one (*B. trigonocephalum*) decline with age, leading to an associated decline in alpha diversity with age. This is consistent with other wild studies but different from studies in farmed animals where anthelmintic use may lower GIN diversity in youngstock. Further I found that GIN abundances and alpha-diversity in adults increase at times of physiological stress and that this effect is not confined to *Te. circumcincta*. The high *B. trigonocephalum* sFECs I observed in adults throughout the year also suggests the immune response against this poorly described species is more limited and less seasonal than that against other GIN.

In Chapter 4 I then used the species-specific estimates derived in Chapter 3 to predict future bodyweight and survival. This analysis identified relationships with lamb bodyweight and survival that would not have been possible with unspeciated data. This showed that the higher levels of *Tr. vitrinus* in lambs in July were associated with lower August bodyweight, a key predictor of survival and fitness in the St Kilda Soay sheep population. *Tr. vitrinus* sFECs in November were also significantly associated with a decreased probability of overwinter

survival. Both these analyses indicated that sFECs provide additional power to reveal impacts on the host that are obscured in total unspeciated FECs. Further, I identified a robust, significant, positive association between *Tr. axei* sFECs in July and lamb bodyweight in August that suggests the impacts of parasites in natural co-infections may be more complex than simply the additive effects of their individual pathological contributions.

In Chapter 5 I then explored the immunological mechanisms that could mediate interactions between coinfecting GIN. Through controlled experiments, I showed that infection with the intestinal GIN *Tr. colubriformis* induces production of antibodies into the abomasal mucus that are capable of cross-reacting with *Te. circumcincta*. However, cross-reactive IgA was confined to the site of *Tr. colubriformis* infection, and the IgG that was present upstream in the abomasum principally bound highly conserved structural and metabolic proteins that are unlikely to provide cross-protection *in vivo* (as they will be shielded within the impermeable cuticle). At the same time, these *Tr. colubriformis* infections also induced transcriptomic responses in the abomasal mucosa associated with parasite detection and Th2 immunity. This suggests that antagonistic interactions between coinfecting GIN may be mediated by increased barriers to larval establishment and more rapid initiation of immune responses as a consequence of the upstream mucosal immune system being place on 'high alert' to subsequent co-infections.

Limitations of approaches

Throughout this thesis, I have attempted to account for methodical limitations in my analyses, and to discuss the implications of any limitations of any limitations that I was unable to account for. Nonetheless it remains important to reiterate some of the most important

limitations in each chapter to guide the interpretation of the key findings above, and to guide the direction of potential future explorations discussed below.

Chapter 2

Meta-analyses are naturally limited by the body of literature upon which they are based. In Chapter 2 it was possible to account for potential publication bias in the source literature using statistical techniques developed by Nagagawa et al. (2022). However, geographical bias in the location of the studies performed was clear from limited countries of origin for the experimental studies compared with the observational studies of GIN retrieved during the systematic literature review (Figure 9) and the global distribution of sheep production (Gilbert et al. 2018). Clustering of effects within geographical region by research group was accounted for in the nested random-effects structure of the meta-analytic mixed models; however, it is important to emphasise that caution should be taken when extending the results to nematode species or geographic regions not included in the meta-analysis.

The meta-analysis was also limited in its scope by the number of experimental pairwise coinfection experiments present in the literature. This impacted the statistical power of the analyses and prevented testing for interactions between specific pairs of parasite species. In addition, the meta-analysis analysed the effect of co-infection upon total parasite count. This measure was used as it was the only measure reported by all studies. However, it is not the only metric of interest and there may be interactions of biological importance between coinfecting GIN species that this meta-analysis was unable to identify, for example: the development of parasites (e.g. the ratio of larvae to adults); the fecundity of parasites (e.g. number of eggs *in utero* in female parasites); the immune response against parasites (e.g. antibody titres or immune cell counts); the impact on the host (e.g. daily liveweight gain).

Chapter 3

In Chapter 3, species-corrected faecal egg counts (sFECs) were estimated by combining faecal egg counting with ITS-2 sequence-based (nemabiome) speciation. This combined methodology contains a large number of processing steps, with the potential for errors to be compounded at each stage. Each step will have therefore introduced numerical noise amongst the true biological signals. This noise is well accounted for using the negative-binomial models utilised in Chapter 3, provided no systematic biases are introduced. One point of potential systematic bias introduction is during the coproculture stage, during which the eggs present in faeces are cultured to the third larval stage (L_3). In the time since the experimental work for Chapter 3 began and this thesis was written, methods for applying the nemabiome technique to DNA direct from nematode eggs have been developed (Francis and Šlapeta 2022). However, coproculture conditions were carefully controlled to avoid bias toward the development of certain species, and that same study demonstrated that when coproculture conditions are controlled, the nemabiome results obtained from eggs, L_1 and L_3 are comparable.

In addition, nemabiome proportions obtained from faecal samples with low egg counts are less accurate than those obtained from faecal samples with higher egg counts (Šlapeta et al. 2025). In order to compensate for this, we utilised as much faeces as possible for each coproculture and rejected samples with low larval yields after coproculture. In addition, there was no evidence that the higher rate of dropout of samples with low egg counts introduced any species-level biases (Figure 15). Further, whilst additional noise at low FEC can have relatively large impacts on species proportions, when these are multiplied by those low FECs, they equate to small absolute differences in sFEC.

Another potential point at which systematic biases may be introduced in the nemabiome pipeline is during the PCRs. This can be due to variation in cell number between species and developmental stage, variation in rDNA copy number between species, and variation in PCR efficiency between sequence variants. These potential biases were controlled for using correction factors that were established by comparing the sequencing results for pools of nematodes which contained known species proportions (Chambers 2020; Redman et al. 2019).

Chapter 3 was also limited in its ability to describe the seasonal dynamics of GIN epidemiology as only four sampling points were performed each year. There are always logistic and economic reasons limiting the collection of samples and the total number that can be processed. Many of the findings of Chapters 3 and 4 would not have been possible without sampling a large number of individuals and sampling over multiple years; however, this came at the expense of sampling frequency. Sampling at higher frequency would have allowed more accurate description of the seasonal changes in sFEC during the first year of a lamb's life and the unexpected epidemiology of *Tr. vitrinus*. More frequent sampling during the periparturient period would have also allowed an estimation of the 'area under the curve' for the periparturient peak in sFECs of *Tr. vitrinus* and *Te. circumcincta*. This area under the curve would have more accurately represented the total number of eggs shed during the periparturient period and informed the epidemiological significance of the observed sFECs.

Chapter 4

The greatest limitation of Chapter 4 is that observational study design precludes the attribution of causation to the correlational findings. Consequently, I am unable to conclude that *Tr. vitrinus* causes lower bodyweights and increased mortality, rather than merely being

associated with lower bodyweights and increased mortality. The suggestion that these relationships are causal are supported by experimental treatments within this study system (Gulland, 1992). However, those treatments were broad-spectrum (i.e. all GIN species were killed) and they hence attribute causation to GIN in general and not *Tr. vitrinus* specifically. The most likely reasons for the correlational relationships I observed to not be causal are either that there is 'reverse causation', or there are 'third variables' that are correlated with both *Tr. vitrinus* sFECs and bodyweight or survival. The likelihood of reverse causation is low, as the parasitological measures were used to predict future events (sFECs in July were used to predict bodyweight in August, and sFECs in November used to predict survival until the following spring). However, reverse causation could still be possible, for example animals that weigh less in July have weaker immune systems, leading to higher *Tr. vitrinus* burdens; those animals are still smaller in August, hence the negative relationship between July *Tr. vitrinus* and August bodyweight. An example of a third variable that could explain the positive relationship between *Tr. axei* and August bodyweight could be space-use and pasture quality. For example: lambs that graze the lushest areas of pasture gain the most weight, those lush areas of pasture also favour *Tr. axei* larval development and hence the animals that graze in those areas have higher *Tr. axei* burdens.

Another limitation of both Chapters 3 and 4 is the assumption that egg count and sFECs correspond to the burden of each parasite species. sFECs more accurately represent the product of both the burden of parasites within an individual and the cumulative fecundity of those parasites. As discussed in the General Introduction, the GIN species vary in their relative fecundities; however, this is unlikely to have impacted the results, with each species fitted to separate statistical models in Chapter 3 and as separate terms in the models in Chapter 4. However, the conclusions of Chapter 4 do need to be considered alongside the possibility that

parasite fecundity may vary between individual hosts, and that variation may be correlated with pathology. A clear example of this is for *Te. circumcincta* (as was discussed in Chapter 4). In domestic sheep *Te. circumcincta* has been shown to exhibit density dependent fecundity (Bishop and Stear, 2000), therefore the assumed correlation between *Te. circumcincta* sFEC and *Te. circumcincta* burdens may be weakened at the highest burdens, where the greatest pathology would be predicted. In addition, Stear et al (1995) and Stear, Strain and Bishop (1999) demonstrated a specific effect of host immunity on *Te. circumcincta* fecundity. Given that the pathology in *Te. circumcincta* infections is predominately immune-mediated (Greer et al., 2008), this may mean that more resistant individuals will have lower *Te. circumcincta* fecundity but higher associated pathology. Together these two phenomena may help explain why I found no association between that important parasite of domestic lambs and bodyweight and survival in lambs on St Kilda.

Chapter 5

One of the key findings of Chapter 5 was that, although *Te. circumcincta*-cross-reactive IgG is present in the abomasal mucus during a *Tr. colubriformis* infection, the antigen targets for those cross-reactive antibodies are mostly internal structural proteins that are unlikely to provide cross-protection *in vivo*. However, it is possible that antibodies against internal structural antigens could provide protection, if cellular mechanisms (e.g. chitinases), first breakdown the parasite cuticle and expose those internal antigens. In addition, the function of some of the proposed *Te. circumcincta* target proteins are not known and these proteins (annotated as 'hypothetical proteins' in the *Te. circumcincta* genome), may provide cross-protection. Further, antigen-precipitation and proteomic analysis was only performed for those antibodies that cross-reacted against somatic antigens of the third larval stage. The

mucus ELISA results (Figure 22) indicate that those are the most common cross-reactive targets, but it is possible that cross-reactive antibodies against targets from the fourth-larval stage or from excretory-secretory products could provide a protective effect *in vivo*. Finally, antigen targets were filtered to include only targets bound by IgG isolated from infected animals and not from uninfected animals. If quantitative proteomic analysis had been performed, antibodies that are more numerous in infected animals than uninfected animals could also have been considered.

It may also seem surprising to the reader that *Tr. colubriformis* was chosen for the experiments in Chapter 5, rather than *Tr. vitrinus*. This limitation was a result of the need for the experiment to be planned before the results of the earlier chapters were achieved, and also because the experiments were co-funded by an Australian funding body (MLA), and *Tr. colubriformis* has major economic impacts on sheep production in Australia. The use of a single parasite species in this chapter means the findings may have limited generalisability to infections with other species of GIN. Nonetheless, it provides a valuable proof in principal that the infection of sheep with an intestinal GIN can lead to changes in gene expression in the abomasum associated with parasite-sensing and Th2-type immunity.

Finally, arguably the greatest limitation of Chapter 5 within the narrative of the thesis is that it did not demonstrate that the proposed mechanisms are effective in a coinfection. If there had been greater time and funding for more experimental groups it would have been extremely valuable to have subsequently challenged some of the lambs with *Te. circumcincta*. This would have potentially confirmed whether the proposed mechanisms provide cross-protection, and also allowed me to examine dynamic changes in the cellular and antibody responses within the abomasum.

Future Directions

A key finding of Chapters 3 and 4 was the unexpectedly high levels of *Tr. vitrinus* in lambs in July and the association this had with future bodyweight in wild Soay sheep. In those chapters I discussed how this could be driven by pasture contamination with eggs from the previous year's lambs and from adults with increased egg counts during times of physiological stress. The relative magnitudes of these contributions could have important demographic feedback and influence how parasites may mediate the effects of environmental conditions between generations. The relative contributions of these sources of pasture contamination could be assessed by combing demographic data for the population within Village Bay and mechanistic models of nematode larval development and survival. As part of the St Kilda Soay Sheep Project, ten censuses of the Village Bay study area are completed in spring, summer and autumn of each year. The data from these censuses allows the estimation of the number of animals in the study area, including their age class and sex/reproductive status. These data could be combined with literature estimates for total faecal volumes produced by sheep of different ages to provide an estimate of total faeces deposited by each demographic subgroup of sheep (Krysl et al. 1988; Field, Sykes, and Gunn 1974). These could then be multiplied by the estimated marginal mean *Tr. vitrinus* sFECs (for each age class and sex/reproductive class in each season), which I presented in Chapter 3. This would give an estimate for the total number of *Tr. vitrinus* eggs deposited on the pasture in each of the seasons by each of the demographic subgroups. After that, a mechanistic model could be used to predict the larval development and survival of the *Tr. vitrinus* on pasture, using climatic data from the weather station present in Village Bay (Rose Vineer, Wang, et al. 2015).

Together this would then allow the estimation of the proportion of the infectious *Tr. vitrinus* L_3 present on the pasture in the spring that were derived from eggs shed by each of the demographic classes.

Understanding the relative epidemiological contributions of youngstock and periparturient females is also utilised in the veterinary management of GIN infections in domestic sheep (Gascoigne et al. 2018; Evans and Sargison 2019; Sargison, Bartram, and Wilson 2012). This has often focussed on the particular role of the PPRI in the epidemiology of *Te. circumcincta* (E. G. Williams et al. 2024; Houdijk 2008; Coop et al. 1990); however, Chapter 3 demonstrated that on St Kilda, rises in multiple GIN sFEC are also seen in non-reproductive females and males in March, and in males in November. In that Chapter, I hypothesised that this may be due to Soay sheep experiencing more extreme variation in nutritional constraints than domestic sheep, but it is worth bearing in mind that studies of the PPRI in domestic sheep have been largely confined to lowground settings and St Kilda may be more reflective of some extensive upland systems.

Body condition is not assessed in Soay sheep in spring, but among reproductive females the above hypothesis could be tested by comparing GIN community between singleton-bearing and twin-bearing ewes (as twin-bearing ewes have both higher metabolic demands and reduced rumen capacity (Crilly, Phythian, and Evans 2021)). As a consequence, I would hypothesise that the PPRI in single-bearing ewes would lead to large increases in *Te. circumcincta* sFEC and small increases in sFEC for the other GIN species, whereas in twin-bearing ewes there would be large increases in sFEC for all GIN species. Alternatively, a more accurate estimate of maternal condition could be generated by leveraging the 40-year dataset of pedigree and birthweight gathered by the St Kilda Soay Sheep Project. This would entail

using an ‘animal model’ to generate estimated breeding values (EBVs) for birthweight, as has been performed previously in Soay sheep (A. J. Wilson et al. 2007). The maternal and paternal identities of a lamb would then be combined with these EBVs to predict the combined birthweight of a litter of lambs. The deviation in observed ‘litter-weight’ from the model prediction would then provide an estimate for maternal condition (i.e. ewes that gave birth to lambs that weighed less than predicted by the EBVs are judged to have been in poor nutritional condition, whilst ewes that gave birth to lambs that weighed more than the EBVs predicted are judged to have been in good nutritional condition). As for the difference between single-bearing and twin-bearing ewes, I would hypothesise that ewes in good nutritional condition would express a PPRI mostly restricted to *Te. circumcincta*, whereas ewes in poor nutritional condition would show elevations in sFEC for all GIN species, including *Tr. vitrinus*.

In addition to the differences associated with reproduction in females, I identified that *B. trigonocephalum* sFECs were high for males through most of the year, and that sFECs for *Te. circumcincta* and *C. ovina* were higher in males in November. Males of many host species appear to have higher helminth burdens than females do (Sellau et al. 2024). A large driver in this is the suppressive effect of testosterone on cell-mediated immunity (Foo et al. 2017; Trumble et al. 2016). Seasonal elevation of testosterone in male sheep around the time of rutting (Dufour, Fahmy, and Minvielle 1984) may therefore be responsible for those results in Chapter 3. Sex-based differences in infectious disease can also be influenced by differences in behaviour between males and females (Guerra-Silveira and Abad-Franch 2013). These differences in behaviour are often focused on altered patterns of exposure, however sexual behaviour also affects metabolic status and this may in turn influence immune defences (Kilpimaa, Alatalo, and Siitari 2004).

Soay sheep are notable for the intense inter-male sexual competition in November, and the metabolic demands of this may also be responsible for the for higher *Te. circumcincta* and *C. ovina* sFECs in males at that time of year. The relative contributions of behaviour and testosterone on the individual species within the nemabiome of males could be further investigating using the data presented in Chapter 3, combined with additional data from the Soay Sheep Project. Males captured during the August catch have their scrotal circumference measured, and this value is positively correlated with serum testosterone levels in both domestic and Soay sheep (Preston et al. 2012; Matos and Thomas 1992). This could therefore be added to models predicting sFECs for males in November as a way of estimating the impact of testosterone. If testosterone is responsible for nemabiome differences in males, I would predict that scrotal circumference would positively predict sFECs in November. Male Soay sheep also possess two distinct horn phenotypes: large horns or scurred (small, ineffectual) horns. Scurred males are unable to compete with horned males in head-butting contests, therefore pursue a 'coursing' reproductive strategy rather than a 'consorting' strategy (Stevenson et al. 2003). This difference in sexual phenotype and strategy clearly entails physiological costs as males with large horns have reduced longevity (Robinson et al. 2006). If nutritional and metabolic stress drive the changes in the nemabiome in males in November, I would therefore hypothesise that the changes would be more pronounced in horned males than scurred males. The proportion of the variance explained by scrotal circumference and horn type could then be directly compared in a single model, and differences in the proportion of variance explained contrasted between the GIN species.

These indirect epidemiological impacts of GIN egg shedding by adults on the health and survival of younger generations are fascinating, but the dataset described in Chapters 3 and 4 also offers the potential to investigate unexplored direct impacts of the nemabiome on

adults. Froy et al. (2019) showed that senescence in immunity against GIN predicted adult mortality in Soay sheep on St Kilda. That study utilised antibodies that bound *Te. circumcincta* L3-SA as a measure of immunity against GIN. The results of Chapter 5 suggest that those antibodies are unlikely to be a direct mechanism of immunity, but may represent the efficacy of a cell-mediated response in the mucosa to kill worms and thereby expose the host to internal structural or metabolic parasite proteins. At the same time, Chapter 2 indicated that GIN species vary in their susceptibility to cross-protective immune responses. Is this age-related decline in immunity identified by Froy *et al.* a decline in non-specific anti-nematode immunity? Of the species examined in the meta-analysis in Chapter 2, *Tr. vitrinus* was the most vulnerable to the impact of a co-infecting species. A decline in cross-protective immunity might therefore favour *Tr. vitrinus*, which could suggest that it is responsible for adult mortality, as well as the lamb mortality identified in Chapter 4. Alternatively, specific changes in other GIN species in the years preceding death might suggest that species-specific immunity against different GIN senesces at different rates.

I have already discussed how physiological variation may lead to changes in anti-GIN immunity and consequent changes in the nemabiome, and in chapter 5 I identified a mechanism that may mediate inter-GIN immune interactions. The project that funded the sample collection and nemabiome sequencing in Chapter 3, also used blood samples collected in August of each of the study years to measure markers of T-helper cell phenotype (Corripio-Miyar et al. 2022). These two datasets could be combined to investigate the role of the immune system as a mediator both of GIN community composition and of its impacts. Specifically, it could further interrogate the positive relationship between *Tr. axei* in lambs in July and their August bodyweights. For example, is *Tr. axei* infection in young lambs associated with a shift in T-helper cell phenotype towards a Th2 response that could reduce the impacts

of a subsequently coinfecting species of greater pathogenicity? A meta-analysis of helminth infections in mouse models suggests that a common protective response to helminth infection is the up regulation of IL-4 and IL-33 expression in whole blood transcriptomics (Zhou et al. 2016), therefore similar changes could also be tested in the lambs captured in August, using a pilot dataset of whole blood transcriptomics obtained in the same years as my data in Chapter 3.

This leads on to the unanswered question of whether the data from Chapter 3 contains evidence of cross-protective immunity and antagonistic interactions between coinfecting GIN. My analysis in Chapter 4 did not identify an interaction between *Tr. axei* in July and *Tr. vitrinus* in July to explain the positive association between *Tr. axei* and bodyweight, but I intend to conduct a more exhaustive exploration of potential interactions between coinfecting species using this dataset. There are two potential approaches to this, either looking at the relative abundances within single time points or looking at the relative abundances between time points. The first of these could be approached using multi-response models (with sFECs of each of the GIN as response variables) in order to calculate the covariance within individuals versus the covariance between individuals (Stutz et al. 2018). I would hypothesis that an individual's inherent susceptibility would lead to a positive covariance in the different GIN species between individuals (i.e., sheep that have high burdens for one species will likely have high burdens for other species). In contrast the antagonistic interactions between coinfecting GIN would lead to a negative covariance within individuals (i.e., if a sheep has a particularly high abundance of one parasite relative to at other timepoints, the abundances of other species will be relatively low). It would not be possible to estimate these covariances using the negative binomial models I used in Chapter 3, therefore I would fit these using Poisson models with random effects for individual sheep

identity and for individual faecal sample identity. Fitting such models in Chapter 3 proved challenging, and negative binomial models were selected instead for that chapter. This difficulty may have been because the frequentist fitting method assumed the random effects should be drawn from a normal distribution (Brooks et al. 2017). Parasite distributions are commonly highly overdispersed and the raw data distributions in Chapter 3 were highly skewed, therefore this problem might be overcome by using a Bayesian model fitting with random effects with gamma-distributed priors (Molenberghs, Verbeke, and Demétrio 2007). This approach would also allow estimation of the repeatability of nemabiome composition, and whether heritability in resistance against GIN as measured by unspeciated FECs (Hayward 2022) leads to heritable differences in nemabiome composition. Given the GIN species vary in their impacts upon the host, such heritable variation would have important implications for the evolution of wild populations and the selective breeding of livestock.

That first approach offers enormous potential if it can be achieved; however, the alternative approach exploring interactions between time points may have a higher chance of success given Chapter 2 showed antagonistic interactions to be greatest in sequential coinfections. Work in wild mice also found the most effective observational method for identifying coinfection interactions to be comparing samples one month apart (Fenton et al. 2014). That study examined a pairwise relationship between two groups of parasites (nematodes and coccidia), which is much simpler than the multiple GIN species present in my data. Testing every pairwise combination of parasites between two time points could risk false discovery, therefore I would focus initially on any potential interaction between *Tr. axei* and *Tr. vitrinus* given Chapter 4 provided evidence of significant impacts of these parasites in lambs. In addition, Chapter 2 suggested that *Tr. axei* exerts particularly strong antagonistic effects and *Tr. vitrinus* is particularly vulnerable to the antagonism of a coinfecting species.

Keegan, Pedersen and Fenton (2024) showed that the impact of parasite coinfections on transmission dynamics also varies with spatial scale, therefore it would be important to incorporate spatial overlap between individuals into this analysis. This could be achieved using kernel density estimation to estimate home ranges for individual sheep and host density distributions, using the locations of animals during each of the censuses conducted under the Soay Sheep Project (Kie et al. 2010; Albery et al. 2024). These data could then be used to control for spatial autocorrelation in the analyses of GIN co-abundance, but may also address interesting questions in themselves. Among lambs on St Kilda, preference for certain vegetation types appears to drive GIN infection in lambs via local host density, whereas GIN abundances in older sheep seem less influenced by spatial variation (Wiersma et al. 2023). However, that study utilised unspeciated egg counts taken in August – are adults equally unaffected by space use at times when their anti-GIN immunity is reduced (e.g. around parturition in females and during the rut in males)? Further, Chapter 3 demonstrated that adults have relatively higher *B. trigonocephalum* sFECs. This species is capable of infecting via the skin so, unlike the other GIN species, transmission is not confined to areas of grazing. Soay sheep on St Kilda use stone structures (*cleits* or *cleitean*) for shelter in poor weather – is *cleit* sharing an indicator of social connectedness and does this predict correlations in *B. trigonocephalum* abundance between individuals? It may be possible to address this question in the next few years as a new project is in the process of applying radiofrequency ID tags to some of the sheep on St Kilda and using these to estimate the social networks of the sheep, including via *cleit* sharing.

Space use is clearly also important in the management of GIN infections in farmed sheep, with the infectious risk of individual fields an important hazard to be analysed when planning grazing (Gascoigne et al. 2018). Mechanistic models of epidemiology can now be used to help

assess this risk at an individual field level (McFarland et al. 2022). The GLOWORM- FL model was initially developed focusing solely on the effect of temperature on the development and survival of the free-living stages of parasites (Rose Vineer, Wang, et al. 2015), with a subsequent expansion to allow for the effects of moisture (T. Wang et al. 2022). The GLOWORM-PARA model then expanded upon this, incorporating the acquisition and waning of immunity to reflect their importance to transmission dynamics (Rose Vineer, Verschave, et al. 2020; Claerebout and Vercruysse 2000). The results of this thesis additionally demonstrate the importance of both host physiology and cross-protective immunity to transmission dynamics of individual GIN species. The GLOWORM-PARA model could be expanded to allow for a cross-protective immune responses that builds as a result of exposure to any species, in addition to a species-specific response. In addition, it may be valuable for the model component that represents the waning of immunity to be allowed to vary according to physiological state (e.g., reproductive status and nutrition). Fully incorporating these effects into the model would require extensive parameterisation and validation. However, the values for interactions between coinfecting species obtained in the meta-analysis in Chapter 2 and the estimates for the PPRI for each of the species in Chapter 3 could provide an initial range of parameters. These could then be used to test the impact they have on the model output, and assess the value of more accurately parameterising a complex multi-species epidemiological model.

The results of this thesis have shown the value of combining meta-analysis, experimental infections and longitudinal observations to investigate interactions between coinfecting GIN and immunity. It would be of great additional value to complete this suite of investigative methods with longitudinal experimental coinfection studies, and targeted GIN community perturbations. A longitudinal coinfection study could utilise abomasal cannulation (W. D.

Smith et al. 1984; Liu et al. 2022) to longitudinally monitor transcriptomic changes in response to challenge with *Te. circumcincta* in animals that did or did not have a pre-existing *Tr. colubriformis* infection, thereby testing the mechanism of interaction I proposed in Chapter 5. Using abomasal cannulated animals would also allow simultaneous sampling of mucus for measurement of upstream IgA production in the presence of an upstream coinfection. Chapter 5 indicated that cross-reactive IgA was not produced upstream in the abomasum in response to an intestinal infection, but the upregulation of the Th2 immune response there could result in a more rapid release of a protective IgA during a co-infection scenario. Such a study could also compare the longitudinal immune response in a co-infection between animals vaccinated with vaccines developed against single species, to see if they provide any cross-species-protection.

To my knowledge, experimental perturbations of specific species within GIN communities have never previously been performed, presumably due to the broad-spectrum activity of anthelmintic drugs. However, faecal egg count reduction tests (FECRTs) are commonly performed on sheep farms to test for anthelmintic resistance. Molecular speciation has been used in conjunction with these tests in order to identify species-specific anthelmintic resistance (McIntyre et al. 2018; Leathwick et al. 2025); however, their potential value as community perturbation experiments has not yet been explored. Owing to their pharmacokinetic properties, salicylanilide drugs such as closantel only treat blood-feeding GIN (Rothwell and Sangster 1997), therefore they could be used in FECRTs to as a targeted perturbation of *Haemonchus contortus* or possibly *B. trigonocephalum* (Garedaghi, Saber, and Attaremadraki 2011). Similarly, FECRTs in populations of GIN where anthelmintic resistance is present could also function as a perturbation experiment. In either of these scenarios the experiment would require three experimental groups, each comprising individual hosts from

a population with established mixed GIN burdens. One group would be treated with an anthelmintic effective against all species (untargeted-treatment); one group would be treated with an anthelmintic known to only be effective against a subgroup of the GIN (targeted-treatment); and a third group would remain untreated (control). Individual sFECs would be calculated using faecal samples from individuals immediately prior to treatment, and again a set time after treatment (as for a standard FECRT (Abbott, Taylor, and Stubbings 2012)). The difference in sFECs for the surviving species between the targeted-treated and control animals could be used to assess for an immediate 'competitive release' (an increase in the sFEC of the surviving species in the targeted-treated animals vs the control animals). The sFECs of the same individuals would then be monitored regularly for several weeks after treatment to assess for 'competitive exclusion' (decreased re-establishment of the susceptible species in the targeted-treated group compared to the untargeted-treated group). These results would provide additional evidence for the presence of interactions between coinfecting GIN. Longer-term monitoring of the health and fitness outcomes of the individuals would also provide additional insight into the impacts of these coinfections upon the host. These findings could also have important implications for the evolution of anthelmintic resistance, with competitive release increasing the rate at which resistant eggs are shed, and competitive exclusion decreasing the efficiency of reestablishment of susceptible GIN from *refugia* (Van Wyk 2001). Additionally, performing these experiments across animals with a range of anti-GIN resistance (e.g. through age, genotype or prior exposure) would allow assessment of the impact of resistance upon these coinfection dynamics.

Conclusion

In summary, this thesis has demonstrated the power of combining scientific approaches across scales. Specifically, distinguishing coinfecting gastrointestinal nematode species from one another has illuminated their distinct-yet-interconnected epidemiologies, with consequences for the host that have both evolutionary and translational relevance. Returning to controlled experiments in light of these findings revealed a clear mechanism for interactions between these coinfecting parasites and emphasises the complementarity between scientific approaches. This combination of methodologies has provided me with broad and rigorous scientific training, and has also revealed key insights into the interactions between coinfecting parasites and immunity, with impacts for both veterinary medicine and evolutionary ecology. Finally, the data I have collected provide great potential for further analyses of the underlying drivers of variation in GIN community composition and have opened new avenues for further research into these globally important parasite coinfections.

References

Abbas, G., A. Ghafar, A. Beasley, M. A. Stevenson, J. Bauquier, A. V. Koehler, E. J. A. Wilkes, et al. 2024. 'Understanding Temporal and Spatial Distribution of Intestinal Nematodes of Horses Using Faecal Egg Counts and DNA Metabarcoding'. *Veterinary Parasitology* 325 (January): 110094.

Abbott, K. A., and I. J. McFarland. 1991. 'Trichostrongylus Axei Infection as a Cause of Deaths and Loss of Weight in Sheep'. *Australian Veterinary Journal* 68 (11). https://doi.org/10.1111/j.1751-0813.1991.tb00741.x.

Abbott, K. A., M. Taylor, and L. A. Stubbings. 2012. 'Sustainable Worm Control Strategies for Sheep 4th Edition'. http://www.scops.org.uk/workspace/pdfs/scopstechnicalmanual4theditionjune2012130720 12175521.pdf.

Aboshady, H. M., M. J. Stear, A. Johansson, E. Jonas, and J. C. Bambou. 2020. 'Immunoglobulins as Biomarkers for Gastrointestinal Nematodes Resistance in Small Ruminants: A Systematic Review'. *Scientific Reports* 10 (1): 7765.

Abuzeid, A. M. I., X. Zhou, Y. Huang, and G. Li. 2020. 'Twenty-Five-Year Research Progress in Hookworm Excretory/Secretory Products'. *Parasites & Vectors* 13 (1): 136.

Agace, W. W., and K. D. McCoy. 2017. 'Regionalized Development and Maintenance of the Intestinal Adaptive Immune Landscape'. *Immunity* 46 (4): 532–48.

Ahn, S., E. Redman, S. Gavriliuc, J. Bellaw, J. Gilleard, Philip D. McLoughlin, and J. Poissant. 2024. 'Mixed Strongyle Parasite Infections Vary across Host Age and Space in a Population of Feral Horses'. *Parasitology* 151 (October): 1299–1316.

Albery, G. F., A. R. Sweeny, Y. Corripio-Miyar, M. J. Evans, A. D. Hayward, J. M. Pemberton, J. Pilkington, and D. H. Nussey. 2024. 'Local and Global Density Have Distinct and Parasite-Dependent Effects on Infection in Wild Sheep'. *BioRxiv* 152 (May): 715–23.

Allen, J. E., T. E. Sutherland, and D. Rückerl. 2015. 'IL-17 and Neutrophils: Unexpected Players in the Type 2 Immune Response'. *Current Opinion in Immunology* 34 (June): 99–106.

Amarante, A. F., T. M. Craig, W. S. Ramsey, S. K. Davis, and F. W. Bazer. 1999. 'Nematode Burdens and Cellular Responses in the Abomasal Mucosa and Blood of Florida Native, Rambouillet and Crossbreed Lambs'. *Veterinary Parasitology* 80 (4): 311–24.

Andrews, S. 2010. 'FastQC: A Quality Control Tool for High Throughput Sequence Data'. 2010. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Andronicos, N. M., M. R. Knox, J. McNally, and P. W. Hunt. 2025. 'Direct Comparison of Host Resistance Status and Barbervax Vaccination to Control Parasitism in Sheep Subjected to a Mixed Parasite Field Challenge'. *Veterinary Parasitology*, no. 110552 (July): 110552.

Anello, C., and J. L. Fleiss. 1995. 'Exploratory or Analytic Meta-Analysis: Should We Distinguish between Them?' *Journal of Clinical Epidemiology* 48 (1): 109–16; discussion 117-8.

Anthony, R. M., L. I. Rutitzky, J. F. Urban Jr, M. J. Stadecker, and W. C. Gause. 2007. 'Protective Immune Mechanisms in Helminth Infection'. *Nature Reviews. Immunology* 7 (12): 975–87.

Antonopoulos, A., J. S. Gilleard, and J. Charlier. 2024. 'Next-Generation Sequencing Technologies for Helminth Diagnostics and Surveillance in Ruminants: Shifting Diagnostic Barriers'. *Trends in Parasitology* 40 (6): 511–26.

Aragaw, K., and G. Gebreegziabher. 2014. 'Small Intestinal Helminth Parasites in Slaughtered Sheep and Goats in Hawassa, Southern Ethiopia'. *African Journal of Basic & Applied Sciences* 6 (2): 25–29.

Atayev, A. M., M. M. Zubairova, N. T. Karsakov, and S. S. Mutuev. 2022. 'Biodiversity of Gastrointestinal Strongylates in Sheep on Pastures of Different Ecological Types in Lowland Dagestan'. *Rossiiskii Parazitologicheskii Zhurnal* 1: 11–16.

Avramenko, R. W., A. Bras, E. M. Redman, M. R. Woodbury, B. Wagner, T. Shury, S. Liccioli, M. C. Windeyer, and J. S. Gilleard. 2018. 'High Species Diversity of Trichostrongyle Parasite Communities within and between Western Canadian Commercial and Conservation Bison Herds Revealed by Nemabiome Metabarcoding'. *Parasites & Vectors* 11 (1): 299.

Avramenko, R. W., E. M. Redman, R. Lewis, M. A. Bichuette, B. M. Palmeira, T. A. Yazwinski, and J. S. Gilleard. 2017. 'The Use of Nemabiome Metabarcoding to Explore Gastro-Intestinal Nematode Species Diversity and Anthelmintic Treatment Effectiveness in Beef Calves'. *International Journal for Parasitology* 47 (13): 893–902.

Avramenko, R. W., E. M. Redman, R. Lewis, T. A. Yazwinski, J. D. Wasmuth, and J. S. Gilleard. 2015. 'Exploring the Gastrointestinal "Nemabiome": Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities'. Edited by Emmanuel Serrano Ferron. *PloS One* 10 (12): e0143559.

Bailey, J. N., L. P. Kahn, and S. W. Walkden-Brown. 2009. 'The Relative Contributions of T. Colubriformis, T. Vitrinus, T. Axei and T. Rugatus to Sheep Infected with Trichostrongylus Spp. on the Northern Tablelands of New South Wales'. *Veterinary Parasitology* 165 (1–2): 88–95.

Bairden, K., J. Armour, and J. L. Duncan. 1995. 'A 4-Year Study of the Effectiveness of Alternate Grazing of Cattle and Sheep in the Control of Bovine Parasitic Gastro-Enteritis'. *Veterinary Parasitology* 60 (1–2): 119–32.

Baltrušis, Paulius, Peter Halvarsson, and Johan Höglund. 2022. 'Estimation of the Impact of Three Different Bioinformatic Pipelines on Sheep Nemabiome Analysis'. *Parasites & Vectors* 15 (1): 290.

Barger, I. A. 1984. 'Correlations between Numbers of Enteric Nematode Parasites in Grazing Lambs'. *International Journal for Parasitology* 14 (6): 587–89.

———. 1987. 'Population Regulation in Trichostrongylids of Ruminants'. *International Journal for Parasitology* 17 (2): 531–40.

Beaumelle, C., E. M. Redman, J. de Rijke, J. Wit, S. Benabed, F. Debias, J. Duhayer, et al. 2021. 'Metabarcoding in Two Isolated Populations of Wild Roe Deer (Capreolus Capreolus) Reveals Variation in Gastrointestinal Nematode Community Composition between Regions and among Age Classes'. *Parasites & Vectors* 14 (1): 594.

Beaumelle, C., E. M. Redman, H. Verheyden, P. Jacquiet, N. Bégoc, F. Veyssière, S. Benabed, et al. 2022. 'Generalist Nematodes Dominate the Nemabiome of Roe Deer in Sympatry with Sheep at a Regional Level'. *International Journal for Parasitology* 52 (12): 751–61.

Berger, A. 2000. 'Th1 and Th2 Responses: What Are They?' *BMJ (Clinical Research Ed.)* 321 (7258): 424.

Beveridge, I., A. L. Pullman, P. H. Phillips, R. R. Martin, A. Barelds, and R. Grimson. 1989. 'Comparison of the Effects of Infection with Trichostrongylus Colubriformis, T. Vitrinus and T. Rugatus in Merino Lambs'. *Veterinary Parasitology* 32 (2–3): 229–45.

Bhatt, G. N., V. K. Srivastava, and G. Subramanian. 1969. 'Studies on Bunostomum Trigonocephalum (Rudolphi, 1808). VI. Percutaneous Invasion in Normal Hosts'. https://doi.org/10.5555/19702205286.

Bishop, S. C., and M. J. Stear. 2000. 'The Use of a Gamma-Type Function to Assess the Relationship between the Number of Adult Teladorsagia Circumcincta and Total Egg Output'. *Parasitology* 121 (Pt 4) (October): 435–40.

Blackburn, P. J., I. H. Carmichael, and S. W. Walkden-Brown. 2015. 'Effects of Chronic Infection with Trichostrongylus Vitrinus and Immune Suppression with Corticosteroid on Parasitological, Immune and Performance Variables in Crossbred Meat Lambs'. *Research in Veterinary Science* 100 (June): 138–47.

Blanchard, J. L., and R. B. Wescott. 1985. 'Enhancement of Resistance of Lambs to Haemonchus Contortus by Previous Infection with Ostertagia Circumcincta'. *American Journal of Veterinary Research* 46 (10): 2136–40.

Boag, B., and R. J. Thomas. 1975. 'Epidemiological Studies on Nematodirus Species in Sheep'. *Research in Veterinary Science* 19 (3): 263–68.

———. 1977. 'Epidemiological Studies on Gastro-Intestinal Nematode Parasites of Sheep: The Seasonal Number of Generations and Succession of Species'. *Research in Veterinary Science* 22 (1): 62–67.

Bordes, F., and S. Morand. 2009. 'Coevolution between Multiple Helminth Infestations and Basal Immune Investment in Mammals: Cumulative Effects of Polyparasitism?' *Parasitology Research* 106 (1): 33–37.

———. 2011. 'The Impact of Multiple Infections on Wild Animal Hosts: A Review'. *Infection Ecology & Epidemiology* 1 (September). https://doi.org/10.3402/iee.v1i0.7346.

Bourgoin, G., J. Poissant, J. Wit, K. Ruckstuhl, C. Beaumelle, G. Hough, C. Letain, J. S. Gilleard, and S. Kutz. 2021. 'Parasitic Nematode Abundance and Diversity Variation in Bighorn Sheep Populations across Southwestern Canada'. In *28th International Conference of the World Association for the Advancement of Veterinary Parasitology*. https://hal.science/hal-04629830.

Brachmann, Jenny, Stefan Fiedler, Hannah Fischer, Jennifer S. Schmidt, Renate Radek, Georg von Samson-Himmelstjerna, and Jürgen Krücken. 2025. 'Old World Camels in Germany: Parasitic Nematode Communities Characterized by Nemabiome Analysis Showed Reduced Anthelmintic Efficacy According to the Fecal Egg Count Reduction Test'. *Parasites & Vectors* 18 (1): 294.

Brand, T. van den. 2025. 'Ggh4x: Hacks for "Ggplot2"'. https://CRAN.R-project.org/package=ggh4x.

Bray, Nicolas L., Harold Pimentel, Páll Melsted, and Lior Pachter. 2016. 'Near-Optimal Probabilistic RNA-Seq Quantification'. *Nature Biotechnology* 34 (5): 525–27.

Brooker, Simon. 2010. 'Estimating the Global Distribution and Disease Burden of Intestinal Nematode Infections: Adding up the Numbers--a Review'. *International Journal for Parasitology* 40 (10): 1137–44.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. 'glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling'. *The R Journal*. https://doi.org/10.32614/RJ-2017-066.

Brown, H., and D. Esterházy. 2021. 'Intestinal Immune Compartmentalization: Implications of Tissue Specific Determinants in Health and Disease'. *Mucosal Immunology* 14 (6): 1259–70.

Brunsdon, R. V. 1970. ‘Seasonal Changes in the Level and Composition of Nematode Worm Burdens in Young Sheep’. *New Zealand Journal of Agricultural Research* 13 (1): 126–48.

———. 1988. ‘The Economic Impact of Nematode Infection in Sheep: Implications for Future Research and Control’. In *Heath ACG (Ed) The Economic Importance of Parasites of Livestock in New Zealand. New Zealand Society for Parasitology*, 4–16.

Brunsdon, R. V., and A. Vlassoff. 1971. ‘The Post-Parturient Rise: A Comparison of the Pattern and Relative Generic Composition of Faecal Strongyle Egg Counts in Ewes and Wethers’. *New Zealand Veterinary Journal* 19 (3): 32–37.

Bull, K. E., J. Hodgkinson, K. Allen, J. Poissant, and L. E. Peachey. 2025. ‘Quantitative DNA Metabarcoding Reveals Species Composition of a Macrocyclic Lactone and Pyrantel Resistant Cyathostomin Population in the UK’. *International Journal for Parasitology, Drugs and Drug Resistance* 27 (100576): 100576.

Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. PDF. Edited by Kenneth P. Burnham and David R. Anderson. 2nd edn. New York, NY: Springer.

Cabaret, J., and H. Hoste. 1998. ‘Comparative Analysis of Two Methods Used to Show Interspecific Associations in Naturally Acquired Parasite Nematode Communities from the Abomasum of Ewes’. *Veterinary Parasitology* 76 (4): 275–85.

Cai, E., R. Wu, Y. Wu, Y. Gao, Y. Zhu, and J. Li. 2024. ‘A Systematic Review and Meta-Analysis on the Current Status of Anthelmintic Resistance in Equine Nematodes: A Global Perspective’. *Molecular and Biochemical Parasitology* 257 (111600): 111600.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. 'DADA2: High-Resolution Sample Inference from Illumina Amplicon Data'. *Nature Methods*. https://doi.org/10.1038/nmeth.3869.

Callinan, A. P. 1978a. 'The Ecology of the Free-Living Stages of Ostertagia Circumcincta'. *International Journal for Parasitology* 8 (3): 233–37.

———. 1978b. 'The Ecology of the Free-Living Stages of Trichostrongylus Axei'. *International Journal for Parasitology* 8 (6): 453–56.

———. 1979. 'The Ecology of the Free-Living Stages of Trichostrongylus Vitrinus'. *International Journal for Parasitology* 9 (2): 133–36.

Cameron, T. W. M. 1923a. 'On the Intestinal Parasites of Sheep and Other Ruminants in Scotland'. *Journal of Helminthology* 1 (2): 53–60.

———. 1923b. 'On the Biology of the Infective Larva of Monodontus Trigonocephalus (Rud.) of Sheep'. *Journal of Helminthology* 1 (5): 205–14.

Cardia, D. F. F., R. A. Rocha-Oliveira, M. H. Tsunemi, and A. F. T. Amarante. 2011. 'Immune Response and Performance of Growing Santa Ines Lambs to Artificial Trichostrongylus Colubriformis Infections'. *Veterinary Parasitology* 182 (2–4): 248–58.

Chambers, A. K. 2020. 'Study of Gastrointestinal Nematodes Co-Infecting Feral Soay Sheep on St Kilda'. PhD, University of Edinburgh. https://era.ed.ac.uk/handle/1842/37170.

Chan, M. S. 1997. 'The Global Burden of Intestinal Nematode Infections--Fifty Years On'. *Parasitology Today* 13 (11): 438–43.

Charlier, J., D. J. Bartley, S. Sotiraki, M. Martinez-Valladares, E. Claerebout, G. von Samson-Himmelstjerna, S. M. Thamsborg, H. Hoste, E. R. Morgan, and L. Rinaldi. 2022. 'Anthelmintic Resistance in Ruminants: Challenges and Solutions'. Edited by David Rollinson and Russell Stothard. *Advances in Parasitology* 115 (February): 171–227.

Charlier, J., L. Rinaldi, E. R. Morgan, E. Claerebout, D. J. Bartley, S. Sotiraki, M. Mickiewicz, et al. 2024. 'Sustainable Worm Control in Ruminants in Europe: Current Perspectives'. *Animal Frontiers* 14 (5): 13–23.

Charlier, J., L. Rinaldi, V. Musella, H. W. Ploeger, C. Chartier, H. Rose Vineer, B. Hinney, et al. 2020. 'Initial Assessment of the Economic Burden of Major Parasitic Helminth Infections to the Ruminant Livestock Industry in Europe'. *Preventive Veterinary Medicine* 182 (September): 105103.

Christensen, N. Ø., P. Nansen, B. O. Fagbemi, and J. Monrad. 1987. 'Heterologous Antagonistic and Synergistic Interactions between Helminths and between Helminths and Protozoans in Concurrent Experimental Infection of Mammalian Hosts'. *Parasitology Research* 73 (5): 387–410.

Claerebout, E., and J. Vercruysse. 2000. 'The Immune Response and the Evaluation of Acquired Immunity against Gastrointestinal Nematodes in Cattle: A Review'. *Parasitology* 120 Suppl (7): S25-42.

Clutton-Brock, T. H., and J. M. Pemberton, eds. 2004. *Soay Sheep: Dynamics and Selection in an Island Population*. Cambridge University, Cambridge, United Kingdom: Cambridge University Press.

Clutton-Brock, T. H., O. F. Price, S. D. Albon, and P. A. Jewell. 1992. 'Early Development and Population Fluctuations in Soay Sheep'. *The Journal of Animal Ecology* 61 (2): 381.

Clutton-Brock, T. H., I. R. Stevenson, P. Marrow, A. D. MacColl, A. I. Houston, and J. M. McNamara. 1996. 'Population Fluctuations, Reproductive Costs and Life-History Tactics in Female Soay Sheep'. *The Journal of Animal Ecology* 65 (6): 675.

Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. 'Parasite-Mediated Selection against Inbred Soay Sheep in a Free-Living Island Populaton'. *Evolution; International Journal of Organic Evolution* 53 (4): 1259–67.

Coltman, D. W., J. Pilkington, L. E. Kruuk, K. Wilson, and J. M. Pemberton. 2001. 'Positive Genetic Correlation between Parasite Resistance and Body Size in a Free-Living Ungulate Population'. *Evolution; International Journal of Organic Evolution* 55 (10): 2116–25.

Coop, R. L., A. C. Field, R. B. Graham, K. W. Angus, and F. Jackson. 1986. 'Effect of Concurrent Infection with Ostertagia Circumcincta and Trichostrongylus Vitrinus on the Performance of Lambs'. *Research in Veterinary Science* 40 (2): 241–45.

Coop, R. L., F. Jackson, R. B. Graham, and K. W. Angus. 1988. 'Influence of Two Levels of Concurrent Infection with Ostertagia Circumcincta and Trichostrongylus Vitrinus on the Growth Performance of Lambs'. *Research in Veterinary Science* 45 (3): 275–80.

Coop, R. L., and I. Kyriazakis. 1999. 'Nutrition-Parasite Interaction'. *Veterinary Parasitology* 84 (3–4): 187–204.

Coop, R. L., D. J. Mellor, E. Jackson, F. Jackson, D. J. Flint, and R. G. Vernon. 1990. 'Teladorsagia Circumcincta Egg Output at the Onset of Natural and Induced Lactation in Ewes'. *Veterinary Parasitology* 35 (4): 295–305.

Cooper, Max D., and Matthew N. Alder. 2006. 'The Evolution of Adaptive Immune Systems'. *Cell* 124 (4): 815–22.

Corripio-Miyar, Y., A. D. Hayward, H. Lemon, A. R. Sweeny, X. Bal, F. Kenyon, J. G. Pilkington, J. M. Pemberton, D. H. Nussey, and T. N. McNeilly. 2022. 'Functionally Distinct T-Helper Cell Phenotypes Predict Resistance to Different Types of Parasites in a Wild Mammal'. *Scientific Reports* 12 (1). https://doi.org/10.1038/s41598-022-07149-9.

Coulson, G., J. K. Cripps, S. Garnick, V. Bristow, and I. Beveridge. 2018. 'Parasite Insight: Assessing Fitness Costs, Infection Risks and Foraging Benefits Relating to Gastrointestinal Nematodes in Wild Mammalian Herbivores'. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 373 (1751). https://doi.org/10.1098/rstb.2017.0197.

Cox, F. E. 2001. 'Concomitant Infections, Parasites and Immune Responses'. *Parasitology* 122 Suppl: S23-38.

Coyne, M. J., G. Smith, and C. Johnstone. 1991. 'Fecundity of Gastrointestinal Trichostrongylid Nematodes of Sheep in the Field'. *American Journal of Veterinary Research* 52 (7): 1182–88.

Craig, B. H. 2005. 'Parasite Diversity in a Free-Living Host Population'. PhD, University of Edinburgh.

Craig, B. H., O. R. Jones, J. G. Pilkington, and J. M. Pemberton. 2009. 'Re-Establishment of Nematode Infra-Community and Host Survivorship in Wild Soay Sheep Following Anthelmintic Treatment'. *Veterinary Parasitology* 161 (1–2): 47–52.

Craig, B. H., J. G. Pilkington, and J. M. Pemberton. 2006. 'Gastrointestinal Nematode Species Burdens and Host Mortality in a Feral Sheep Population'. *Parasitology* 133 (Pt 4): 485–96.

Craig, B. H., L. J. Tempest, J. G. Pilkington, and J. M. Pemberton. 2008. 'Metazoan-Protozoan Parasite Co-Infections and Host Body Weight in St Kilda Soay Sheep'. *Parasitology* 135 (4): 433–41.

Crilly, J. P., C. Phythian, and M. J. Evans. 2021. 'Advances in Managing Pregnancy Toxaemia in Sheep'. *In Practice* 43 (2): 79–94.

Cripps, A. W., A. J. Husband, and A. K. Lascelles. 1974. 'The Origin of Immunoglobulins in Intestinal Secretion of Sheep'. *The Australian Journal of Experimental Biology and Medical Science* 52 (4): 711–16.

Crofton, H. 1963. 'Nematode Parasite Population in Sheep and on Pasture'. *Commonewealth Bur. Helminthol.* https://www.semanticscholar.org/paper/Nematode-parasite-population-in-sheep-and-on-Crofton/2e6563acc0cbfa40d6e4dae50bb2aa26b4f7a38a.

Crofton, H. D. 1957. 'Nematode Parasite Populations in Sheep on Lowland Farms. III. The Seasonal Incidence of Species'. *Parasitology* 47 (3–4): 304–18.

Cuquerella, M., M. T. Gómez-Muñoz, L. Carrera, C. de la Fuente, and J. M. Alunda. 1994. 'Cross Antigenicity among Ovine Trichostrongyloidea. Preliminary Report'. *Veterinary Parasitology* 53 (3–4): 243–51.

Dash, K. M. 1981. 'Interaction between Oesophagostomum Columbianum and Oesophagostomum Venulosum in Sheep'. *International Journal for Parasitology* 11 (3): 201–7.

Davey, Marie L., Stefaniya Kamenova, Frode Fossøy, Erling J. Solberg, Rebecca Davidson, Atle Mysterud, and Christer M. Rolandsen. 2023. 'Faecal Metabarcoding Provides Improved Detection and Taxonomic Resolution for Non-Invasive Monitoring of Gastrointestinal Nematode Parasites in Wild Moose Populations'. *Parasites & Vectors* 16 (1): 19.

De Cisneros, J. P. J., L. Matthews, C. Mair, T. Stefan, and M. J. Stear. 2014. 'The Transfer of IgA from Mucus to Plasma and the Implications for Diagnosis and Control of Nematode Infections'. *Parasitology* 141 (7): 875–79.

De Seram, E. L., E. M. Redman, F. K. Wills, C. de Queiroz, J. R. Campbell, C. L. Waldner, S. E. Parker, R. W. Avramenko, J. S. Gilleard, and F. D. Uehlinger. 2022. 'Regional Heterogeneity and Unexpectedly High Abundance of Cooperia Punctata in Beef Cattle at a Northern Latitude Revealed by ITS-2 RDNA Nemabiome Metabarcoding'. *Parasites & Vectors* 15 (1): 17.

Dean, Andrew D., Dylan Z. Childs, Yolanda Corripio-Miyar, Mike Evans, Adam Hayward, Fiona Kenyon, Luke McNally, et al. 2024. 'Host Resources and Parasite Traits Interact to Determine the Optimal Combination of Host Parasite-Mitigation Strategies'. *Ecology and Evolution* 14 (6): e11310.

Debray, R., R. A. Herbert, A. L. Jaffe, A. Crits-Christoph, M. E. Power, and B. Koskella. 2022. 'Priority Effects in Microbiome Assembly'. *Nature Reviews. Microbiology* 20 (2): 109–21.

Desai, P., M. S. Diamond, and L. B. Thackray. 2021. 'Helminth-Virus Interactions: Determinants of Coinfection Outcomes'. *Gut Microbes* 13 (1): 1961202.

Diez-Baños, N., J. Cabaret, and P. Diez-Baños. 1992. 'Interspecific Interactions in Naturally Acquired Nematode Communities from Sheep Abomasum in Relation to Age of Host and Season in Four Areas of León (Spain)'. *International Journal for Parasitology* 22 (3): 327–34.

Dijk, J. van, and E. R. Morgan. 2008. 'The Influence of Temperature on the Development, Hatching and Survival of Nematodirus Battus Larvae'. *Parasitology* 135 (2): 269–83.

———. 2010. 'Variation in the Hatching Behaviour of Nematodirus Battus: Polymorphic Bet Hedging?' *International Journal for Parasitology* 40 (6): 675–81.

Dobson, R. J., and E. H. Barnes. 1995. 'Interaction between Ostertagia Circumcincta and Haemonchus Contortus Infection in Young Lambs'. *International Journal for Parasitology* 25 (4): 495–501.

Dobson, R. J., P. J. Waller, and A. D. Donald. 1990a. 'Population Dynamics of Trichostrongylus Colubriformis in Sheep: The Effect of Host Age on the Establishment of Infective Larvae'. *International Journal for Parasitology* 20 (3): 353–57.

———. 1990b. 'Population Dynamics of Trichostrongylus Colubriformis in Sheep: The Effect of Infection Rate on Loss of Adult Parasites'. *International Journal for Parasitology* 20 (3): 359–63.

———. 1990c. 'Population Dynamics of Trichostrongylus Colubriformis in Sheep: The Effect of Infection Rate on the Establishment of Infective Larvae and Parasite Fecundity'. *International Journal for Parasitology* 20 (3): 347–52.

Donohue, R. E., Z. K. Cross, and E. Michael. 2019. 'The Extent, Nature, and Pathogenic Consequences of Helminth Polyparasitism in Humans: A Meta-Analysis'. *PLoS Neglected Tropical Diseases* 13 (6): e0007455.

Douch, P. G., G. B. Harrison, L. L. Buchanan, and R. V. Brunsdon. 1984. 'Relationship of Histamine in Tissues and Antiparasitic Substances in Gastrointestinal Mucus to the Development of Resistance to Trichostrongyle Infections in Young Sheep'. *Veterinary Parasitology* 16 (3–4): 273–88.

Downey, N. E. 1962. 'The Establishment of Ostertagia Spp. in Lambs Previously Infected with Trichostrongylus Axei'. *The Veterinary Record* 74: 1321.

Dufour, J. J., M. H. Fahmy, and F. Minvielle. 1984. 'Seasonal Changes in Breeding Activity, Testicular Size, Testosterone Concentration and Seminal Characteristics in Rams with Long or Short Breeding Season'. *Journal of Animal Science* 58 (2): 416–22.

Emery, D. L., B. M. Wagland, and S. J. McClure. 1993. 'Rejection of Heterologous Nematodes by Sheep Immunized with Larval or Adult Trichostrongylus Colubriformis'. *International Journal for Parasitology* 23 (7): 841–46.

Eranga, L., F. D. Uehlinger, Queiroz C. De, E. M. Redman, J. R. Campbell, and D. Nooyen. 2023. 'Integration of ITS-2 RDNA Nemabiome Metabarcoding with Fecal Egg Count Reduction Testing (FECRT) Reveals Ivermectin Resistance in Multiple Gastrointestinal Nematode Species, Including Hypobiotic Ostertagia Ostertagi, in Western Canadian Beef Cattle'. *International Journal for Parasitology: Drugs and Drug Resistance* 22: 27–35.

Evans, M. J., U. N. Chaudhry, L. M. Costa-Júnior, K. Hamer, S. R. Leeson, and N. D. Sargison. 2021. 'A 4 Year Observation of Gastrointestinal Nematode Egg Counts, Nemabiomes and the Benzimidazole Resistance Genotypes of Teladorsagia Circumcincta on a Scottish Sheep Farm'. *International Journal for Parasitology* 51 (5): 393–403.

Evans, M. J., Y. Corripio-Miyar, A. D. Hayward, F. Kenyon, T. N. McNeilly, and D. H. Nussey. 2023. 'Antagonism between Co-Infecting Gastrointestinal Nematodes: A Meta-Analysis of Experimental Infections in Sheep'. *Veterinary Parasitology* 323 (November): 110053.

Evans, M. J., and N. D. Sargison. 2019. 'Planning Anthelmintic Treatments to Control Gastrointestinal Nematode Infections in Sheep'. *Livestock* 24 (Sup2): 4–8.

Eysker, M. 1978. 'Inhibition of the Development of Trichostrongylus Spp. as Third Stage Larvae in Sheep'. *Veterinary Parasitology* 4 (1): 29–33.

———. 1987. 'Regulation of Trichostrongylus Vitrinus and T Colubriformis Populations in Naturally Infected Sheep in the Netherlands'. *Research in Veterinary Science* 42 (3): 267–71.

Ezenwa, V. O., and A. E. Jolles. 2011. 'From Host Immunity to Pathogen Invasion: The Effects of Helminth Coinfection on the Dynamics of Microparasites'. *Integrative and Comparative Biology* 51 (4): 540–51.

———. 2015. 'Epidemiology. Opposite Effects of Anthelmintic Treatment on Microbial Infection at Individual versus Population Scales'. *Science (New York, N.Y.)* 347 (6218): 175–77.

Faber, M. N., D. Smith, D. R. G. Price, P. Steele, K. A. Hildersley, L. J. Morrison, N. A. Mabbott, A. J. Nisbet, and T. N. McNeilly. 2022. 'Development of Bovine Gastric Organoids as a Novel In Vitro Model to Study Host-Parasite Interactions in Gastrointestinal Nematode Infections'. *Frontiers in Cellular and Infection Microbiology*, 829.

Fenton, A., S. C. L. Knowles, O. L. Petchey, and A. B. Pedersen. 2014. 'The Reliability of Observational Approaches for Detecting Interspecific Parasite Interactions: Comparison with Experimental Results'. *International Journal for Parasitology* 44 (7): 437–45.

Field, A. C., A. R. Sykes, and R. G. Gunn. 1974. 'Effects of Age and State of Incisor Dentition on Faecal Output of Dry Matter and on Faecal and Urinary Output of Nitrogen and Minerals, of Sheep Grazing Hill Pastures'. *The Journal of Agricultural Science* 83 (1): 151–60.

Fitzpatrick, J. L. 2013. 'Global Food Security: The Impact of Veterinary Parasites and Parasitologists'. *Veterinary Parasitology* 195 (3–4): 233–48.

Flajnik, M. F., and M. Kasahara. 2010. 'Origin and Evolution of the Adaptive Immune System: Genetic Events and Selective Pressures'. *Nature Reviews. Genetics* 11 (1): 47–59.

Foo, Y. Z., S. Nakagawa, G. Rhodes, and L. W. Simmons. 2017. 'The Effects of Sex Hormones on Immune Function: A Meta-Analysis'. *Biological Reviews of the Cambridge Philosophical Society* 92 (1). https://doi.org/10.1111/brv.12243.

Fox, J., and S. Weisberg. 2019. 'An R Companion to Applied Regression'. Thousand Oaks CA: Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Francis, Emily Kate, and Jan Šlapeta. 2022. 'A New Diagnostic Approach to Fast-Track and Increase the Accessibility of Gastrointestinal Nematode Identification from Faeces: FECPAKG2 Egg Nemabiome Metabarcoding'. *International Journal for Parasitology* 52 (6): 331–42.

———. 2023. 'Refugia or Reservoir? Feral Goats and Their Role in the Maintenance and Circulation of Benzimidazole-Resistant Gastrointestinal Nematodes on Shared Pastures'. *Parasitology* 150 (8): 672–82.

Froy, H., A. M. Sparks, K. A. Watt, R. Sinclair, F. Bach, J. G. Pilkington, J. M. Pemberton, T. N. McNeilly, and D. H. Nussey. 2019. 'Senescence in Immunity against Helminth Parasites Predicts Adult Mortality in a Wild Mammal'. *Science* 365 (6459): 1296–98.

Garedaghi, Y., A. Saber, and R. Attaremadraki. 2011. 'Efficacy of Closantel 5% against Cattle Gastrointestinal Parasites'. *American Journal of Animal and Veterinary Sciences* 6 (October): 112–16.

Gascoigne, E., E. R. Morgan, F. Lovatt, and H. Rose Vineer. 2018. 'Controlling Nematode Infections in Sheep: Application of HACCP'. *In Practice* 40 (8): 334–47.

Gasser, Robin B., Nathan J. Bott, Neil B. Chilton, Peter Hunt, and Ian Beveridge. 2008. 'Toward Practical, DNA-Based Diagnostic Methods for Parasitic Nematodes of Livestock - Bionomic and Biotechnological Implications'. *Biotechnology Advances*. Elsevier. https://doi.org/10.1016/j.biotechadv.2008.03.003.

Gasser, Robin B., Neil B. Chilton, Hervé Hoste, and Ian Beveridge. 1993. 'Rapid Sequencing of RDNA from Single Worms and Eggs of Parasitic Helminths'. *Nucleic Acids Research* 21 (10): 2525–26.

Geddes, E., C. Morgan-Davies, A. McLaren, P. J. Skuce, J. M. Duncan, N. D. Sargison, and F. Kenyon. 2024. 'Investigating the Perceived versus Actual Gastrointestinal Nematode Challenge on Extensive Sheep Farms'. *Veterinary Parasitology* 327 (110148): 110148.

Geiger, S. M., I. R. Caldas, B. E. Mc Glone, A. C. Campi-Azevedo, L. M. De Oliveira, S. Brooker, D. Diemert, R. Corrêa-Oliveira, and J. M. Bethony. 2007. 'Stage-Specific Immune Responses in Human Necator Americanus Infection'. *Parasite Immunology* 29 (7): 347–58.

Geldhof, P., E. Claerebout, D. P. Knox, J. Jagneessens, and J. Vercruysse. 2000. 'Proteinases Released in Vitro by the Parasitic Stages of the Bovine Abomasal Nematode Ostertagia Ostertagi'. *Parasitology* 121 Pt 6 (December): 639–47.

Gerardo, N. M., K. L. Hoang, and K. S. Stoy. 2020. 'Evolution of Animal Immunity in the Light of Beneficial Symbioses'. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 375 (1808): 20190601.

Gerbe, F., E. Sidot, D. J. Smyth, M. Ohmoto, I. Matsumoto, V. Dardalhon, P. Cesses, L. Garnier, M. Pouzolles, and B. Brulin. 2016. 'Intestinal Epithelial Tuft Cells Initiate Type 2 Mucosal Immunity to Helminth Parasites'. *Nature* 529 (7585): 226–30.

Gibson, T., and G. Everett. 1963. 'The Development of Resistance by Sheep to the Nematode Nematodirus Battus'. *The British Veterinary Journal* 119 (May): 214–18.

Gilbert, M., G. Nicolas, G. Cinardi, T. P. Van Boeckel, S. O. Vanwambeke, G. R. W. Wint, and T. P. Robinson. 2018. 'Global Distribution Data for Cattle, Buffaloes, Horses, Sheep, Goats, Pigs, Chickens and Ducks in 2010'. *Scientific Data* 5 (October): 180227.

Gossner, Anton, Hazel Wilkie, Anagha Joshi, and John Hopkins. 2013. 'Exploring the Abomasal Lymph Node Transcriptome for Genes Associated with Resistance to the Sheep Nematode Teladorsagia Circumcincta'. *Veterinary Research* 44 (1): 68.

Graham, A. L. 2008. 'Ecological Rules Governing Helminth–Microparasite Coinfection'. *Proceedings of the National Academy of Sciences* 105 (2): 566–70.

Graham, E. G., T. J. Harris, and C. B. Ollerenshaw. 1984. 'Some Observations on the Epidemiology of Nematodirus Battus in Anglesey'. *Agricultural and Forest Meteorology* 32 (2): 121–32.

Gravdal, M., I. D. Woolsey, L. J. Robertson, J. Höglund, C. Chartier, and S. Stuen. 2024. 'Occurrence of Gastrointestinal Nematodes in Lambs in Norway, as Assessed by Copromicroscopy and Droplet Digital Polymerase Chain Reaction'. *Acta Veterinaria Scandinavica* 66 (1): 22.

Greer, A. W., J. F. Huntley, A. Mackellar, R. W. McAnulty, N. P. Jay, R. S. Green, M. Stankiewicz, and A. R. Sykes. 2008. 'The Effect of Corticosteroid Treatment on Local Immune Responses, Intake and Performance in Lambs Infected with Teladorsagia Circumcincta'. *International Journal for Parasitology* 38 (14): 1717–28.

Guerra-Silveira, F., and F. Abad-Franch. 2013. 'Sex Bias in Infectious Disease Epidemiology: Patterns and Processes'. *PloS One* 8 (4): e62390.

Gulland, F. M. 1991. 'The Role of Parasites in the Population Dynamics of Soay Sheep on St Kilda'. PhD, University of Cambridge.

———. 1992. 'The Role of Nematode Parasites in Soay Sheep (Ovis Aries L.) Mortality during a Population Crash'. *Parasitology* 105 (Pt 3) (December): 493–503.

Gulland, F. M., and M. Fox. 1992. 'Epidemiology of Nematode Infections of Soay Sheep (Ovis Aries L.) on St Kilda'. *Parasitology* 105 (Pt 3) (December): 481–92.

Gupta, N., and P. A. Pevzner. 2009. 'False Discovery Rates of Protein Identifications: A Strike against the Two-Peptide Rule'. *Journal of Proteome Research* 8 (9): 4173–81.

Habermann, R. T. 1946. 'The Probable Origin of Some Unusually Heavy Infections with Common Sheep Hookworm (Bunostomum Trigonocephalum)'. *Proceedings of the Helminthological Society of Washington* 13 (January): 11.

Halliday, F. W., R. M. Penczykowski, B. Barrès, J. L. Eck, E. Numminen, and A. Laine. 2020. 'Facilitative Priority Effects Drive Parasite Assembly under Coinfection'. *Nature Ecology & Evolution* 4 (11): 1510–21.

Halvarsson, P., P. Baltrušis, P. Kjellander, and J. Höglund. 2022. 'Parasitic Strongyle Nemabiome Communities in Wild Ruminants in Sweden'. *Parasites & Vectors* 15 (1): 341.

Halvarsson, P., G, Giulio, S. Hägglund, and J. Höglund. 2024. 'Gastrointestinal Parasite Community Structure in Horses after the Introduction of Selective Anthelmintic Treatment Strategies'. *Veterinary Parasitology* 326 (110111): 110111.

Halvarsson, P., and J. Höglund. 2021. 'Sheep Nemabiome Diversity and Its Response to Anthelmintic Treatment in Swedish Sheep Herds'. *Parasites & Vectors* 14 (1): 114.

Hamer, K., J. McIntyre, A. A. Morrison, A. Jennings, R. F. Kelly, S. Leeson, D. J. Bartley, U. N. Chaudhry, V. Busin, and N. D. Sargison. 2019. 'The Dynamics of Ovine Gastrointestinal Nematode Infections within Ewe and Lamb Cohorts on Three Scottish Sheep Farms'. *Preventive Veterinary Medicine* 171 (November): 104752.

Hananeh, W. M., A. Radhi, R. M. Mukbel, and Z. B. Ismail. 2022. 'Effects of Parasites Coinfection with Other Pathogens on Animal Host: A Literature Review'. *Veterinary World* 15 (10): 2414–24.

Harrison, G. B. L., H. D. Pulford, E. E. Doolin, A. Pernthaner, C. B. Shoemaker, and W. R. Hein. 2008. 'Antibodies to Surface Epitopes of the Carbohydrate Larval Antigen CarLA Are Associated with Passive Protection in Strongylid Nematode Challenge Infections'. *Parasite Immunology* 30 (11-12): 577–84.

Harrison, G. B. L., H. D. Pulford, T. K. Gatehouse, R. J. Shaw, A. Pfeffer, and C. B. Shoemaker. 1999. 'Studies on the Role of Mucus and Mucosal Hypersensitivity Reactions during Rejection of Trichostrongylus Colubriformis from the Intestine of Immune Sheep Using an Experimental Challenge Model'. *International Journal for Parasitology* 29 (3): 459–68.

Harrison, G. B. L., H. D. Pulford, W. R. Hein, T. K. Barber, R. J. Shaw, M. McNeill, St J Wakefield, and C. B. Shoemaker. 2003. 'Immune Rejection of Trichostrongylus Colubriformis in Sheep; a Possible Role for Intestinal Mucus Antibody against an L3-specific Surface Antigen'. *Parasite Immunology* 25 (1): 45–53.

Harrison, G. B. L., H. D. Pulford, W. R. Hein, W. B. Severn, and C. B. Shoemaker. 2003a. 'Characterization of a 35-kDa Carbohydrate Larval Antigen (CarLA) from Trichostrongylus Colubriformis; a Potential Target for Host Immunity'. *Parasite Immunology* 25 (2): 79–86.

———. 2003b. 'Characterization of a 35-KDa Carbohydrate Larval Antigen (CarLA) from Trichostrongylus Colubriformis; a Potential Target for Host Immunity'. *Parasite Immunology* 25 (2): 79–86.

Hartig, F. 2022. 'DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models'. https://CRAN.R-project.org/package=DHARMa.

Hawkins, H. J. 2017. 'A Global Assessment of Holistic Planned Grazing™ Compared with Season-Long, Continuous Grazing: Meta-Analysis Findings'. *African Journal of Range & Forage Science* 34 (2): 65–75.

Hayward, A. D. 2022. 'Genetic Parameters for Resistance to Gastrointestinal Nematodes in Sheep: A Meta-Analysis'. *International Journal for Parasitology* 52 (13–14): 843–53.

Hayward, A. D., D. H. Nussey, A. J. Wilson, C. Berenos, J. G. Pilkington, K. A. Watt, J. M. Pemberton, and A. L. Graham. 2014. 'Natural Selection on Individual Variation in Tolerance of Gastrointestinal Nematode Infection'. *PLoS Biology* 12 (7): e1001917.

Hayward, A. D., J. G. Pilkington, J. M. Pemberton, and L. E. B. Kruuk. 2010. 'Maternal Effects and Early-Life Performance Are Associated with Parasite Resistance across Life in Free-Living Soay Sheep'. *Parasitology* 137 (8): 1261–73.

Hayward, A. D., J. G. Pilkington, K. Wilson, T. N. McNeilly, and K. A. Watt. 2019. 'Reproductive Effort Influences Intra-seasonal Variation in Parasite-specific Antibody Responses in Wild Soay Sheep'. *Functional Ecology* 33 (7): 1307–20.

Hayward, A. D., A. J. Wilson, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and L. E. B. Kruuk. 2011. 'Natural Selection on a Measure of Parasite Resistance Varies across Ages and Environmental Conditions in a Wild Mammal'. *Journal of Evolutionary Biology* 24 (8): 1664–76.

Heath, M. F., and R. M. Connan. 1991. 'Interaction of Ostertagia and Nematodirus Species in Sheep and the Potential of Serum Fructosamine Determination in Monitoring Gastrointestinal Parasitism'. *Research in Veterinary Science* 51 (3): 322–26.

Hedges, L. V. 1981. 'Distribution Theory for Glass's Estimator of Effect Size and Related Estimators'. *Journal of Educational and Behavioral Statistics: A Quarterly Publication*

Sponsored by the American Educational Research Association and the American Statistical Association 6 (2): 107–28.

———. 1982. 'Estimation of Effect Size from a Series of Independent Experiments'. *Psychological Bulletin* 92 (2): 490–99.

Herd, R. P. 1971. 'The Parasitic Life Cycle of Chabertia Ovina (Fabricius, 1788) in Sheep'. *International Journal for Parasitology* 1 (2): 189–99.

Herlich, H. 1965. 'Immunity and Cross Immunity to Cooperia Oncophora and Cooperia Pectinata in Calves and Lambs'. *American Journal of Veterinary Research* 26 (114): 1037–41.

Hildersley, K. A., T. N. McNeilly, V. Gillan, T. D. Otto, S. Löser, F. Gerbe, P. Jay, R. M. Maizels, E. Devaney, and C. Britton. 2021. 'Tuft Cells Increase Following Ovine Intestinal Parasite Infections and Define Evolutionarily Conserved and Divergent Responses'. *Frontiers in Immunology*, 4979.

Hirano, M., Rl S. Davis, W. D. Fine, S. Nakamura, K. Shimizu, H. Yagi, K. Kato, R. P. Stephan, and M. D. Cooper. 2007. 'IgEb Immune Complexes Activate Macrophages through FcgammaRIV Binding'. *Nature Immunology* 8 (7): 762–71.

Hoagland, K. E., and G. A. Schad. 1978. 'Necator Americanus and Ancylostoma Duodenale: Life History Parameters and Epidemiological Implications of Two Sympatric Hookworms of Humans'. *Experimental Parasitology* 44 (1): 36–49.

Holmstad, P. R., P. J. Hudson, and A. Skorping. 2005. 'The Influence of a Parasite Community on the Dynamics of a Host Population: A Longitudinal Study on Willow Ptarmigan and Their Parasites'. *Oikos* 111 (2): 377–91.

Horton, J. 2003. 'Human Gastrointestinal Helminth Infections: Are They Now Neglected Diseases?' *Trends in Parasitology* 19 (11): 527–31.

Hoste, H., and J. Cabaret. 1992. 'Intergeneric Relations between Nematodes of the Digestive Tract in Lambs: A Multivariate Approach'. *International Journal for Parasitology* 22 (2): 173–79.

Hothorn, Torsten, Frank Bretz, and Peter Westfall. 2008. 'Simultaneous Inference in General Parametric Models'. *Biometrical Journal. Biometrische Zeitschrift* 50 (3): 346–63.

Houdijk, J. G. M. 2008. 'Influence of Periparturient Nutritional Demand on Resistance to Parasites in Livestock: Nutritional Demand and Immunity to Parasites'. *Parasite Immunology* 30 (2): 113–21.

Howitt, M. R., S. Lavoie, M. Michaud, A. M. Blum, S. V. Tran, J. V. Weinstock, C. A. Gallini, K. Redding, R. F. Margolskee, and L. C. Osborne. 2016. 'Tuft Cells, Taste-Chemosensory Cells, Orchestrate Parasite Type 2 Immunity in the Gut'. *Science* 351 (6279): 1329–33.

Hulsen, Tim. 2022. 'DeepVenn -- a Web Application for the Creation of Area-Proportional Venn Diagrams Using the Deep Learning Framework Tensorflow.Js'. *ArXiv [Cs.HC]*. arXiv. http://arxiv.org/abs/2210.04597.

Huntley, J. F., G. Newlands, and H. R. Miller. 1984. 'The Isolation and Characterization of Globule Leucocytes: Their Derivation from Mucosal Mast Cells in Parasitized Sheep'. *Parasite Immunology* 6 (4): 371–90.

Iijima, H., I. Takahashi, and H. Kiyono. 2001. 'Mucosal Immune Network in the Gut for the Control of Infectious Diseases'. *Reviews in Medical Virology* 11 (2): 117–33.

Ilík, V., J. Kreisinger, D. Modrý, E. M. Schwarz, N. Tagg, D. Mbohli, I. C. Nkombou, K. Judita Petrželková, and B. Pafčo. 2023. 'High Diversity and Sharing of Strongylid Nematodes in Humans and Great Apes Co-Habiting an Unprotected Area in Cameroon'. *PLoS Neglected Tropical Diseases* 17 (8): e0011499.

Illius, A. W., S. D. Albon, J. M. Pemberton, I. J. Gordon, and T. H. Clutton-Brock. 1995. 'Selection for Foraging Efficiency during a Population Crash in Soay Sheep'. *The Journal of Animal Ecology* 64 (4): 481.

Israf, D. A., R. L. Coop, F. Jackson, and E. Jackson. 1996. 'Effect of Dietary Protein on the Regulation of Populations of Nematodirus Battus by Lambs'. *Research in Veterinary Science* 60 (3): 276–77.

Israf, D. A., R. L. Coop, L. M. Stevenson, D. G. Jones, F. Jackson, E. Jackson, A. MacKellar, and J. F. Huntley. 1996. 'Dietary Protein Influences upon Immunity to Nematodirus Battus Infection in Lambs'. *Veterinary Parasitology* 61 (3–4): 273–86.

Jackson, F. 1974. 'New Technique for Obtaining Nematode Ova from Sheep Faeces'. *Laboratory Practice* 23 (2): 65–66.

Jackson, F., E. Jackson, R. L. Coop, and J. Huntley. 1992. 'Interactions between Teladorsagia Circumcincta and Trichostrongylus Vitrinus Infections in Young Lambs'. *Research in Veterinary Science* 53 (3): 363–70.

Jackson, F., E. Jackson, and J. T. Williams. 1988. 'Susceptibility of the Pre-Parturient Ewe to Infection with Trichostrongylus Vitrinus and Ostertagia Circumcincta'. *Research in Veterinary Science* 45 (2): 213–18.

Jenkins, T. P., D. I. Pritchard, R. Tanasescu, G. Telford, M. Papaiakovou, R. Scotti, A. Cortés, C. S. Constantinescu, and C. Cantacessi. 2021. 'Experimental Infection with the Hookworm, Necator Americanus, Is Associated with Stable Gut Microbial Diversity in Human Volunteers with Relapsing Multiple Sclerosis'. *BMC Biology* 19 (1): 74.

Johnson, Pieter T. J., and Ian D. Buller. 2011. 'Parasite Competition Hidden by Correlated Coinfection: Using Surveys and Experiments to Understand Parasite Interactions'. *Ecology* 92 (3): 535–41.

Jouffroy, S., C. Girard, E. Giraud, C. Beaumelle, G. Bourgoin, L. Bordes, C. Grisez, et al. 2025. 'Transhumance and Eprinomectin Resistance of Haemonchus Contortus in Dairy Sheep Flocks of the French Pyrenees'. *Veterinary Parasitology* 340 (110618): 110618.

Kamenov, Y., and K. Kanchev. 2022. 'On the Pathogenic Role of Larvae and Mature Stages of Bunostomum Trigonocephalum (Nematoda: Ancylostomatidae)'. *Comptes Rendus de l'Academie Bulgare Des Sciences: Sciences Mathematiques et Naturelles* 75 (12): 1848–51.

Kaplan, R. M., M. J. Denwood, M. K. Nielsen, S. M. Thamsborg, P. R. Torgerson, J. S. Gilleard, R. J. Dobson, J. Vercruysse, and B. Levecke. 2023. 'World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guideline for Diagnosing Anthelmintic Resistance Using the Faecal Egg Count Reduction Test in Ruminants, Horses and Swine'. *Veterinary Parasitology* 318 (109936): 109936.

Kaplan, R. M., and A. N. Vidyashankar. 2012. 'An Inconvenient Truth: Global Worming and Anthelmintic Resistance'. *Veterinary Parasitology* 186 (1–2): 70–78.

Karvonen, A., A. M. Bagge, and E. T. Valtonen. 2007. 'Interspecific and Intraspecific Interactions in the Monogenean Communities of Fish: A Question of Study Scale?' *Parasitology* 134 (9): 1237–42.

Kates, K. C. 1943. 'Overwinter Survival on Pasture of Preparasitic Stages of Some Nematodes Parasitic in Sheep' 10: 23–25.

Kates, K. C., and J. H. Turner. 1953. 'A Comparison of the Pathogenicity and Course of Infection of Two Nematodes of Sheep, Nematodirus Spathiger and Trichostrongylus Colubriformis, in Pure and Mixed Infections'. *Proceedings of the Helminthological Society of Washington* 20 (2): 117–24.

———. 1960a. ‘An Experiment on the Combined Pathogenic Effects of Haemonchus Contortus and Nematodirus Spathiger on Lambs’. *Proceedings of the Helminthological Society of Washington* 27: 62.

———. 1960b. ‘Experimental Trichostrongylosis (Axei) in Lambs, with a Discussion of Recent Research on This Disease in Ruminants’. *American Journal of Veterinary Research* 21 (March): 254–61.

Kebeta, M. M., B. C. Hine, S. W. Walkden-Brown, L. P. Kahn, and E. K. Doyle. 2021. ‘Protective Efficacy of Barbervax® in Merino Weaner Sheep Trickle Infected with Five Doses of Haemonchus Contortus Infective Larvae’. *Veterinary Parasitology* 292 (109386): 109386.

Keegan, S. P., A. B. Pedersen, and A. Fenton. 2024. ‘The Impact of Within-Host Coinfection Interactions on between-Host Parasite Transmission Dynamics Varies with Spatial Scale’. *Proceedings. Biological Sciences* 291 (2021): 20240103.

Kelly, R. F., E. Galbraith, O. Zahid, U. N. Chaundhry, and N. D. Sargison. 2025. ‘The Dynamics of Gastrointestinal Nematodes Present in Co-Grazed Host Species Kept in a Scottish Zoological Collection’. *Veterinary Parasitology (Amsterdam: Online)* 59 (101227): 101227.

Kenyon, F., Jan Dick, Ron Smith, Drew Coulter, David McBean, and Philip Skuce. 2013. ‘Reduction in Greenhouse Gas Emissions Associated with Worm Control in Lambs’. *Collection FAO: Agriculture* 3 (2): 271–84.

Kenyon, F., N. D. Sargison, P. J. Skuce, and F. Jackson. 2009. ‘Sheep Helminth Parasitic Disease in South Eastern Scotland Arising as a Possible Consequence of Climate Change’. *Veterinary Parasitology* 163 (4): 293–97.

Khare, R. K., A. K. Dixit, R. Kumar, G. Das, D. Bhinsara, S. Ghosh, R. Singh, D. Chandra, and M. Sankar. 2018. 'Prevalence of Bunostomum Trigonocephalum Infection in Sheep and Goats in Madhya Pradesh'. *Indian Journal of Veterinary Sciences and Biotechnology* 14 (2): 78–81.

Kidane, A., J. Houdijk, S. Athanasiadou, B. Tolkamp, and I. Kyriazakis. 2010. 'Nutritional Sensitivity of Periparturient Resistance to Nematode Parasites in Two Breeds of Sheep with Different Nutrient Demands'. *The British Journal of Nutrition* 104 (10): 1477–86.

Kidane, A., J. G. M. Houdijk, B. J. Tolkamp, S. Athanasiadou, and I. Kyriazakis. 2009. 'Consequences of Infection Pressure and Protein Nutrition on Periparturient Resistance to Teladorsagia Circumcincta and Performance in Ewes'. *Veterinary Parasitology* 165 (1–2): 78–87.

Kie, J. G., J. Matthiopoulos, J. Fieberg, R. A. Powell, F. Cagnacci, M. S. Mitchell, J. M. Gaillard, and P. R. Moorcroft. 2010. 'The Home-Range Concept: Are Traditional Estimators Still Relevant with Modern Telemetry Technology?' *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 365 (1550): 2221–31.

Kilpimaa, J., R. V. Alatalo, and H. Siitari. 2004. 'Trade-Offs between Sexual Advertisement and Immune Function in the Pied Flycatcher (Ficedula Hypoleuca)'. *Proceedings. Biological Sciences* 271 (1536): 245–50.

Kipp, Kaylee R., Elizabeth M. Redman, Joe L. Luksovsky, Dani Claussen, John S. Gilleard, and Guilherme G. Verocai. 2025. 'High Frequency of Benzimidazole Resistance Polymorphisms and Age-Class Differences in Trichostrongyle Nematodes of Ranched Bison from the South-Central United States'. *International Journal for Parasitology, Drugs and Drug Resistance* 28 (100594): 100594.

Kolberg, L., U. Raudvere, I. Kuzmin, P. Adler, J. Vilo, and H. Peterson. 2023. 'G:Profiler-Interoperable Web Service for Functional Enrichment Analysis and Gene Identifier Mapping (2023 Update)'. *Nucleic Acids Research* 51 (W1): W207–12.

Kreider, Timothy, Robert M. Anthony, Joseph F. Urban Jr, and William C. Gause. 2007. 'Alternatively Activated Macrophages in Helminth Infections'. *Current Opinion in Immunology* 19 (4): 448–53.

Krücken, J., P. Ehnert, S. Fiedler, F. Horn, C. S. Helm, S. Ramünke, T. Bartmann, et al. 2024. 'Faecal Egg Count Reduction Tests and Nemabiome Analysis Reveal High Frequency of Multi-Resistant Parasites on Sheep Farms in North-East Germany Involving Multiple Strongyle Parasite Species'. *International Journal for Parasitology, Drugs and Drug Resistance* 25 (100547): 100547.

Krysl, L. J., M. L. Galyean, R. E. Estell, and B. F. Sowell. 1988. 'Estimating Digestibility and Faecal Output in Lambs Using Internal and External Markers'. *The Journal of Agricultural Science* 111 (1): 19–25.

Leathwick, D., P. Green, C. Bouchet, A. Chambers, T. Waghorn, and C. Sauermann. 2025. 'The Faecal Egg Count Reduction Test: Will Identification of Larvae to Species Improve Its Utility?' *International Journal for Parasitology, Drugs and Drug Resistance* 27 (100589): 100589.

Leivesley, J. A., L. F. Bussière, J. M. Pemberton, J. G. Pilkington, K. Wilson, and A. D. Hayward. 2019. 'Survival Costs of Reproduction Are Mediated by Parasite Infection in Wild Soay Sheep'. *Ecology Letters* 22 (8): 1203–13.

Lello, J., B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson. 2004. 'Competition and Mutualism among the Gut Helminths of a Mammalian Host'. *Nature* 428 (6985): 840–44.

Lello, J., B. Boag, and P. J. Hudson. 2005. 'The Effect of Single and Concomitant Pathogen Infections on Condition and Fecundity of the Wild Rabbit (Oryctolagus Cuniculus)'. *International Journal for Parasitology* 35 (14): 1509–15.

Lello, J., and T. Hussell. 2008. 'Functional Group/Guild Modelling of Inter-Specific Pathogen Interactions: A Potential Tool for Predicting the Consequences of Co-Infection'. *Parasitology* 135 (7): 825–39.

Lello, J., S. J. McClure, K. Tyrrell, and M. E. Viney. 2018. 'Predicting the Effects of Parasite Co-Infection across Species Boundaries'. *Proceedings of the Royal Society B: Biological Sciences* 285 (1874): 20172610.

Liu, W., T. N. McNeilly, M. Mitchell, S. T. G. Burgess, A. J. Nisbet, J. B. Matthews, and S. A. Babayan. 2022. 'Vaccine-Induced Time- and Age-Dependent Mucosal Immunity to Gastrointestinal Parasite Infection'. *Npj Vaccines* 7 (1): 78.

Lok, J. B., and T. R. Unnasch. 2018. *Transgenesis in Animal Parasitic Nematodes: Strongyloides Spp. and Brugia Spp*. Wormbook Press.

Longhi-Browne, C. W. 2014. 'Using Caenorhabditis Elegans as a Novel Expression System for the Generation of Recombinant Teladorsagia Circumcincta Vaccine Candidates'. PhD, University of Glasgow. https://theses.gla.ac.uk/5547/.

Love, M. I., W. Huber, and S. Anders. 2014. 'Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data with DESeq2'. *Genome Biology*. https://doi.org/10.1186/s13059-014-0550-8.

Lu, M. R., C. K. Lai, B. Y. Liao, and I. J. Tsai. 2020. 'Comparative Transcriptomics across Nematode Life Cycles Reveal Gene Expression Conservation and Correlated Evolution in Adjacent Developmental Stages'. *Genome Biology and Evolution* 12 (7): 1019–30.

Lucker, J., and E. Neumayer. 1944. 'The Production of Anemia in Lambs by Hookworms, Bunostomum Trigonocephalum'. *The Journal of Parasitology* 30. https://doi.org/10.5555/19440800657.

Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. 'Performance: An R Package for Assessment, Comparison and Testing of Statistical Models'. *Journal of Open Source Software*. https://doi.org/10.21105/joss.03139.

Ma, J., S. W. He, H. Li, Q. C. Guo, W. W. Pan, X. J. Wang, J. Zhang, L. Z. Liu, W. Liu, and Y. Liu. 2014. 'First Survey of Helminths in Adult Goats in Hunan Province, China'. *Tropical Biomedicine* 31 (2): 261–69.

MAFF. 1986. 'Manual of Veterinary Parasitological Laboratory Techniques HMSO'.

Mapes, C. J., and R. L. Coop. 1970. 'The Interaction of Infections of Haemonchus Contortus and Nematodirus Battus in Lambs. I. The Effect of Massive Infections of Haemonchus on Subsequent Infections of Nematodirus'. *Journal of Comparative Pathology* 80 (1): 123–36.

———. 1971. 'Effect of Concurrent and Terminated Infections of Haemonchus Contortus on the Development and Reproductive Capacity of Nematodirus Battus'. *Journal of Comparative Pathology* 81 (4): 479–92.

Mathers, Colin D., Majid Ezzati, and Alan D. Lopez. 2007. 'Measuring the Burden of Neglected Tropical Diseases: The Global Burden of Disease Framework'. *PLoS Neglected Tropical Diseases* 1 (2): e114.

Matos, C. A. P., and D. L. Thomas. 1992. 'Physiology and Genetics of Testicular Size in Sheep: A Review'. *Livestock Production Science* 32 (1): 1–30.

Mavrot, F., H. Hertzberg, and P. Torgerson. 2015. 'Effect of Gastro-Intestinal Nematode Infection on Sheep Performance: A Systematic Review and Meta-Analysis'. *Parasites and Vectors* 8 (1). https://doi.org/10.1186/s13071-015-1164-z.

McArdle, A. J., A. Turkova, and A. J. Cunnington. 2018. 'When Do Co-Infections Matter?' *Current Opinion in Infectious Diseases* 31 (3): 209–15.

McFarland, C., H. Rose Vineer, L. Chesney, N. Henry, C. Brown, P. Airs, C. Nicholson, et al. 2022. 'Tracking Gastrointestinal Nematode Risk on Cattle Farms through Pasture Contamination Mapping'. *International Journal for Parasitology* 52 (10): 691–703.

McIntyre, J., K. Hamer, A. A. Morrison, D. J. Bartley, N. D. Sargison, E. Devaney, and R. Laing. 2018. 'Hidden in Plain Sight - Multiple Resistant Species within a Strongyle Community'. *Veterinary Parasitology*. https://doi.org/10.1016/j.vetpar.2018.06.012.

McKean, P. G., and D. I. Pritchard. 1989. 'The Action of a Mast Cell Protease on the Cuticular Collagens of Necator Americanus'. *Parasite Immunology* 11 (3): 293–97.

McKenna, P. B. 1981. 'The Diagnosis Value and Interpretation of Faecal Egg Counts in Sheep'. *New Zealand Veterinary Journal* 29 (8): 129–32.

McKenzie, G. J., A. Bancroft, R. K. Grencis, and A. N. McKenzie. 1998. 'A Distinct Role for Interleukin-13 in Th2-Cell-Mediated Immune Responses'. *Current Biology: CB* 8 (6): 339–42.

McLeod, R. S. 1995. 'Costs of Major Parasites to the Australian Livestock Industries'. *International Journal for Parasitology* 25 (11): 1363–67.

McNeilly, T. N., E. Devaney, and J. B. Matthews. 2009. 'Teladorsagia Circumcincta in the Sheep Abomasum: Defining the Role of Dendritic Cells in T Cell Regulation and Protective Immunity'. *Parasite Immunology* 31 (7): 347–56.

McNeilly, T. N., S. W. Naylor, M. C. Mitchell, S. McAteer, A. Mahajan, D. G. E. Smith, D. L. Gally, J. C. Low, and J. F. Huntley. 2007. 'Simple Methods for Measurement of Bovine Mucosal Antibody Responses in Vivo'. *Veterinary Immunology and Immunopathology* 118 (1–2): 160–67.

McNeilly, T. N., and A. J. Nisbet. 2014. 'Immune Modulation by Helminth Parasites of Ruminants: Implications for Vaccine Development and Host Immune Competence'. *Parasite* 21: 51.

McNeilly, T. N., M. Rocchi, Y. Bartley, J. K. Brown, D. Frew, C. W. Longhi-Browne, L. McLean, et al. 2013. 'Suppression of Ovine Lymphocyte Activation by Teladorsagia Circumcincta Larval Excretory-Secretory Products'. *Veterinary Research* 44 (1): 70.

McRae, K. M., M. J. Stear, B. Good, and O. M. Keane. 2015. 'The Host Immune Response to Gastrointestinal Nematode Infection in Sheep'. *Parasite Immunology* 37 (12): 605–13.

Meeusen, E. N., and A. Balic. 2000. 'Do Eosinophils Have a Role in the Killing of Helminth Parasites?' *Parasitology Today (Personal Ed.)* 16 (3): 95–101.

Metz, M, and M. Maurer. 2007. 'Mast Cells--Key Effector Cells in Immune Responses'. *Trends in Immunology* 28 (5): 234–41.

Midha, A., J. Schlosser, and S. Hartmann. 2017. 'Reciprocal Interactions between Nematodes and Their Microbial Environments'. *Frontiers in Cellular and Infection Microbiology* 7 (April): 144.

Milner, J. M., S. D. Albon, A. W. Illius, J. M. Pemberton, and T. H. Clutton-Brock. 1999. 'Repeated Selection of Morphometric Traits in the Soay Sheep on St Kilda'. *The Journal of Animal Ecology* 68 (3): 472–88.

Molenberghs, G., G. Verbeke, and C. G. B. Demétrio. 2007. 'An Extended Random-Effects Approach to Modeling Repeated, Overdispersed Count Data'. *Lifetime Data Analysis* 13 (4): 513–31.

Morales, G., L. A. Pino, and E. Sandoval. 2006. 'Intestinal Strongylosis in Grazing Sheep in Venezuela'. *REDVET* 7 (11): 110619.

Morgan, E. R., N. A. A. Aziz, A. Blanchard, J. Charlier, C. Charvet, E. Claerebout, P. Geldhof, et al. 2019. '100 Questions in Livestock Helminthology Research'. *Trends in Parasitology* 35 (1): 52–71.

Morgan, E. R., and J. van Dijk. 2012. 'Climate and the Epidemiology of Gastrointestinal Nematode Infections of Sheep in Europe'. *Veterinary Parasitology* 189 (1): 8–14.

Mowat, A. M., and W. W. Agace. 2014. 'Regional Specialization within the Intestinal Immune System'. *Nature Reviews. Immunology* 14 (10): 667–85.

Murall, Carmen Lia, Jessica L. Abbate, Maximilian Puelma Touzel, Emma Allen-Vercoe, Samuel Alizon, Remy Froissart, and Kevin McCann. 2017. 'Invasions of Host-Associated Microbiome Networks'. Edited by David A. Bohan, Alex J. Dumbrell, and François Massol. *Advances in Ecological Research* 57: 201–81.

Naem, S., and T. Gorgani. 2011. 'Gastrointestinal Parasitic Infection of Slaughtered Sheep (Zel Breed) in Fereidoonkenar City, Iran'. *Veterinary Research Forum* 2 (December): 238–41.

Nakagawa, S., M. Lagisz, M. D. Jennions, J. Koricheva, D. W. A. Noble, T. H. Parker, A. Sánchez-Tójar, Y. Yang, and R. E. O'Dea. 2022. 'Methods for Testing Publication Bias in Ecological and Evolutionary Meta-analyses'. *Methods in Ecology and Evolution / British Ecological Society* 13 (1): 4–21.

Nakagawa, S., M. Lagisz, R. E. O'Dea, J. Rutkowska, Y. Yang, D. W. A. Noble, and A. M. Senior. 2021. 'The Orchard Plot: Cultivating a Forest Plot for Use in Ecology, Evolution, and Beyond'. *Research Synthesis Methods*. https://doi.org/10.1002/jrsm.1424.

Ndjim, M., I. Gasmi, F. Herbert, C. Joséphine, J. Bas, A. Lamrani, N. Coutry et al. 2024. 'Tuft Cell Acetylcholine Is Released into the Gut Lumen to Promote Anti-Helminth Immunity'. *Immunity* 57 (6): 1260-1273.e7.

Neumayer, J. T., and E. Lucker. 1947. 'An Experiment on the Relationship of Diet to Hookworm Disease in Lambs'. *American Journal of Veterinary Research* 8: 400–412.

Newton, P., N. Civita, L. Frankel-Goldwater, K. Bartel, and C. Johns. 2020. 'What Is Regenerative Agriculture? A Review of Scholar and Practitioner Definitions Based on Processes and Outcomes'. *Frontiers in Sustainable Food Systems* 4 (October): 577723.

Nielsen, M. K., A. E. Steuer, H. P. Anderson, S. Gavriliuc, A. B. Carpenter, E. M. Redman, J. S. Gilleard, C. R. Reinemeyer, and J. Poissant. 2022. 'Shortened Egg Reappearance Periods of Equine Cyathostomins Following Ivermectin or Moxidectin Treatment: Morphological and Molecular Investigation of Efficacy and Species Composition'. *International Journal for Parasitology* 52 (12): 787–98.

Nieuwhof, G. J., and S. C. Bishop. 2005. 'Costs of the Major Endemic Diseases of Sheep in Great Britain and the Potential Benefits of Reduction in Disease Impact'. *Animal Science* 81 (1): 23–29.

Nisbet, A. J., D. P. Knox, C. M. McNair, L. I. Meikle, S. K. Smith, L. A. Wildblood, and J. B. Matthews. 2009. 'Immune Recognition of the Surface Associated Antigen, Tc-SAA-1, from Infective Larvae of Teladorsagia Circumcincta'. *Parasite Immunology* 31 (1): 32–40.

Nisbet, A. J., T. N. McNeilly, D. R. G. Price, Y. Bartley, M. Oliver, D. McBean, L. Andrews, et al. 2024. 'Field Testing of Recombinant Subunit Vaccines against Teladorsagia Circumcincta in Lambing Ewes Demonstrates a Lack of Efficacy in the Face of a Multi-Species Parasite Challenge'. *Frontiers in Parasitology* 3 (March): 1360029.

Nisbet, A. J., T. N. McNeilly, Louise A. Wildblood, Alison A. Morrison, David J. Bartley, Yvonne Bartley, Cassandra Longhi, Iain J. McKendrick, Javier Palarea-Albaladejo, and Jacqueline B. Matthews. 2013. 'Successful Immunization against a Parasitic Nematode by Vaccination with Recombinant Proteins'. *Vaccine* 31 (37): 4017–23.

Nussey, D. H., K. A. Watt, A. Clark, J. G. Pilkington, J. M. Pemberton, A. L. Graham, and T. N. McNeilly. 2014. 'Multivariate Immune Defences and Fitness in the Wild: Complex but Ecologically Important Associations among Plasma Antibodies, Health and Survival'. *Proceedings. Biological Sciences* 281 (1779): 20132931.

Nyström, E. E. L., L. Arike, E. Ehrencrona, G. C. Hansson, and M. E. V. Johansson. 2019. 'Calcium-Activated Chloride Channel Regulator 1 (CLCA1) Forms Non-Covalent Oligomers in Colonic Mucus and Has Mucin 2-Processing Properties'. *The Journal of Biological Chemistry* 294 (45): 17075–89.

O'Connor, L. J., S. W. Walkden-Brown, and L. P. Kahn. 2006. 'Ecology of the Free-Living Stages of Major Trichostrongylid Parasites of Sheep'. *Veterinary Parasitology*. https://doi.org/10.1016/j.vetpar.2006.08.035.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, et al. 2022. 'Vegan: Community Ecology Package'. https://CRAN.R-project.org/package=vegan.

Ortlepp, R. J., and P. J. Du Toit. 1939. 'Observations on the Life-History of Bunostomum Trigonocephalum, a Hookworm of Sheep and Goats'. *The Onderstepoort Journal of Veterinary Science and Animal Industry* 12: 305–18.

Overall, A. D. J., K. A. Byrne, J. G. Pilkington, and J. M. Pemberton. 2005. 'Heterozygosity, Inbreeding and Neonatal Traits in Soay Sheep on St Kilda'. *Molecular Ecology* 14 (11): 3383–93.

Page, M. J., J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L. Shamseer, et al. 2021. 'The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews'. *BMJ* 372 (March): n71.

Page, M. J., D. Moher, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L. Shamseer, et al. 2021. 'PRISMA 2020 Explanation and Elaboration: Updated Guidance and Exemplars for Reporting Systematic Reviews'. *BMJ* 372 (March): n160.

Parnell, I. W., C. Rayski, A. M. Dunn, and G. M. Mackintosh. 1954. 'A Survey of the Helminths of Scottish Hill Sheep'. *Journal of Helminthology* 28 (1–2): 53–110.

Paton, G., R. J. Thomas, and P. J. Waller. 1984. 'A Prediction Model for Parasitic Gastro-Enteritis in Lambs'. *International Journal for Parasitology* 14 (5): 439–45.

Pedersen, A. B., and J. Antonovics. 2013. 'Anthelmintic Treatment Alters the Parasite Community in a Wild Mouse Host'. *Biology Letters* 9 (4): 20130205.

Pedersen, A. B., and A. Fenton. 2007. 'Emphasizing the Ecology in Parasite Community Ecology'. *Trends in Ecology & Evolution* 22 (3): 133–39.

Poissant, J., S. Gavriliuc, J. Bellaw, E. M. Redman, R. W. Avramenko, D. Robinson, M. L. Workentine, et al. 2021. 'A Repeatable and Quantitative DNA Metabarcoding Assay to Characterize Mixed Strongyle Infections in Horses'. *International Journal for Parasitology* 51 (2–3): 183–92.

Poulin, R. 2001. ‘Interactions between Species and the Structure of Helminth Communities’. *Parasitology* 122 Suppl: S3-11.

Powers, T. O., T. C. Todd, A. M. Burnell, P. C. Murray, C. C. Fleming, A. L. Szalanski, B. A. Adams, and T. S. Harris. 1997. ‘The RDNA Internal Transcribed Spacer Region as a Taxonomic Marker for Nematodes’. *Journal of Nematology* 29 (4): 441–50.

Premvati, G., and B. Narain. 1969. ‘Effect of Temperature on Hatching and Viability of the Eggs of Bunostomum Trigonocephalum Rudolphi, 1808’. *Parasitology* 59 (1): 185–91.

Preston, B. T., I. R. Stevenson, G. A. Lincoln, S. L. Monfort, J. G. Pilkington, and K. Wilson. 2012. ‘Testes Size, Testosterone Production and Reproductive Behaviour in a Natural Mammalian Mating System: Testes Size and Testosterone in Soay Sheep’. *The Journal of Animal Ecology* 81 (1): 296–305.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, D. W. Coltman, and K. Wilson. 2003. ‘Overt and Covert Competition in a Promiscuous Mammal: The Importance of Weaponry and Testes Size to Male Reproductive Success’. *Proceedings. Biological Sciences* 270 (1515): 633–40.

Pullan, R., and S. Brooker. 2008. ‘The Health Impact of Polyparasitism in Humans: Are We under-Estimating the Burden of Parasitic Diseases?’ *Parasitology* 135 (7): 783–94.

Pustovoi, I. F. 1972. ‘Interspecies Relationships of Gastro-Intestinal Strongyles of Sheep. [Russian]’. In *Trudy VII Vsesoyuznoi Konferentsii Po Prirodnoi Ochagovosti Boleznei i Obshchim Voprosam Parazitologii Zhivotnykh*, 143–47. Tajik Veterinary Research Institute.

Queiroz, C., M. Levy, R. W. Avramenko, E. M. Redman, K. Kearns, and L. Swain. 2020. ‘The Use of ITS-2 RDNA Nemabiome Metabarcoding to Enhance Anthelmintic Resistance

Diagnosis and Surveillance of Ovine Gastrointestinal Nematodes'. *International Journal for Parasitology: Drugs and Drug Resistance* 14: 105–17.

R Core Team. 2023. 'R: A Language and Environment for Statistical Computing'. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rainbird, M. A., D. Macmillan, and E. N. T. Meeusen. 1998. 'Eosinophil-mediated Killing of Haemonchus Contortus Larvar: Effect of Eosinophil Activation and Role of Antobody, Complement and Interleukin-5: Parasite Immunology'. *Parasite Immunology* 20 (2): 93–103.

Ramos, A.C.S., L.M. Oliveira, Y.L.D.C.O. SantoS, M.C.S. Dantas, C.I.B. Walker, A.M.C. Faria, L.L. Bueno, S.S. Dolabella and R.T. Fujiwara. 2022. 'The Role of IgA in Gastrointestinal Helminthiasis: A Systematic Review'. *Immunology Letters* 249 (September): 12–22.

Redman, E. M., C. Queiroz, D. J. Bartley, M. Levy, R. W. Avramenko, and J. Stuart Gilleard. 2019. 'Validation of ITS-2 RDNA Nemabiome Sequencing for Ovine Gastrointestinal Nematodes and Its Application to a Large Scale Survey of UK Sheep Farms'. *Veterinary Parasitology* 275 (November). https://doi.org/10.1016/j.vetpar.2019.108933.

Redman, E. M., F. Whitelaw, A. Tait, C. Burgess, Y. Bartley, P. Skuce, F. Jackson, and J. S. Gilleard. 2015. 'The Emergence of Resistance to the Benzimidazole Anthlemintics in Parasitic Nematodes of Livestock Is Characterised by Multiple Independent Hard and Soft Selective Sweeps'. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0003494.

Rehbein, S., T. Lindner, M. Kollmannsberger, R. Winter, and M. Visser. 1997. '[Helminth infection of slaughtered sheep in Upper Bavaria. 3. Distribution of colonization of nematodes in the large intestine of sheep]'. *Berliner und Munchener tierarztliche Wochenschrift* 110 (6): 223–28.

Reid, J. F., and J. Armour. 1972. ‘Seasonal Fluctuations and Inhibited Development of Gastro-Intestinal Nematodes of Sheep’. *Research in Veterinary Science* 13 (3): 225–29.

———. 1975. ‘Seasonal Variations in the Gastro-Intestinal Nematode Populations of Scottish Hill Sheep’. *Research in Veterinary Science* 18 (3): 307–13.

Reinecke, R. K. 1974. ‘Studies on Haemonchus Contortus. I. The Influence of Previous Exposure to Trichostrongylus Axei on Infestation with H. Contortus’. *The Onderstepoort Journal of Veterinary Research* 41 (4): 213–15.

Reinecke, R. K., C. Bruckner, and I. L. De Villiers. 1981. ‘Studies on Haemonchus Contortus. IV. The Effect of Trichostrongylus Axei and Ostertagia Circumcincta on Challenge with H. Contortus’. *The Onderstepoort Journal of Veterinary Research* 48 (4): 229–34.

Reinecke, R. K., C. Brückner, and I. L. De Villiers. 1982. ‘Studies on Haemonchus Contortus. VII. The Effect of Treatment of Trichostrongylus Axei Prior to Challenge with H. Contortus’. *The Onderstepoort Journal of Veterinary Research* 49 (1): 69.

Reinecke, R. K., C. M. Bruckner, and I. L. De Villiers. 1980. ‘Studies on Haemonchus Contortus. III. Titration of Trichostrongylus Axei and Explusion of H. Contortus’. *The Onderstepoort Journal of Veterinary Research* 47 (1): 35–44.

Reinecke, R. K., I. L. De Villiers, and C. Brückner. 1982. ‘Studies on Haemonchus Contortus. VI. Attempts to Stimulate Immunity to Abomasal Trichostrongylids in Merino Sheep’. *The Onderstepoort Journal of Veterinary Research* 49 (1): 3–6.

Reinecke, R. K., I. L. De Villiers, and G. Joubert. 1982. ‘Studies on Haemonchus Contortus. VIII. Attempts to Protect Suckling Lambs against Infestation with H. Contortus’. *The Onderstepoort Journal of Veterinary Research* 49 (3): 149–50.

Reinecke, R. K., H. M. Snyman, and H. Seaman. 1979. 'Studies on Haemonchus Contortus. II. The Effect of Abomasal Nematodes on Subsequent Challenge with H. Contortus'. *The Onderstepoort Journal of Veterinary Research* 46 (4): 199–205.

Rijal, S., P. Neuhaus, J. Thorley, N. Caulkett, S. Kutz, and K. E. Ruckstuhl. 2024. 'Patterns of Gastrointestinal Parasite Infections in Bighorn Sheep, Ovis Canadensis, with Respect to Host Sex and Seasonality'. *International Journal for Parasitology. Parasites and Wildlife* 24 (100950): 100950.

Roberts, J. L., and R. A. Swan. 1981. 'Quantitative Studies of Ovine Haemonchosis. I. Relationship between Faecal Egg Counts and Total Worm Counts'. *Veterinary Parasitology* 8 (2): 165–71.

Robinson, M. R., J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and L. E. B. Kruuk. 2006. 'Live Fast, Die Young: Trade-Offs between Fitness Components and Sexually Antagonistic Selection on Weaponry in Soay Sheep'. *Evolution; International Journal of Organic Evolution* 60 (10): 2168–81.

Roeber, Florian, Alison Morrison, Stijn Casaert, Lee Smith, Edwin Claerebout, and Philip Skuce. 2017. 'Multiplexed-Tandem PCR for the Specific Diagnosis of Gastrointestinal Nematode Infections in Sheep: An European Validation Study'. *Parasites & Vectors* 10 (1): 226.

Rogozynski, N. P., and B. Dixon. 2024. 'The Th1/Th2 Paradigm: A Misrepresentation of Helper T Cell Plasticity'. *Immunology Letters* 268 (106870): 106870.

Rose Vineer, H., E. R. Morgan, H. Hertzberg, D. J. Bartley, A. Bosco, J. Charlier, C. Chartier, et al. 2020. 'Increasing Importance of Anthelmintic Resistance in European Livestock: Creation and Meta-Analysis of an Open Database'. *Parasite (Paris, France)* 27 (December): 69.

Rose Vineer, H., L. Rinaldi, A. Bosco, F. Mavrot, T. de Waal, P. Skuce, J. Charlier, et al. 2015. 'Widespread Anthelmintic Resistance in European Farmed Ruminants: A Systematic Review'. *The Veterinary Record* 176 (21): 546.

Rose Vineer, H., S. H. Verschave, E. Claerebout, J. Vercruysse, D. J. Shaw, J. Charlier, and E. R. Morgan. 2020. 'GLOWORM-PARA: A Flexible Framework to Simulate the Population Dynamics of the Parasitic Phase of Gastrointestinal Nematodes Infecting Grazing Livestock'. *International Journal for Parasitology* 50 (2): 133–44.

Rose Vineer, H., T. Wang, J. van Dijk, and E. R. Morgan. 2015. 'GLOWORM-FL: A Simulation Model of the Effects of Climate and Climate Change on the Free-Living Stages of Gastro-Intestinal Nematode Parasites of Ruminants'. *Ecological Modelling* 297 (February): 232–45.

Ross, J. G., D. A. Purcell, C. Dow, and J. R. Todd. 1967. 'Experimental Infections of Calves with Trichostrongylus Axei; the Course and Development of Infection and Lesions in Low Level Infections'. *Research in Veterinary Science* 8 (2): 201–6.

Rothwell, J., and N. Sangster. 1997. 'Haemonchus Contortus: The Uptake and Metabolism of Closantel'. *International Journal for Parasitology* 27 (3): 313–19.

Roy, E. A., H. Hoste, and I. Beveridge. 2004. 'The Effects of Concurrent Experimental Infections of Sheep with Trichostrongylus Colubriformis and T. Vitrinus on Nematode Distributions, Numbers and on Pathological Changes'. *Parasite* 11 (3): 293–300.

Ruppert, D. 2014. 'Trimming and Winsorization'. In *Wiley StatsRef: Statistics Reference Online*. Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118445112.stat01887.

Sargison, N. D., D. J. Bartram, and D. J. Wilson. 2012. 'Use of a Long Acting Injectable Formulation of Moxidectin to Control the Periparturient Rise in Faecal Teladorsagia Circumcincta Egg Output of Ewes'. *Veterinary Parasitology* 189 (2–4): 274–83.

Sargison, N. D., A. Chambers, U. N. Chaudhry, L. Costa Júnior, S. R. Doyle, A. Ehimiyein, M. J. Evans, et al. 2022. 'Faecal Egg Counts and Nemabiome Metabarcoding Highlight the Genomic Complexity of Equine Cyathostomin Communities and Provide Insight into Their Dynamics in a Scottish Native Pony Herd'. *International Journal for Parasitology* 52 (12): 763–74.

Sargison, N. D., D. J. Wilson, and P. R. Scott. 2012. 'Observations on the Epidemiology of Autumn Nematodirosis in Weaned Lambs in a Scottish Sheep Flock'. *The Veterinary Record* 170 (15): 391.

Sauerborn, M., V. Brinks, W. Jiskoot, and H. Schellekens. 2010. 'Immunological Mechanism Underlying the Immune Response to Recombinant Human Protein Therapeutics'. *Trends in Pharmacological Sciences* 31 (2): 53–59.

Seaton, D. S., F. Jackson, W. D. Smith, and K. W. Angus. 1989a. 'Development of Immunity to Incoming Radiolabelled Larvae in Lambs Continuously Infected with Trichostrongylus Vitrinus'. *Research in Veterinary Science* 46 (1): 22–26.

———. 1989b. 'Development of Immunity to Incoming Radiolabelled Larvae in Lambs Continuously Infected with Ostertagia Circumcincta'. *Research in Veterinary Science* 46 (2): 241–46.

Seguel, M., S. A. Budischak, A. E. Jolles, and V. O. Ezenwa. 2023. 'Helminth-Associated Changes in Host Immune Phenotype Connect Top-down and Bottom-up Interactions during Co-Infection'. *Functional Ecology* 37 (4): 860–72.

Sellau, J., C. S. Hansen, R. I. Gálvez, L. Linnemann, B. Honecker, and H. Lotter. 2024. 'Immunological Clues to Sex Differences in Parasitic Diseases'. *Trends in Parasitology* 40 (11): 1029–41.

Shaw, D. J., and A. P. Dobson. 1995. 'Patterns of Macroparasite Abundance and Aggregation in Wildlife Populations: A Quantitative Review'. *Parasitology* 111 Suppl: S111-27.

Shaw, R. J., C. A. Morris, and M. Wheeler. 2013. 'Genetic and Phenotypic Relationships between Carbohydrate Larval Antigen (CarLA) IgA, Parasite Resistance and Productivity in Serial Samples Taken from Lambs after Weaning'. *International Journal for Parasitology* 43 (8): 661–67.

Shaw, R. J., C. A. Morris, M. Wheeler, M. Tate, and I. A. Sutherland. 2012. 'Salivary IgA: A Suitable Measure of Immunity to Gastrointestinal Nematodes in Sheep'. *Veterinary Parasitology* 186 (1–2): 109–17.

Signorell, A. 2025. 'DescTools: Tools for Descriptive Statistics'. https://CRAN.R-project.org/package=DescTools.

Sinclair, Rona, Lynsey Melville, Fiona Sargison, Fiona Kenyon, Dan Nussey, Kathryn Watt, and Neil Sargison. 2016. 'Gastrointestinal Nematode Species Diversity in Soay Sheep Kept in a Natural Environment without Active Parasite Control'. *Veterinary Parasitology* 227 (August): 1–7.

Sinn, R., J. Ketzis, and T. Chen. 1999. 'The Role of Woman in the Sheep and Goat Sector'. *Small Ruminant Research: The Journal of the International Goat Association* 34 (3): 259–69.

Šlapeta, J., J. Krücken, A. Rojas, A. Chambers, L.A. Melville, M. Martínez-Valladares, C. Canton et al. 2025. 'Ten Simple Rules for Implementing Deep Amplicon Sequencing in Parasitology'. *International Journal for Parasitology*, November. https://doi.org/10.1016/j.ijpara.2025.11.003.

Smith, G., B. T. Grenfell, and R. M. Anderson. 1987. 'The Regulation of Ostertagia Ostertagi Populations in Calves: Density-Dependent Control of Fecundity'. *Parasitology* 95 (October): 373–88.

Smith, W. D. 2007. 'Some Observations on Immunologically Mediated Inhibited Teladorsagia Circumcincta and Their Subsequent Resumption of Development in Sheep'. *Veterinary Parasitology* 147 (1–2): 103–9.

Smith, W. D., F. Jackson, E. Jackson, J. Williams, and H. R. Miller. 1984. 'Manifestations of Resistance to Ovine Ostertagiasis Associated with Immunological Responses in the Gastric Lymph'. *Journal of Comparative Pathology* 94 (4): 591–601.

Smith, W. D., D. Pettit, and S. K. Smith. 2001. 'Cross-Protection Studies with Gut Membrane Glycoprotein Antigens from Haemonchus Contortus and Teladorsagia Circumcincta'. *Parasite Immunology* 23 (4): 203–11.

Smith, W. D., S. K. Smith, and D. Pettit. 2000. 'Evaluation of Immunization with Gut Membrane Glycoproteins of Ostertagia Ostertagi against Homologous Challenge in Calves and against Haemonchus Contortus in Sheep'. *Parasite Immunology* 22 (5): 239–47.

Soneson, Charlotte, Michael I. Love, and Mark D. Robinson. 2015. 'Differential Analyses for RNA-Seq: Transcript-Level Estimates Improve Gene-Level Inferences'. *F1000Research*. https://doi.org/10.12688/f1000research.7563.1.

Sparks, A. M., A. D. Hayward, K. A. Watt, J. G. Pilkington, J. M. Pemberton, S. E. Johnston, T. N. McNeilly, and D. H. Nussey. 2020. 'Maternally Derived Anti-Helminth Antibodies Predict Offspring Survival in a Wild Mammal'. *Proceedings. Biological Sciences* 287 (1939): 20201931.

Sparks, A. M., K. A. Watt, R. Sinclair, J. G. Pilkington, J. M. Pemberton, S. E. Johnston, T. N. McNeilly, and D. H. Nussey. 2018. 'Natural Selection on Antihelminth Antibodies in a Wild Mammal Population'. *The American Naturalist* 192 (6): 745–60.

Sparks, A. M., K. A. Watt, R. Sinclair, J. G. Pilkington, J. M. Pemberton, T. N. McNeilly, D. H. Nussey, and S. E. Johnston. 2019. 'The Genetic Architecture of Helminth-Specific Immune Responses in a Wild Population of Soay Sheep (Ovis Aries)'. *PLoS Genetics* 15 (11): e1008461.

Spedding, C. R. 1954. 'Effect of a Sub-Clinical Worm-Burden on the Digestive Efficiency of Sheep'. *Journal of Comparative Pathology* 64 (1): 5–14.

Stear, M. J., K. Bairden, S. C. Bishop, G. Gettinby, Q. A. McKellar, M. Park, S. Strain, and D. S. Wallace. 1998. 'The Processes Influencing the Distribution of Parasitic Nematodes among Naturally Infected Lambs'. *Parasitology* 117 (Pt 2) (August): 165–71.

Stear, M. J., S. C. Bishop, M. Doligalska, J. L. Duncan, P. H. Holmes, J. Irvine, L. McCririe, Q. A. McKellar, E. Sinski, and Max Murray. 1995. 'Regulation of Egg Production, Worm Burden, Worm Length and Worm Fecundity by Host Responses in Sheep Infected with Ostertagia Circumcincta'. *Parasite Immunology* 17 (12): 643–52.

Stear, M. J., S. Strain, and S. C. Bishop. 1999. 'Mechanisms Underlying Resistance to Nematode Infection'. *International Journal for Parasitology* 29 (1): 51–56.

Steel, J. W., W. O. Jones, and L. E. A. Symons. 1982. 'Effects of a Concurrent Infection of Trichostrongylus Colubriformis on the Productivity and Physiological and Metabolic Responses of Lambs Infected with Ostertagia Circumcincta'. *Australian Journal of Agricultural Research* 33 (1): 131–40.

Stepek, Gillian, David J. Buttle, Ian R. Duce, and Jerzy M. Behnke. 2006. 'Human Gastrointestinal Nematode Infections: Are New Control Methods Required?: Control

of Human Gastrointestinal Nematodes'. *International Journal of Experimental Pathology* 87 (5): 325–41.

Stevenson, I. R., P. Marrow, B. T. Preston, J. M. Pemberton, and K. Wilson. 2003. 'Adaptive Reproductive Strategies'. In *Soay Sheep*, edited by T. H. Clutton-Brock and J. M. Pemberton, 243–75. Cambridge: Cambridge University Press.

Stien, A., R. J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, and S. D. Albon. 2002. 'The Impact of Gastrointestinal Nematodes on Wild Reindeer: Experimental and Cross-sectional Studies'. *The Journal of Animal Ecology* 71 (6): 937–45.

Stoffel, M. A., S. E. Johnston, J. G. Pilkington, and J. M. Pemberton. 2021. 'Genetic Architecture and Lifetime Dynamics of Inbreeding Depression in a Wild Mammal'. *Nature Communications* 12 (1): 2972.

Stroud, J. T., B. M. Delory, E. M. Barnes, J. M. Chase, L. De Meester, J. Dieskau, T. N. Grainger, et al. 2024. 'Priority Effects Transcend Scales and Disciplines in Biology'. *Trends in Ecology & Evolution* 39 (7): 677–88.

Stubbings, L., D. Bartley, V. Busin, F. Lovatt, P. Page, H. Rose Vineer, and P. Skuce. 2022. 'SCOPS Technical Manual'. Open Science Framework. https://doi.org/10.17605/OSF.IO/SQA4E.

Stutz, William E., Andrew R. Blaustein, Cheryl J. Briggs, Jason T. Hoverman, Jason R. Rohr, and Pieter T. J. Johnson. 2018. 'Using Multi-Response Models to Investigate Pathogen Coinfections across Scales: Insights from Emerging Diseases of Amphibians'. *Methods in Ecology and Evolution* 9 (4): 1109–20.

Swales, W. E. 1940. 'The Helminth Parasites and Parasitic Diseases of Sheep in Canada: I. A Survey and Some Preliminary Studies on Existing Problems'. *Canadian Journal of Research* 18d (1): 29–48.

Sweeny, A. R., Y. Corripio-Miyar, X. Bal, A. D. Hayward, J. G. Pilkington, T. N. McNeilly, D. H. Nussey, and F. Kenyon. 2022. 'Longitudinal Dynamics of Co-Infecting Gastrointestinal Parasites in a Wild Sheep Population'. *Parasitology* 149 (5): 1–12.

Sweeny, A. R., C. A. Thomason, E. A. Carbajal, C. B. Hansen, A. L. Graham, and A. B. Pedersen. 2020. 'Experimental Parasite Community Perturbation Reveals Associations between Sin Nombre Virus and Gastrointestinal Nematodes in a Rodent Reservoir Host'. *Biology Letters* 16 (12): 20200604.

Sweeny, Joshua P. A., Una M. Ryan, Ian D. Robertson, and Caroline Jacobsen. 2012. 'Molecular Identification of Naturally Acquired Strongylid Infections in Lambs--an Investigation into How Lamb Age Influences Diagnostic Sensitivity'. *Veterinary Parasitology* 187 (1–2): 227–36.

Sykes, A. R., R. L. Coop, and K. W. Angus. 1979. 'Chronic Infection with Trichostrongylus Vitrinus in Sheep. Some Effects on Food Utilisation, Skeletal Growth and Certain Serum Constituents'. *Research in Veterinary Science* 26 (3): 372–77.

Sykes, A. R., D. P. Poppi, and D. C. Elliot. 1988. 'Effect of Concurrent Infection with Ostertagia Circumcincta and Trichostrongylus Colubriformis on the Performance of Growing Lambs Consuming Fresh Herbage'. *The Journal of Agricultural Science* 110 (3): 531–41.

Taylor, D. M., and R. J. Thomas. 1986. 'The Development of Immunity to Nematodirus Battus in Lambs'. *International Journal for Parasitology* 16 (1): 43–46.

Taylor, M. A., R. L. Coop, and R. L. Wall. 2015. 'Parasites of Sheep and Goats'. In *Veterinary Parasitology, 4th Edition*, edited by M. A. Taylor, R. L. Coop, and R. L. Wall, 436–523. Hoboken: Wiley-Blackwell.

Taylor, S. M., and G. R. Pearson. 1979. 'Trichostrongylus Vitrinus in Sheep. I. The Location of Nematodes during Parasitic Development and Associated Pathological Changes in the Small Intestine'. *Journal of Comparative Pathology* 89 (3): 397–403.

Tennekes, M. 2018. 'Tmap: Thematic Maps in R'. *Journal of Statistical Software* 84 (6): 1–39.

Tetley, J. H. 1935. 'Ecological Studies on Nematodirus Species in Sheep in Manawatu District, New Zealand'. *Journal of Helminthology* 13 (1): 41–58.

Thomas, R. J. 1959. 'Field Studies on the Seasonal Incidence of Nematodirus Battus and N. Filicollis in Sheep'. *Parasitology* 49 (November): 387–410.

Thumbi, S. M., B. M. de C Bronsvoort, E. J. Poole, H. Kiara, P. Toye, M. Ndila, I. Conradie, et al. 2013. 'Parasite Co-Infections Show Synergistic and Antagonistic Interactions on Growth Performance of East African Zebu Cattle under One Year'. *Parasitology* 140 (14): 1789–98.

Tombak, K. J., C. B. Hansen, J. M. Kinsella, J. Pansu, R. M. Pringle, and D. I. Rubenstein. 2021. 'The Gastrointestinal Nematodes of Plains and Grevy's Zebras: Phylogenetic Relationships and Host Specificity'. *International Journal for Parasitology. Parasites and Wildlife* 16 (December): 228–35.

Tompkins, D. M., and M. Begon. 1999. 'Parasites Can Regulate Wildlife Populations'. *Parasitology Today (Personal Ed.)* 15 (8): 311–13.

Trumble, B. C., A. D. Blackwell, J. Stieglitz, M. E. Thompson, I. M. Suarez, H. Kaplan, and M. Gurven. 2016. 'Associations between Male Testosterone and Immune Function in a Pathogenically Stressed Forager-Horticultural Population'. *American Journal of Physical Anthropology* 161 (3): 494–505.

Tummers, B. 2016. 'DataThief III'. 2016. https://www.datathief.org/.

Turner, J. H., and M. L. Colglazier. 1954. ‘Control of Pasture-Acquired Infections of Nematodirus Spathiger And’. *American Journal of Veterinary Research* 15 (57): 564–73.

Turner, J. H., K. C. Kates, and G. I. Wilson. 1962. ‘The Interaction of Concurrent Infections of the Abomasal Nematpdes, Haemonchus Contortus, Ostertagia Circumcincta, and Trichostrongylus Axei (Trichostrongylidae), in Lambs’. *Proceedings of the Helminthological Society of Washington* 29 (2): 210–16.

Van Wyk, J. A. 2001. ‘Refugia--Overlooked as Perhaps the Most Potent Factor Concerning the Development of Anthelmintic Resistance’. *The Onderstepoort Journal of Veterinary Research* 68 (1): 55–67.

Van Wyk, J. A., and E. Mayhew. 2013. ‘Morphological Identification of Parasitic Nematode Infective Larvae of Small Ruminants and Cattle: A Practical Lab Guide’. *The Onderstepoort Journal of Veterinary Research* 80 (1): 539.

Vannella, K.M., T.R. Ramalingam, K.M. Hart, R. de Queiroz Prado, J. Sciurba, L. Barron, L.A. Borthwick et al. 2016. ‘Acidic Chitinase Primes the Protective Immune Response to Gastrointestinal Nematodes’. *Nature Immunology* 17 (5): 538–44.

Veritas Health Information. 2022. ‘Covidence Systematic Review Software’. Melbourne. 2022. www.covidence.org.

Viechtbauer, Wolfgang. 2010. ‘Conducting Meta-Analyses in R with the Metafor Package’. *Journal of Statistical Software* 36 (August): 1–48.

Vlassoff, A., and P. B. McKenna. 1994. ‘Nematode Parasites of Economic Importance in Sheep in New Zealand’. *New Zealand Journal of Zoology* 21 (1): 1–8.

Waghorn, T., A. Chambers, C. Bouchet, R. Hannaford, M. Jones, C. Miller, C. Sauermann, and D. Leathwick. 2025. ‘The Seasonality of Gastrointestinal Nematode Species

Abundance (Excluding *Nematodirus*) in Sheep of Different Classes across New Zealand'. *New Zealand Journal of Agricultural Research* 68 (7): 2436–55.

Waller, P. J., and R. J. Thomas. 1978. 'Nematode Parasitism in Sheep in North-East England: The Epidemiology of Ostertagia Species'. *International Journal for Parasitology* 8 (4): 275–83.

Wang, H., K. Barry, A. Zaini., G. Coakley, M. Moyat, C.P. Daunt, L.C. Wickramasinghe et al. 2024. 'Helminth Infection Driven Gastrointestinal Hypermotility Is Independent of Eosinophils and Mediated by Alterations in Smooth Muscle Instead of Enteric Neurons'. *PLoS Pathogens* 20 (8): e1011766.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. 'Naive Bayesian Classifier for Rapid Assignment of RRNA Sequences into the New Bacterial Taxonomy'. *Applied and Environmental Microbiology* 73 (16): 5261–67.

Wang, T., E. M. Redman, A. Morosetti, R. Chen, S. Kulle, N. Morden, C. McFarland, et al. 2021. 'Seasonal Epidemiology of Gastrointestinal Nematodes of Cattle in the Northern Continental Climate Zone of Western Canada as Revealed by Internal Transcribed Spacer-2 Ribosomal DNA Nemabiome Barcoding'. *Parasites & Vectors* 14 (1): 604.

Wang, T., Hannah Rose Vineer, Elizabeth Redman, Arianna Morosetti, Rebecca Chen, Christopher McFarland, Douglas D. Colwell, Eric R. Morgan, and John S. Gilleard. 2022. 'An Improved Model for the Population Dynamics of Cattle Gastrointestinal Nematodes on Pasture: Parameterisation and Field Validation for Ostertagia Ostertagi and Cooperia Oncophora in Northern Temperate Zones'. *Veterinary Parasitology* 310 (109777): 109777.

Wernersson, S., and G. Pejler. 2014. 'Mast Cell Secretory Granules: Armed for Battle'. *Nature Reviews. Immunology* 14 (7): 478–94.

Westen, H. 1967. ‘The Life-Cycle and Pathogenesis of Bunostomum Trigonocephalum Rudolphi 1808 (Ancylostomidae) in Goats’. https://doi.org/10.5555/19680800299.

Wickham, H. 2009. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

———. 2017. ‘Tidyverse: Easily Install and Load the “Tidyverse”’. https://cran.r-project.org/package=tidyverse.

Wiersma, E., R. J. Pakeman, X. Bal, J. G. Pilkington, J. M. Pemberton, D. H. Nussey, and A. R. Sweeny. 2023. ‘Age-Specific Impacts of Vegetation Functional Traits on Gastrointestinal Nematode Parasite Burdens in a Large Herbivore’. *The Journal of Animal Ecology* 92 (9): 1869–80.

Wilke, C. O. 2018. ‘Cowplot: Streamlined Plot Theme and Plot Annotations for “Ggplot2”’. https://cran.r-project.org/package=cowplot.

Williams, A. R., D. G. Palmer, I. H. Williams, P. E. Vercoe, and L. J. E. Karlsson. 2010. ‘Faecal Dry Matter, Inflammatory Cells and Antibodies in Parasite-Resistant Sheep Challenged with Either Trichostrongylus Colubriformis or Teladorsagia Circumcincta’. *Veterinary Parasitology* 170 (3–4): 230–37.

Williams, E. G., H. W. Williams, P. M. Brophy, S. R. Evans, H. McCalman, and R. A. Jones. 2024. ‘Assessing Periparturient Ewe Characteristics and Nemabiome Composition to Guide Targeted Selective Treatment for Sustainable Gastrointestinal Nematodes Control in Sheep’. *Animal: An International Journal of Animal Bioscience* 18 (6): 101156.

Wilson, A. J., J. M. Pemberton, J. G. Pilkington, T. H. Clutton-Brock, D. W. Coltman, and L. E. B. Kruuk. 2007. ‘Quantitative Genetics of Growth and Cryptic Evolution of Body Size in an Island Population’. *Evolutionary Ecology* 21 (3): 337–56.

Wilson, D. J., N. D. Sargison, P. R. Scott, and C. D. Penny. 2008. 'Epidemiology of Gastrointestinal Nematode Parasitism in a Commercial Sheep Flock and Its Implications for Control Programmes'. *The Veterinary Record* 162 (17): 546–50.

Wilson, D. S., and E. Sober. 1989. 'Reviving the Superorganism'. *Journal of Theoretical Biology* 136 (3): 337–56.

Wilson, K., B. T. Grenfell, J. G. Pilkington, H. E. G. Boyd, and F. M. Gulland. 2004. 'Parasites and Their Impact'. In *Soay Sheep: Dynamics and Selection in an Island Population*, edited by J. M. Pemberton and T. H. Clutton-Brock, 113–65. Cambridge: Cambridge University Press.

Wong, Koon Ho, Yi Jin, and Zarmik Moqtaderi. 2013. 'Multiplex Illumina Sequencing Using DNA Barcoding'. *Et al [Current Protocols in Molecular Biology]* Chapter 7 (1): Unit 7.11.

Yacob, H. T., C. Duranton-Grisez, F. Prevot, J. P. Bergeaud, C. Bleuart, Ph Jacquiet, Ph Dorchies, and H. Hoste. 2002. 'Experimental Concurrent Infection of Sheep with Oestrus Ovis and Trichostrongylus Colubriformis: Negative Interactions between Parasite Populations and Related Changes in the Cellular Responses of Nasal and Digestive Mucosae'. *Veterinary Parasitology* 104 (4): 307–17.

Zahid, O., N. D. Sargison, U. N. Chaudhry, and J. P. Crilly. 2023. 'O-161 Nemabiome Metabarcoding Shows Varying Levels of Genetic Diversity in Anthelmintic-Resistant Gastrointestinal Nematodes'. *Animal - Science Proceedings* 14 (1): 176–77.

Zhao, A., J. McDermott, J.F. Urban Jr, W.C. Gause, K.B. Madden, K.A. Yeung, S.C. Morris, F.D. Finkelman, and T. Shea-Donohue. 2003. 'Dependence of IL-4, IL-13, and Nematode-Induced Alterations in Murine Small Intestinal Smooth Muscle Contractility on Stat6 and Enteric Nerves'. *Journal of Immunology (Baltimore, Md.: 1950)* 171 (2): 948–54.

Zhao, A., J.F. Urban Jr, R.M. Anthony, R. Sun, J. Stiltz, N. van Rooijen, T.A. Wynn, W.C. Gause, and T. Shea-Donohue. 2008. 'Th2 Cytokine-Induced Alterations in Intestinal Smooth Muscle Function Depend on Alternatively Activated Macrophages'. *Gastroenterology* 135 (1): 217-225.e1.

Zhou, G., M. M. Stevenson, T. G. Geary, and J. Xia. 2016. 'Comprehensive Transcriptome Meta-Analysis to Characterize Host Immune Responses in Helminth Infections'. *PLoS Neglected Tropical Diseases* 10 (4): e0004624.

Zilber-Rosenberg, Ilana, and Eugene Rosenberg. 2008. 'Role of Microorganisms in the Evolution of Animals and Plants: The Hologenome Theory of Evolution'. *FEMS Microbiology Reviews* 32 (5): 723–35.

Appendix I

PDF of the publication arising from Chapter 2.

Veterinary Parasitology 323 (2023) 110053

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Antagonism between co-infecting gastrointestinal nematodes: A meta-analysis of experimental infections in Sheep☆

M.J. Evans [a,b,c,*], Y. Corripio-Miyar [b], A. Hayward [b], F. Kenyon [b], T.N. McNeilly [b,1], D.H. Nussey [c,1]

[a] Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK
[b] Department for Disease Control, Moredun Research Institute, Penicuik, UK
[c] Institute for Ecology and Evolution, University of Edinburgh, Edinburgh, UK

ARTICLE INFO

Keywords:
Meta-analysis
Co-infection
Parasite
Helminth
Nematode
Gastro-intestinal
Sheep

ABSTRACT

Gastrointestinal nematodes (GIN) have enormous global impacts in humans, wildlife and grazing livestock. Within grazing livestock, sheep are of particular global importance and the economics and sustainability of sheep production are greatly constrained by GIN infections. Natural infections are composed of co-infections with multiple species, and while some past work suggests species can interact negatively with one another within the same host, there is wide variation in reported patterns. Here, we undertook a systematic literature search and meta-analysis of experimental GIN co-infections of sheep to determine whether these experimental studies support the hypothesis of antagonistic interactions between different co-infecting GIN, and test whether aspects of parasite biology or experimental design influence the observed effects. A systematic search of the literature yielded 4848 studies, within which, we identified 19 experimental sheep studies comparing *post-mortem* worm counts across two co-infecting GIN species. Meta-analysis of 67 effects obtained from these studies provides strong evidence for interactions between GIN species. There was wide variation in the strength and direction of these interactions, but the global effect was significantly antagonistic. On average, there was a decrease in the number of worms of one species when a co-infecting species was also present, relative to a mono-infection with that species alone. This effect was dependent on the infectious dose and was rapidly lost after anthelmintic treatment, suggesting that live worms are required for the effect to occur. Individual parasite species varied in the extent to which they both exerted, and were subject to, these interspecies interactions, and these differences are more complex than simply co-localisation within the gastrointestinal tract. Antagonistic interactions between co-infecting GIN may feedback into their epidemiology as well as potentially affecting the clinical impacts of infection. Furthermore, the consequences of these interactions may be heightened when clinical interventions affect only one species within the co-infecting network. Whilst it was not possible to identify the causes of variation between GIN species in the impact of co-infection, these findings point to new avenues for epidemiological, clinical and mechanistic research on GIN co-infections.

1. Introduction

Gastrointestinal nematodes (GIN) infect over half the world's human population (Chan, 1997; Horton, 2003) and are near ubiquitous parasites in wildlife and grazing livestock. Within grazing livestock, sheep are the most numerous species globally (Gilbert et al., 2018) and are highly important to rural economies both in higher income countries and in lower income countries, where, alongside goats, they are particularly relied upon by people living in poverty, especially women (Sinn et al., 1999). However, the economics and sustainability of sheep production are greatly constrained by GIN infections (Charlier et al., 2020; Fitzpatrick, 2013; Mavrot et al., 2015; McLeod, 1995; Nieuwhof and Bishop, 2005) and are further threatened by the widespread development of anthelmintic resistance (Kaplan and Vidyashankar,

* Corresponding author at: Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK.
E-mail address: mike.evans@ed.ac.uk (M.J. Evans).
[1] Joint-Last Authors.

https://doi.org/10.1016/j.vetpar.2023.110053
Received 25 July 2023; Received in revised form 8 October 2023; Accepted 11 October 2023
Available online 20 October 2023

2012; Rose et al., 2015a). These challenges have pushed the livestock industry to develop host genetics and farm management techniques that are less dependent on anthelmintics, and to invest in research that has generated enormous advances in GIN epidemiology, vaccines and immunology (Morgan et al., 2019). However, most research considers GIN species in isolation, whereas most natural infections are in fact complex co-infections of multiple species, affecting multiple sites within the GI tract. In temperate climates, the composition of these communities vary seasonally (Boag and Thomas, 1977) and geographically (Redman et al., 2019); however, variation in GIN community composition may also occur between age classes and between years even within a single farm (Evans et al., 2021).

Epidemiological models of livestock GIN continue to improve (McFarland et al., 2022; Rose et al., 2015b; Rose Vineer et al., 2020) but an understanding of interactions between co-infecting GIN species is vital for the construction of holistic multi-species models of GIN. Between-species differences in the ecologies of GIN parasites' free-living larval stages will undoubtedly contribute to observed variation in co-infection composition (O'Connor et al., 2006). However, interactions between species may also contribute to epidemiological patterns, as was postulated by Jackson et al. (1992) for *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* - two species with similar free-living ecologies, but markedly different seasonal epidemiologies.

Interactions between co-infecting GIN could also have impacts on the effectiveness of clinical interventions, and the evolution of anthelmintic resistance. Lello et al. (2004) demonstrated in rabbits that the removal of a single species from a network of interacting parasites can have unexpected effects on the remaining species. Such situations may readily arise in veterinary practice, for example targeted treatment of *Haemonchus contortus* with salicylanilide drugs, use of monovalent anti-nematode vaccines, or use of broad spectrum anthelmintics where anthelmintic resistance is present in only some of the co-infecting species. If such interactions are antagonistic they could also have impacts on the evolution of anthelmintic resistance, via both competitive release (increased fecundity or survival of those worms surviving treatment) and subsequent competitive exclusion (reduced establishment of susceptible worms from *refugia*).

Interactions between GIN of sheep are therefore of clear importance, and there has been an increase in scientific interest in the composition of ovine GIN communities following the development of a metabarcoded ITS-2 sequence-based GIN speciation platform ('the Nemabiome') (Avramenko et al., 2015; Redman et al., 2019). However, this platform has almost exclusively been applied to cross-sectional studies utilising samples pooled from multiple individuals, which are ill-suited to identifying inter-specific interactions (Fenton et al., 2014). Experimental approaches are more powerful in that regard but the results can be hard to extrapolate beyond the specific experimental conditions. The review of co-infection experiments by Christensen et al. (1987) showed that interactions between ovine GIN were predominately antagonistic, but examples of synergistic interactions do exist (Kates and Turner, 1960; Lello et al., 2018; Turner et al., 1962; Turner and Colglazier, 1954). We therefore aimed to perform a meta-analysis of GIN co-infection experiments in sheep to test the hypothesis of antagonistic interactions between different co-infecting GIN, and determine whether the broad range of experimental results reported were affected by the parasite species, or by experimental design details.

Pederson and Fenton (2007) described how co-infecting parasites may interact with each other negatively (via competition for space, consumption of resources, or stimulation of non-specific host responses) or positively (via mechanical facilitation, immunosuppression, or immune-polarisation). In general, interactions are predicted to be strongest for species occupying similar ecological niches, and Lello et al. (2004, 2018) and Lello and Hussell (2008) showed that by defining the ecological niches of GIN as a combination of their feeding habit and their predilection site, the strength and direction of co-infection interactions could be predicted. However, within the GIN species routinely studied in sheep, *H. contortus* is unique in feeding on blood, with the other species all considered 'mucosal browsers'. Due to the lack of replication across feeding habits, we therefore chose to also test whether the co-infection interactions were affected simply by the relationship between the parasites' anatomic predilection sites.

2. Methods

2.1. Systematic literature review

Our systematic literature review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (Page et al., 2021b, 2021a) (see Fig. 1 for PRISMA diagram). The systematic literature search was performed in September 2022 (last search 2022–09–22) using CABabstracts, CABabstracts archive, MEDLINE, SCOPUS and Web of Science databases, searching in all fields. The search string was composed of four elements:

1. Synonyms for sheep:
   Sheep OR Lamb* OR Ovine OR "Ovis aries"
2. Synonyms for co-infections:
   "co-infect* " OR "co infect* " OR "coinfect* " OR "concomitant* " OR "concurrent* " OR synerg* OR antagonis* OR compet* OR interact* OR interspecific OR influenc* OR heterologous OR "cross-resistan* " OR "cross resistan* " OR "cross-immun* " OR "cross immun* "
3. Genera of species reported to infect sheep (including historic names) (Taylor et al., 2015), truncated in order to find references to both the genus and the associated clinical syndrome (e.g. *Haemonchus* and haemonchosis), or a truncation of the word 'nematode'. Following a scoping search specific exclusions were added to the truncation of '*Capillaria*' in order to avoid irrelevant references to 'capillary' and 'capillaries':
   Nematod* OR Bunostom* OR Camelostrongyl* OR (Capillar* NOT capillary NOT capillaries) OR Chabert* OR Cooper* OR Gaiger* OR Gongylone* OR Haemonch* OR Marshallag* OR Mecistocirr* OR Monodont* OR Nematodir* OR Oesophagostom* OR Ostertag* OR Oxyur* OR Parabrone* OR Skrjabine* OR Strongyloid* OR Teladorsag* OR Trichocephal* OR Trichostrongyl* OR Trichur*
4. Synonyms for nematode community diversity (adjacency operators dependent on database):

Nemabiome* or (Nematod* adj4\NEAR4\W/4 diversity) or (Nematod* adj4\NEAR4\W/4 community).

These four terms were linked to create the complete search string:

Sheep_synonyms AND ((Co-infection_synonyms AND Nematode_synonyms) OR Nematode_diversity_synonyms).

This search strategy yielded a library of 4157 studies. Backward and forward citation searching (using SCOPUS, Web of Science and Google Scholar) of all final eligible included papers yielded an additional 690 studies which were fed back into the library to give a total of 4847 studies. These were uploaded into the online Covidence application, which screened for duplicates automatically (Veritas Health Information, 2022). After final manual curation, this resulted in the removal of 2210 duplicate studies. The remaining 2637 studies were screened for relevance against their title and abstract (including any study that reported GIN species composition from co-infected sheep), resulting in the exclusion of 2496 studies. The full texts for the remaining 141 studies were then assessed for eligibility. 82 of these studies reported natural co-infections rather than experimental infection; 8 were secondary reports or conference abstracts without data, and 25 did not have the right study design. Those studies were excluded from the meta-analysis, but their meta-data were recorded, in order to assess the temporal and geographical representativeness of the included studies relative to the wider literature. This left 24 eligible studies that compared *post-mortem* worm counts of sheep infected with two GIN species (referred to hereafter as the 'principal species' and the 'co-infecting species') against

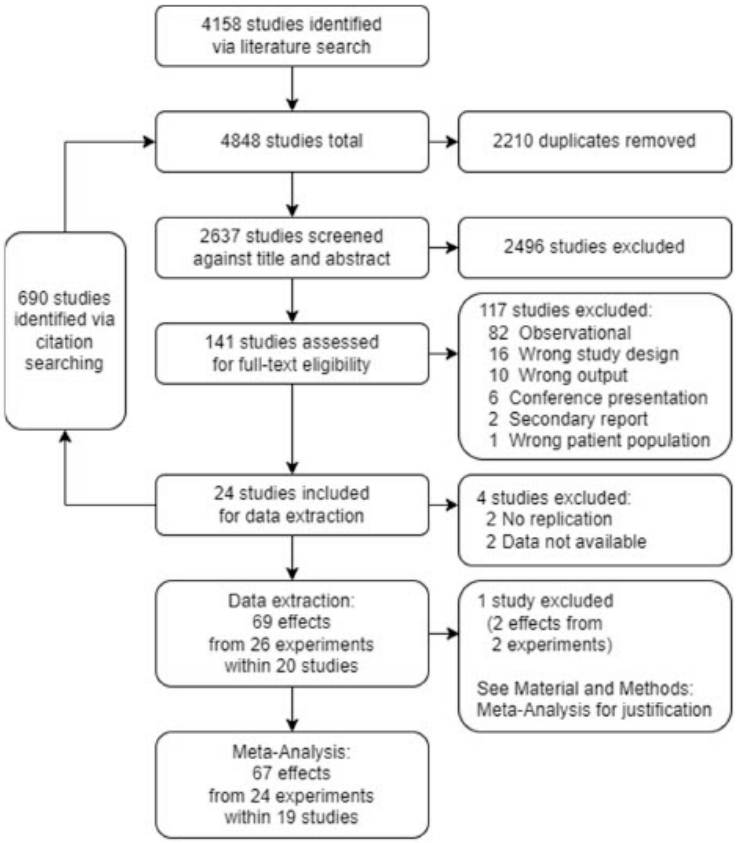


Fig. 1. PRISMA diagram illustrating study selection. All screening and data extraction was performed by the lead author (ME).

control sheep mono-infected with just a single species (i.e., the principal species) (Fig. 1).

2.2. Data extraction, effect size calculation

The standardized mean differences (SMD or Hedges' *g*) in *post-mortem* (principal species) total worm count (all worm life-stages) between the co-infected sheep and the mono-infected sheep were calculated from the mean, standard deviation (SD) and number of sheep (n) in each experimental group using the 'escalc(., measure = "SMD")' command in the 'metafor' package: (Hedges, 1981; Viechtbauer, 2010)

$$g = \frac{\bar{y}_1 - \bar{y}_2}{s_p}, \text{ where } \quad s_p = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{(n_1-1)+(n_2-1)}}$$

That command also generated the associated sampling variances using the large-sample approximation method (Hedges, 1982, equation 8). Where the SD was not available, it was back-calculated using the reported Standard Error (SE) and sample size (n). Where the mean and SD/SE were not available, raw data was used to calculate them (extracted from data tables or graphs using DataThief III software (Tummers, 2016)).

Of the 24 eligible studies, it was not possible to calculate effect sizes for four studies. In two this was due to a lack of replication (n = 1 in some groups) arising from either differing study objective (Kates and Turner, 1953) or the host death mid-experiment (Kates and Turner, 1960), and in the other two this was because neither the raw data, nor the SD/SE were reported, and no author contact details were available to trace the raw data (Blanchard and Wescott, 1985; Dash, 1981). Therefore, effect sizes were calculated from a total of 20 studies. As many studies contained multiple experimental groups (see below) a total of 69 effect sizes were obtained from 26 experiments within those 20 studies.

2.3. Experimental design metadata

During data extraction, the following methodological details were also recorded: the principal species; the co-infecting species; the breed of sheep (if stated); age at first infection (recorded in days, months assumed to contain 30 days, midpoint used if a range given); inter-infection period in days; and post-infection period (days from last infection until necropsy). In addition, for both the principal and the co-infecting species, the total infectious dose (total number of third stage larvae (L_3) administered - always identical in experimental and control groups) and the duration of the infection administration (number of days from first administration to final administration) were recorded. Experimental methods were then classified into four categories according to their design (Fig. 2). As only one experiment utilized the

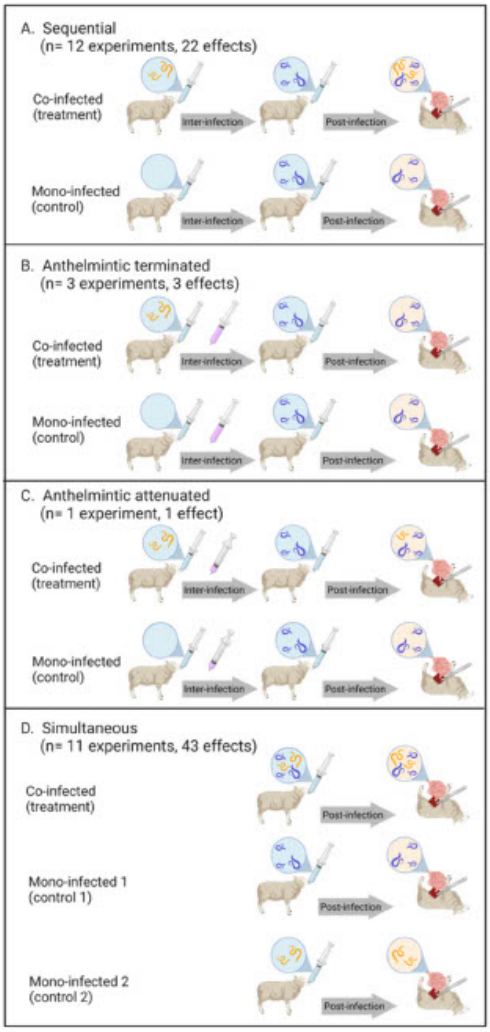


Fig. 2. Experimental design classifications (created with BioRender.com). A: Sequential. Co-infected sheep were infected with the co-infecting species (yellow) and subsequently infected with the principal species (blue); control sheep were mono-infected with the principal species (blue) only. *Post-mortem* worm counts of the principal species (blue) in the co-infected group were compared against those in the mono-infected group. B: Anthelmintic terminated. Design as per A, except with a therapeutic dose of anthelmintic administered between the co-infecting species (yellow) and the principal species (blue) in order to eliminate the co-infecting species. C: Anthelmintic attenuated. As per B, except using a subtherapeutic dose of anthelmintic intended to reduce the intensity of the co-infecting species without eliminating it. Note B & C were combined for analysis into a single 'Anthelmintic' group. D: Simultaneous. Co-infected sheep were infected with the principal species and the co-infecting species simultaneously; control sheep were mono-infected with the principal species only. In some cases, mono-infections with both species were performed, therefore two effects could be obtained (co-infected vs mono-infected 1 - blue worms are the principal species; or co-infected vs mono-infected 2 - yellow worms are the principal species).

'anthelmintic attenuated' design, this category was collapsed with the 'anthelmintic terminated' design, giving a total of three experimental design categories: sequential, simultaneous and anthelmintic.

GIN species were then classified according to their predilection for either the abomasum (stomach) (*Haemonchus contortus, Teladorsgia circumcincta, Trichostrongylus axei)* or intestines (*Nematodirus battus, Nematodirus spathiger, Trichostrongylus colubriformis, Trichostrongylus vitrinus*). An additional variable 'Anatomic Direction' with three levels was then assigned: 'Within Organ' (both species infect the same site); 'Downstream' (co-infecting species infects the abomasum and the principal species infects the intestines); and 'Upstream' (co-infecting species infects the intestines and the principal species infects the abomasum).

In some experiments, sheep were euthanized for necropsy at various time points after the last infection. These animals provided separate effect sizes, although they are clearly not entirely independent of each other. Similarly, some studies compared multiple experimental groups against a common control group (e.g. Species A co-infected with Species B vs Species A mono-infection; and Species A co-infected with Species C vs Species A mono-infection). Also, some studies compared a single experimental group against multiple control groups (Fig. 2D). To account for this interdependence, individual studies were divided into separate experiments, with effect sizes considered to be derived from the same experiment if they fitted any of the examples given above. (More in-depth descriptions of the original authors' methodologies are included in the complete dataframe, accessible in the supplementary materials.) Further interdependence may exist between studies due to the use of similar sheep breeds, parasite strains, necropsy protocols etc. by authors operating within wider research groups. Studies were therefore classified into 'research groups' if they shared any author with any other study. The following hierarchy of independence was therefore produced: Research Group (10) > Study (20) > Experiment (26) > Effect (69) (Table S1).

2.4. Meta-analysis

All analysis was conducted in RStudio v2022.12.0 using R v4.2.2 (R Core Team, 2022). Meta-analytic modelling was performed using the 'metafor' package (Viechtbauer, 2010). Plots were created using 'orchaRd' (Nakagawa et al., 2021), 'tidyverse' (Wickham, 2017), 'cowplot' (Wilke, 2018) and 'tmap' (Tennekes, 2018) packages. The dataset and R scripts can be accessed at DOI: 10.17632/275d8w3x3j.1.

We conducted our meta-analysis using multi-level mixed-effects maximum likelihood models. A random-effects model was first constructed by fitting research group, study and experiment as a three-tier nested random effect, in order to account for interdependence between multiple effects. This random effects model was used to estimate the global effect and the 95% confidence interval (CI) and 95% prediction interval around the global effect. In addition, the proportion of heterogeneity explained by the random effects structure was estimated by calculating I^2 for the random-effects model using the 'i2_ml' command from the 'orchaRd' package (Nakagawa et al., 2021) and comparing it against I^2 for a null model with no random effects.

The effects of 'Experimental Design', 'Co-Infecting Parasite Dose', 'Co-infecting Species', 'Principal Species' and 'Anatomic Direction' were then tested by adding each to the random effects model as a single moderator. Each moderator was judged to be significant where the Wald-type χ^2 test for moderators (Q_M) provided a P-value < 0.05. A more normal distribution for 'Co-Infecting Parasite Dose' was achieved using a natural logarithm (ln) transformation prior to fitting.

To assess the significance of potential confounding methodological variables on SMD, the following experimental design data were also tested as single moderators: 'Co-infection Duration' (as a continuous moderator in days); 'Co-Infection Duration' (discretised into 'single bolus' or 'multiple bolus/trickle infection'); and 'Inter-Infection Interval' (as a continuous moderator in days). The following methodological

details were not tested as for each effect they were controlled for by identical conditions in the within-experiment control group: the principal parasite dose; the principal parasite infection duration; the age at experiment onset; and the post-infection period.

All significant single moderators were then fitted together into a multiple moderator model. Moderators were considered significant after controlling for the other moderators if the Wald-type χ^2 test (Q_M) provided a P-value < 0.05. To control for potential publication bias 'Publication Year' and $\sqrt{1/\tilde{n}_i}$ $where \tilde{n}_i = \frac{n_{1i}n_{2i}}{n_{1i}+n_{2i}}$ were also fitted to the multiple moderator model (Nakagawa et al., 2022). As the $\sqrt{1/\tilde{n}_i}$ term was statistically significant it was replaced in the model by $1/\tilde{n}_i$ as recommended by Nakagawa et al. (2022). That replacement had no effect on the significance of any of the other moderators, therefore only the latter is reported in the results (both versions are present in the Supplementary Material).

Pairwise post hoc testing for all levels of categorical moderators that were significant in the multiple moderator model was performed with Benjamini-Hochberg correction using the 'multcomp' package (Hothorn et al., 2008).

One study (Herlich, 1965) had very large SEs and was therefore considered a potential outlier. It also had a much longer co-infection duration and utilised a pair of parasites (*Cooperia oncophora* and *Cooperia pectinata*) not represented in any of the other studies, therefore its inclusion would have prevented fitting the multiple moderator meta-regression. Consequently, this study (two effects) was excluded from all the meta-analyses reported below. For completeness the single moderator meta-regressions were repeated without excluding that study and no change to the effects or conclusions were seen (Supplementary Materials).

3. Results

Multi-level meta-analysis indicates that sheep co-infected with two GIN species usually have significantly fewer worms of the principal species than sheep mono-infected with just the principal species (Fig. 3). The global estimate for the standardized mean difference (SMD) in *post-mortem* worm counts of co-infected sheep compared to mono-infected control sheep (β_{global}) was − 0.732 (95% confidence interval (CI) = −1.03 - − 0.431, $Z = -4.78$, $P < 0.001$). Total heterogeneity was substantial (I^2 = 62.60%), whilst heterogeneity in the random-effects model was moderate (I^2 = 52.31%), indicating that the nested random effects structure accounted for 20.3% of the inter-effect heterogeneity.

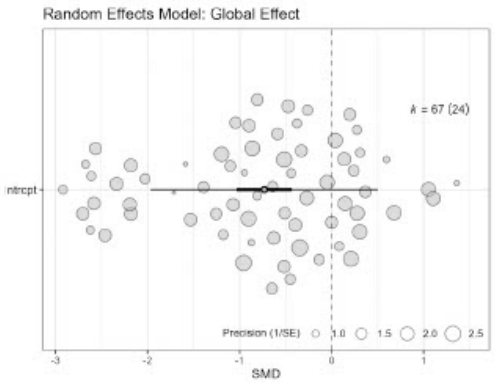


Fig. 3. Orchard plot showing significant effect of GIN co-infection on the Standardised Mean Difference (SMD) in *post-mortem* worm count of the principal GIN. Circles show individual effects, with their diameter inversely proportional to the standard error (SE) of the SMD; central circle shows the global estimate from the random effects model, with the thick lines showing the 95% confidences intervals associated with that estimate; the thin lines show the 95% prediction interval for the model; k shows the number of effects, with the number of experiments in brackets.

Single moderator meta-regression analysis showed the anatomic direction of the co-infection had no significant effect on *post-mortem* worm counts ($Q_M = 1.30$, df = 2, $P = 0.523$) (Fig. 4). Co-infection duration had no significant effect either as a continuous moderator ($Q_M = 2.18$, df = 1, $P = 0.140$) or as a two-level discrete moderator (bolus vs trickle) ($Q_M = 1.22$, df = 2, $P = 0.269$). Similarly, the inter-infection interval in days had no significant effect ($Q_M = 1.64$, df = 1, $P = 0.201$). Significant single moderator effects were identified for: experimental design ($Q_M = 8.68$, df = 2, $P = 0.013$); ln(co-infecting parasite dose) ($Q_M = 5.18$, df = 2, $P = 0.023$); co-infecting species ($Q_M = 13.46$, df = 5, $P = 0.019$); and principal species ($Q_M = 13.27$, df = 6, $P = 0.039$). Therefore these variables were carried forward into the multiple moderator meta-regression, where they all remained significant (Table 1).

Sequential co-infection experiments without the use of anthelmintics had significantly more negative effects on worm count than sequential co-infection experiments where an anthelmintic was administered between infections (post hoc $t = -3.28$, $P_{(BH\ adjusted)} = 0.005$). Simultaneously administered co-infections occupied an intermediate position relative to those other experimental designs, although the 95% CI did not cross the zero, suggesting simultaneous GIN co-infections also interact negatively (Fig. 5A). There was also a significant negative effect of ln(Co-Infecting Dose), indicating that higher doses of co-infecting worms exerted greater antagonism on the principal infection (Fig. 5B).

GIN species differed significantly in both the degree to which they inhibited a co-infecting GIN (Fig. 5C) and the degree to which they were inhibited by a co-infecting GIN (Fig. 5D). No significant pairwise comparisons between GIN species were found on Benjamini-Hochberg adjusted post hoc testing; however visual examination of the 95% CIs suggests *T. vitrinus*, *T. axei* and *H. contortus* may have more negative effects on co-infecting GIN (Fig. 5C), and at the same time *T. vitrinus* and *H. contortus* may be more greatly affected by a co-infecting GIN compared to the potentially more resilient *T. axei* (Fig. 5D).

There is no evidence that these results were significantly affected by publication bias, given publication year and $1/\tilde{n}_i$were non-significant in the multiple moderator meta-regression (Table 1 and Fig. S1).

The majority of inter-effect heterogeneity was explained together by experimental design, co-infecting species, principal species and co-infecting parasite dose, whilst controlling for publication year, effective sample size, research group and study. I^2 for the multiple moderator model was 13.18%, indicating a 74.81% reduction in the heterogeneity present in the random-effects model.

There were marked differences in the temporal and the geographic distributions of experimental studies identified during the literature search, compared to the excluded observational reports. The observational reports were globally distributed across 41 countries (Fig. 6A), whereas the experimental infections were performed in only 5 countries (Fig. 6B). The cumulative number of experimental studies increased approximately linearly through the 1960 s to the 1990 s but plateaued after the millennium; in contrast, the cumulative number of observational studies has expanded more exponentially with the greatest number of annual publications (7) in 2021 (Fig. 6C).

4. Discussion

There is strong evidence from this meta-analysis that GIN species co-infecting sheep interact with each other, and although there is wide variation the general effect is predominately antagonistic, leading to a decrease in the number of worms of one species, relative to a mono-infection. This effect is also dose dependent, with greater antagonism seen at greater infectious doses. Further, GIN species vary in both their effect on co-infecting species and their susceptibility to such effects, but these interactions appear more complex than simply the relationship

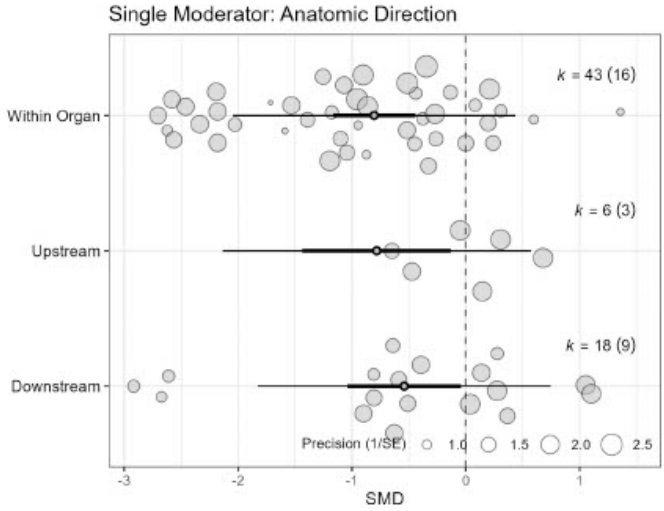


Fig. 4. Orchard plot showing the lack of effect of anatomical direction on the Standardised Mean Difference (SMD) in *post-mortem* worm count of the principal GIN.

Table 1
Results from the multiple-moderator meta-regression. Q_M, DF and P refer to the test for significant differences in effect size due to the moderator. Within moderators the estimates, 95% CIs, Z and P are relative to the reference level. Significantly different groups of moderator levels according to post hoc pairwise testing are indicated by different letters.

Variable	Level	Q_M	DF	*P*	Estimate	95% CI	*Z*	*P*	*Post hoc*
Intercept	Intercept				21.17	-87.33–129.67	0.382	0.702	
Experimental design	Anthelmintic	10.91	2	0.004					a
	Sequential				-1.39	-2.27 - – 0.565	-3.30	0.001	b
	Simultaneous				-0.700	-2.00–0.607	-1.05	0.295	ab
Co-infecting species	*H. contortus*	13.35	5	0.020					a
	N. spathiger				0.396	-0.857–1.65	0.619	0.536	a
	T. circumcincta				0.498	-0.487–1.48	0.991	0.322	a
	T. axei				-0.355	-1.46–0.754	-0.623	0.530	a
	T. colubriformis				0.879	-0.367–2.13	1.38	0.167	a
	T. vitrinus				-0.934	-1.95–0.085	-1.80	0.072	a
Principal species	*H. contortus*	15.19	6	0.019					a
	N. battus				0.926	-1.06–2.92	0.912	0.362	a
	N. spathiger				1.07	-0.082–2.23	1.82	0.069	a
	T. circumcincta				1.08	0.285–1.88	2.66	0.008	a
	T. axei				2.66	0.775–4.54	2.77	0.006	a
	T. colubriformis				0.709	-0.613–2.03	1.05	0.293	a
	T. vitrinus				-0.155	-0.904–0.594	-0.405	0.686	a
ln(Co-infecting dose)		4.62	1	0.032	-0.343	-0.655–0.030	-2.15	0.032	
Year		0.095	1	0.759	-0.009	-0.063–0.046	-0.307	0.759	
$1/\tilde{n}_0$		3.29	1	0.070	-2.39	-4.98–0.192	-1.81	0.070	

between their anatomic locations, given that factor was not significant in the single moderator analysis. This finding may simply reflect a lack of power given the small number of experiments examining upstream effects. However, it could also suggest that the mechanisms responsible for interactions among GIN (e.g. immunity, resource competition) are not local, but operate across the GI tract. This could have important implications for the development of immunity against natural mixed infections across multiple sites. Combinations of other life history factors may also be driving the observed variation between species, and specific parasite pair combinations may be important; however, unfortunately there were insufficient studies to fit an interaction between the co-infecting and principal species in the multiple moderator model.

Our results are unable to definitively state how individual species vary from each other in their co-infection interactions but the observed differences provide interesting points for discussion. Lello et al. (2018) proposed that *H. contortus* has a facilitative effect on co-infecting *T. colubriformis* (mediated via immunosuppression); however, the multiple moderator model suggests *H. contortus* has antagonistic effects on co-infecting GIN in general, raising questions about how the host response against *T. colubriformis* may differ from other species. Lello et al. (2018) also suggested that blood-feeding by *H. contortus* may make it particularly vulnerable to serum antibodies raised against a co-infecting species. That idea is supported by previous vaccine trials against *H. contortus* using antigens from *Ostertagia ostertagi* (Smith et al., 2000) and *T. circumcincta* (Smith et al., 2001), and receives further support from the strongly negative estimate for *H. contortus* as the principal parasite in our meta-analysis (Fig. 5D). *T. vitrinus* was negatively affected by co-infection with *T. circumcincta* (an abomasal species), and the authors of those experiments proposed that this was due to a vulnerability of this species to pH changes induced by co-infection

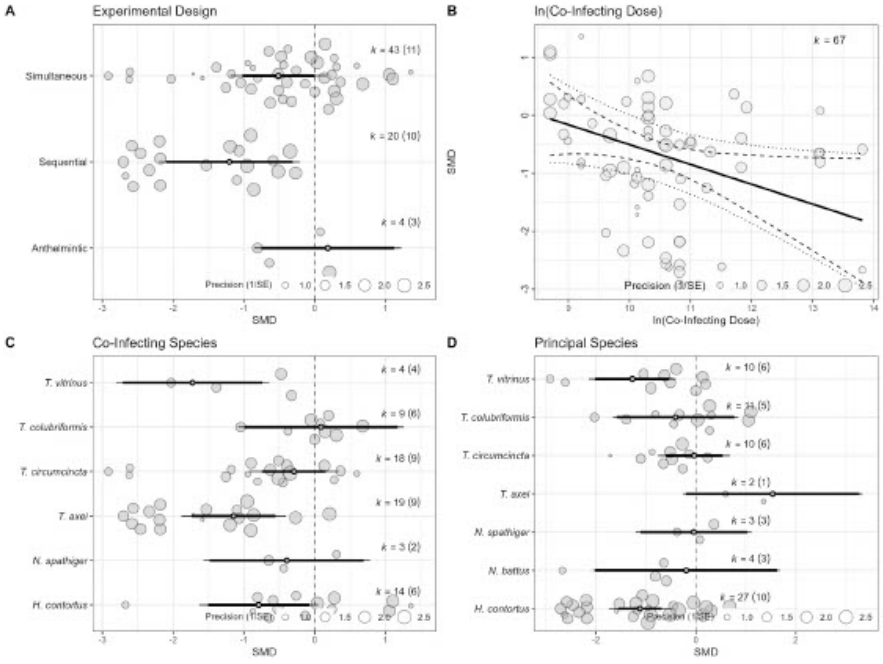


Fig. 5. Outputs from the multiple moderator meta-regression (Table 1) describing effects of experimental design and GIN species on *post-mortem* worm count of the principal GIN. Orchard plots (A, C & D) as described for Fig. 3. B: Bubble plot as per Orchard plots but with the solid line representing the estimated effect, the dashed lines representing the 95%CI around the effect, and the dotted lines representing the 95% prediction interval. SMD = Standardised Mean Difference in *post-mortem* worm count of the principal GIN.

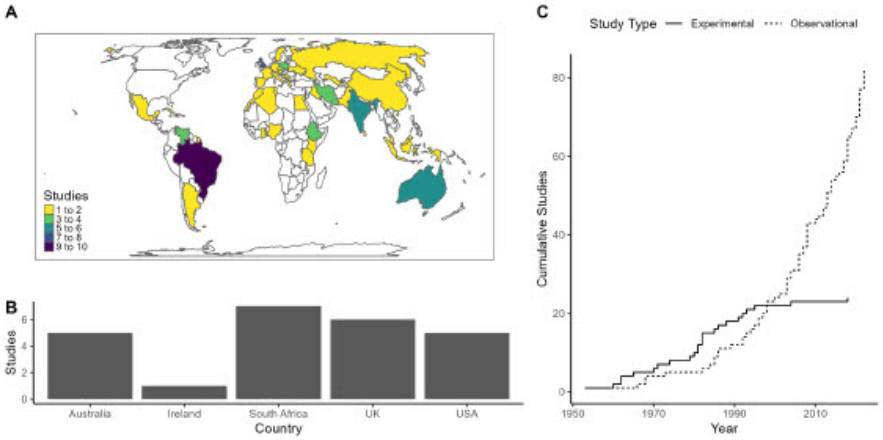


Fig. 6. Choropleth map (A) and bar chart (B) showing total number of observational (A) and experimental (B) studies from each country identified during the systematic literature review. C: Cumulate frequencies of the studies in A and B against time.

with abomasal parasites (Coop et al., 1988; Jackson et al., 1992). This hypothesis is potentially supported by the fact that Roy et al. (2004) experiment co-infecting *T. vitrinus* with *T. colubriformis* (another intestinal species) provided three effect sizes close to zero (Fig. 5D). Our meta-analysis suggests that *T. axei* may be more resilient to the effects of a co-infecting GIN, albeit based on data from a single experiment. This species is arguably the most generalist of GIN species, capable of infecting many host species and potentially infecting the duodenum in

addition to its abomasal predilection site. This generalist role may give it a wide ecological niche and the flexibility to potentially modify its site of infection and thereby mitigate the antagonism of a co-infecting species, as suggested by Pustovoi (1972).

A further striking finding of our meta-analysis was that the effects of a co-infection were lost following anthelmintic treatment. This suggests that either live parasites are necessary to mediate their effects, or that any indirect mechanisms are quickly lost after the clearance of the first species. Experimental design was significant both as a single and as a multiple moderator, and the pairwise comparison between sequential and anthelmintic-treated designs was significant on post hoc testing. Simultaneous co-infections also had smaller effect sizes than sequential co-infections. Whilst post hoc testing of this pairwise comparison was not significant, it is intuitive that effects would be greater if one species has first either modified its environment to suit its own niche, or has pre-stimulated non-specific host immune or physiological responses. The indication that live parasites are necessary for antagonistic effects to occur raises important questions about the underlying mechanisms through which parasites may mediate antagonistic effects (e.g. via parasite excretory-secretory products) and why mucosal immune responses may be so short lived as to be lost quicky after anthelmintic treatment.

Although this meta-analysis has provided strong support for several important findings, the ability to investigate species-specific factors was limited due to the low number of reports relative to the number of potential pairwise combinations of parasites. The potential range of species interactions is even greater when the limited geographical distribution of the included studies is considered against the globally distributed observational reports. Although it would be interesting to expand the range of species studied, the number of pairwise comparisons would expand exponentially, and it would be unfeasible to test so many pairwise co-infections in vivo. Further, two-species co-infections are only a single step towards the biological reality of the multi-species co-infections that occur in the field. There is therefore value in studies utilising complex natural infections. The scope of this meta-analysis was also limited, in that it was only possible to assess the effect of co-infection on worm number, rather than fecundity or pathology. There is evidence that GIN co-infections may reduce worm egg production (Dobson and Barnes, 1995; Jackson et al., 1992; Mapes and Coop, 1971) and that their pathologic impact on the host may be positive or negative (Coop et al., 1986; Steel et al., 1982; Sykes et al., 1988). There is hence also a need for further work on the impacts of co-infection interactions.

Two clear avenues for future research therefore open from this meta-analysis: firstly, studies of natural GIN co-infections able to identify multi-species interactions and quantify their impacts; and secondly, further controlled co-infection experiments aimed at identifying the mechanisms underlying them. The development of high throughput techniques for quantifying GIN species offers the potential for observational studies of natural co-infection dynamics (across the global breadth of host biomes). In contrast to the findings of this meta-analysis, observational studies of natural infections have generally reported positive correlations between co-infecting species (Barger, 1984; Cabaret and Hoste, 1998; Diez-Baños et al., 1992; Hoste and Cabaret, 1992; Morales et al., 2006; Rehbein et al., 1997; Stear et al., 1998; Sweeny et al., 2012). However, those studies were all cross-sectional, a study design which Fenton et al. (2014) showed to have limited power to identify interspecies interactions in co-infections. To resolve this issue, future studies should consider longitudinally sampling a large number of individual hosts with high temporal granularity. Within such studies, the collection of good quality long-term measures of host fitness/production (e.g. lamb growth and survival, and ewe rearing success and longevity) would provide the most meaningful measure of the impact of co-infection dynamics, and the contextualisation alongside species-specific pasture larval counts would enable the greatest epidemiological inference. Analysing the effects of anthelmintic treatment or monovalent vaccines on GIN community composition would provide further insight and could be designed as deliberate perturbation experiments or could utilise clinical samples from faecal egg count reduction tests or vaccine trials. Once these studies have identified significant interaction pathways, it would be valuable to return to controlled co-infection experiments targeting the causative mechanisms. Such studies could be in vivo, for example focussing on the influence of co-infections on mucosal immune responses along sites in the GI tract. Alternatively, they could be in vitro, perhaps looking for evidence of direct communication between nematode species, or using organoid models to examine whether changes in epithelial phenotypes (e.g. gastric remodelling (Faber et al., 2022)) are evidence of niche alterations that may affect the invasion success of a subsequent heterologous infection.

CRediT authorship contribution statement

M.J. Evans, Y. Corripio-Miyar, A. Hayward, F. Kenyon, T.N. McNeilly, D.H. Nussey: Conceptualization, Methodology, Writing – review & editing. **M.J. Evans, A. Hayward, D.H. Nussey**: Formal analysis. **M.J. Evans**: Investigation, Data curation, Writing – original draft. **M.J. Evans, A. Hayward**: Visualization. **Y. Corripio-Miyar, A. Hayward, F. Kenyon, T.N. McNeilly, D.H. Nussey**: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding Source: This work was primarily supported by ME's ECAT-V Fellowship, awarded by the Wellcome Trust and University of Edinburgh. AH is supported by a Moredun Foundation Research Fellowship; AH, FK and TNM receive funding from the Scottish Government Rural and Environment Science and Analytical Services (RESAS). YCM is supported by the large NERC grant (NE/R016801/1), awarded to DHN and TNM, which underpinned the conceptualisation of this study.

Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2023.110053.

References

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J. S., 2015. Exploring the gastrointestinal "nematbiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One 10, e0143559.

Barger, I.A., 1984. Correlations between numbers of enteric nematode parasites in grazing lambs. Int. J. Parasitol. 14, 587–589.

Blanchard, J.L., Wescott, R.B., 1985. Enhancement of resistance of lambs to *Haemonchus contortus* by previous infection with *Ostertagia circumcincta*. Am. J. Vet. Res 46, 2136–2140.

Boag, B., Thomas, R.J., 1977. Epidemiological studies on gastro-intestinal nematode parasites of sheep: the seasonal number of generations and succession of species. Res. Vet. Sci. 22, 62–67.

Cabaret, J., Hoste, H., 1998. Comparative analysis of two methods used to show interspecific associations in naturally acquired parasite nematode communities from the abomasum of ewes. Vet. Parasitol. 76, 275–285.

Chan, M.S., 1997. The global burden of intestinal nematode infections–fifty years on. Parasitol. Today 13, 438–443.

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H.W., Chartier, C., Vineer, H.R., Hinney, B., von Samson-Himmelstjerna, G., Băcescu, B., Mickiewicz, M., Mateus, T.L., Martinez-Valladares, M., Quealy, S., Azaizeh, H., Sekovska, B., Akkari, H., Petkevicius, S., Hektoen, L., Höglund, J., Morgan, E.R., Bartley, D.J., Claerebout, E., 2020. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Prev. Vet. Med. 182, 105103.

Christensen, N.Ø., Nansen, P., Fagbemi, B.O., Monrad, J., 1987. Heterologous antagonistic and synergistic interactions between helminths and between helminths

and protozoans in concurrent experimental infection of mammalian hosts. Parasitol. Res. 73, 387–410.
Coop, R.L., Field, A.C., Graham, R.B., Angus, K.W., Jackson, F., 1986. Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus vitrinus* on the performance of lambs. Res. Vet. Sci. 40, 241–245.
Coop, R.L., Jackson, F., Graham, R.B., Angus, K.W., 1989. Influence of two levels of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus vitrinus* on the growth performance of lambs. Res. Vet. Sci. 45, 275–280.
Dash, K.M., 1981. Interaction between *Oesophagostomum columbianum* and *Oesophagostomum venulosum* in sheep. Int. J. Parasitol. 11, 201–207.
Diez-Baños, N., Cabaret, J., Diez-Baños, P., 1992. Interspecific interactions in naturally acquired nematode communities from sheep abomasum in relation to age of host and season in four areas of León (Spain). Int. J. Parasitol. 22, 327–334.
Dobson, R.J., Barnes, E.H., 1995. Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. Int. J. Parasitol. 25, 495–501.
Evans, M.J., Chaudhry, U.N., Costa-Júnior, L.M., Hamer, K., Leeson, S.R., Sargison, N.D., 2021. A 4 year observation of gastrointestinal nematode egg counts, nemabiomes and the benzimidazole resistance genotypes of Teladorsagia circumcincta on a Scottish sheep farm. Int. J. Parasitol. 51, 393–403.
Faber, M.N., Smith, D., Price, D.R.G., Steele, P., Hildersley, K.A., Morrison, L.J., Mabbott, N.A., Nisbet, A.J., McNeilly, T.N., 2022. Development of bovine gastric organoids as a novel in vitro model to study host-parasite interactions in gastrointestinal nematode infections. Front. Cell. Infect. Microbiol. 829.
Fenton, A., Knowles, S.C.L., Petchey, O.L., Pedersen, A.B., 2014. The reliability of observational approaches for detecting interspecific parasite interactions: comparison with experimental results. Int. J. Parasitol. 44, 437–445.
Fitzpatrick, J.L., 2013. Global food security: the impact of veterinary parasites and parasitologists. Vet. Parasitol. 195, 233–248.
Gilbert, M., Nicolas, G., Cinardi, G., Van Boeckel, T.P., Vanwambeke, S.O., Wint, G.R.W., Robinson, T.P., 2018. Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. Sci. Data 5, 180227.
Hedges, L.V., 1982. Estimation of effect size from a series of independent experiments. Psychol. Bull. 92, 490–499.
Hedges, L.V., 1981. Distribution Theory for Glass's Estimator of Effect size and Related Estimators. J. Educ. Behav. Stat. 6, 107–128.
Herlich, H., 1965. Immunity and cross immunity to *Cooperia oncophora* and *Cooperia pectinata* in calves and lambs. Am. J. Vet. Res 26, 1037–1041.
Horton, J., 2003. Human gastrointestinal helminth infections: are they now neglected diseases? Trends Parasitol. 19, 527–531.
Hoste, H., Cabaret, J., 1992. Intergeneric relations between nematodes of the digestive tract in lambs: A multivariate approach. Int. J. Parasitol. 22, 173–179.
Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. Biom. J. 50, 346–363.
Jackson, F., Jackson, E., Coop, R.L., Huntley, J., 1992. Interactions between *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* infections in young lambs. Res. Vet. Sci. 53, 363–370.
Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: Global worming and anthelmintic resistance. Vet. Parasitol. 186, 70–78.
Kates, K.C., Turner, J.H., 1960. An experiment on the combined pathogenic effects of *Haemonchus contortus* and *Nematodirus spathiger* on lambs. Proc. Helminthol. Soc. Wash. 27, 62.
Kates, K.C., Turner, J.H., 1953. A comparison of the pathogenicity and course of infection of two nematodes of sheep, *Nematodirus spathiger* and *Trichostrongylus colubriformis*, in pure and mixed infections. Proc. Helminthol. Soc. Wash. 20, 117–124.
Lello, J., Boag, B., Fenton, A., Stevenson, I.R., Hudson, P.J., 2004. Competition and mutualism among the gut helminths of a mammalian host. Nature 428, 840–844.
Lello, J., Hussell, T., 2008. Functional group/guild modelling of inter-specific pathogen interactions: A potential tool for predicting the consequences of co-infection. Parasitology 135, 825–839.
Lello, J., McClure, S.J., Tyrrell, K., Viney, M.E., 2018. Predicting the effects of parasite co-infection across species boundaries. Proc. R. Soc. B: Biol. Sci. 285, 20172610.
Mapes, C.J., Coop, R.L., 1971. Effect of concurrent and terminated infections of *Haemonchus contortus* on the development and reproductive capacity of *Nematodirus battus*. J. Comp. Pathol. 81, 479–492.
Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep performance: A systematic review and meta-analysis. Parasites Vectors 8. https://doi.org/10.1186/s13071-015-1164-z.
McFarland, C., Rose Vineer, H., Chesney, L., Henry, N., Brown, C., Airs, P., Nicholson, C., Scollan, N., Lively, F., Kyriazakis, I., Morgan, E.R., 2022. Tracking gastrointestinal nematode risk on cattle farms through pasture contamination mapping. Int. J. Parasitol. 52, 691–703.
McLeod, R.S., 1995. Costs of major parasites to the Australian livestock industries. Int. J. Parasitol. 25, 1363–1367.
Morales, G., Pino, L.A., Sandoval, E., 2006. Intestinal strongylosis in grazing sheep in Venezuela. REDVET 7, 110619.
Morgan, E.R., Aziz, N.-A.A., Blanchard, A., Charlier, J., Charvet, C., Claerebout, E., Geldhof, P., Greer, A.W., Hertzberg, H., Hodgkinson, J., Höglund, J., Hoste, H., Kaplan, R.M., Martínez-Valladares, M., Mitchell, S., Ploeger, H.W., Rinaldi, L., von Samson-Himmelstjerna, G., Sotiraki, S., Schnyder, M., Skuce, P., Bartley, D., Kenyon, F., Thamsborg, S.M., Vineer, H.R., de Waal, T., Williams, A.R., van Wyk, J. A., Vercruysse, J., 2019. 100 Questions in Livestock Helminthology Research. Trends Parasitol. 35, 52–71.
Nakagawa, S., Lagisz, M., Jennions, M.D., Koricheva, J., Noble, D.W.A., Parker, T.H., Sánchez-Tójar, A., Yang, Y., O'Dea, R.E., 2022. Methods for testing publication bias in ecological and evolutionary meta-analyses. Methods Ecol. Evol. 13, 4–21.
Nakagawa, S., Lagisz, M., O'Dea, R.E., Rutkowska, J., Yang, Y., Noble, D.W.A., Senior, A. M., 2021. The orchard plot: Cultivating a forest plot for use in ecology, evolution, and beyond. Res. Synth. Methods. https://doi.org/10.1002/jrsm.1424.
Nieuwhof, G.J., Bishop, S.C., 2005. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. Anim. Sci. 81, 23–29.
O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. https://doi.org/10.1016/j.vetpar.2006.08.035.
Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021a. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372 n71.
Page, M.J., Moher, D., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., McKenzie, J.E., 2021b. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ 372 n160.
Pedersen, A.B., Fenton, A., 2007. Emphasizing the ecology in parasite community ecology. Trends Ecol. Evol. 22, 133–139.
Pustovoi, I.P., 1972, Interspecies relationships of gastro-intestinal strongyles of sheep. [Russian], in: Trudy VII Vsesoyuznoi Konferentsii Po Prirodnoi Ochagovosti Boleznei i Obshchim Voprosam Parazitologii Zhivotnykh. Presented at the Konferentsii po Prirodnoi Ochagovosti Boleznei i Obshchim Voprosam Parazitologii Zhivotnykh, Tajik Veterinary Research Institute, pp. 143–147.
R Core Team, 2022, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Redman, E., Queiroz, C., Bartley, D.J., Levy, M., Avramenko, R.W., Gilleard, J.S., 2019. Validation of ITS-2 rDNA nemabiome sequencing for ovine gastrointestinal nematodes and its application to a large scale survey of UK sheep farms. Vet. Parasitol. 275 https://doi.org/10.1016/j.vetpar.2019.108933.
Rehbein, S., Lindner, T., Kollmannsberger, M., Winter, R., Visser, M., 1997. [Helminth infection of slaughtered sheep in Upper Bavaria. 3. Distribution of colonization of nematodes in the large intestine of sheep]. Berl. Munch. Tierarztl. Wochenschr. 110, 223–228.
Rose, H., Rinaldi, L., Bosco, A., Mavrot, F., de Waal, T., Skuce, P., Charlier, J., Torgerson, P.R., Hertzberg, H., Hendrickx, G., Vercruysse, J., Morgan, E.R., 2015a. Widespread anthelmintic resistance in European farmed ruminants: a systematic review. Vet. Rec. 176, 546.
Rose, H., Wang, T., van Dijk, J., Morgan, E.R., 2015b. GLOWORM-FL: A simulation model of the effects of climate and climate change on the free-living stages of gastrointestinal nematode parasites of ruminants. Ecol. Modell. 297, 232–245.
Rose Vineer, H., Verschave, S.H., Claerebout, E., Vercruysse, J., Shaw, D.J., Charlier, J., Morgan, E.R., 2020. GLOWORM-PARA: a flexible framework to simulate the population dynamics of the parasitic phase of gastrointestinal nematodes infecting grazing livestock. Int. J. Parasitol. 50, 133–144.
Roy, E.A., Hoste, H., Beveridge, I., 2004. The effects of concurrent experimental infections of sheep with *Trichostrongylus colubriformis* and *T. vitrinus* on nematode distributions, numbers and on pathological changes. Parasite 11, 293–300.
Sinn, R., Ketzis, J., Chen, T., 1999. The role of woman in the sheep and goat sector. Small Rumin. Res. 34, 259–269.
Smith, W.D., Pettit, D., Smith, S.K., 2001. Cross-protection studies with gut membrane glycoprotein antigens from *Haemonchus contortus* and *Teladorsagia circumcincta*. Parasite Immunol. 23, 203–211.
Smith, W.D., Smith, S.K., Pettit, D., 2000. Evaluation of immunization with gut membrane glycoproteins of *Ostertagia ostertagi* against homologous challenge in calves and against *Haemonchus contortus* in sheep. Parasite Immunol. 22, 239–247.
Stear, M.J., Bairden, K., Bishop, S.C., Gettinby, G., McKellar, Q.A., Park, M., Strain, S., Wallace, D.S., 1998. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. Parasitology 117 (Pt 2), 165–171.
Steel, J.W., Jones, W.O., Symons, L.E.A., 1982. Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. Aust. J. Agric. Res. 33, 131–140.
Sweeny, J.P.A., Ryan, U.M., Robertson, I.D., Jacobsen, C., 2012. Molecular identification of naturally acquired strongylid infections in lambs–an investigation into how lamb age influences diagnostic sensitivity. Vet. Parasitol. 187, 227–236.
Sykes, A.R., Poppi, D.P., Elliot, D.C., 1988. Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus colubriformis* on the performance of growing lambs consuming fresh herbage. J. Agric. Sci. 110, 531–541.
Parasites of sheep and goats. In: Taylor, M.A., Coop, R.L., Wall, R.L. (Eds.), 2015. Veterinary Parasitology, 4th edition.,. Wiley-Blackwell, Hoboken, pp. 436–523.
Tennekes, M., 2018. tmap: Thematic maps in R. J. Stat. Softw. 84, 1–39.
Tummers, B., 2016, DataThief III [WWW Document]. URL (https://www.datathief.org/) **(accessed 22).**
Turner, J.H., Colglazier, M.L., 1954. Control of pasture-acquired infections of *Nematodirus spathiger* and. Am. J. Vet. Res 15, 564–573.
Turner, J.H., Kates, K.C., Wilson, G.I., 1962. The interaction of concurrent infections of the abomasal nematodes, *Haemonchus contortus*, *Ostertagia circumcincta*, and *Trichostrongylus axei* (*Trichostrongylidae*), in lambs. Proc. Helminthol. Soc. Wash. 29, 210–216.
Veritas Health Information, 2022, Covidence systematic review software [WWW Document]. URL www.covidence.org **(accessed 22).**

Viechtbauer, W., 2010. Conducting Meta-Analyses in R with the metafor Package. J. Stat. Softw. 36, 1–48.
Wickham, H., 2017, tidyverse: Easily Install and Load the 'Tidyverse'. URL (https://cran.r-project.org/package=tidyverse).
Wilke, C.O., 2018, cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. URL (https://cran.r-project.org/package=cowplot).

Appendix II

Differentially Expressed Genes from the RNA sequence analysis in Chapter 5.

Location	ID	log2FoldChange	padj	external_gene_name
Pylorus	ENSOARG00020039041	-18.30676408	8.36E-11	
Pylorus	ENSOARG00020014019	-2.028264748	3.14E-07	SLC9A3
Pylorus	ENSOARG00020007616	-1.283828064	1.27E-05	CDKN2B
Pylorus	ENSOARG00020020313	-1.468811473	3.13E-05	BAMBI
Pylorus	ENSOARG00020026161	-2.079682009	1.26E-04	FRMD1
Pylorus	ENSOARG00020017093	-1.302654591	1.45E-04	ADRA2A
Pylorus	ENSOARG00020039510	-1.142501566	2.59E-04	
Pylorus	ENSOARG00020038683	-1.57633238	4.92E-04	
Pylorus	ENSOARG00020000199	-1.006201211	6.86E-04	EFEMP1

	ENSOARG0002000098			
Pylorus	1	-1.948440502	8.32E-04	SEMA3E
	ENSOARG0002001769			
Pylorus	0	-1.344026585	8.46E-04	CCN3
	ENSOARG0002000111		0.00210	
Pylorus	4	-1.624983894	3	ADAM22
	ENSOARG0002002114			
Pylorus	4	-1.009051587	0.00225	CLDN3
	ENSOARG0002002477		0.00258	
Pylorus	7	-5.306268304	5	APOA4
	ENSOARG0002002930		0.00262	
Pylorus	4	-1.513567926	6	NCMAP
	ENSOARG0002003000		0.00269	
Pylorus	5	-2.789441396	2	
	ENSOARG0002001645		0.00302	
Pylorus	8	-1.214924229	2	PKHD1
	ENSOARG0002002274		0.00624	
Pylorus	6	-1.311557794	3	PLEKHS1
	ENSOARG0002000771		0.00850	
Pylorus	0	-1.071453948	9	DPT
	ENSOARG0002000425			
Pylorus	3	-1.153931755	0.00921	FGF9

	ENSOARG00020002017		0.01005	
Pylorus		-1.340689686	4	PRRX2
	ENSOARG00020018312			
Pylorus		-2.072533997	0.01141	LYPD8
	ENSOARG00020004709		0.01197	
Pylorus		-2.639108456	7	
	ENSOARG00020032111		0.01301	
Pylorus		-1.611798485	1	
	ENSOARG00020022225			
Pylorus		-1.870537049	0.01443	GDPD2
	ENSOARG00020013398		0.01470	
Pylorus		-3.01023631	9	SCEL
	ENSOARG00020011063		0.01736	
Pylorus		-1.057333636	4	VSIG8
	ENSOARG00020020457		0.01920	
Pylorus		-1.244302575	3	
	ENSOARG00020022681		0.02667	
Pylorus		-1.511193078	9	TM4SF20
	ENSOARG00020025289		0.02667	
Pylorus		-1.352378361	9	NPB
	ENSOARG00020001627		0.02785	
Pylorus		-1.114387643	9	NRXN1

	ENSOARG00020008398		0.02854	
Pylorus		-1.272589435	5	MFAP5
	ENSOARG00020019343		0.02991	
Pylorus		-1.902377153	6	VNN1
	ENSOARG00020034875			
Pylorus		-1.032611003	0.03123	FIBIN
	ENSOARG00020029769			
Pylorus		-2.382326869	0.03215	MMP13
	ENSOARG00020004455		0.03338	
Pylorus		-1.676961308	3	
	ENSOARG00020012857		0.03463	
Pylorus		-1.82024229	8	CRIP1
	ENSOARG00020014036		0.03483	
Pylorus		-1.134694291	3	MTTP
	ENSOARG00020012512			
Pylorus		-1.614617539	0.03544	FAT3
	ENSOARG00020011714		0.03673	
Pylorus		-1.352435174	6	ABCA9
	ENSOARG00020033374		0.03735	
Pylorus		-1.552466351	5	
	ENSOARG00020022088		0.04044	
Pylorus		-1.443080962	4	NPY1R

	ENSOARG0002001184		0.04621	
Pylorus	1	-1.562892827	3	GPR83
	ENSOARG0002001856		0.04702	
Pylorus	4	-1.307595596	1	PCDH19
	ENSOARG0002002699			
Pylorus	3	36.49694703	1.07E-63	
	ENSOARG0002002110			
Pylorus	4	3.859140675	1.60E-31	ALPL
	ENSOARG0002000220			
Pylorus	1	7.929744775	1.19E-30	CLCA1
	ENSOARG0002001003			
Pylorus	7	10.58922841	1.19E-28	BPIFB1
	ENSOARG0002002623			
Pylorus	5	12.13554333	9.48E-24	
	ENSOARG0002001500			
Pylorus	4	4.985856872	1.32E-16	IL5RA
	ENSOARG0002001913			
Pylorus	9	5.235817811	3.35E-13	COL6A5
	ENSOARG0002001653			
Pylorus	6	1.876889989	3.19E-11	CBLIF
	ENSOARG0002003116			
Pylorus	6	1.716844283	3.49E-10	GCNT1

	ENSOARG0002000622			
Pylorus	3	1.34951827	4.04E-10	F10
	ENSOARG0002003103			
Pylorus	3	2.280358848	2.09E-09	
	ENSOARG0002002927			
Pylorus	4	2.904538758	4.63E-09	ZBED2
	ENSOARG0002003329			
Pylorus	9	4.385041427	1.11E-08	MCP1
	ENSOARG0002001837			
Pylorus	5	2.815489613	4.72E-08	SH2D7
	ENSOARG0002001676			
Pylorus	3	2.489960323	4.92E-08	
	ENSOARG0002001107			
Pylorus	1	5.731096669	1.21E-07	
	ENSOARG0002002683			
Pylorus	7	6.786555541	1.77E-07	
	ENSOARG0002000446			
Pylorus	6	1.563578791	2.15E-07	IRAG2
	ENSOARG0002001199			
Pylorus	8	1.559378283	2.15E-07	PIGR
	ENSOARG0002002921			
Pylorus	9	9.867864064	2.29E-07	

	ENSOARG0002000461			
Pylorus	4	2.885455943	5.52E-07	SPTA1
	ENSOARG0002001528			
Pylorus	7	1.560049776	5.52E-07	KIT
	ENSOARG0002000742			
Pylorus	3	2.676692981	7.27E-07	TH
	ENSOARG0002002338			
Pylorus	5	1.445984638	8.55E-07	ALOX5
	ENSOARG0002000295			
Pylorus	1	2.18007969	1.11E-06	SH2D6
	ENSOARG0002000163			
Pylorus	1	2.241271369	1.37E-06	HES6
	ENSOARG0002002150			
Pylorus	7	1.694716677	1.57E-06	IL17RB
	ENSOARG0002000209			
Pylorus	5	1.169397698	1.77E-06	ODF2L
	ENSOARG0002001098			
Pylorus	5	1.565548479	3.41E-06	HK3
	ENSOARG0002000636			
Pylorus	9	2.060548981	7.54E-06	TRPM5
	ENSOARG0002000985			
Pylorus	1	3.222206291	7.96E-06	

	ENSOARG0002003980			
Pylorus	7	2.932088705	1.03E-05	ADGRE3
	ENSOARG0002000571			
Pylorus	0	2.619211269	1.35E-05	CPA3
	ENSOARG0002001365			
Pylorus	5	3.157590697	1.78E-05	
	ENSOARG0002000828			
Pylorus	7	1.171747279	2.53E-05	PFKP
	ENSOARG0002000951			
Pylorus	4	2.575314287	2.96E-05	FCER1A
	ENSOARG0002003526			
Pylorus	4	3.074095988	3.74E-05	
	ENSOARG0002000985			
Pylorus	6	1.271316209	4.43E-05	PLCB2
	ENSOARG0002000308			
Pylorus	1	4.121585167	6.51E-05	
	ENSOARG0002002272			
Pylorus	0	1.61252088	6.51E-05	SERPINF2
	ENSOARG0002001538			
Pylorus	3	1.768437422	7.81E-05	
	ENSOARG0002002396			
Pylorus	8	2.755599096	8.02E-05	RAB44

	ENSOARG0002000678			
Pylorus	2	2.798003094	8.73E-05	
	ENSOARG0002001408			
Pylorus	3	3.87116098	8.73E-05	SLC1A2
	ENSOARG0002001617			
Pylorus	5	7.061232063	9.78E-05	LGALS15
	ENSOARG0002003452			
Pylorus	4	3.211582901	9.84E-05	
	ENSOARG0002002459			
Pylorus	8	4.530035282	1.00E-04	MCP-3
	ENSOARG0002000018			
Pylorus	2	4.300573223	1.03E-04	IL13
	ENSOARG0002000672			
Pylorus	7	1.259137651	1.26E-04	KRT23
	ENSOARG0002002622			
Pylorus	5	2.018311734	1.28E-04	
	ENSOARG0002001038			
Pylorus	9	7.800748793	1.30E-04	LGALS15
	ENSOARG0002001548			
Pylorus	5	4.125692094	1.48E-04	CEBPE
	ENSOARG0002002280			
Pylorus	2	3.172781262	1.48E-04	HMX3

	ENSOARG0002002501			
Pylorus	3	5.403832166	2.21E-04	PNLIPRP2
	ENSOARG0002001968			
Pylorus	3	1.43821655	2.86E-04	AVIL
	ENSOARG0002002815			
Pylorus	9	1.00020177	3.50E-04	TENT5C
	ENSOARG0002002518			
Pylorus	2	2.324211984	3.74E-04	CCR3
	ENSOARG0002001685			
Pylorus	9	2.478980468	3.97E-04	
	ENSOARG0002003194			
Pylorus	0	2.833286067	4.92E-04	
	ENSOARG0002000986			
Pylorus	8	1.310987044	4.99E-04	SRGN
	ENSOARG0002000863			
Pylorus	8	1.227127207	6.34E-04	BMX
	ENSOARG0002000928			
Pylorus	4	1.617664023	6.81E-04	ALOX5AP
	ENSOARG0002002977			
Pylorus	5	1.690274612	7.03E-04	RGS13
	ENSOARG0002001472			
Pylorus	9	1.775039901	7.24E-04	

	ENSOARG0002000949			
Pylorus	5	1.099358906	8.10E-04	PARPBP
	ENSOARG0002000321			
Pylorus	8	2.691323021	8.46E-04	TFF3
	ENSOARG0002000181			
Pylorus	7	1.585683291	9.17E-04	
	ENSOARG0002004090		0.00119	
Pylorus	6	2.306560016	6	
	ENSOARG0002000349		0.00127	
Pylorus	2	3.512329847	7	GNLY
	ENSOARG0002001686		0.00127	
Pylorus	6	1.246146826	9	BTK
	ENSOARG0002002604		0.00148	
Pylorus	5	3.33566186	3	
	ENSOARG0002000229		0.00156	
Pylorus	9	1.948200352	8	PDZK1IP1
	ENSOARG0002000264		0.00208	
Pylorus	0	1.029981224	5	IL1RL1
	ENSOARG0002001663		0.00261	
Pylorus	2	3.274338765	5	GFI1B
	ENSOARG0002001510		0.00295	
Pylorus	7	2.19834256	5	CSF3R

	ENSOARG0002003788		0.00309	
Pylorus	3	1.165159682	6	
	ENSOARG0002002093		0.00318	
Pylorus	6	1.036346206	7	PIK3R5
	ENSOARG0002001539		0.00354	
Pylorus	2	2.146016101	7	POU2AF2
	ENSOARG0002002740		0.00400	
Pylorus	6	1.461760182	4	
	ENSOARG0002003081		0.00452	
Pylorus	5	1.445223542	2	
	ENSOARG0002002527		0.00496	
Pylorus	6	1.468050303	1	KANK4
	ENSOARG0002000298		0.00507	
Pylorus	8	1.774965195	3	
	ENSOARG0002002595		0.00507	
Pylorus	5	1.009877201	3	
	ENSOARG0002002171		0.00510	
Pylorus	3	1.423544465	8	TRIM66
	ENSOARG0002000455		0.00528	
Pylorus	6	1.998788608	9	
	ENSOARG0002001386		0.00528	
Pylorus	0	1.670948103	9	

	ENSOARG0002000105		0.00539	
Pylorus	2	1.172304589	6	
	ENSOARG0002003306			
Pylorus	9	1.1764774	0.00551	
	ENSOARG0002002340		0.00551	
Pylorus	1	3.349740771	5	CCL26
	ENSOARG0002000928		0.00558	
Pylorus	0	4.562553029	9	BBOX1
	ENSOARG0002001140			
Pylorus	5	1.976378737	0.00632	NMUR1
	ENSOARG0002001503		0.00635	
Pylorus	4	1.157120646	4	CHAT
	ENSOARG0002001800		0.00646	
Pylorus	6	1.542954522	9	SLC23A1
	ENSOARG0002001844		0.00646	
Pylorus	9	1.145899319	9	ZCWPW1
	ENSOARG0002001715		0.00664	
Pylorus	2	1.802313078	4	MS4A2
	ENSOARG0002000222			
Pylorus	7	2.25595465	0.00711	CLCA4
	ENSOARG0002003406		0.00740	
Pylorus	2	1.01321237	9	CARD6

	ENSOARG0002000662		0.00754	
Pylorus	1	1.027993524	6	SNX20
	ENSOARG0002000890		0.00850	
Pylorus	8	2.504589532	9	C9
	ENSOARG0002001311		0.00850	
Pylorus	3	1.623819848	9	MGAT3
	ENSOARG0002002432		0.00876	
Pylorus	0	1.80297527	7	ALOX15
	ENSOARG0002000474			
Pylorus	3	1.507978345	0.00921	
	ENSOARG0002002519			
Pylorus	6	1.306516767	0.00921	NEIL3
	ENSOARG0002000971		0.00944	
Pylorus	9	2.342566255	6	
	ENSOARG0002001791		0.00964	
Pylorus	0	1.841075993	4	
	ENSOARG0002002551		0.00970	
Pylorus	1	2.887199352	1	HRH4
	ENSOARG0002003023		0.01117	
Pylorus	4	1.211563428	2	APOD
	ENSOARG0002000523		0.01170	
Pylorus	6	1.554879493	2	HDC

	ENSOARG0002000597		0.01170	
Pylorus	3	2.041464746	2	CCL28
	ENSOARG0002000339		0.01176	
Pylorus	2	3.387372695	1	
	ENSOARG0002001887		0.01262	
Pylorus	6	1.556197683	8	SLC28A1
	ENSOARG0002002855		0.01278	
Pylorus	5	1.48441207	2	
	ENSOARG0002000674		0.01286	
Pylorus	1	1.457246236	4	PLA2G5
	ENSOARG0002002021		0.01286	
Pylorus	1	1.128920693	4	CRTAM
	ENSOARG0002002109		0.01368	
Pylorus	1	1.058241177	6	FGR
	ENSOARG0002002378		0.01452	
Pylorus	4	1.09087973	7	NAIP
	ENSOARG0002000899		0.01599	
Pylorus	9	1.679773537	9	RHEX
	ENSOARG0002000370		0.01629	
Pylorus	6	1.146995542	1	CDKL2
	ENSOARG0002002048		0.01717	
Pylorus	1	3.970002969	9	LPO

	ENSOARG0002000058		0.01759	
Pylorus	7	1.268251725	3	
	ENSOARG0002001839		0.01857	
Pylorus	0	1.153613178	8	NCF1
	ENSOARG0002002982		0.02037	
Pylorus	1	2.240483615	1	GNG13
	ENSOARG0002001740		0.02088	
Pylorus	1	1.14657067	4	LY75
	ENSOARG0002002169		0.02236	
Pylorus	8	3.634168334	1	
	ENSOARG0002003321		0.02260	
Pylorus	2	2.699720251	1	
	ENSOARG0002000897		0.02304	
Pylorus	1	1.354329351	2	
	ENSOARG0002001566		0.02378	
Pylorus	3	1.188449511	8	P2RX1
	ENSOARG0002003871			
Pylorus	3	1.877377515	0.0243	
	ENSOARG0002000716		0.02564	
Pylorus	4	3.321323898	5	AURKC
	ENSOARG0002002335		0.02572	
Pylorus	7	1.36915685	5	CCR10

	ENSOARG00020001658		0.02614	
Pylorus		1.154793657	1	SLC4A4
	ENSOARG00020035929		0.02663	
Pylorus		2.844833612	5	
	ENSOARG00020013237		0.02785	
Pylorus		1.844107185	9	SLC6A12
	ENSOARG00020020932		0.02916	
Pylorus		1.079361792	7	CRTAC1
	ENSOARG00020005602		0.03005	
Pylorus		3.3448234	8	CAPN14
	ENSOARG00020018910		0.03117	
Pylorus		1.570189245	8	CELA1
	ENSOARG00020024540			
Pylorus		1.057246983	0.03123	EGR2
	ENSOARG00020001000		0.03231	
Pylorus		1.33922559	2	
	ENSOARG00020028454		0.03284	
Pylorus		1.157885641	4	GCSAM
	ENSOARG00020014807		0.03362	
Pylorus		1.391210699	6	NR4A1
	ENSOARG00020022716		0.03465	
Pylorus		1.120887607	1	TMEM156

	ENSOARG0002000292		0.03483	
Pylorus	8	1.294194713	3	
	ENSOARG0002000786		0.03483	
Pylorus	6	2.331696818	3	ITPRID1
	ENSOARG0002001781		0.03551	
Pylorus	4	2.83695967	6	COLEC10
	ENSOARG0002000606			
Pylorus	0	2.559311546	0.03598	OXT
	ENSOARG0002003269		0.03737	
Pylorus	6	1.903371037	6	
	ENSOARG0002000959		0.03739	
Pylorus	5	1.393814369	2	
	ENSOARG0002001319		0.03739	
Pylorus	4	2.402550732	2	TRPC3
	ENSOARG0002000519		0.03766	
Pylorus	8	1.630434552	2	NR1H4
	ENSOARG0002003316		0.03896	
Pylorus	8	1.089744649	8	CYCS
	ENSOARG0002000069		0.04360	
Pylorus	3	1.05043487	4	NFAM1
	ENSOARG0002002827		0.04360	
Pylorus	3	2.204794068	4	

	ENSOARG0002001722		0.04361	
Pylorus	5	1.03386525	1	CD244
	ENSOARG0002001968		0.04365	
Pylorus	7	2.53514273	6	GATA1
	ENSOARG0002001026		0.04440	
Pylorus	4	1.06763124	1	IGSF9B
	ENSOARG0002003403		0.04594	
Pylorus	0	2.561460744	5	
	ENSOARG0002002184		0.04702	
Pylorus	9	1.256914685	1	CHDH
	ENSOARG0002002699			
Fundus	3	20.26031864	9.60E-18	
	ENSOARG0002002623		0.00269	
Fundus	5	5.093409864	4	
	ENSOARG0002000220		0.00670	
Fundus	1	3.153589265	7	CLCA1
	ENSOARG0002001617		0.00670	
Fundus	5	6.730613045	7	LGALS15
	ENSOARG0002000326		0.04416	
Fundus	1	2.599489401	5	SFTPB
	ENSOARG0002001287		0.04416	
Fundus	9	2.0144077	5	CATSPERB

	ENSOARG0002003526		0.04840	
Fundus	4	2.661012898	3	
	ENSOARG0002001500		0.04961	
Fundus	4	2.256438891	4	IL5RA
	ENSOARG0002002553		0.04961	
Fundus	7	4.780558742	4	LUZP2
Small	ENSOARG0002001279			
Intestine	7	-1.171400529	9.79E-13	SLC38A7
Small	ENSOARG0002001864			
Intestine	8	-3.959140429	1.27E-11	FGF19
Small	ENSOARG0002000488			
Intestine	7	-1.214550436	5.49E-11	DGAT1
Small	ENSOARG0002001882			
Intestine	8	-2.296692132	3.53E-09	FAM151A
Small	ENSOARG0002001611			
Intestine	5	-2.468593289	8.07E-09	GAL3ST1
Small	ENSOARG0002004043			
Intestine	2	-1.621041986	1.44E-08	
Small	ENSOARG0002002052			
Intestine	0	-1.106559421	2.38E-08	PCTP
Small	ENSOARG0002001464			
Intestine	3	-1.594346881	5.20E-08	MS4A18

Small	ENSOARG0002001336			
Intestine	6	-1.105813129	1.33E-07	FBXO44
Small	ENSOARG0002002458			
Intestine	4	-1.199510851	1.33E-07	ABCD1
Small	ENSOARG0002000134			
Intestine	3	-2.071940045	2.17E-07	CGREF1
Small	ENSOARG0002001497			
Intestine	5	-1.778468528	2.29E-07	PANX
Small	ENSOARG0002000556			
Intestine	4	-1.597875023	3.36E-07	BST1
Small	ENSOARG0002002182			
Intestine	7	-1.136458936	5.10E-07	MYLIP
Small	ENSOARG0002001576			
Intestine	0	-1.016910769	5.12E-07	AdpR2
Small	ENSOARG0002003236			
Intestine	2	-1.680524604	5.70E-07	STYK1
Small	ENSOARG0002000020			
Intestine	0	-1.989645257	6.36E-07	ABCA4
Small	ENSOARG0002001546			
Intestine	6	-3.444370896	6.45E-07	CPO
Small	ENSOARG0002002079			
Intestine	2	-1.199002844	8.47E-07	GRAMD1B

Small	ENSOARG0002001934			
Intestine	3	-3.038765389	9.91E-07	VNN1
Small	ENSOARG0002001946			
Intestine	1	-1.51806887	2.01E-06	TULP2
Small	ENSOARG0002000259			
Intestine	4	-1.06016105	2.34E-06	IGFBP3
Small	ENSOARG0002001324			
Intestine	7	-1.953826041	2.41E-06	PMP22
Small	ENSOARG0002001305			
Intestine	1	-1.328028447	2.76E-06	PLIN3
Small	ENSOARG0002001597			
Intestine	0	-1.48749263	2.92E-06	CNIH4
Small	ENSOARG0002000462			
Intestine	6	-1.754460747	4.24E-06	TMEM150B
Small	ENSOARG0002001891			
Intestine	4	-1.008608932	5.43E-06	LDHD
Small	ENSOARG0002002056			
Intestine	5	-1.66463298	6.60E-06	
Small	ENSOARG0002001203			
Intestine	3	-1.559238537	6.74E-06	WIPF3
Small	ENSOARG0002000625			
Intestine	2	-1.141995888	6.79E-06	PLEKHG6

Small	ENSOARG0002002652			
Intestine	0	-1.474965461	6.79E-06	
Small	ENSOARG0002000869			
Intestine	7	-1.420714346	7.65E-06	SLC27A4
Small	ENSOARG0002002397			
Intestine	1	-1.319110169	7.65E-06	PFKFB4
Small	ENSOARG0002003591			
Intestine	4	-1.266324631	7.65E-06	
Small	ENSOARG0002003271			
Intestine	9	-1.469512052	8.41E-06	RAB30
Small	ENSOARG0002002479			
Intestine	6	-1.960345243	1.02E-05	APOA1
Small	ENSOARG0002001145			
Intestine	8	-1.119839279	1.14E-05	TMEM45B
Small	ENSOARG0002000080			
Intestine	2	-1.375269197	1.53E-05	
Small	ENSOARG0002001530			
Intestine	7	-1.697222138	1.53E-05	SEC14L2
Small	ENSOARG0002002012			
Intestine	6	-1.967289102	1.53E-05	SMPDL3B
Small	ENSOARG0002002969			
Intestine	6	-1.066231223	1.56E-05	TFEC

Small	ENSOARG0002001211			
Intestine	4	-1.26906464	1.88E-05	CAT
Small	ENSOARG0002001605			
Intestine	6	-1.750119251	2.05E-05	ANKRD24
Small	ENSOARG0002001029			
Intestine	9	-1.792810515	2.05E-05	XYLB
Small	ENSOARG0002002831			
Intestine	3	-2.136339837	2.16E-05	
Small	ENSOARG0002001715			
Intestine	4	-2.813007618	2.19E-05	SLC34A2
Small	ENSOARG0002000136			
Intestine	4	-1.509112345	2.25E-05	ABHD1
Small	ENSOARG0002001257			
Intestine	2	-1.458203831	2.41E-05	
Small	ENSOARG0002002007			
Intestine	9	-1.107864536	2.63E-05	XKR8
Small	ENSOARG0002001334			
Intestine	8	-1.308562984	2.80E-05	ACSL1
Small	ENSOARG0002001896			
Intestine	5	-2.658422474	2.83E-05	KLKB1
Small	ENSOARG0002002275			
Intestine	5	-1.182616164	3.20E-05	GGACT

Small	ENSOARG0002000655			
Intestine	8	-2.085475483	3.57E-05	DCST1
Small	ENSOARG0002001293			
Intestine	0	-1.186244399	3.67E-05	TEDC1
Small	ENSOARG0002001761			
Intestine	7	-1.270493241	3.92E-05	ACSL5
Small	ENSOARG0002001225			
Intestine	1	-1.345876521	4.40E-05	
Small	ENSOARG0002001214			
Intestine	1	-1.174884088	5.15E-05	IL22RA1
Small	ENSOARG0002003320			
Intestine	5	-1.220234187	5.27E-05	
Small	ENSOARG0002001726			
Intestine	2	-2.255027845	5.32E-05	NOX4
Small	ENSOARG0002001206			
Intestine	5	-1.381317888	6.14E-05	PLPPR3
Small	ENSOARG0002001318			
Intestine	2	-1.64686117	6.51E-05	PLA2G7
Small	ENSOARG0002000618			
Intestine	6	-1.002073547	8.52E-05	
Small	ENSOARG0002001361			
Intestine	2	-1.588808233	1.16E-04	ENTPD5

Small	ENSOARG0002000223			
Intestine	2	-2.299864448	1.19E-04	SDR9C7
Small	ENSOARG0002000328			
Intestine	7	-1.292193704	1.19E-04	PHYH
Small	ENSOARG0002000541			
Intestine	1	-2.263138748	1.25E-04	
Small	ENSOARG0002000128			
Intestine	4	-1.843974157	1.26E-04	CYP4B1
Small	ENSOARG0002003914			
Intestine	4	-2.299034774	1.38E-04	
Small	ENSOARG0002000117			
Intestine	3	-1.525861849	1.46E-04	SULT1B1
Small	ENSOARG0002000005			
Intestine	8	-1.137833683	1.49E-04	CTPS1
Small	ENSOARG0002001694			
Intestine	8	-3.317571421	1.49E-04	PRSS56
Small	ENSOARG0002002596			
Intestine	0	-1.328231907	1.87E-04	MFSD2A
Small	ENSOARG0002001201			
Intestine	7	-1.648378099	1.90E-04	KCNK17
Small	ENSOARG0002002238			
Intestine	9	-1.842503004	1.96E-04	NR0B2

Small	ENSOARG0002002589			
Intestine	1	-1.204227103	1.97E-04	ACAA1
Small	ENSOARG0002001207			
Intestine	9	-1.434314068	2.15E-04	DPP4
Small	ENSOARG0002000805			
Intestine	3	-1.75231222	2.22E-04	SOAT2
Small	ENSOARG0002001115			
Intestine	1	-2.715805027	2.22E-04	SLC6A18
Small	ENSOARG0002002224			
Intestine	4	-1.10675557	2.32E-04	HSD17B11
Small	ENSOARG0002000957			
Intestine	7	-1.446030217	2.33E-04	
Small	ENSOARG0002002865			
Intestine	1	-1.189536603	2.35E-04	RNF186
Small	ENSOARG0002000304			
Intestine	7	-1.899297828	2.54E-04	PDZK1
Small	ENSOARG0002002039			
Intestine	6	-2.297365474	2.72E-04	
Small	ENSOARG0002001843			
Intestine	6	-3.048098141	2.74E-04	DLK1
Small	ENSOARG0002003728			
Intestine	6	-1.594996151	2.83E-04	

Small	ENSOARG0002003985			
Intestine	7	-1.104159825	2.92E-04	
Small	ENSOARG0002001863			
Intestine	9	-1.715325954	3.05E-04	AFMID
Small	ENSOARG0002003251			
Intestine	3	-3.104724842	3.05E-04	
Small	ENSOARG0002001965			
Intestine	3	-1.265225095	3.07E-04	SEC16B
Small	ENSOARG0002002910			
Intestine	3	-1.104056047	3.22E-04	
Small	ENSOARG0002000698			
Intestine	3	-4.707936341	3.23E-04	GKN1
Small	ENSOARG0002003984			
Intestine	5	-1.173685926	3.47E-04	
Small	ENSOARG0002002115			
Intestine	8	-1.138190197	3.80E-04	TLCD1
Small	ENSOARG0002000623			
Intestine	0	-1.083123346	3.92E-04	UGT1A6
Small	ENSOARG0002002344			
Intestine	7	-1.100789951	4.28E-04	GPD1
Small	ENSOARG0002001393			
Intestine	4	-1.078032885	4.29E-04	UNC5CL

Small	ENSOARG0002002333			
Intestine	7	-1.082264851	4.52E-04	
Small	ENSOARG0002003357			
Intestine	2	-1.255159309	4.58E-04	
Small	ENSOARG0002001285			
Intestine	7	-2.363159088	4.61E-04	CRIP1
Small	ENSOARG0002001985			
Intestine	4	-1.343358178	4.61E-04	INPP5J
Small	ENSOARG0002003069			
Intestine	1	-1.039438578	4.61E-04	
Small	ENSOARG0002000147			
Intestine	6	-3.902597319	4.86E-04	
Small	ENSOARG0002000563			
Intestine	3	-1.017738901	5.06E-04	SLC25A1
Small	ENSOARG0002000243			
Intestine	1	-1.003805074	5.33E-04	SLC5A9
Small	ENSOARG0002001991			
Intestine	3	-1.472030092	5.43E-04	QPRT
Small	ENSOARG0002002089			
Intestine	8	-1.894395966	5.83E-04	
Small	ENSOARG0002002115			
Intestine	2	-1.601344414	6.22E-04	LEAP2

Small	ENSOARG0002002582			
Intestine	9	-3.155089062	6.22E-04	ANGPTL3
Small	ENSOARG0002000666			
Intestine	0	-1.505577316	6.67E-04	ITGA7
Small	ENSOARG0002000500			
Intestine	8	-1.082898383	7.20E-04	TMEM86B
Small	ENSOARG0002001719			
Intestine	8	-2.822579564	7.51E-04	CHRND
Small	ENSOARG0002000869			
Intestine	8	-1.190387761	8.49E-04	PM20D1
Small	ENSOARG0002001011			
Intestine	4	-4.077213307	9.50E-04	SLC22A14
Small	ENSOARG0002001335			
Intestine	5	-1.719192448	9.60E-04	ANPEP
Small	ENSOARG0002000627			
Intestine	4	-1.214197296	9.74E-04	TBX1
Small	ENSOARG0002002508			
Intestine	5	-2.973484826	9.77E-04	CIDEC
Small	ENSOARG0002001470		0.00102	
Intestine	5	-1.206702511	7	MS4A15
Small	ENSOARG0002001445		0.00105	
Intestine	1	-1.284571426	9	AHRR

Small	ENSOARG0002001577		0.00105	
Intestine	6	-1.306331544	9	NAPSA
Small	ENSOARG0002003166		0.00119	
Intestine	1	-1.66668071	1	
Small	ENSOARG0002003795		0.00122	
Intestine	4	-1.293856749	6	
Small	ENSOARG0002001126		0.00124	
Intestine	1	-1.554291063	2	DLX4
Small	ENSOARG0002000630			
Intestine	3	-1.188108607	0.00125	PLS1
Small	ENSOARG0002001883		0.00136	
Intestine	2	-1.120802944	8	CYP4V2
Small	ENSOARG0002002034		0.00136	
Intestine	3	-3.081094763	9	
Small	ENSOARG0002003515		0.00144	
Intestine	1	-1.825638148	8	
Small	ENSOARG0002000190		0.00145	
Intestine	7	-1.660131841	7	LSMEM1
Small	ENSOARG0002000710		0.00146	
Intestine	1	-4.031958015	4	GKN2
Small	ENSOARG0002003957		0.00154	
Intestine	4	-2.127532132	4	

Small	ENSOARG0002003021		0.00164	
Intestine	1	-1.154900187	4	RETSAT
Small	ENSOARG0002003947		0.00168	
Intestine	0	-1.123264151	1	
Small	ENSOARG0002002083		0.00170	
Intestine	0	-1.094540903	2	CYSTM1
Small	ENSOARG0002001315		0.00178	
Intestine	1	-1.797105675	1	TEKT3
Small	ENSOARG0002003408		0.00179	
Intestine	6	-2.109450364	2	
Small	ENSOARG0002001170		0.00180	
Intestine	6	-1.044347341	5	
Small	ENSOARG0002003189		0.00194	
Intestine	6	-1.345973132	7	
Small	ENSOARG0002001100			
Intestine	2	-1.149218869	0.00196	CCL25
Small	ENSOARG0002002078			
Intestine	5	-2.041497038	0.00196	GSTA1
Small	ENSOARG0002002216			
Intestine	3	-1.404494299	0.00196	CLSTN3
Small	ENSOARG0002002308		0.00197	
Intestine	9	-1.479795449	6	

Small	ENSOARG0002001032		0.00211	
Intestine	3	-1.084462836	5	SLC43A2
Small	ENSOARG0002002492		0.00211	
Intestine	7	-2.429701597	5	DSG3
Small	ENSOARG0002002537		0.00215	
Intestine	5	-1.124609854	7	AQP11
Small	ENSOARG0002003450		0.00215	
Intestine	7	-2.074437519	7	
Small	ENSOARG0002000360		0.00218	
Intestine	1	-2.617991735	7	
Small	ENSOARG0002001299		0.00219	
Intestine	8	-1.088677832	2	AGTRAP
Small	ENSOARG0002003276		0.00219	
Intestine	9	-1.172564938	2	
Small	ENSOARG0002003985		0.00224	
Intestine	9	-1.376253208	1	
Small	ENSOARG0002000722		0.00226	
Intestine	1	-1.223099575	1	TSKU
Small	ENSOARG0002002544		0.00241	
Intestine	2	-1.197649999	4	INCA1
Small	ENSOARG0002004101			
Intestine	0	-1.697994139	0.00242	C3orf85

Small	ENSOARG0002000517		0.00245	
Intestine	5	-1.434974861	7	MCF2L2
Small	ENSOARG0002001769		0.00257	
Intestine	6	-1.070958954	1	TNFSF15
Small	ENSOARG0002003282		0.00257	
Intestine	7	-1.681385011	5	
Small	ENSOARG0002003228		0.00262	
Intestine	7	-1.100138116	6	
Small	ENSOARG0002002562			
Intestine	6	-1.399948916	0.00264	GIMD1
Small	ENSOARG0002000434		0.00273	
Intestine	8	-1.34570055	6	KISS1
Small	ENSOARG0002004072		0.00301	
Intestine	2	-1.257039146	2	
Small	ENSOARG0002000446		0.00313	
Intestine	2	-1.126790159	9	ID1
Small	ENSOARG0002002053		0.00313	
Intestine	2	-1.166068378	9	MBNL3
Small	ENSOARG0002003473		0.00316	
Intestine	4	-2.473612541	1	
Small	ENSOARG0002000423		0.00317	
Intestine	6	-1.055842939	7	CARD9

Small	ENSOARG0002002377		0.00319	
Intestine	7	-1.628126199	5	
Small	ENSOARG0002001480		0.00321	
Intestine	2	-1.908294251	4	C14H19orf33
Small	ENSOARG0002001350		0.00322	
Intestine	8	-1.39607752	7	CR2
Small	ENSOARG0002001626			
Intestine	6	-1.102879931	0.00327	TCN2
Small	ENSOARG0002002154		0.00338	
Intestine	7	-1.263347169	5	
Small	ENSOARG0002003094		0.00338	
Intestine	4	-1.096529153	5	LBHD1
Small	ENSOARG0002000366		0.00342	
Intestine	2	-2.498007545	9	PTK6
Small	ENSOARG0002001113		0.00349	
Intestine	8	-1.367162385	1	ADA
Small	ENSOARG0002002636		0.00358	
Intestine	8	-2.070002199	7	SLC5A10
Small	ENSOARG0002001975		0.00362	
Intestine	2	-5.975592106	3	RDH12
Small	ENSOARG0002003269		0.00374	
Intestine	4	-1.028856758	6	

Small	ENSOARG0002002350			
Intestine	6	-2.496825053	0.00378	LYPD3
Small	ENSOARG0002003913			
Intestine	6	-1.307969762	0.00379	
Small	ENSOARG0002003514		0.00379	
Intestine	8	-1.78554854	4	
Small	ENSOARG0002002036		0.00398	
Intestine	3	-1.646879506	8	
Small	ENSOARG0002003970		0.00417	
Intestine	0	-1.00494602	3	
Small	ENSOARG0002002273		0.00448	
Intestine	9	-1.16537573	2	KIF17
Small	ENSOARG0002001468		0.00451	
Intestine	7	-1.053223179	2	NAALADL1
Small	ENSOARG0002000190		0.00452	
Intestine	0	-1.24015052	3	STEAP1
Small	ENSOARG0002001014		0.00452	
Intestine	2	-1.273278578	3	RHOD
Small	ENSOARG0002003817		0.00455	
Intestine	0	-1.077573279	8	
Small	ENSOARG0002001014		0.00494	
Intestine	3	-1.308276473	3	PHOSPHO1

Small	ENSOARG0002000531		0.00518	
Intestine	0	-1.63043958	6	
Small	ENSOARG0002000717			
Intestine	7	-1.154711997	0.0053	SOX6
Small	ENSOARG0002002222			
Intestine	5	-1.679124342	0.0053	GDPD2
Small	ENSOARG0002002277			
Intestine	2	-1.683773863	0.00535	
Small	ENSOARG0002000328		0.00548	
Intestine	0	-1.254703347	4	FST
Small	ENSOARG0002000423			
Intestine	1	-1.25801842	0.00551	CEACAM20
Small	ENSOARG0002002031		0.00578	
Intestine	2	-2.607537491	6	
Small	ENSOARG0002000828		0.00581	
Intestine	0	-1.522462624	5	CIDEB
Small	ENSOARG0002004035		0.00591	
Intestine	4	-1.125156831	2	
Small	ENSOARG0002003895		0.00598	
Intestine	2	-1.28822055	2	
Small	ENSOARG0002001791		0.00600	
Intestine	8	-1.68317149	1	CES3

Small	ENSOARG0002001175		0.00603	
Intestine	6	-1.076425268	3	EPHX2
Small	ENSOARG0002001307		0.00630	
Intestine	7	-1.242322068	3	DRAXIN
Small	ENSOARG0002003639		0.00636	
Intestine	6	-1.127320595	1	
Small	ENSOARG0002003410		0.00662	
Intestine	2	-1.207484734	3	
Small	ENSOARG0002000639		0.00667	
Intestine	4	-1.735740522	7	ASAH2
Small	ENSOARG0002002329		0.00671	
Intestine	8	-1.870961303	6	F2
Small	ENSOARG0002003560		0.00674	
Intestine	2	-1.516801548	2	GPR55
Small	ENSOARG0002002930		0.00689	
Intestine	2	-1.103229608	9	
Small	ENSOARG0002001166		0.00712	
Intestine	9	-2.271008646	3	
Small	ENSOARG0002001041		0.00721	
Intestine	0	-1.821922564	2	FMO3
Small	ENSOARG0002000349		0.00756	
Intestine	8	-2.924213282	9	SRMS

Small	ENSOARG0002001318		0.00758	
Intestine	9	-1.035879801	7	
Small	ENSOARG0002001724		0.00787	
Intestine	5	-1.361522392	3	ATOX1
Small	ENSOARG0002003211			
Intestine	1	-1.500163035	0.00806	
Small	ENSOARG0002000261			
Intestine	2	-4.445558223	0.00815	WDR49
Small	ENSOARG0002001223		0.00877	
Intestine	3	-1.111464861	8	TEX52
Small	ENSOARG0002003650		0.00892	
Intestine	4	-1.455988157	2	
Small	ENSOARG0002003176			
Intestine	5	-1.90111718	0.00895	
Small	ENSOARG0002003868			
Intestine	7	-1.29765076	0.00895	TMEM238
Small	ENSOARG0002001540		0.00900	
Intestine	7	-2.4666602	2	SDSL
Small	ENSOARG0002000883		0.00913	
Intestine	7	-1.007019834	3	
Small	ENSOARG0002004013		0.00913	
Intestine	7	-1.077225679	3	

Small	ENSOARG0002004022		0.00938	
Intestine	4	-1.124975765	2	DNAJB7
Small	ENSOARG0002001433		0.00945	
Intestine	4	-1.930859114	6	ISG15
Small	ENSOARG0002001087			
Intestine	6	-1.188189154	0.00951	SLC4A5
Small	ENSOARG0002003629		0.00958	
Intestine	6	-1.229260752	3	
Small	ENSOARG0002000439		0.00964	
Intestine	6	-1.044144252	6	GPR171
Small	ENSOARG0002000612		0.00964	
Intestine	9	-1.068130804	6	DNAJC15
Small	ENSOARG0002002547		0.00965	
Intestine	8	-1.619762737	7	ACOX2
Small	ENSOARG0002001282		0.00971	
Intestine	1	-1.135784398	9	PEX11A
Small	ENSOARG0002001489		0.00978	
Intestine	7	-1.497012908	3	
Small	ENSOARG0002000534		0.00983	
Intestine	7	-1.553450129	8	
Small	ENSOARG0002003336			
Intestine	7	-1.186415481	0.01003	

Small	ENSOARG0002003579		0.01024	
Intestine	9	-1.800339294	9	
Small	ENSOARG0002002998		0.01032	
Intestine	1	-2.255483529	5	
Small	ENSOARG0002003252		0.01069	
Intestine	3	-1.857330932	1	
Small	ENSOARG0002001214		0.01083	
Intestine	7	-1.697320631	3	
Small	ENSOARG0002000817		0.01113	
Intestine	2	-1.564869531	4	ATP5MK
Small	ENSOARG0002003971		0.01124	
Intestine	9	-1.080647312	8	
Small	ENSOARG0002002674			
Intestine	0	-1.275420898	0.01131	
Small	ENSOARG0002002899		0.01143	
Intestine	1	-1.4863108	9	
Small	ENSOARG0002000127		0.01148	
Intestine	5	-1.103397992	9	AGBL5
Small	ENSOARG0002002867		0.01169	
Intestine	8	-1.800578203	2	
Small	ENSOARG0002002519		0.01186	
Intestine	7	-1.50656123	3	

Small	ENSOARG0002003088		0.01203	
Intestine	3	-1.361689627	4	
Small	ENSOARG0002002655		0.01206	
Intestine	2	-1.089742016	6	
Small	ENSOARG0002003950		0.01206	
Intestine	3	-1.042919063	6	
Small	ENSOARG0002001780		0.01221	
Intestine	7	-1.12866806	8	XPNPEP2
Small	ENSOARG0002000847		0.01238	
Intestine	9	-1.517393035	6	
Small	ENSOARG0002001709			
Intestine	4	-1.43575051	0.0125	ACOT12
Small	ENSOARG0002001371			
Intestine	2	-1.195293861	0.0127	ADGRF1
Small	ENSOARG0002002385		0.01285	
Intestine	7	-1.012923693	3	SCD
Small	ENSOARG0002002147		0.01313	
Intestine	2	-1.076099457	6	COL4A3
Small	ENSOARG0002000490		0.01314	
Intestine	7	-1.346181413	9	
Small	ENSOARG0002002109		0.01369	
Intestine	2	-1.184024685	6	CLEC10A

Small	ENSOARG0002001341		0.01376	
Intestine	9	-1.144416649	2	FBXO2
Small	ENSOARG0002001488		0.01381	
Intestine	3	-1.648909623	4	SLC6A4
Small	ENSOARG0002002787		0.01382	
Intestine	4	-1.245766874	8	
Small	ENSOARG0002003885		0.01430	
Intestine	1	-1.054243504	9	
Small	ENSOARG0002001120		0.01432	
Intestine	2	-1.092292692	5	DAD1
Small	ENSOARG0002003487		0.01432	
Intestine	6	-1.445592255	5	
Small	ENSOARG0002003669		0.01447	
Intestine	3	-2.464433709	2	
Small	ENSOARG0002000123		0.01454	
Intestine	1	-1.035738003	4	
Small	ENSOARG0002000663		0.01455	
Intestine	3	-1.527280764	4	SLC38A8
Small	ENSOARG0002003521		0.01466	
Intestine	5	-1.51820852	1	
Small	ENSOARG0002001152		0.01486	
Intestine	9	-1.194120377	2	

Small	ENSOARG0002003493		0.01496	
Intestine	0	-1.578348113	8	
Small	ENSOARG0002001374		0.01498	
Intestine	6	-1.007404101	7	MGST2
Small	ENSOARG0002000298		0.01512	
Intestine	4	-3.385506857	3	SLC2A9
Small	ENSOARG0002001403		0.01540	
Intestine	6	-1.088180421	1	MTTP
Small	ENSOARG0002001226		0.01566	
Intestine	2	-1.695313793	6	
Small	ENSOARG0002000799		0.01595	
Intestine	3	-1.712153158	4	RPL37
Small	ENSOARG0002000864		0.01616	
Intestine	0	-1.008493694	5	APOBEC1
Small	ENSOARG0002000663		0.01620	
Intestine	8	-1.066861336	2	ABCG5
Small	ENSOARG0002002382		0.01621	
Intestine	5	-2.120898751	8	CYP1A1
Small	ENSOARG0002003202		0.01622	
Intestine	0	-1.709477625	8	
Small	ENSOARG0002002893		0.01696	
Intestine	8	-1.017464159	3	

Small	ENSOARG0002000345		0.01709	
Intestine	1	-6.113662385	8	
Small	ENSOARG0002003013		0.01715	
Intestine	9	-1.177464137	1	
Small	ENSOARG0002000961		0.01725	
Intestine	0	-1.039488695	6	DEPDC7
Small	ENSOARG0002002147			
Intestine	5	-1.00870437	0.01758	
Small	ENSOARG0002002793			
Intestine	6	-2.401324416	0.01764	
Small	ENSOARG0002000058		0.01769	
Intestine	0	-2.183053621	2	FETUB
Small	ENSOARG0002002181		0.01769	
Intestine	0	-1.051527253	2	DLX6
Small	ENSOARG0002003296		0.01769	
Intestine	8	-1.009758903	2	
Small	ENSOARG0002002569		0.01769	
Intestine	7	-1.177494393	3	UPB1
Small	ENSOARG0002001805		0.01810	
Intestine	4	-1.460009419	3	RPL37A
Small	ENSOARG0002002138		0.01810	
Intestine	1	-1.273418222	3	HEATR9

Small	ENSOARG0002003130		0.01826	
Intestine	4	-2.119649553	6	
Small	ENSOARG0002002906		0.01834	
Intestine	3	-2.694364131	8	
Small	ENSOARG0002002265		0.01841	
Intestine	0	-3.172590057	8	ALOXE3
Small	ENSOARG0002001537		0.01854	
Intestine	1	-1.819491971	1	TVP23A
Small	ENSOARG0002004089		0.01894	
Intestine	4	-1.575660074	3	
Small	ENSOARG0002001752		0.01896	
Intestine	8	-1.588465509	8	TTC21A
Small	ENSOARG0002001629			
Intestine	2	-2.974548153	0.019	
Small	ENSOARG0002001066			
Intestine	8	-2.825636133	0.01927	
Small	ENSOARG0002001657		0.01937	
Intestine	5	-1.104263824	7	
Small	ENSOARG0002000759		0.01945	
Intestine	3	-2.093648498	5	
Small	ENSOARG0002001463		0.01995	
Intestine	4	-1.353734581	1	SLC35F3

Small	ENSOARG0002002079		0.01998	
Intestine	4	-1.021525895	7	SGK1
Small	ENSOARG0002003837		0.01998	
Intestine	7	-1.142189371	7	
Small	ENSOARG0002001531		0.02001	
Intestine	0	-1.134419639	4	ZBTB37
Small	ENSOARG0002002659		0.02010	
Intestine	3	-1.154535794	6	
Small	ENSOARG0002000374		0.02011	
Intestine	2	-1.434854023	4	SHC3
Small	ENSOARG0002004044		0.02011	
Intestine	5	-2.485324058	4	
Small	ENSOARG0002001651		0.02017	
Intestine	4	-1.300757726	1	TMEM82
Small	ENSOARG0002003573		0.02121	
Intestine	1	-1.25998495	2	
Small	ENSOARG0002001612			
Intestine	1	-1.189697109	0.02161	TNF
Small	ENSOARG0002001057		0.02170	
Intestine	8	-1.697867029	6	
Small	ENSOARG0002002641		0.02211	
Intestine	8	-4.031856014	6	

Small	ENSOARG0002002023		0.02229	
Intestine	6	-1.267303534	1	
Small	ENSOARG0002003592		0.02291	
Intestine	9	-1.960146019	4	
Small	ENSOARG0002003903		0.02294	
Intestine	5	-1.203015725	6	
Small	ENSOARG0002000415		0.02301	
Intestine	0	-1.115753502	7	CAMK2B
Small	ENSOARG0002004067		0.02304	
Intestine	6	-1.961435803	3	
Small	ENSOARG0002003387		0.02304	
Intestine	6	-1.354783969	4	
Small	ENSOARG0002003899		0.02322	
Intestine	9	-1.161235746	8	
Small	ENSOARG0002003695		0.02323	
Intestine	8	-1.354555078	8	
Small	ENSOARG0002000357			
Intestine	1	-1.216686362	0.02335	NPC1L1
Small	ENSOARG0002000032		0.02352	
Intestine	9	-1.761636467	3	MGAM
Small	ENSOARG0002000503		0.02352	
Intestine	9	-1.368725085	3	SLIRP

Small	ENSOARG0002003707		0.02375	
Intestine	0	-1.057512943	8	
Small	ENSOARG0002004101		0.02416	
Intestine	8	-1.625093866	1	
Small	ENSOARG0002002989		0.02477	
Intestine	0	-1.47470448	1	GGN
Small	ENSOARG0002002606		0.02479	
Intestine	3	-1.650114044	6	
Small	ENSOARG0002003255		0.02490	
Intestine	7	-1.091309038	5	
Small	ENSOARG0002003225		0.02548	
Intestine	0	-1.014271397	4	
Small	ENSOARG0002002393		0.02552	
Intestine	4	-1.096160563	4	ANKRD61
Small	ENSOARG0002002121		0.02582	
Intestine	0	-1.497421737	2	HRC
Small	ENSOARG0002002517		0.02585	
Intestine	9	-1.882421245	4	GCGR
Small	ENSOARG0002003674		0.02708	
Intestine	9	-1.438534953	5	
Small	ENSOARG0002000190		0.02754	
Intestine	8	-1.3159821	9	AGMO

Small	ENSOARG0002002231		0.02775	
Intestine	4	-1.66147286	8	F13B
Small	ENSOARG0002001564		0.02822	
Intestine	9	-1.084012044	7	TMEM253
Small	ENSOARG0002000438		0.02898	
Intestine	1	-1.483745031	6	PLB1
Small	ENSOARG0002001554		0.02933	
Intestine	1	-1.86162647	3	
Small	ENSOARG0002002208		0.02937	
Intestine	0	-1.012238136	1	SEMA5A
Small	ENSOARG0002003933		0.02937	
Intestine	0	-1.079089617	1	PRR15
Small	ENSOARG0002000838			
Intestine	8	-1.150647384	0.02961	
Small	ENSOARG0002001512		0.02994	
Intestine	8	-1.466088332	3	CRISP2
Small	ENSOARG0002002651		0.03013	
Intestine	0	-1.048459148	2	
Small	ENSOARG0002001519		0.03039	
Intestine	8	-1.608804309	2	
Small	ENSOARG0002001611		0.03061	
Intestine	1	-1.02645418	5	LYVE1

Small	ENSOARG0002000369		0.03172	
Intestine	5	-1.495005619	8	
Small	ENSOARG0002000876		0.03199	
Intestine	2	-1.151875939	2	ACE2
Small	ENSOARG0002001094			
Intestine	9	-1.352831342	0.03235	STAB2
Small	ENSOARG0002000309		0.03239	
Intestine	5	-1.117379693	8	
Small	ENSOARG0002000129		0.03246	
Intestine	4	-1.655390455	9	TRPV6
Small	ENSOARG0002002791		0.03273	
Intestine	7	-1.228460968	1	
Small	ENSOARG0002002585		0.03358	
Intestine	6	-1.064529211	8	TIMP4
Small	ENSOARG0002001502		0.03365	
Intestine	8	-1.822596171	8	
Small	ENSOARG0002001345		0.03402	
Intestine	7	-1.144176008	3	PCK1
Small	ENSOARG0002000490		0.03454	
Intestine	6	-2.290300747	6	SRD5A2
Small	ENSOARG0002000454		0.03460	
Intestine	5	-3.179055874	2	

Small	ENSOARG0002001002		0.03463	
Intestine	9	-1.080989972	4	
Small	ENSOARG0002002169		0.03516	
Intestine	9	-1.315128866	3	SEM1
Small	ENSOARG0002000932			
Intestine	6	-2.111968601	0.03578	
Small	ENSOARG0002001905		0.03594	
Intestine	2	-2.072866745	6	
Small	ENSOARG0002002723			
Intestine	4	-1.025246001	0.0366	ERICH4
Small	ENSOARG0002000484			
Intestine	3	-2.184825868	0.03669	
Small	ENSOARG0002000303			
Intestine	1	-1.677810133	0.03746	
Small	ENSOARG0002001183		0.03761	
Intestine	1	-1.278796022	7	PKLR
Small	ENSOARG0002000378		0.03780	
Intestine	0	-1.253495355	8	
Small	ENSOARG0002001687		0.03841	
Intestine	0	-1.18835176	9	ZFR2
Small	ENSOARG0002001993		0.03896	
Intestine	6	-1.197301307	1	

Small	ENSOARG0002003216		0.03999	
Intestine	5	-1.032872839	1	
Small	ENSOARG0002002311		0.04002	
Intestine	2	-1.031924457	5	IZUMO4
Small	ENSOARG0002004079		0.04045	
Intestine	7	-1.305573407	3	B3GALNT1
Small	ENSOARG0002000201		0.04107	
Intestine	3	-1.206664894	7	COX17
Small	ENSOARG0002003425		0.04133	
Intestine	3	-1.28773307	3	
Small	ENSOARG0002000565		0.04188	
Intestine	9	-1.346600781	2	SNRPG
Small	ENSOARG0002003795		0.04200	
Intestine	1	-1.153270648	6	
Small	ENSOARG0002000966			
Intestine	6	-1.433945811	0.04241	
Small	ENSOARG0002001739		0.04280	
Intestine	6	-1.088947091	5	AK5
Small	ENSOARG0002000951		0.04385	
Intestine	2	-1.106414923	2	C16orf74
Small	ENSOARG0002001494		0.04402	
Intestine	6	-1.026811733	8	GPX4

Small	ENSOARG0002000722		0.04426	
Intestine	2	-1.29403591	3	SCN2A
Small	ENSOARG0002000392		0.04447	
Intestine	4	-1.227436239	3	ACACB
Small	ENSOARG0002002438		0.04501	
Intestine	2	-2.299212998	2	
Small	ENSOARG0002000567		0.04597	
Intestine	7	-1.003392684	1	RDH5
Small	ENSOARG0002004070		0.04629	
Intestine	0	-1.566856912	8	
Small	ENSOARG0002002229		0.04632	
Intestine	1	-2.433991992	6	
Small	ENSOARG0002001256		0.04672	
Intestine	6	-1.829212782	2	LCT
Small	ENSOARG0002002916		0.04672	
Intestine	2	-1.295358958	2	
Small	ENSOARG0002002927		0.04675	
Intestine	4	-1.188996698	3	ZBED2
Small	ENSOARG0002001260		0.04696	
Intestine	1	-1.610976046	5	
Small	ENSOARG0002000523		0.04706	
Intestine	3	-1.972305695	5	

Small	ENSOARG0002001353		0.04707	
Intestine	1	-2.670253396	1	
Small	ENSOARG0002003618		0.04772	
Intestine	9	-2.60714115	3	
Small	ENSOARG0002004009		0.04776	
Intestine	5	-1.980067294	8	
Small	ENSOARG0002000860		0.04782	
Intestine	7	-1.607802207	8	WDR38
Small	ENSOARG0002001210		0.04790	
Intestine	8	-1.301877718	7	
Small	ENSOARG0002002101		0.04840	
Intestine	5	-1.642952414	2	IYD
Small	ENSOARG0002003982		0.04882	
Intestine	9	-1.042195191	5	
Small	ENSOARG0002003144		0.04917	
Intestine	4	-1.275675837	9	
Small	ENSOARG0002001462		0.04986	
Intestine	1	-1.201583737	5	MRPS18C
Small	ENSOARG0002001511			
Intestine	2	2.996324559	4.38E-17	A4GNT
Small	ENSOARG0002000679			
Intestine	9	1.483297065	3.93E-15	PTPRN2

Small Intestine	ENSOARG0002001329 6	1.230130649	4.23E-14	COL3A1
Small Intestine	ENSOARG0002001514 8	1.29223198	1.13E-12	DZIP1L
Small Intestine	ENSOARG0002002574 7	1.380994885	1.27E-11	GNAZ
Small Intestine	ENSOARG0002000105 8	1.461811545	1.46E-11	UNC13B
Small Intestine	ENSOARG0002000114 8	1.068550582	3.13E-11	COL15A1
Small Intestine	ENSOARG0002002258 5	1.173387063	8.05E-11	ITGB1
Small Intestine	ENSOARG0002001540 6	1.242860749	1.24E-10	ARHGEF40
Small Intestine	ENSOARG0002000874 3	1.060984057	2.42E-10	SPTBN2
Small Intestine	ENSOARG0002001995 9	2.678740548	2.42E-10	ETV4
Small Intestine	ENSOARG0002002110 4	2.419280687	1.06E-09	ALPL
Small Intestine	ENSOARG0002000881 8	1.20446074	1.48E-09	AEBP1

Small	ENSOARG0002000854			
Intestine	0	1.432778799	3.23E-09	
Small	ENSOARG0002003880			
Intestine	7	1.25354219	4.89E-09	
Small	ENSOARG0002002568			
Intestine	4	1.474445781	7.49E-09	CA8
Small	ENSOARG0002000435			
Intestine	9	3.94942697	1.83E-08	THBS4
Small	ENSOARG0002001340			
Intestine	1	1.695767891	1.83E-08	ITGA8
Small	ENSOARG0002002274			
Intestine	3	1.004995499	1.83E-08	POMGNT2
Small	ENSOARG0002001691			
Intestine	9	1.216484905	2.88E-08	PDZRN3
Small	ENSOARG0002001257			
Intestine	6	1.394187369	4.73E-08	CORO2B
Small	ENSOARG0002000218			
Intestine	4	1.096587166	4.97E-08	MECOM
Small	ENSOARG0002001407			
Intestine	4	1.082242333	5.01E-08	BCAS1
Small	ENSOARG0002002212			
Intestine	8	2.047082537	6.33E-08	KCNJ3

Small	ENSOARG0002002448			
Intestine	2	1.535848577	7.80E-08	VLDLR
Small	ENSOARG0002001001			
Intestine	0	1.635759541	7.86E-08	COL12A1
Small	ENSOARG0002001578			
Intestine	2	1.701736183	1.33E-07	SORBS2
Small	ENSOARG0002001921			
Intestine	1	2.103975178	1.33E-07	HGFAC
Small	ENSOARG0002001968			
Intestine	3	1.865122495	2.26E-07	AVIL
Small	ENSOARG0002000096			
Intestine	7	5.841871201	3.14E-07	PROM2
Small	ENSOARG0002000672			
Intestine	7	1.571812124	3.79E-07	KRT23
Small	ENSOARG0002003502			
Intestine	5	1.461661974	3.79E-07	CAMK1D
Small	ENSOARG0002000887			
Intestine	1	2.056522976	4.09E-07	RGL3
Small	ENSOARG0002000114			
Intestine	3	1.015923465	4.23E-07	COL5A3
Small	ENSOARG0002000396			
Intestine	8	2.526171889	4.23E-07	CHRDL2

Small	ENSOARG0002002479			
Intestine	4	1.740918458	4.64E-07	SNAP25
Small	ENSOARG0002000814			
Intestine	0	1.137659158	5.10E-07	KITLG
Small	ENSOARG0002003976			
Intestine	5	1.119016909	6.36E-07	C1QTNF1
Small	ENSOARG0002002173			
Intestine	0	1.018026785	9.34E-07	CREB3L1
Small	ENSOARG0002000131			
Intestine	2	1.44894418	9.91E-07	ASPN
Small	ENSOARG0002000568			
Intestine	3	2.124501133	1.01E-06	TNC
Small	ENSOARG0002001653			
Intestine	6	1.432620899	1.18E-06	CBLIF
Small	ENSOARG0002002507			
Intestine	4	2.596237021	1.39E-06	LTF
Small	ENSOARG0002000197			
Intestine	0	1.109227504	1.42E-06	FBLN1
Small	ENSOARG0002002997			
Intestine	3	2.3123725	1.45E-06	
Small	ENSOARG0002000109			
Intestine	7	1.399794238	1.63E-06	SLC12A8

Small	ENSOARG0002000100			
Intestine	9	2.379385406	2.01E-06	OLFML2B
Small	ENSOARG0002000286			
Intestine	9	1.331403509	2.01E-06	BMAL2
Small	ENSOARG0002003554			
Intestine	0	1.313272171	2.23E-06	GPR63
Small	ENSOARG0002001500			
Intestine	4	2.767129822	2.37E-06	IL5RA
Small	ENSOARG0002000499			
Intestine	2	1.760667206	2.41E-06	POSTN
Small	ENSOARG0002000384			
Intestine	8	1.200405961	2.55E-06	ZSWIM5
Small	ENSOARG0002000016			
Intestine	7	3.315056269	2.82E-06	SEPTIN3
Small	ENSOARG0002002510			
Intestine	1	1.211634508	2.99E-06	ENTPD2
Small	ENSOARG0002001481			
Intestine	2	1.823153601	2.99E-06	FBXO32
Small	ENSOARG0002002077			
Intestine	5	1.636587187	3.02E-06	SYTL5
Small	ENSOARG0002000766			
Intestine	0	2.230204435	3.08E-06	ST3GAL4

Small	ENSOARG0002000548			
Intestine	4	1.093841082	3.17E-06	PLOD2
Small	ENSOARG0002002483			
Intestine	9	1.201131539	3.48E-06	FN1
Small	ENSOARG0002000862			
Intestine	7	1.224406917	4.16E-06	COL6A2
Small	ENSOARG0002002623			
Intestine	5	5.375678175	4.20E-06	
Small	ENSOARG0002001968			
Intestine	8	1.798523844	4.23E-06	FHOD3
Small	ENSOARG0002001044			
Intestine	0	1.17456783	4.85E-06	HK1
Small	ENSOARG0002000242			
Intestine	1	1.167720278	5.04E-06	COL6A3
Small	ENSOARG0002001130			
Intestine	7	2.370011642	5.51E-06	PITPNM3
Small	ENSOARG0002002527			
Intestine	6	2.022376963	5.51E-06	KANK4
Small	ENSOARG0002001829			
Intestine	1	1.549181333	5.55E-06	MYRIP
Small	ENSOARG0002000928			
Intestine	8	1.375797696	6.01E-06	KRT18

Small	ENSOARG0002000645			
Intestine	9	1.519955893	6.27E-06	LOX
Small	ENSOARG0002001453			
Intestine	9	1.125974997	7.65E-06	IRAG1
Small	ENSOARG0002002216			
Intestine	0	3.314495136	8.95E-06	SHH
Small	ENSOARG0002002086			
Intestine	0	1.606147103	9.83E-06	SORBS1
Small	ENSOARG0002000975			
Intestine	3	1.096969295	1.02E-05	
Small	ENSOARG0002000310			
Intestine	8	1.025055726	1.06E-05	ABCG1
Small	ENSOARG0002002424			
Intestine	9	1.955439171	1.06E-05	COL21A1
Small	ENSOARG0002002646			
Intestine	9	1.012554525	1.12E-05	GLIS2
Small	ENSOARG0002002383			
Intestine	6	1.368833119	1.20E-05	PLCB1
Small	ENSOARG0002001724			
Intestine	7	1.05321621	1.26E-05	ACSM3
Small	ENSOARG0002000669			
Intestine	4	1.125280144	1.28E-05	FAM174B

Small	ENSOARG0002001099			
Intestine	9	1.202174761	1.29E-05	RASSF10
Small	ENSOARG0002000868			
Intestine	3	1.203452483	1.44E-05	
Small	ENSOARG0002001054			
Intestine	8	1.404046359	1.59E-05	SLC1A5
Small	ENSOARG0002001550			
Intestine	9	3.630968135	1.72E-05	ST6GALNAC1
Small	ENSOARG0002000174			
Intestine	7	1.4879142	1.97E-05	TMOD1
Small	ENSOARG0002001797			
Intestine	5	1.480350944	2.05E-05	JPH2
Small	ENSOARG0002003789			
Intestine	7	2.60723476	2.58E-05	MMP3
Small	ENSOARG0002001548			
Intestine	0	1.297933349	2.61E-05	POU2AF3
Small	ENSOARG0002001421			
Intestine	0	1.103662586	2.80E-05	PALLD
Small	ENSOARG0002001548			
Intestine	7	2.319379686	2.83E-05	OCA2
Small	ENSOARG0002000388			
Intestine	8	1.067660849	3.22E-05	COL4A2

Small Intestine	ENSOARG0002000541 6	1.011236201	3.30E-05	ELAPOR1
Small Intestine	ENSOARG0002000287 9	1.18708178	3.42E-05	ITGB8
Small Intestine	ENSOARG0002000444 1	1.372375563	3.88E-05	SLC7A4
Small Intestine	ENSOARG0002001982 2	2.525719937	5.06E-05	CFAP100
Small Intestine	ENSOARG0002002204 0	1.187586991	5.25E-05	CFP
Small Intestine	ENSOARG0002000251 3	1.530815357	5.86E-05	AGPAT2
Small Intestine	ENSOARG0002002272 0	1.526860234	5.86E-05	SERPINF2
Small Intestine	ENSOARG0002001165 6	6.563630492	6.51E-05	DUOXA1
Small Intestine	ENSOARG0002001187 3	4.059615354	6.51E-05	DUOX2
Small Intestine	ENSOARG0002000880 7	2.466087394	6.83E-05	EPOR
Small Intestine	ENSOARG0002003303 8	1.39789138	7.84E-05	TENT5B

Small Intestine	ENSOARG0002001768 2	1.011611896	8.18E-05	PTK7
Small Intestine	ENSOARG0002000614 5	1.386974581	9.28E-05	RGMA
Small Intestine	ENSOARG0002001546 4	1.72171812	1.22E-04	GPX2
Small Intestine	ENSOARG0002001176 6	4.186408335	1.26E-04	DUOXA2
Small Intestine	ENSOARG0002001644 4	1.905511999	1.26E-04	PDIA2
Small Intestine	ENSOARG0002001078 4	1.967640817	1.27E-04	TMEM178A
Small Intestine	ENSOARG0002001723 5	6.455625381	1.35E-04	TNN
Small Intestine	ENSOARG0002001004 4	1.118134589	1.38E-04	ADGRG6
Small Intestine	ENSOARG0002001004 5	2.294125934	1.64E-04	KCNF1
Small Intestine	ENSOARG0002003609 2	1.754213765	1.71E-04	
Small Intestine	ENSOARG0002001506 4	1.503777334	1.74E-04	CNTN4

Small	ENSOARG0002000225			
Intestine	5	1.182698175	1.76E-04	SEMA3C
Small	ENSOARG0002002290			
Intestine	6	2.133699031	1.87E-04	RNF208
Small	ENSOARG0002002051			
Intestine	6	1.526984151	1.87E-04	
Small	ENSOARG0002001211			
Intestine	7	1.327846662	1.89E-04	RRAGD
Small	ENSOARG0002002179			
Intestine	5	1.387294333	1.90E-04	
Small	ENSOARG0002002414			
Intestine	6	1.201846613	1.90E-04	P3H2
Small	ENSOARG0002002639			
Intestine	0	2.1220794	1.90E-04	
Small	ENSOARG0002003317			
Intestine	3	1.353258445	1.90E-04	LRRC55
Small	ENSOARG0002001779			
Intestine	9	1.675617527	2.04E-04	SHANK1
Small	ENSOARG0002001121			
Intestine	3	1.172323133	2.29E-04	FAP
Small	ENSOARG0002000981			
Intestine	1	2.407905867	2.33E-04	

Small	ENSOARG0002001959			
Intestine	9	1.054952507	2.37E-04	SLC2A10
Small	ENSOARG0002001455			
Intestine	0	1.139042294	2.41E-04	FGF11
Small	ENSOARG0002000985			
Intestine	1	2.580389205	2.49E-04	
Small	ENSOARG0002003568			
Intestine	9	1.026079292	2.85E-04	
Small	ENSOARG0002001286			
Intestine	6	1.072062186	2.87E-04	FERMT2
Small	ENSOARG0002003442			
Intestine	0	1.092241984	3.05E-04	SERTAD4
Small	ENSOARG0002000298			
Intestine	8	1.961490034	3.09E-04	
Small	ENSOARG0002002079			
Intestine	5	1.705393797	3.47E-04	MMP28
Small	ENSOARG0002000044			
Intestine	5	1.478264072	3.61E-04	SLC2A1
Small	ENSOARG0002000086			
Intestine	8	1.003887622	3.84E-04	MET
Small	ENSOARG0002001243			
Intestine	2	1.070163208	4.05E-04	COL1A1

Small Intestine	ENSOARG0002001539 2	2.268335024	4.05E-04	POU2AF2
Small Intestine	ENSOARG0002001299 5	1.05051219	4.16E-04	DAAM2
Small Intestine	ENSOARG0002001296 7	1.649986903	4.41E-04	NOX5
Small Intestine	ENSOARG0002002543 9	1.03330612	4.58E-04	IGFBP5
Small Intestine	ENSOARG0002003103 3	1.421728351	4.59E-04	
Small Intestine	ENSOARG0002000339 2	4.098441708	4.69E-04	
Small Intestine	ENSOARG0002001879 1	4.51741958	4.71E-04	FGF21
Small Intestine	ENSOARG0002000563 7	1.323970068	4.76E-04	EHD3
Small Intestine	ENSOARG0002001711 8	1.25808657	4.85E-04	GATA2
Small Intestine	ENSOARG0002003612 5	1.074909059	4.86E-04	
Small Intestine	ENSOARG0002002940 7	2.010725519	4.96E-04	

Small	ENSOARG0002002445			
Intestine	4	1.969751299	5.04E-04	PDZD7
Small	ENSOARG0002000400			
Intestine	8	1.956896343	5.06E-04	ADGRG3
Small	ENSOARG0002000162			
Intestine	0	1.660889509	5.09E-04	FAM131B
Small	ENSOARG0002003851			
Intestine	1	1.655271757	5.09E-04	
Small	ENSOARG0002000447			
Intestine	1	2.496533743	5.73E-04	
Small	ENSOARG0002001322			
Intestine	0	1.022532324	6.03E-04	PALD1
Small	ENSOARG0002000636			
Intestine	9	1.571330406	6.22E-04	TRPM5
Small	ENSOARG0002002039			
Intestine	9	1.459998089	6.48E-04	SH3RF2
Small	ENSOARG0002000367			
Intestine	2	4.127322296	6.57E-04	MROH5
Small	ENSOARG0002001556			
Intestine	8	1.658308594	7.20E-04	
Small	ENSOARG0002000139			
Intestine	4	1.26864172	7.22E-04	PHGDH

Small	ENSOARG0002001617			
Intestine	5	5.854851648	8.08E-04	LGALS15
Small	ENSOARG0002002027			
Intestine	8	1.544888934	8.29E-04	
Small	ENSOARG0002000176			
Intestine	5	1.514926412	8.40E-04	PIK3C2G
Small	ENSOARG0002000191			
Intestine	1	1.575366445	8.46E-04	LRRC31
Small	ENSOARG0002002001			
Intestine	9	1.050941288	8.75E-04	
Small	ENSOARG0002001528			
Intestine	7	1.061335924	9.27E-04	KIT
Small	ENSOARG0002001883			
Intestine	6	1.106179059	9.27E-04	ADAMTS12
Small	ENSOARG0002000687			
Intestine	6	1.125237942	9.32E-04	ANTXR1
Small	ENSOARG0002001965			
Intestine	2	4.436294104	9.74E-04	TPD52L1
Small	ENSOARG0002003322			
Intestine	9	1.12463999	9.81E-04	ALPK3
Small	ENSOARG0002003652		0.00103	
Intestine	7	1.949448372	1	RASL10B

Small	ENSOARG0002000463		0.00105	
Intestine	4	1.017254952	5	GEM
Small	ENSOARG0002001244		0.00105	
Intestine	8	1.299031681	9	C4BPA
Small	ENSOARG0002001577		0.00105	
Intestine	4	1.794615656	9	RAB15
Small	ENSOARG0002000461		0.00117	
Intestine	4	1.851230644	5	SPTA1
Small	ENSOARG0002002247		0.00119	
Intestine	8	1.09402466	1	DTNA
Small	ENSOARG0002002478		0.00119	
Intestine	1	1.253214201	9	SLIT2
Small	ENSOARG0002001153		0.00125	
Intestine	7	1.243529083	9	SLC7A5
Small	ENSOARG0002004002		0.00131	
Intestine	9	1.586866167	6	FOXO6
Small	ENSOARG0002001921		0.00135	
Intestine	4	1.021186968	1	CDO1
Small	ENSOARG0002001450			
Intestine	4	1.305276817	0.00136	PPARGC1A
Small	ENSOARG0002002424		0.00143	
Intestine	5	1.438109478	8	

Small	ENSOARG0002002918		0.00152	
Intestine	7	1.646900272	6	
Small	ENSOARG0002000235		0.00153	
Intestine	6	1.773534532	2	PSAT1
Small	ENSOARG0002002223		0.00158	
Intestine	2	2.037180005	5	GALNT13
Small	ENSOARG0002001882		0.00173	
Intestine	7	1.036814952	4	KIF12
Small	ENSOARG0002000179		0.00175	
Intestine	6	1.009909825	7	MAP3K20
Small	ENSOARG0002001858		0.00180	
Intestine	3	1.172128425	6	NEXN
Small	ENSOARG0002001600		0.00186	
Intestine	7	2.211744957	3	PPM1E
Small	ENSOARG0002001800		0.00189	
Intestine	4	1.57735663	5	Hoxc8
Small	ENSOARG0002002158		0.00199	
Intestine	4	4.746804064	7	STMND1
Small	ENSOARG0002001124		0.00203	
Intestine	4	4.153748969	3	GREB1
Small	ENSOARG0002001676			
Intestine	3	1.49566742	0.00204	

Small	ENSOARG0002001394		0.00207	
Intestine	9	1.048539462	5	B4GALNT3
Small	ENSOARG0002001410		0.00207	
Intestine	5	2.150237723	5	TLL2
Small	ENSOARG0002001756		0.00211	
Intestine	5	1.200012864	1	ME1
Small	ENSOARG0002001566		0.00216	
Intestine	3	1.282007651	5	P2RX1
Small	ENSOARG0002002551		0.00221	
Intestine	6	1.196179629	9	NANOS1
Small	ENSOARG0002002290			
Intestine	2	1.477982084	0.00242	TMEM151A
Small	ENSOARG0002000077		0.00257	
Intestine	8	1.417613642	5	COL8A1
Small	ENSOARG0002001266		0.00258	
Intestine	7	2.368324852	7	ARSI
Small	ENSOARG0002001038		0.00268	
Intestine	9	5.985340608	7	LGALS15
Small	ENSOARG0002001107		0.00284	
Intestine	1	3.340527075	7	
Small	ENSOARG0002003869		0.00296	
Intestine	9	2.312145244	8	

Small	ENSOARG0002002350		0.00301	
Intestine	8	1.848841266	5	CEMIP
Small	ENSOARG0002001745		0.00316	
Intestine	4	1.172524523	1	KCNS3
Small	ENSOARG0002001117		0.00327	
Intestine	0	3.882249085	9	
Small	ENSOARG0002000031		0.00328	
Intestine	8	1.263134291	6	ASNS
Small	ENSOARG0002000857		0.00328	
Intestine	4	1.6419376	6	ADM2
Small	ENSOARG0002002163		0.00345	
Intestine	1	1.223191521	3	FLNA
Small	ENSOARG0002002462		0.00345	
Intestine	3	3.304959627	7	SCNN1G
Small	ENSOARG0002001918		0.00350	
Intestine	7	1.859703734	1	KLK1
Small	ENSOARG0002002559		0.00351	
Intestine	0	4.308524466	2	RUFY4
Small	ENSOARG0002000463		0.00356	
Intestine	8	2.407014491	1	GDF15
Small	ENSOARG0002001415		0.00367	
Intestine	0	1.583537754	9	ALDH1L2

Small	ENSOARG0002000312		0.00373	
Intestine	4	2.491893233	4	BHMT
Small	ENSOARG0002000269			
Intestine	3	1.093124286	0.00407	FAM3B
Small	ENSOARG0002000538			
Intestine	5	1.013540831	0.00408	CBS
Small	ENSOARG0002002086		0.00419	
Intestine	4	1.306443872	5	ADGRL3
Small	ENSOARG0002002895		0.00420	
Intestine	7	1.16615662	1	RASD2
Small	ENSOARG0002000872		0.00421	
Intestine	4	1.257969078	1	MAP1A
Small	ENSOARG0002003722		0.00471	
Intestine	1	1.711588609	1	
Small	ENSOARG0002000326		0.00473	
Intestine	4	2.659954476	4	TFF2
Small	ENSOARG0002001567		0.00480	
Intestine	4	1.382526516	3	TMEM38A
Small	ENSOARG0002003077		0.00480	
Intestine	3	1.023213361	6	
Small	ENSOARG0002000579		0.00480	
Intestine	3	1.427723648	7	FLNC

Small	ENSOARG0002000584		0.00483	
Intestine	6	1.957615287	5	PRDM6
Small	ENSOARG0002000013		0.00484	
Intestine	1	1.057484773	2	LZTS1
Small	ENSOARG0002000043		0.00508	
Intestine	1	1.841033771	9	PDZRN4
Small	ENSOARG0002000295		0.00508	
Intestine	1	1.317931463	9	SH2D6
Small	ENSOARG0002001259		0.00512	
Intestine	9	1.425179907	8	ATP1A2
Small	ENSOARG0002002378		0.00516	
Intestine	2	1.441192655	3	RBPMS2
Small	ENSOARG0002000942		0.00531	
Intestine	0	1.087189982	6	SLC29A4
Small	ENSOARG0002000226		0.00540	
Intestine	7	1.270003518	6	GPR156
Small	ENSOARG0002002676		0.00547	
Intestine	8	1.306784584	4	HSPB7
Small	ENSOARG0002001466		0.00548	
Intestine	6	1.195874541	4	ACHE
Small	ENSOARG0002001409		0.00548	
Intestine	8	1.605102276	8	PLIN5

Small	ENSOARG0002001585		0.00548	
Intestine	0	4.825069742	8	GABRP
Small	ENSOARG0002001812			
Intestine	5	1.081186399	0.00568	RAB38
Small	ENSOARG0002002026			
Intestine	8	1.323264385	0.00568	HSPB6
Small	ENSOARG0002000382			
Intestine	5	1.408708516	0.00574	FMOD
Small	ENSOARG0002002118		0.00574	
Intestine	0	1.44681094	1	PRKAA2
Small	ENSOARG0002002462		0.00590	
Intestine	8	1.261775563	7	LRCH2
Small	ENSOARG0002002483		0.00591	
Intestine	7	3.633939592	2	DUSP9
Small	ENSOARG0002000903		0.00598	
Intestine	3	1.083376368	2	NDRG4
Small	ENSOARG0002000878		0.00599	
Intestine	0	1.79959708	8	SLC26A9
Small	ENSOARG0002003783		0.00599	
Intestine	8	1.462922482	8	C4BPB
Small	ENSOARG0002001830		0.00607	
Intestine	3	1.32788998	9	EPOP

Small	ENSOARG0002001773		0.00612	
Intestine	6	1.588622152	4	PRSS35
Small	ENSOARG0002001366			
Intestine	5	1.063702596	0.00644	EPM2A
Small	ENSOARG0002003112		0.00648	
Intestine	8	2.343345318	5	DIO2
Small	ENSOARG0002002757		0.00674	
Intestine	4	3.409853644	2	
Small	ENSOARG0002002539		0.00674	
Intestine	2	1.092021247	2	PYCR1
Small	ENSOARG0002003676		0.00677	
Intestine	0	1.137795753	6	
Small	ENSOARG0002002624		0.00712	
Intestine	9	1.123559602	3	PERCC1
Small	ENSOARG0002000538		0.00721	
Intestine	6	1.26609908	2	CILP
Small	ENSOARG0002002949		0.00734	
Intestine	3	1.216596046	1	CCDC9B
Small	ENSOARG0002001026		0.00734	
Intestine	4	3.335078499	1	IGSF9B
Small	ENSOARG0002001194		0.00745	
Intestine	8	1.083680368	5	DMPK

Small	ENSOARG0002002217		0.00756	
Intestine	7	1.044356767	9	PGM5
Small	ENSOARG0002001150		0.00758	
Intestine	4	1.236365499	3	SPOCK1
Small	ENSOARG0002002499		0.00762	
Intestine	5	1.52059704	4	ANK1
Small	ENSOARG0002001654		0.00802	
Intestine	1	1.047341794	1	MYOCD
Small	ENSOARG0002003284		0.00830	
Intestine	5	2.294991081	6	
Small	ENSOARG0002002192		0.00851	
Intestine	0	3.98192575	3	TMPRSS9
Small	ENSOARG0002000587		0.00865	
Intestine	0	1.371397612	5	RNF180
Small	ENSOARG0002000560		0.00867	
Intestine	4	1.139410222	3	AKAP6
Small	ENSOARG0002000513		0.00889	
Intestine	2	1.911246414	7	CEACAM16
Small	ENSOARG0002000849		0.00905	
Intestine	2	1.787248666	6	TRIM46
Small	ENSOARG0002000181		0.00908	
Intestine	7	1.200873681	6	

Small	ENSOARG0002001188		0.00908	
Intestine	2	1.689384487	6	SYNPO2
Small	ENSOARG0002002173		0.00929	
Intestine	9	1.209125533	2	LMOD1
Small	ENSOARG0002002409		0.00948	
Intestine	1	1.417804834	8	SPP1
Small	ENSOARG0002001650		0.00974	
Intestine	0	3.249233617	8	
Small	ENSOARG0002001699		0.00979	
Intestine	2	1.083534426	5	CACNA1C
Small	ENSOARG0002003294		0.00991	
Intestine	2	1.04105077	7	KCNMB1
Small	ENSOARG0002001715		0.01027	
Intestine	2	1.411753239	7	MS4A2
Small	ENSOARG0002001744		0.01032	
Intestine	8	1.081079781	7	SLC2A4
Small	ENSOARG0002000951			
Intestine	3	2.606690246	0.01056	APOH
Small	ENSOARG0002001340			
Intestine	4	3.424474594	0.0107	CDH8
Small	ENSOARG0002001375			
Intestine	0	1.302439779	0.01116	RASL11B

Small	ENSOARG0002000595		0.01148	
Intestine	6	1.170765032	9	FNDC1
Small	ENSOARG0002001959		0.01164	
Intestine	1	1.150467105	4	
Small	ENSOARG0002000944		0.01186	
Intestine	0	1.687332931	3	
Small	ENSOARG0002002020		0.01257	
Intestine	6	1.239953176	4	BMPR1B
Small	ENSOARG0002001447		0.01260	
Intestine	7	1.12207233	9	NRCAM
Small	ENSOARG0002003585		0.01269	
Intestine	0	2.916677442	9	
Small	ENSOARG0002000771		0.01313	
Intestine	1	3.754278513	6	
Small	ENSOARG0002000901		0.01313	
Intestine	1	1.111949166	6	TPM1
Small	ENSOARG0002000047		0.01360	
Intestine	3	1.027780294	3	F3
Small	ENSOARG0002002269		0.01360	
Intestine	2	1.004935284	3	GASK1A
Small	ENSOARG0002002284		0.01360	
Intestine	9	1.594000789	3	MYH11

Small	ENSOARG0002001728		0.01375	
Intestine	1	1.048466331	4	EPHB1
Small	ENSOARG0002002501		0.01380	
Intestine	3	2.292008586	8	PNLIPRP2
Small	ENSOARG0002003316		0.01415	
Intestine	6	1.026301011	8	
Small	ENSOARG0002000897		0.01454	
Intestine	1	1.211629408	4	
Small	ENSOARG0002002268		0.01505	
Intestine	8	1.714845862	5	SERPINF1
Small	ENSOARG0002000356		0.01552	
Intestine	5	1.520659371	2	NTRK2
Small	ENSOARG0002000523		0.01568	
Intestine	6	1.288796745	5	HDC
Small	ENSOARG0002000369		0.01582	
Intestine	8	1.212356567	6	APOLD1
Small	ENSOARG0002000675		0.01595	
Intestine	8	1.98930216	4	ST8SIA2
Small	ENSOARG0002000699		0.01599	
Intestine	9	1.761252439	4	GRID2IP
Small	ENSOARG0002002443		0.01602	
Intestine	9	1.409674644	1	MDGA1

Small	ENSOARG0002000354		0.01613	
Intestine	1	1.054078562	4	TUBB3
Small	ENSOARG0002002574		0.01619	
Intestine	3	1.807562041	1	CLVS1
Small	ENSOARG0002001533		0.01632	
Intestine	3	1.29712431	7	CASQ1
Small	ENSOARG0002002313		0.01644	
Intestine	5	3.682924228	3	TMEM210
Small	ENSOARG0002001535		0.01645	
Intestine	8	2.565237737	7	ZFHX4
Small	ENSOARG0002002254		0.01651	
Intestine	1	1.181610242	7	RTN4RL1
Small	ENSOARG0002002548		0.01700	
Intestine	6	1.116537857	3	TNS1
Small	ENSOARG0002002512		0.01764	
Intestine	5	1.325689556	4	ASB5
Small	ENSOARG0002002049		0.01795	
Intestine	1	1.355624692	5	CTHRC1
Small	ENSOARG0002001710		0.01850	
Intestine	0	1.351082907	9	FRZB
Small	ENSOARG0002002480		0.01897	
Intestine	9	1.609286022	3	KIF5C

Small	ENSOARG0002002280		0.01900	
Intestine	2	1.866379386	9	HMX3
Small	ENSOARG0002002059		0.01902	
Intestine	9	1.130266505	2	PPP1R12B
Small	ENSOARG0002002746		0.01909	
Intestine	0	1.114144377	6	
Small	ENSOARG0002000547		0.01915	
Intestine	5	1.178024274	8	COLGALT2
Small	ENSOARG0002001450		0.01930	
Intestine	1	1.004539508	4	IL9R
Small	ENSOARG0002001747		0.01930	
Intestine	9	3.194497191	4	
Small	ENSOARG0002000760		0.01937	
Intestine	0	2.824244631	7	GABRA2
Small	ENSOARG0002002040		0.01971	
Intestine	8	1.896103776	8	NRIP3
Small	ENSOARG0002001901		0.01998	
Intestine	2	1.022869302	7	BNC2
Small	ENSOARG0002000370			
Intestine	6	1.026418605	0.02027	CDKL2
Small	ENSOARG0002000018			
Intestine	2	2.428510484	0.02046	IL13

Small	ENSOARG0002001574		0.02114	
Intestine	1	1.852101108	8	SFRP4
Small	ENSOARG0002002056			
Intestine	1	3.482142562	0.02148	SYT2
Small	ENSOARG0002002419		0.02200	
Intestine	9	1.700694387	4	STRA6
Small	ENSOARG0002001082		0.02223	
Intestine	4	1.821045989	3	CTXN1
Small	ENSOARG0002000784		0.02258	
Intestine	4	2.06018556	1	TMEFF2
Small	ENSOARG0002001479		0.02258	
Intestine	2	3.277425848	1	PTPRT
Small	ENSOARG0002000057		0.02298	
Intestine	4	1.05086248	6	LPL
Small	ENSOARG0002001803		0.02301	
Intestine	6	1.286744478	7	HOXC9
Small	ENSOARG0002000500			
Intestine	2	1.031922234	0.02334	CNMD
Small	ENSOARG0002001943		0.02352	
Intestine	2	1.170600546	3	RAB6B
Small	ENSOARG0002004084		0.02444	
Intestine	6	1.178531367	1	CHRM2

Small	ENSOARG0002002519		0.02451	
Intestine	5	4.575108818	8	ALKAL1
Small	ENSOARG0002001118		0.02493	
Intestine	3	1.180147909	5	NEUROG3
Small	ENSOARG0002001837		0.02529	
Intestine	5	1.295236077	1	SH2D7
Small	ENSOARG0002002886		0.02536	
Intestine	6	4.07756127	7	
Small	ENSOARG0002002148		0.02585	
Intestine	6	1.504172087	3	RBM24
Small	ENSOARG0002001107		0.02604	
Intestine	9	3.150332284	9	RTBDN
Small	ENSOARG0002000885		0.02608	
Intestine	8	3.914269828	6	
Small	ENSOARG0002000776		0.02658	
Intestine	8	1.481603573	4	RTN4R
Small	ENSOARG0002001635		0.02663	
Intestine	0	3.825530231	5	GP2
Small	ENSOARG0002000794		0.02740	
Intestine	9	1.145081715	3	
Small	ENSOARG0002003881		0.02754	
Intestine	2	1.01318957	1	

Small	ENSOARG0002000408		0.02764	
Intestine	8	1.211529966	5	MATCAP2
Small	ENSOARG0002001187			
Intestine	6	1.20014544	0.02874	ANKRD6
Small	ENSOARG0002002347		0.02908	
Intestine	8	1.055893452	7	CFI
Small	ENSOARG0002002214		0.02914	
Intestine	6	2.116908816	8	TMEM252
Small	ENSOARG0002000376		0.02922	
Intestine	3	1.653088725	6	P4HA3
Small	ENSOARG0002001526		0.02990	
Intestine	0	1.674799124	5	PCDHB4
Small	ENSOARG0002001968		0.02994	
Intestine	9	1.394257085	3	EYA2
Small	ENSOARG0002000421		0.03039	
Intestine	1	2.712377182	2	CRLF1
Small	ENSOARG0002002554		0.03039	
Intestine	6	1.944568298	2	ATP2B2
Small	ENSOARG0002002486		0.03139	
Intestine	1	1.804170043	7	ATP2B3
Small	ENSOARG0002000052		0.03140	
Intestine	3	1.414892771	5	COL28A1

Small	ENSOARG0002000894		0.03178	
Intestine	6	2.939111769	7	ADCY8
Small	ENSOARG0002001119		0.03185	
Intestine	1	2.565969931	6	
Small	ENSOARG0002001995		0.03235	
Intestine	0	1.073456378	6	CTNND2
Small	ENSOARG0002002432		0.03239	
Intestine	0	1.335424104	8	ALOX15
Small	ENSOARG0002001103		0.03243	
Intestine	8	2.154746625	1	TACR2
Small	ENSOARG0002002976		0.03253	
Intestine	9	2.088530357	6	MMP13
Small	ENSOARG0002001834		0.03289	
Intestine	9	1.602862638	1	SRCIN1
Small	ENSOARG0002000838		0.03330	
Intestine	9	1.029208742	2	KCNMA1
Small	ENSOARG0002002278		0.03343	
Intestine	8	2.021737629	7	CNNM1
Small	ENSOARG0002001623		0.03350	
Intestine	3	1.03356975	4	CTTNBP2
Small	ENSOARG0002000548		0.03383	
Intestine	5	1.778139304	3	DHRS9

Small	ENSOARG0002003098		0.03492	
Intestine	4	1.907966998	6	PPP1R14C
Small	ENSOARG0002002055		0.03516	
Intestine	5	2.288618784	3	PLA2G3
Small	ENSOARG0002002155		0.03516	
Intestine	7	2.062201856	3	
Small	ENSOARG0002000199			
Intestine	0	1.112306141	0.03578	MEOX2
Small	ENSOARG0002001417		0.03591	
Intestine	3	1.893228355	2	
Small	ENSOARG0002000145		0.03667	
Intestine	6	2.195791817	7	
Small	ENSOARG0002001613			
Intestine	9	3.908866187	0.03791	FAM131C
Small	ENSOARG0002000959		0.03893	
Intestine	5	1.156476762	9	
Small	ENSOARG0002001489			
Intestine	2	2.236685012	0.03902	
Small	ENSOARG0002000888		0.03917	
Intestine	4	2.591341545	9	ALX3
Small	ENSOARG0002003519		0.03941	
Intestine	3	3.424305446	6	CCDC180

Small	ENSOARG0002002574		0.04025	
Intestine	4	1.314889798	3	RSPH14
Small	ENSOARG0002000056		0.04045	
Intestine	6	3.17071812	3	HRG
Small	ENSOARG0002001794		0.04112	
Intestine	3	2.040067162	9	TMEM91
Small	ENSOARG0002002404		0.04112	
Intestine	3	1.488483667	9	TRMT9B
Small	ENSOARG0002002561		0.04193	
Intestine	6	1.082572229	3	LAMA3
Small	ENSOARG0002002074		0.04208	
Intestine	3	1.224952163	4	HPGDS
Small	ENSOARG0002000323		0.04220	
Intestine	1	1.183251255	9	CCDC85A
Small	ENSOARG0002002282		0.04305	
Intestine	8	1.928057242	9	HMX2
Small	ENSOARG0002000176		0.04418	
Intestine	7	1.133340614	2	FRRS1L
Small	ENSOARG0002002246		0.04422	
Intestine	8	1.386057742	7	DKK1
Small	ENSOARG0002001161		0.04430	
Intestine	3	1.227164082	6	

Small	ENSOARG0002000351		0.04577	
Intestine	7	1.774070942	5	SLITRK5
Small	ENSOARG0002002453		0.04696	
Intestine	7	3.564995045	5	
Small	ENSOARG0002000371		0.04739	
Intestine	2	1.267000275	2	TRIB3
Small	ENSOARG0002003269		0.04903	
Intestine	6	1.353486187	5	
Small	ENSOARG0002000448		0.04981	
Intestine	6	1.197813562	5	KLHL23