QUANTIFICATION OF HOST–PARASITE INTERACTIONS: SHEEP AND THEIR NEMATODES

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Abstract

The objective of this dissertation is to use modelling and statistical approaches to expand our knowledge of the immune responses against gastrointestinal nematode infections, to assess the impact of nematode infection, and to use our improved knowledge to examine novel means of selective breeding in farm animals (sheep) as a control strategy.

To expand the knowledge of the host immune response against infection, Chapter 2 of this dissertation focuses on immunoglobulin A (IgA), an antibody that binds nematode molecules, and its transfer through the body from the abomasal mucus (i.e. site of infection, where it is produced) to the blood plasma, where it is typically measured. These findings have been published in Parasitology [1].

The implications of low levels of infection in adult milking ewes, which are more resistant than lambs, were also studied. A relationship is generated between infection levels using parasitological data and production data. There were however limitations in the dataset, which are discussed at the end of Chapter 3.

Parasite resistance in adults sheep at low levels of infection was also studied, especially since the most common parasitological marker of disease is the faecal egg count (i.e. number of nematode eggs in the animals faeces) which is subject to substantial measurement error, among other limitations. Chapter 4 analyses a dataset of adult animals with low infection levels using a zero inflated binomial model (ZINB) and extends the model by including other evidence of parasite resistance to discriminate between exposed and unexposed animals.

To examine selective breeding, an individual-based data-driven immunogenetically explicit mathematical model was developed. One application of this model is to compare the efficacy of two selective breeding schemes, each based on a different marker for disease, namely faecal egg counts and plasma IgA. This work has been published in Journal of the Royal Society Interface [2].

The model can be extended to create a distribution for the variation in larval intake that best fits the field data. This allows the partitioning of the variation in adult worm burden into different components. The purpose is to quantify the contribution of the immune response and larval intake to determine which of the two accounts for more of the variation in the level of infection.

The model can be also extended to explore selection schemes in the two components of the immune response (i.e. namely the IgA mediated and IgE mediated immune response) and estimate animal size at the end of the grazing season.

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For Aleksandra

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Chapter 1

General introduction

1.1 Background

Gastrointestinal nematodes have been infecting human and animals since ancient times. Nematodes influence the size of wild ungulate populations [3, 4]. The World Health Organisation has estimated the number of humans infected with gastrointestinal nematodes to be a quarter of the world's population, mainly in the less developed countries in Africa, South America and Asia [5, 6]. In modern farms, gastrointestinal nematode infections result in a loss of productivity [7]. Different nematodes cause different pathologies; in cold, temperate climates, as is found in Scotland, the predominant nematode in sheep is *Teladorsagia circumcincta*, which causes a protein deficiency in the host [8], compromising animal growth and productivity and in some extreme cases even kill the host. The work in the thesis, unless stated otherwise, focuses on the parasite *T. circumcincta* in sheep.

1.1.1 *T. circumcincta* nematode life-cycle

T. circumcincta, of the family *Trichostrongyloidea*, has its predilection site in the ovine abomasum (the sheep's fourth stomach) where the adults mate and the females lay the eggs that will be carried away in the host's faeces. These eggs can survive the winter and will eventually hatch and emerge as first stage larvae (L1) that will develop inside the faeces to infective third stage larvae (L3). The L3 then migrate or are flushed out by rain from the faecal pat into the pasture, and in this way increase their chance of consumption by a potential host. The rate of growth and migration depends mainly on temperature and moisture [9]. Only L3 larvae are infective and they cannot develop further outside a host. Their survival is also limited by hot, dry summers [10].

Once ingested by a suitable host, the L3 ex-sheath from their cuticle in the rumen (first stomach) before arriving to the abomasum (fourth stomach), where they have their tissue

niche. As they invade the host tissue in the gastrointestinal tract, *T. circumcincta* L3 moult into L4 and occupy and distend the abomasal gastric pits. Further development occurs here before they become adults. After approximately 12-60 days, depending upon the immune status of the host, the ingested L3 larvae that successfully established will emerge from the gastric pit as young adults (referred by some authors as L5).

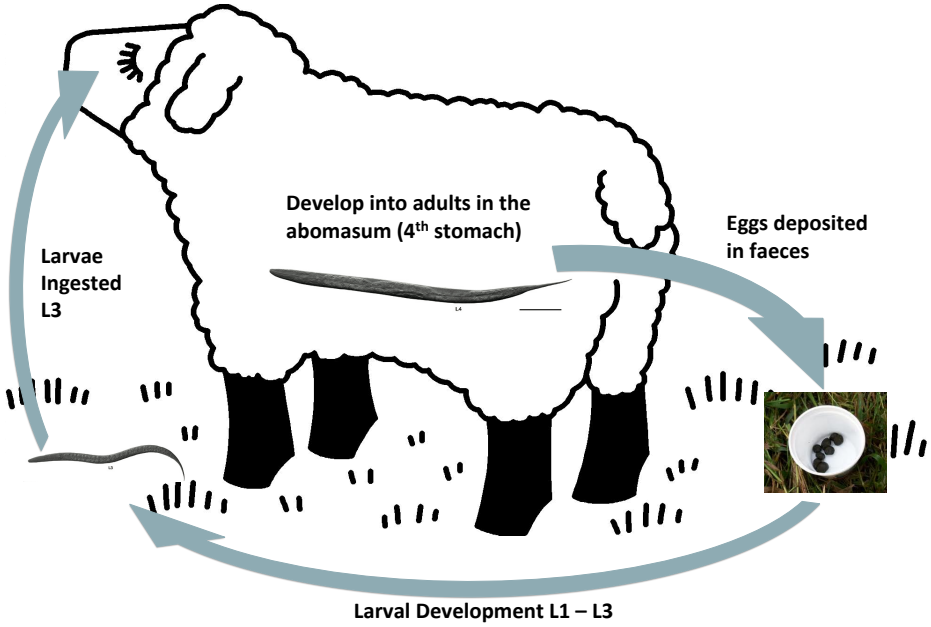

Figure 1.1: Gastrointestinal nematode *T. circumcincta* life cycle. Adults live in the abomasum and their eggs are carried in the host faeces. Larvae are not infective until they reach the L3 stage and cannot develop further outside the host. Ingested L3 that become established can continue their growth to the L4 and subsequently the adult stage.

The traditional control strategy relies on the use of anthelmintic drugs, which, when no resistance is present, kill essentially all parasites inside the host (from L3 stage to adults). However, the recent rise of nematode resistance to the three most common anthelmintic compounds compromises the sustainability of this approach [11, 12]. Alternative methods to control nematodes are therefore urgently needed.

1.2 Current control methods

Current treatments involve dosing sheep with anthelmintics during the season in order to lower the infection intensity and to prevent the fields becoming heavily contaminated with infectious larvae. The intensity and frequency of the treatment vary from countries, and there is no standard in the UK. The rapid development of drug resistance by the parasites and the persistence of the chemical residues in animal products are making the use of chemical drenches only, unsustainable [13]. This anthelmintic resistance is a bigger problem in sheep

because the development is faster than for nematodes affecting other species (e.g. cattle). This problem is accentuated in tropical and sub-tropical areas [13]. Resistant isolates of *T. circumcincta* have been reported worldwide. This is the most important nematode species in sheep in the British Isles, where three classes of anthelmintic were licensed by 2004 (now five classes) for the treatment of nematode infections. Emergence of so called "triple resistant" strains, resistant to all three major anthelmintic classes in common use, poses a major threat to the intensive sheep farming industry [14].

There are currently five potentially effective alternatives to anthelmintic treatment that are starting to be implemented. These are grazing management, biological control, nutritional supplementation, vaccination and genetic approaches, e.g. selective breeding [15]. I will briefly discuss the first four and focus afterwards on selective breeding, which is the alternative examined using the mathematical model in Chapter 5.

The last three control methods revolve around improving the immune response of the host. For this reason, a better understanding of how the immune response interacts with the parasite is crucial. In this thesis, two chapters mainly explore aspects of the immune response, namely IgA transfer from mucus to plama (Chapter 2) and the mathematical model (Chapter 5) in which the main components of the immune response are captured in a simple yet explicit way. The key alternatives to anthelmintics are summarized below, and have been reviewed in detail in the literature [15].

1.3 Alternative control methods

1.3.1 Grazing management

There are multiple strategies revolving around grazing management that could be used to better control the infection. These strategies aim to reduce the number of infective larvae in the field.

One strategy is to reduce the number of animals grazing (i.e. reduce stocking density), since this means fewer hosts shedding nematode eggs. Also, this means that the animals are more likely to succeed in avoiding the heavy infected faecal pats [16]. This however is unsustainable in the long term, since having few animals in large fields limits the profitability for the farmer [15].

Another strategy to reduce pasture contamination is to change the use of a particular field. To an extent, this was happening historically; since most farms had a mixture of different livestock and crops, the field usage would change each season. This takes advantage of the inability of most nematodes to infect multiple hosts, although care must be taken to prevent the rise of multi-host nematodes like *Trichostrongylus axei* that can infect horses, sheep and

cattle [8]. However, the current trend for farms is to be single enterprises, thus allowing the larval numbers to increase when the same field is grazed each season by the same animals.

Adult animals also have lower egg counts, hence a rotation between young and old animals could be considered. However, older animals also produce more faeces, which could outweigh the epidemiological benefit of reduced faecal egg counts [16].

An alternative strategy is to move susceptible animals to a less infected field. However, to avoid the build up of larvae in that field, the animals would have to be treated beforehand which means that any drug resistant larvae that survive the treatment would be more prevalent in the "clean" field [17].

Another option is to divide the pasture into sections and rotate the animals between them leaving the other areas to "rest"; although plausible, it is not very effective [18]. Success with this strategy was reported by Healey *et al*. [19] in protecting against *H. contortus* with minimal use of anthelmintics. Nevertheless, to achieve this very short grazing periods and very long resting periods were used, which is due to the long life expectancy of the infective larval stage in the field [20].

1.3.2 Biological control

Nematodes are a large phylogenetic group with many organisms that prey on them [21]. These predators could be considered for biological control to reduce the number of larvae in the field. Trials with the fungus *Duddingtonia flagrans* have shown a reduction in larval prevalence in the field and lower intensity of infection [22]. For optimal results, the diet has to be supplemented with fungal spores on a daily basis, which is the main drawback of this control method [23].

Alternatively, if the rate of faecal breakdown and burial was altered, it could lead to the exposure of the young free-living larval stages to the weather [24] if the rate was increased or, on the other hand, prevent the movement of the infective larvae [25] if this rate was decreased. Faecal degradation depends on the type of forage, the distribution of faeces in the field and the presence of grazing sheep [24].

Sheep grazing in fields with chicory (*Cichorium intybus*) and birdsfoot trefoil (*Lotus corniculatus*) have lower egg deposition [23]. The possible reasons for this effect are the increased nutritional value of these plants in proteins or trace elements and the negative effect they have on the nematodes reducing their survival and development [26]. The results are quite inconsistent and more research is needed before this approach can be recommended [15].

1.3.3 Nutritional supplementation

One of the consequences of gastrointestinal nematode infection is a relative protein deficiency [27], which is caused by infected animals eating less and digesting proteins less efficiently; it is also exacerbated by protein loss due to breaches in the epithelial barrier and the increased demand for proteins during infection [8]. Feeding extra protein causes a reduction in the frequency of appearance and the intensity of the clinical symptoms [28]. Supplementary feeding has been reported as successful against *H. contortus* [29, 30], *T. colubriformis* [31, 32] and *N. battus* [33] infections. Resilience has been enhanced in animals infected with *T. circumcincta* nematodes by supplementing their diet with urea [34, 35], which suggests that inexpensive feed sources could be used instead of the more expensive protein supplements.

Peri-parturient ewes often show increased levels of disease, which is associated with a relaxation of their immune system. Given that the only two sources of infective larvae in the field at lambing are the overwintered larvae and the ewes' deposition, limiting maternal deposition in the field has an important role in managing infection [36]. This effect, more acute in ewes that produce twins, can be prevented by supplying additional feeding (mainly proteins) to the animals [37]. Supplementing the diet with some trace elements such as iron and zinc also proved beneficial [38].

Although nutritional supplementation has shown some efficacy, its use is still limited by the cost of the extra feeding and the labour required [15].

1.3.4 Vaccination

Research into vaccines against gastrointestinal nematodes has been extensive in the last decade, and three main approaches explored: attenuated vaccines [39], vaccines based on natural immunity [40] and the hidden antigen approach [41].

Irradiated larval vaccines do not generate immunity against gastrointestinal nematodes in lambs, although there are commercially available vaccines against bovine and ovine lungworm [39, 42]. Only older animals are protected against *H. contortus* when vaccinated, which indicates that older animals might mount different responses than younger sheep. These vaccines for older animals are experimental and not commercially available [39].

Research into vaccines based on natural immunity revolves around finding parasite molecules that can be recognized by the immune system during an infection. However, the variety of parasite molecules recognized during a gastrointestinal nematode infection is very large [43, 44, 45], and resistance appears to require the recognition of multiple parasite molecules [46]. Perhaps not surprisingly, results from the use of single natural antigens have not been

successful. The use of multivalent cocktails is more promising; Nisbet *et al*. [47] recently achieved a reduction to up to 75% of the worm burden against *T. circumcincta* infection in 6-7 month old sheep using a cocktail of recombinant proteins.

Another focus of vaccine development has been the search for hidden antigens; these are molecules expressed by the parasite that could trigger an immune response but typically do not because the host is not exposed to them, i.e. protein molecules inside the parasite. For instance, H11 and H-gal-P are proteins from the gastrointestinal tract of the parasite and have been successful in generating effective immunity in field trials [41]. Recombinant molecules could be the basis of less expensive and reliable vaccines [48], this however has been largely unsuccessful. The proposed explanation for the failure to achieve this is that it is often not known whether the native parasite antigens can induce protection and, if that is the case, which epitopes are important [49]. Some experimental vaccines have shown some levels of protection in laboratory conditions against *H. contortus* [50]; yet, few have been tested in the field [51]. However, these vaccines still need many years of development before they are available to farmers (Prof. M. Stear, pers. comm.).

1.3.5 Selective breeding

Breeding for resistance reduces the impact of the disease on animals [52] thereby increasing productivity, which directly benefits farmers. Another benefit to the farmer is that resistant animals also require less frequent treatment. In Australia and New Zealand, selective breeding programs for sheep resistant to gastrointestinal nematodes have been running for many years [52, 53, 54] with successful reduction in the incidence of disease in their flocks selected for resistance compared to the unselected flocks. Breeding for disease resistance is sustainable, feasible and desirable [55].

There were multiple concerns when breeding for disease resistance started. One concern was that the traits for resistance would not be under strong genetic control, thereby making selection ineffective. However, some diseases have similar heritabilities to production traits [56]. This means that because selection is successful in increasing production traits such as milk yield in dairy cows, achieving increases in resistance to disease is also feasible.

Another concern is that selection against parasite resistance could lead to an inability to respond to non-parasite antigens or a reduced productivity. However, Stankiewicz *et al*. [57] concluded there is no major effect on the antibody response against non-parasitic antigens between selected and non-selected sheep for parasite resistance.

On the other hand, there have been reports of selected animals being both less productive [58] and more productive [59] than unselected animals. Even in the case of a negative genetic correlation between traits, a selection index could be developed to maximise the improvement

of the traits. Selection indexes combine all the traits of interest (i.e. production and resistance to disease traits) by assigning a relative weight based on the importance each one is given.

Selective breeding for disease resistance is an important control option for parasitic nematode diseases in livestock due the lack of an effective and sustainable alternative control measure, i.e. lack of vaccines. This has led to selective breeding of sheep more resistant to nematodes, although mostly in Australia and New Zealand [52]. The selection is based on the faecal egg count (FEC) of the animals. The sheep with lower FEC are chosen, although the FEC measure is not an absolute indicator on its own, it is compensated by the pedigree of the animal. The justification for this is that the breeding value (i.e. additive genetic component) is the most important component for selective breeding, since it is the only component of the phenotype that is inherited. In practice, it means that animals with a low egg count that come from a "bad" family (progenitors with high FEC) would not be selected in favour of another with a higher FEC but with a better pedigree. It is important to bear in mind that selection schemes normally use selection indexes that also account for production traits e.g. milk yield or growth.

Quantitative genetic theory assumes that the distribution of the studied trait is normal (Gaussian), which is true for production traits such as growth or milk yield. However, traits related to parasitic nematode infections such as FEC or plasma IgA are not normally distributed and thus require different statistical procedures [56]. The challenge is to reconcile such non-normal distributions with quantitative genetic theory. In addition, the traditional animal breeding equations from genetic theory are not suitable for predicting the impact of selection against parasitic diseases because the improvement in resistance will also affect parasite's abundance. For this reason, there is need of mathematical models to better predict the effects of selective breeding for disease resistance.

1.4 Manifestations and mechanisms of resistance

Sheep continually exposed to *T. circumcincta* in natural conditions develop immunity to the parasite. The evidence comes from the observation that parasite gastroenteritis is a problem of lambs in their first grazing season, and is rarely seen in older animals. There are several manifestations of resistance, which can be broadly divided into regulating parasite numbers, either by reducing larval establishment or by expelling worms, and regulating their growth. The manifestations of resistance are commented below and describe the mechanisms of resistance involved.

1.4.1 Resistance to establishment of new populations

A reduction in the number of larvae establishing in the tissues and developing into adults is a major consequence of acquired immunity against nematode infections [60]. It takes multiple larval challenges to induce immunity; in the field, immunity will develop naturally during the first grazing season as the animals ingest infective larvae from the pasture. However, expulsion of infective larvae may occur over a period of hours to weeks, suggesting that different mechanisms of immunity may be operating [60].

In the first instance, mast cells, which are multi-functional cells of bone-marrow origin that reside and mature in the tissues, and basophils, which are cells with the same precursor as the mast cells that circulate as mature cells in the blood stream, can be quickly mobilised to inflamed tissue sites. They contain various biologically active potent mediators and can be released, when the cell degranulates, within minutes as a rapid response to infection. The activation of mast cells induced by pathogens occurs after direct contact with molecules derived from the pathogen or triggered by non-specific stimuli as part of the innate immune response [61].

At a later stage, discharged mast cells occur in the epithelial layer and are referred to as globule leukocytes. IgE is likely to be involved in this hypersensitivity reaction, and its levels are closely correlated with those of globule leucocytes, and in turn to FEC and worm burdens [62].

High levels of mast cells are present in sheep that are more effective at excluding nematode infection [63]. In addition, two negative correlations of mucosal mast cells and globule leukocytes counts have been demonstrated with FEC in peri-parturient ewes [64].

On the other hand, resistance develops against a particular species but it can offer protection against heterologous species present in the same tissue [65]. For example, hypersensitivity reactions, which expel larvae, are non-specific and will affect all the species that dwell in the same organ or further downstream. Selected sheep with high resistance to a particular nematode are also resistant to other species.

1.4.2 Expulsion of established adult worms

Expulsion of adult nematodes is largely a function of acquired immunity, developed as a consequence of repeated infection [60]. This expulsion of adult nematodes appears to be a hypersensitivity reaction against incoming larvae [66]; therefore it is a non-specific response against the larval stages that seems to expel adult worms. This expulsion of the adult nematodes occurs concurrently with the development of resistance to larval establishment following continuous infection [67]. However, because the rate of development of immunity

against adult sized nematodes is different than the rate of the development of resistance to larval establishment, it would suggest that if expulsion of adult nematodes is a non-specific effect, then the level of immunity required to expel adult nematodes differs from the level needed to prevent larval establishment. On the other hand, Hong *et al*. [68] demonstrated that the rate of loss of adult *T. circumcincta* is also related to the size of the resident population and experimental infections [69] generated sheep immunized by infection with L3 larvae that after two weeks failed to expel the surgically implanted adults.

1.4.3 Reduced fecundity in female worms

The main morphological change in adult nematodes as a manifestation of host acquired immunity is their reduced size [66] and loss of vulval flaps [70], thus reducing their fecundity. The reduced size of adult nematodes is associated with the local IgA response against L4 and with a density-dependent effect of high worm numbers [66]. Thus, the drop in fecundity of adult females is the result of either density-dependent parasite competition or/and acquired immunity.

The mechanism of resistance involves IgA antibodies, which respond to L4 antigens, therefore suggesting that the response is directed against premature nematode stages and does not have a direct effect on established adult nematodes. Eosinophils, which appear to be a stable population, have mature cells created at low levels in the bone marrow. Those cells are then sent through the blood to the tissues where they can survive up to a few weeks and rise dramatically in both blood and tissue after parasite infection. Eosinophils have receptors for IgG, IgA and low affinity receptors for IgE; and the binding of IgA is what provides the most potent stimulus for degranulation [71]. Their major function during helminthic infections is their cytotoxic potential, which can disrupt worm growth [60].

1.4.4 Inhibition of worm development

Arrested development (hypobiosis) of larval stages (L4) of gastrointestinal nematodes in the host mucosa can occur. Hypobiosis is associated with seasonal changes, worm density and nematode strain [72] and also with increased resistance of the host [60]. In studies of *T. circumcincta*, arrested L4 stages were related to production of IgA and mucus IgG1 against L3 stages and adult worm burden [66]. L4 larvae in drenched animals resumed their development to adults [73]. The arrested L4 nematodes are protected from the hostile environment and can persist from one season to another. This maintains the levels of adult worm burden for a short period of time even in resistant hosts [74]; it is therefore a successful survival strategy although adults from arrested larvae are considerably shorter [70]. In a resistant animal, a high number of arrested L4 are expected. This inhibition is dependent on

the worm burden; therefore naïve animals with high worm burdens may also have inhibited L4s.

1.4.5 Cytokines

The identification and manipulation of cytokine responses has had a major impact on the understanding of the immune mechanism involved in parasite infections [75]. The infection of *T. circumcincta* in sheep triggers predominantly a type-2 (Th2) immune response and multiple cytokines (such as interleukin-4) are up-regulated in challenged animals [76]. Cytokine expression can be rapidly up and down-regulated, therefore the measuring time is critical since in less than a day, different levels of cytokines can be observed [60]. It is therefore very difficult to quantify values to include cytokines explicitly in a mechanistic model of parasite infection. Moreover, Cytokines control the production of effector cells, such as eosinophils or mast cells, so modelling them does not offer an advantage over modelling just the main effector cells.

The number and interactions between different mechanisms make the biology very complex, and although there is a good mechanistic understanding of how the immune response to nematode infection works, its dynamics have not been quantified. The immune response was divided, for the purpose of a mechanistic model, into two main components:

1. One component prevents worm establishment and is driven by mast cell degranulation and IgE specific to incoming stage 3 larvae.

2. The other component disrupts worms growth and fecundity and is largely driven by stage 4 larval specific IgA that mediates degranulation of eosinophils.

1.5 Mathematical models

Mathematical models have been used extensively over the last few decades in the field of parasitology. In this section, some of the key models that have been used in the past and the origin of the model presented in this thesis (Chapter 5) are introduced. One of the earliest articles about modelling gastrointestinal nematodes in livestock was published in the mid 80s by Smith and Grenfell [77]. Thirty years later, some of the premises that were stated back then still hold true, such as the first sentence of the paper: "*Ostertagia ostertagi* is one of the most prevalent and harmful nematode parasites of cattle in the temperate world". Although the problems currently faced have not changed and parasitic diseases of humans and livestock are still a serious and economically challenging problem worldwide, the models have greatly evolved and with them the tools to better understand and deal with these problems.

Mathematical models of gastrointestinal nematode infection in grazing animals have evolved in two ways. First, the techniques employed have changed, and with the developments in computing power, much more complex and detailed models can be run in relatively short periods of time. Secondly, the scope of the models has also increased, while the first models were more focused on studying the dynamics and the "equilibrium" scenarios, current models more often try to make specific quantitative predictions and are less generic, with some exceptions [78]. It was argued however, that generic models contribute as much as the first specific models [79]. There are a number of excellent reviews on the evolution of models of parasite infection in grazing animals available [80, 81, 82] and here they will be summarised, commented on and, where appropriate, an update will be offered on what it is said.

1.5.1 Evolution of model features

The first major technical advance in parasite modelling was incorporating stochasticity. Deterministic models such as the ones popularised by Anderson and May [83] were very common but their qualitative behaviour is different from that of stochastic models. Individual-based models (IBMs) track each individual, including birth, death, infection events and infection status. These models are computationally more intensive, but are routinely run using modern computers and can simulate random fluctuations that can alter the end results. The main advantage of stochastic models is that they be used to capture situations with variable outcomes and generate the probabilities of those outcomes. Nevertheless, deterministic models still have the advantage that they offer theoretical results which are easy to interpret and their equilibrium final state should be similar to the mean result of multiple stochastic simulations.

A key feature of gastrointestinal nematode infection is the over-dispersed distribution of parasites among hosts. A key question in the field has been to define how much observed variation in infection burden is due to variation in exposure and how much is due to variation in host susceptibility to infection [84]. Other effects such as clumped infection events and feedback in the reinfection process may explain part of the aggregation [85]. Independently of what causes the aggregation, numerous models have included this effect, usually with a negative binomial distribution [86, 87].

The immune response is another feature that has often been included in models. However, it has been modelled very much as a black box, since it is commonly perceived wrongly, in the view of Roberts [88], as too complicated to be modelled. In most of the models that include the immune response, each host is assumed to have an immune response that increases with exposure and decreases over time [89, 90, 91]. A more biologically realistic and data-driven immune response is one of the key features that needs to be included in a mathematical model in an explicit but simple way, capturing the two arms of the immune

response, which contrasts with the single immune response of these earlier papers. The acquisition of immunity against gastrointestinal nematodes through the grazing season has also been studied [91, 92, 93].

1.5.2 Evolution in focus

Mathematical models have been extensively used to gain insight into the dynamics of host-parasite interactions in humans, wildlife and livestock, and to help identify effective control measures [94, 95, 96, 97]. Since a comprehensive review [77], the dynamics of gastrointestinal parasites of ruminants have received considerable modelling attention. The models developed range in complexity from relatively simple phenomenological models [89, 98, 99] to detailed models which capture the multiple stages of the parasite life cycle within and outwith the host, allowing effects such as temperature, climate, grazing behaviour, nutrition and management to be incorporated [92, 100, 101, 102]. The focus of these models has encompassed the use of grazing management as a control measure [103, 104], the impact of drenching regimes [101], the generation and spread of anthelminthic resistance [92, 105, 106, 107, 108, 109], the implementation of targeted or strategic treatments [110, 111] and selective breeding for disease resistance [112].

Two important themes that recur in these modelling studies are the aggregation of infection loads and the acquisition of immunity by the host. Some studies have characterised the observed aggregation of parasite burdens across numerous host-parasite systems [113, 114, 115, 116], whilst mathematical modelling has been used to investigate the mechanisms and consequences of aggregation [85, 116, 117, 118, 119, 120]. The studies by Cornell *et al.* [87] and Grenfell *et al.* [118] suggest that much of the observed variation in parasite burden between hosts is attributable to some form of host heterogeneity.

In this thesis, a genetically and immunologically explicit model is developed to quantify the sources of aggregation in parasite burdens, commonly divided into aggregation in parasite intake and host heterogeneity [85]. These results are presented in chapter 5. Specifically, the variation in worm number is partitioned between those two components (larval intake and host genetics) and it is shown that most of the aggregation is explained, at least in this system, by the host immune response.

In the following section, the evolution of the key features of the model are described, with particular reference to the model of the development of immunity published by Singleton *et al.* [93], which is refined in this thesis, and to the 1997 paper by Bishop and Stear [112].

1.5.3 Evolution of the model in this thesis

Bishop and Stear [112] described an epidemiological model that can be summarised in two equations, one related to worm burden and another one to larval intake. They used a development of the immune response similar to the approach in Roberts and Grenfell [89]. The authors also included animal genetics and used the model to predict the response to selection for resistance, which in that model of 1997 was selecting for low faecal egg counts. Fecundity in that model was a function of worm number, so it was indirectly related to host immunity.

Singleton *et al*. [93] made important extensions to this model by incorporating fitting to empirical data and, critically, the interactions between development of immunity and both worm number and worm fecundity. Singleton *et al*. also captured the two components of the immune response, the anti-fecundity response against adult worms and the anti-establishment response against incoming larvae. In this model they also explored some of the parameters that were unclear in the literature via a sensitivity analysis, but model fitting was based on a simple least squares approach. The model in this thesis is fully described in Chapter 5 and in the Appendix F, with the main extensions and improvements on the Singleton model summarised below:

- In the Singleton model, larval intake and the rate at which animals develop the immune response through the grazing season is assumed to be the same for the whole flock. this is extended in the model presented in this thesis, with genetic and environmental components in the host immune response and larval intake to create a heterogeneous population of individuals.

- The Singleton model ran through the first grazing season of the lambs (from birth to 6 months of age). This is extended with simulations though multiple years (or generations, normally 10) and accounted for the ram and ewe flocks, allowing selection schemes to be modelled.

- The Singleton model reproduced the worm life quite accurately, but more detail is added in areas that are relevant to the model's use in this thesis (i.e. adding the larval L4 stage explicitly); this allows for a more accurate reproduction in season dynamics of the immune response. Temporal fluctuations in the environment have also been added, which allows for faster development of larvae in the field in the summer months.

- The Singleton model had both an anti-fecundity and anti-establishment immune response, which were both triggered by larval intake. The anti-fecundity immune response is extended (IgA) to include the transfer from the abomasal mucus (which is the site of infection) to the blood (plasma), which allows modelling of an alternative

marker of selection. Also in the model presented in this thesis, the anti-fecundity response is triggered by larvae stage 4 (L4), which is as reported in the literature [66].

- Instead of using a least squares to fit the model, it has been instead fitted using a Bayesian ABC algorithm. The model is now fitted to the means, variances and heritabilities of the field data, allowing it to reproduce observed distributions more accurately.

Chapter 2

The transfer of IgA from mucus to plasma

Immunoglobulin A (IgA) activity has been associated with reduced growth and fecundity of *Teladorsagia circumcincta*. IgA is active at the site of infection in the abomasal mucus. However, while IgA activity in abomasal mucus is not easily measured in live animals without invasive methods, IgA activity can be readily detected in the plasma, making it a potentially valuable tool in diagnosis and control. A Bayesian statistical analysis was used to quantify the relationship between mucosal and plasma IgA in sheep deliberately infected with *T. circumcincta*. The transfer of IgA depends on mucosal IgA activity as well as its interaction with worm number and size; together these account for over 80% of the variation in plasma IgA activity. By quantifying the impact of mucosal IgA and worm number and size on plasma IgA, a tool is provided that allows more meaningful interpretation of plasma IgA measurements and aid the development of efficient control programs.

2.1 Introduction

In sheep, IgA activity against fourth-stage larvae is strongly and consistently associated with reduced worm growth and fecundity following infection with the abomasal parasite *T. circumcincta* [66, 121, 122]. The site of infection is the abomasum, but collecting abomasal tissue or mucus requires invasive surgery or post-mortem sampling. Therefore plasma IgA has been widely used to study the build-up of immunity over the grazing season and the sources of variation among animals [123, 124]. The relationship between mucosal and plasma IgA is consequently of critical importance for the interpretation of IgA activity. In particular, the construction of immunologically explicit models of the host-parasite system is only possible if this relationship is understood. The relationship between mucosal and plasma IgA is influenced by IgA activity in the abomasal mucus and the number of nematodes in the abomasum

[66, 125]. Mucosal IgA will bind to nematodes and excess IgA will move into the lymph and subsequently appear in the plasma.

However, adult *T. circumcincta* differ in size. Some sheep have populations of relatively small worms while other sheep harbour much larger worms [115]. Therefore the influence of adult nematode size in the relationship between mucosal and plasma IgA was also investigated. A three-dimensional surface plot of the relationship between mucosal IgA, worm mass (worm length $\times$ worm number) and plasma IgA has been produced. In addition, this relationship was used to predict the likely distribution of mucosal IgA.

2.2 Materials and methods

2.2.1 Experimental data

The experimental data used in the statistical analysis and to infer the distributions of the parameters are from two published studies. One study monitored the response to deliberate infection in Scottish Blackface sheep [66]. This study measured mucosal and plasma IgA activity against fourth-stage larvae, worm burdens and mean worm lengths. These data were used to quantify the relationship between mucosal and plasma IgA. The second study was based on five successive cohorts, each of 200 lambs from a naturally infected commercial flock in southwest Strathclyde [115], which were monitored for plasma IgA, worm burden and worm length. This second study was used to explore the possible distribution of mucosal IgA.

The deliberate infection study was conducted with 30 six month old female lambs chosen randomly from a flock of 200 Scottish Blackface lambs which had been exposed to natural, mixed, predominantly *T. circumcincta* infection. They were transferred to the Veterinary School and housed together indoors under conditions to minimize extraneous infection. After 3 months, 24 of the 30 lambs were infected with $50,000$ infective third-stage *T. circumcincta* larvae. After 8 weeks, all sheep were treated with two broad-spectrum anthelmintics and 4 weeks later reinfected with $50,000$ larvae. 3 sheep were infected only at the second date and served as infection controls, while the other 3 were not infected and served as uninfected controls. All sheep were monitored for another 8 weeks and necropsied.

Mean worm length was calculated by measuring the length of 100 female adult worms from the abomasal contents of each animal. Only one worm was recovered from one lamb, which was dropped from the analysis. Worm number was calculated in both experiments by counting the worms in a 2% sample of abomasum wash from each animal and multiplying by 50 [126]. The abomasal mucosa from each animal was digested and a 2% sample was used to estimate the number of fourth-stage larvae [27].

Indirect ELISA (enzyme-linked immunosorbent assay) with biotinylated monoclonal antibodies was used to measure mucosal and plasma IgA activity. The cell line used to generate monoclonal IgG anti-sheep IgA ($M1521$) were kindly donated by Dr. S. Hobbes, Dr. P. Bird and Professor I. McConnell (pers. comm.) [126]. Larval antigens were prepared from somatic and excretory-secretory material as described by Sinski *et al*. [125].

2.2.2 Statistical analysis

The relationship between plasma IgA, mucosal IgA and worm biomass was explored in a Bayesian framework. A similar relationship between plasma IgA, mucosal IgA and worm burden, rather than worm biomass, has previously been explored using a general linear model by Stear et al. [66]. As there is no informative prior information, a Bayesian analysis is expected to give similar results to a classical frequentist analysis. However, a Bayesian framework provides a more natural method for future work in this area.

For the purposes of this analysis, the worm mass in individual sheep was estimated as mean adult female worm length multiplied by the number of adult worms. This approximates worm mass, but the actual worm mass would require a knowledge of worm diameter, deviation from a cylindrical shape, the length of adult males and the variation in worm length among individual worms. A logarithmic transformation was used to improve the goodness of fit of the model. The log-transformed distribution of worm mass in the 23 sheep was not significantly different from a normal distribution (Figure 2.1; Shapiro-Wilk test, $P = 0.21$), although with only 23 animals it is not a very powerful test of the distribution.

The intercept and the main effect of worm mass were found not significant after fitting the full model (intercept, the two main effects and the interaction of mucosal IgA and worm mass), so these variables were dropped from the equation. The final model equation, hereafter referred to as the IgA transfer equation, therefore only included the effects of mucosal IgA and the interaction between mucosal IgA and worm mass.

$$\mathrm{IgA_p} = \lambda_1 \cdot \mathrm{IgA_m} + \lambda_2 \cdot \mathrm{IgA_m} \cdot \log_{10}(\mathrm{WM}) + \epsilon \tag{2.1}$$

This model was fitted using a Bayesian approach, which returns the distribution of possible values (called the posterior distribution) for each coefficient; if zero lies inside the 95% credible interval, that coefficient is viewed as non-significant [127]. λ_1 and λ_2 are the regression coefficients being calculated while ϵ is the error term, deviation of the obtained value from the true $\mathrm{IgA_p}$ value, which accounts for the variation that is not explained by the model.

The DIC (deviance information criterion) was used to compare different models [128], with the preferred model being the one with the lowest DIC. The penalized R^2 was also calculated,

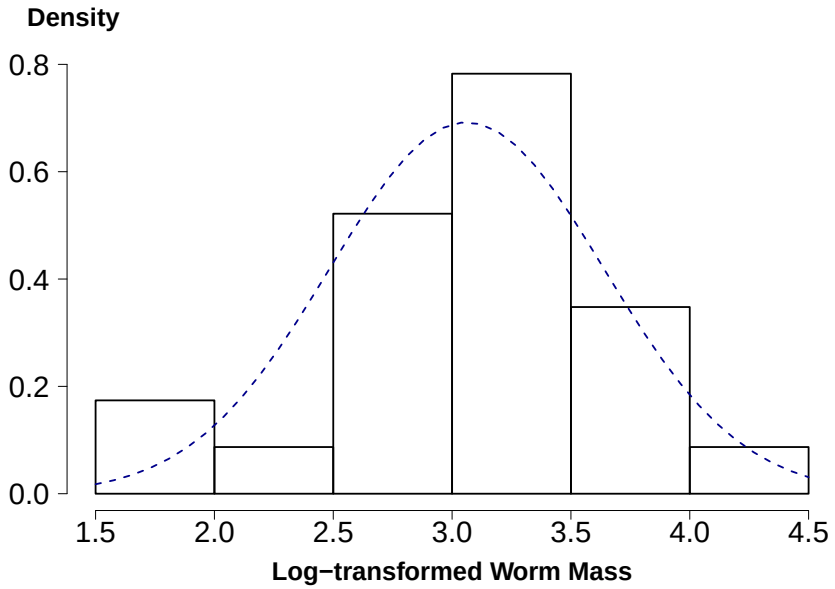

Figure 2.1: Distribution of the log transformed estimated total worm mass in individual sheep ($n = 23$) overlaid with the best-fitting normal distribution estimated by maximum likelihood (mean $= 3.06$; standard deviation $= 0.58$).

which estimates the proportion of the total variation in plasma IgA activity that was explained by the variation in mucosal IgA and worm mass, while accounting for changes in the number of variables being fitted [129].

The statistical distribution of mucosal IgA levels among individual sheep is not known. It was therefore tested whether a normal distribution of mucosal IgA was compatible with the known distributions of plasma IgA and the number of *T. circumcincta* in the host. Plasma IgA follows a gamma distribution [130], the number of *T. circumcincta* follows a negative binomial [115] while worm length is normally distributed [66].The model equation was revised to infer mucosal IgA, hereafter referred as reverse transfer equation, from the plasma IgA and worm mass (calculated again as the product of mean adult female length and number of adult worms) data in the natural infection study [115]. The potential distributions of mucosal IgA were then explored. This dataset has a larger number of animals; therefore tests to explore the distribution are more powerful. Extreme predicted IgA values were discarded because they may have arisen from errors in the estimation of worm number; the Shapiro-Wilk procedure was used to test departures from normality and the Kolmogorov-Smirnov test to compare the fitted gamma distribution to the mucosal IgA distribution among lambs.

Statistical analyses were carried out using the R language [131] and JAGS (a Bayesian Analysis program using Gibbs sampling [132]). The Bayesian regression analysis was run using uninformative priors, two chains were used with $500,000$ iterations and a thinning of 40; the burn-in period was $10,000$. The model was run twice, first as a full model with all the parameters and then a simplified model with only the statistically significant parameters

from the previous fitting. Type III sums of squares (SS) were calculated to obtain the R^2; unlike commonly used type I sums of squares, type III SS do not depend on the order in which effects are specified in the model.

Figures were plotted using the R language [131]. To draw the surface plot, a grid of mucosal IgA activity and worm mass values was created (from 0 to 2 and 0 to $10,000$ respectively) with 50 intermediate nodes on each axis. The plasma IgA values were calculated for each point of the grid from the transfer equation and the surface was then interpolated from these points. Worm mass origin was changed to 200 (the first node) for graphic convenience to avoid artefacts being created at very low worm biomass. Additionally, these three variables were plotted against each other to clarify the model fit and range of values for which the fit is valid (Figure 2.3).

2.3 Results

Using the Bayesian model, plasma IgA activity was dependent on mucosal IgA activity and the interaction between worm mass and mucosal IgA activity (Table 2.1). Dropping the intercept and the main effect of mucosal IgA reduced the DIC from 42.8 to 36.6 indicating that the full model is only 0.045 times as probable as the final model. The coefficient of determination (R^2) for the final model is 0.83: an R^2 of 0.83 implies a correlation of 0.91. This high correlation indicates that the major determinants of IgA activity in the plasma are in fact the two explanatory variables, IgA activity in the abomasal mucus and the mass of worms in the abomasum.

Table 2.1: Regression coefficients and 95% credible intervals for the relationship of plasma IgA activity with mucosal IgA activity, and the interaction of mucosal IgA activity with worm mass.

Regression Term	Mean (95% Credible Interval)
$\mathrm{IgA_m}$	3.97 (2.91; 5.03)
$\mathrm{IgA_m} \cdot \log_{10}(\mathrm{WM})$	-1.02 (-1.37; -0.67)

For a given worm mass, increased mucosal IgA was associated with increased IgA activity in the plasma (Figure 2.2). Similarly, for a given concentration of mucosal IgA, increased worm mass was associated with decreased IgA activity in the plasma. Therefore low plasma IgA activity could be a consequence of low abomasal IgA activity or a high number of worms in the abomasum. Figure 2.2 demonstrates that there is very little IgA activity in the plasma when there is a high mass of nematodes in the abomasum.

The distribution of mucosal IgA activity in the abomasal mucus was predicted using the transfer equation based on values of plasma IgA activity, worm numbers and worm lengths

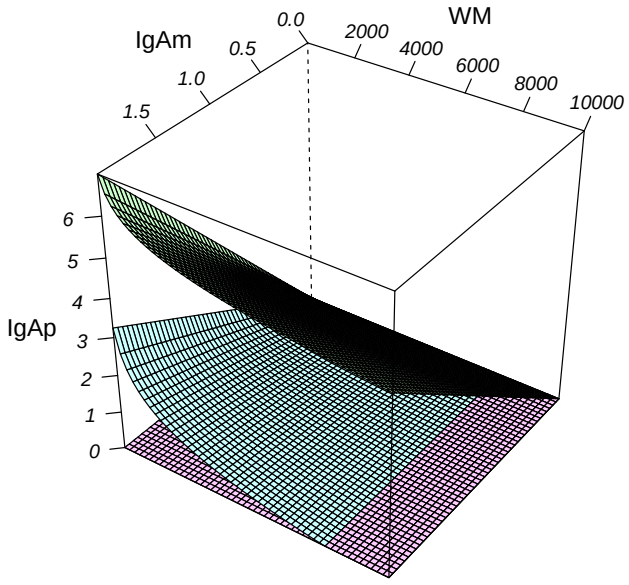

Figure 2.2: 3 Dimensional surface plot of the relationship between plasma IgA (IgA_p), mucosal IgA (IgA_m) and worm mass (WM $-$ number of adult female *T. circumcincta* multiplied by worm length in cm). The aquamarine (middle) surface shows the most probable values, the purple and green (lower and upper) surfaces were plotted with the lower and higher values in the 95% credible interval (Table 2.1) respectively.

from 484 naturally infected lambs from a single farm in Southwest Strathclyde. The predicted distribution of mucosal IgA did not follow a normal distribution (Shapiro-Wilk normality test $P < 2.2 \times 10^{-16}$), but was consistent with a gamma distribution (Kolmogorov-Smirnov test $P = 0.543$, Figure 2.4).

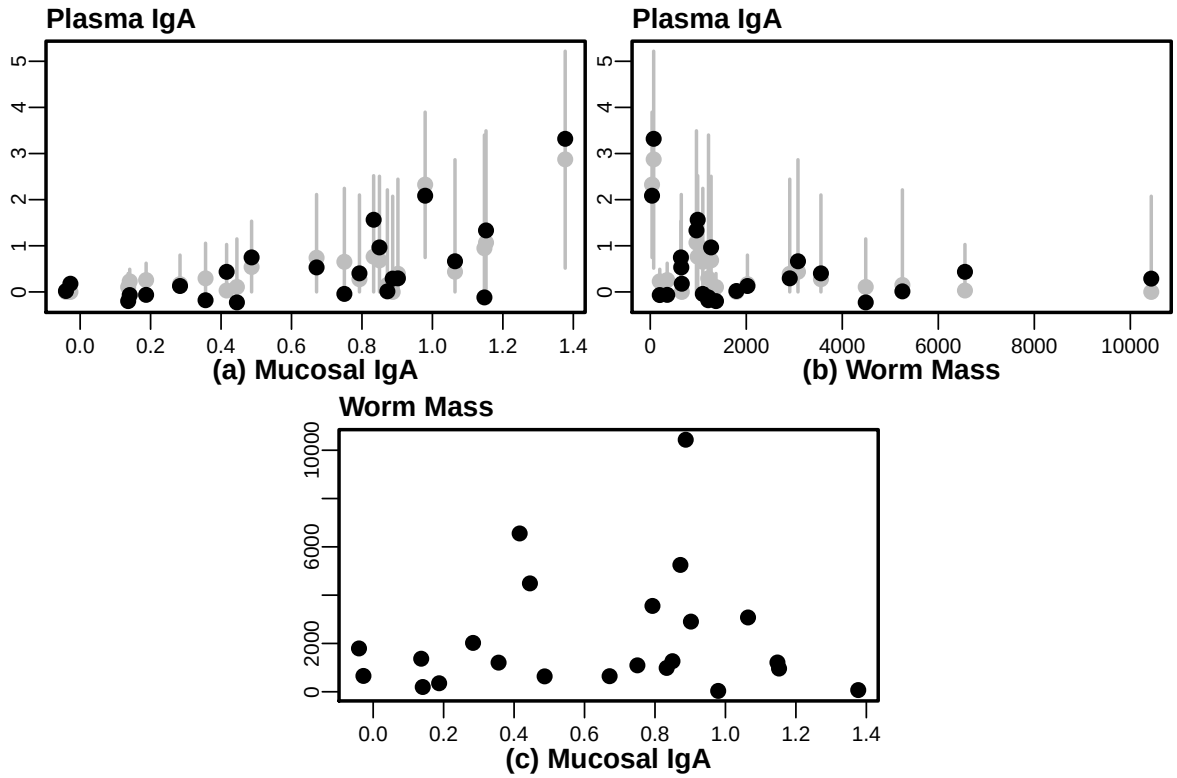

Figure 2.3: Relationship between (a) plasma IgA (IgA_p) and mucosal IgA (IgA_m), (b) plasma IgA and worm mass and (c) mucosal IgA and worm mass. Black dots are data, grey dots represent the fitted value, which corresponds to the aquamarine surface (Figure 2.2). Vertical grey lines show the 95% credible interval, which corresponds to the range between the purple and green surfaces (Figure 2.2).

2.4 Discussion

This study has improved the understanding of the relationship between plasma and mucosal IgA following *T. circumcincta* infection in sheep, confirming that there is a strong, but non-linear, relationship, and has suggested that the distribution of mucosal IgA among naturally infected individuals is highly skewed.

The relationship between nematodes and the antibody response is complex. A strong IgA response against fourth-stage larvae will delay nematode development [66] and nematodes will spend longer as fourth-stage larvae and provide a greater antigenic stimulus. Conversely, a strong IgE response will prevent the establishment of third stage larvae and reduce the antigenic stimulus provided by fourth-stage larvae [66]. In this experiment, sheep received challenges of $50,000$ larvae. In addition to antibody responses affecting the intensity of the antigenic challenge, there will be cross-reactions between different species of nematodes [133] and possibly between different stages of the same parasite. For example, the IgA response against third stage larvae peaked 8-10 days after infection then persisted at a low level [124]. Most third-stage larvae will mature to fourth-stage larvae or be rejected in 4 days

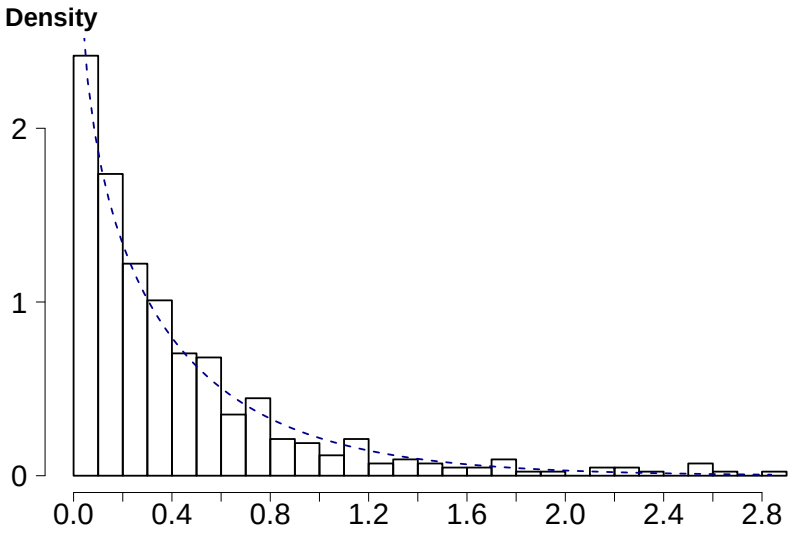

Figure 2.4: Distribution of mucosal IgA from 0 to 3 OD (optical density). 88% of the data values are in this interval (negatives and extreme positives were dropped). Dashed line shows the fitted gamma distribution (shape $= 0.78$; rate $= 1.83$). The Kolmogorov-Smirnov test p-value for the gamma fit was 0.543. The absence of extremely high values of mucosal IgA shortens the tail of the gamma distribution.

[27]. IgA has a short half-life in plasma [134]. Consequently the persistence of antibody to L3 may reflect cross-reactions with molecules from other parasitic stages. In natural infections, there will be continuous challenge with incoming and developing larvae and most of the variation in IgA activity reflects genetic differences among lambs [123] rather than variation in exposure to infection.

An understanding of the relationship between plasma and mucosal IgA is necessary for drawing appropriate conclusions from measurements of plasma IgA. Previous research [135] has shown that in sheep, unlike humans [136], plasma IgA is derived from mucosal surfaces. The simplest explanation for the observed relationship between plasma IgA and mucosal IgA in this study is that abomasal IgA binds to nematodes and excretory-secretory molecules released from nematodes. The unbound IgA then equilibrates between mucus and interstitial fluid, and is then transferred to blood via the lymphatic system. This equilibration is helped by breaches that occur in the epithelial barrier during nematode infection [8], these breaches are caused by mast cell degranulation that break the junctions between the epithelial cells to facilitate the flow of effector cells. This also causes protein loss, which is one of the signs of nematode infections [8].

The transfer equation was simplified from the full model using the DIC values and penalized R^2. However, the model could have also been simplified based on biological grounds. IgA in the plasma must arise from IgA produced in the mucus in this system [135], hence the inter-

cept and the main effect of worm mass have to be zero for a biologically meaningful transfer equation (a deviation from zero for the intercept could be explained by cross-reactions with other molecules).

From an evolutionary perspective, sheep should produce enough IgA to counter an infection but should not invest excess resources in the immune system. Production of excessive IgA would produce large amounts of plasma IgA and a close relationship between mucosal and plasma IgA. The observation that very little IgA appears in the plasma during heavy infection suggests that most IgA is bound to nematodes and a relatively small proportion of nematode-specific IgA is transferred into plasma.

After applying the inverse transfer equation to naturally infected sheep, the mucosal IgA values obtained were consistent with a gamma distribution. The observed gamma distribution implies that a few animals will have high mucosal IgA immune responses while the majority will be low responders. Low plasma IgA values occur in animals with a low intensity infection that triggers a small immune response or a heavily infected animal in which most of the mucosal IgA is bound to parasite antigens. High plasma IgA means that the response to infection is strong. A selection scheme that uses high responders for breeding would be potentially very successful in improving the overall response of the flock since the heritability of IgA responses is high [123]. This is consistent with results obtained independently by Shaw *et al.* [137], where they propose the use of salivary IgA to the nematode molecule CarLA as a measure of immunity to gastrointestinal nematodes. Salivary, plasma and mucosal IgA are correlated, with plasma having the higher correlation with mucosal IgA [122].

One area of concern is whether this type of selection will select for animals investing too many resources on the immune response. However, selection criteria generally include production traits such as growth and the family history. The performance of the animals selected should therefore not decrease.

The transfer equation should provide a valuable component of mathematical models that simulate the behaviour of the immune system in small grazing animals facing infection, e.g. Singleton *et al.* [93]. One advantage of the nematode system is that it is possible to quantify variation among hosts in the number and size of the antigenic challenge. With the appropriate data, a similar study could be conducted in other grazing species and with other nematodes.

In conclusion, the transfer of IgA from the abomasal mucus to the plasma has been quantified. The amount of IgA in the plasma is strongly but non-linearly related to the amount of IgA in the mucus and the interaction between mucus IgA and the parasite size and burden. The nature of this relationship has important implications for the design and outcome of diagnostic and control scenarios.

Chapter 3

Nematode effect on milk production

The effect of gastrointestinal (GI) nematodes in young lambs has been extensively studied, but the effect on production traits in older dairy ewes is unknown. Dairy ewes from the Mediterranean region are used to produce milk and high quality cheese. It is therefore important to know whether nematode infections may reduce significantly the productivity and hence the competitiveness of the sector. This chapter quantifies the effect of parasitism on three production traits: protein and fat percentage in milk, which is the measure of milk quality, and the total milk yield. Three parasitological and immunological traits were used to assess gastrointestinal nematode infection: faecal egg counts (FEC), which are the traditional measure of GI infection in sheep; *T. circumcinta* L4 stage specific serum IgA; and serum pepsinogen. A Bayesian framework was used to model the impact on the protein and the fat percentage in the milk, which affects the quality of the cheese, and on the milk yield. A significant relationship was found in both analyses with high levels of pepsinogen being associated with a reduction in both the protein and fat percentage contained in milk. Serum IgA and pepsinogen are also related to variation in the milk yield. The conclusion is that parasitism can result in reduced productivity in older ewes.

3.1 Introduction

Gastrointestinal nematodes are endemic in small ruminants worldwide. The reduction in growth they produce in young lambs was first reported almost three decades ago [138]. The impact of nematode infection on the health of dairy ewes has been also explored [139], but the impact on production traits has not been studied.

Spain has large populations of Churra dairy sheep which have been the focus of QTL (Quan-

titative trait loci) analyses with the goal of increasing resistance to gastrointestinal nematodes [139] and improving production traits such as milk yield [140]. These flocks historically have low faecal egg counts; hence it is thought that they have low levels of infection (although low faecal egg counts can occur in heavily infected sheep [141]. The low infection levels are assumed to be mainly due to management practices, with some flocks kept indoors during the whole year. Because the faecal egg counts have always been low, the studies in dairy ewes have focused on resistance to infection and disease, but nematode infection has not been viewed as a problem for production. However, to ensure that the needs of the sector in milk and cheese production are met, it is important to quantify the effects of parasitism on productivity by the ewes.

A Bayesian framework was used with models developed to infer the effect of gastrointestinal nematode infection on overall milk production and on the protein and fat contained in the milk, the latter being important determinants of cheese quality.

3.2 Materials and methods

3.2.1 Experimental data

The data was obtained by combining datasets from two different studies. All animals belonged to the selection nucleus of the National Association of Churra Breeders (ANCHE), Spain.

The parasitological and immunological data (faecal egg counts, IgA in the blood plasma and serum pepsinogen) were measured in a study with 1545 records from 928 ewes [142]. The production data, milk yield (MY), protein (PP) and fat (FP) percentage in milk, were measured in a daughter design of 11 half-sib families of Churra sheep, with 1213 ewes distributed across 17 flocks. The lactation time in Churra sheep is around 140 days; the first record was obtained between 31 to 75 days post-partum and each month subsequently [140].

The dataset used in this analyses contains the ewes from the first study that were also part of the second one a total of 309 sheep. Because complete records on pepsinogen and plasma IgA as well as milk yield were only available for 229 ewes, different analyses employed different subsets of the 309 sheep to maximise the number of animals in each analysis.

Plasma IgA measures ranged from zero to 0.35 (Optical Density Ratio, ODR)and followed a gamma distribution (Kolmogorov-Smirnov test $P = 0.71$). Pepsinogen, which is measured in mUTry (milimoles of tyrosine per litre of blood), ranged from -150 to 200 and was close to a normal distribution (Pearson normality test $P = 0.03$). Transformed FEC, $log_{10}(FEC + 1)$, hereafter referred to as FEC for simplicity, ranged from -0.35 to 0.25 and

were normally distributed (Pearson normality test $P = 0.38$. All these variables had previously been examined using a mixed model and corrected by accounting for the effects of flock, sampling month, lambing number, physiological status and sire [142]. MY, PP and FP were calculated as deviations from the population mean that were corrected for the following systematic environmental effects: flock test-day (1294 levels), week of lactation (13 levels) and lambing age (six levels) [143].

3.2.2 Statistical analysis

Two analyses were conducted, with each analysis including several alternative models. The first analysis was to quantify the cost of the immune response in terms of protein and fat content of the milk. The second analysis was to quantify the influence of nematode infection on milk yield. A summary of the alternative models is presented in Table 3.1.

Table 3.1: Summary of the models, estimating protein percentage (PP), fat percentage (FP) and milk yield (MY).

Response variable	Explanatory variables
PP	MY, Pepsinogen
PP	MY, IgA_p
PP	MY, FEC
FP	MY, Pepsinogen
FP	MY, IgA_p
FP	MY, FEC
MY	Intercept, Pepsinogen, IgA_p
MY	Intercept, FEC

The first three models estimated the protein percentage contained in milk as a function of milk yield and each of the three listed markers of infection. In each case, the intercept was removed because it was non-significant. The following three models estimated the fat percentage in milk as a function of the same explanatory variables. All 6 models include milk yield as an explanatory variable since the percentage values are expected to decrease as the milk yield increases. The final two models estimated the influence of three parasitological and immunological markers on milk yield. Originally, all three markers and the interactions between them were modelled together, and the non-significant effects were dropped one at a time (using the least-significant at each step), all interactions between markers were hence dropped and only the three main effects were kept. In this model with the three main effects, the FEC were non-significant (p-value almost $= 1$) and since IgA and pepsinogen are related to two independent immune responses, anti worm fecundity [144] and anti establishment

[8, 145] respectively, they should be modelled together, while FEC is a downstream marker for both. The model was then split in two, to avoid the confounding between the immune responses and the downstream FEC: the first quantifies the relationship between plasma IgA and pepsinogen on the milk yield, while the second quantifies the relationship between faecal eggs count and milk yield.

All models were run in a Bayesian MCMC framework, which yields a distribution of possible values (called a posterior distribution) for each coefficient; if zero is inside the credible interval, that coefficient is statistically not different from zero [127].

Statistical analyses were carried out using the R language [131] and JAGS (a Bayesian Analysis program using Gibbs sampling [132]). The Bayesian regression analyses were run using uninformative priors, two chains of $200,000$ iterations and a thinning of 10; the burn-in period was $10,000$.

3.3 Results

3.3.1 Immunological and parasitological effects on milk protein

The percentage of protein in the milk is negatively related to pepsinogen concentration. Whilst there appears to be a weak negative relationship with plasma IgA levels this is not statistically significant. There was no significant relationship with faecal egg count (Figure 3.1).

As expected in all three models, milk yield is significantly negatively related to protein percentage, The maximum (most likely) value of the regression coefficient is approximately -2×10^{-4}, meaning that a high milk yield could produce a deviation of up to 0.21 in milk yield, which is 25% of the most negative deviation in milk protein percentage seen in the data (-0.83).

The maximum reduction (deviation) in the percentage of protein in milk associated with pepsinogen, obtained using the most probable value of the coefficient is 0.19. Since the most negative deviation in milk protein percentage was -0.83, pepsinogen could be associated with up to 25% of this deviation. On average pepsinogen concentration explains almost 5% of the variation in protein percentage.

Although non-significant, plasma IgA seems to be negatively related with protein percentage in milk. The effect is however small, and the most negative deviation in the data with the most likely value is less than 0.075, which is 9% of the deviation. This model explains 4.5% of the variation.

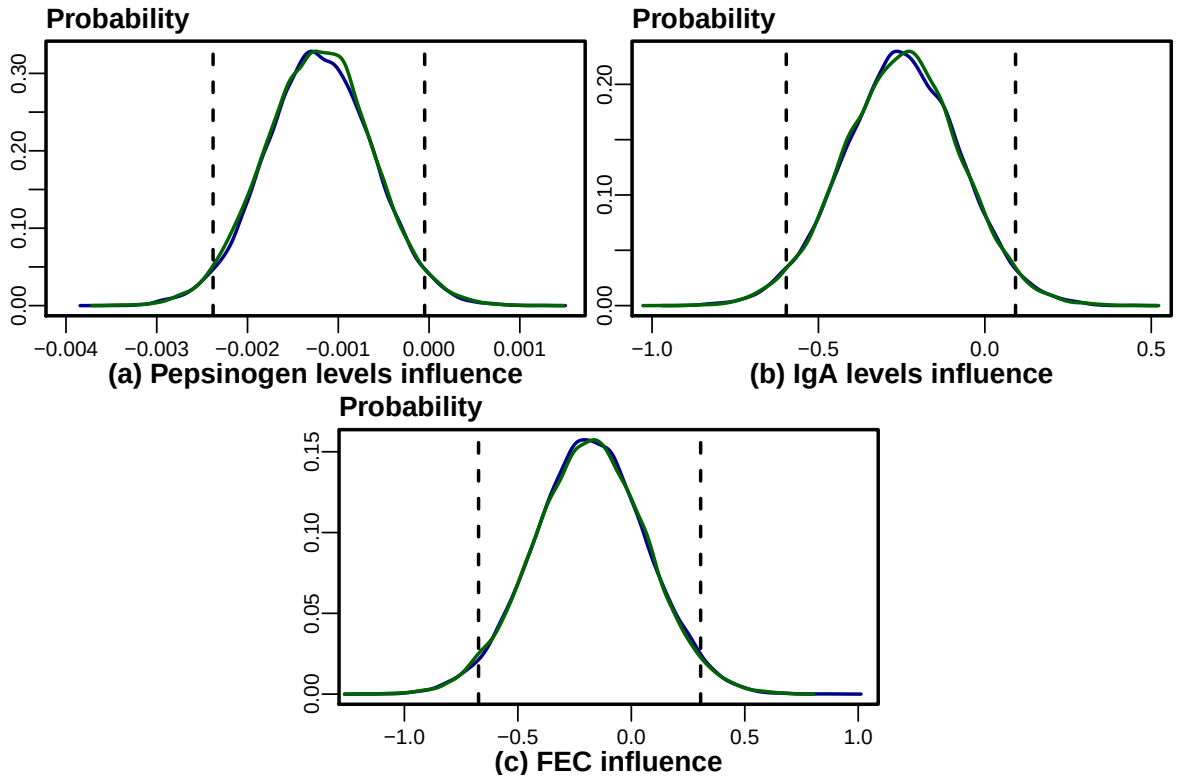

Figure 3.1: Posterior distributions for the influence of pepsinogen levels (a), plasma IgA levels (b) and faecal egg counts (c) on the percentage of protein in milk. Each colour represents one Monte Carlo chain; the chains are independent of each other. Vertical dashed lines indicate the confidence limits (95% credible interval).

3.3.2 Immunological and parasitological effects on milk fat

Pepsinogen is negatively related to milk fat (although this effect is borderline significant), while plasma IgA levels are positively related. Faecal egg counts have no significant relationship with fat percentage in milk (Figure 3.2).

Again, as expected in all three models, the fat percentage was significantly negatively related to milk yield, with a maximum (most likely) value close to -6.5×10^{-4}. Milk yield could be associated with deviation of up to 0.57, which is 31% of the most negative deviation in milk fat percentage seen in the data (-1.85).

Pepsinogen levels have a negative influence on fat percentage with borderline significance; using the most probable value however, the maximum deviation is 0.38, which is 20% of the most negative deviation in fat in the data. Plasma IgA can explain a maximum of 9% of the positive deviation since it can explain a 0.25 deviation from a maximum of 2.75. The pepsinogen and plasma IgA models explain 7.5% of the variation.

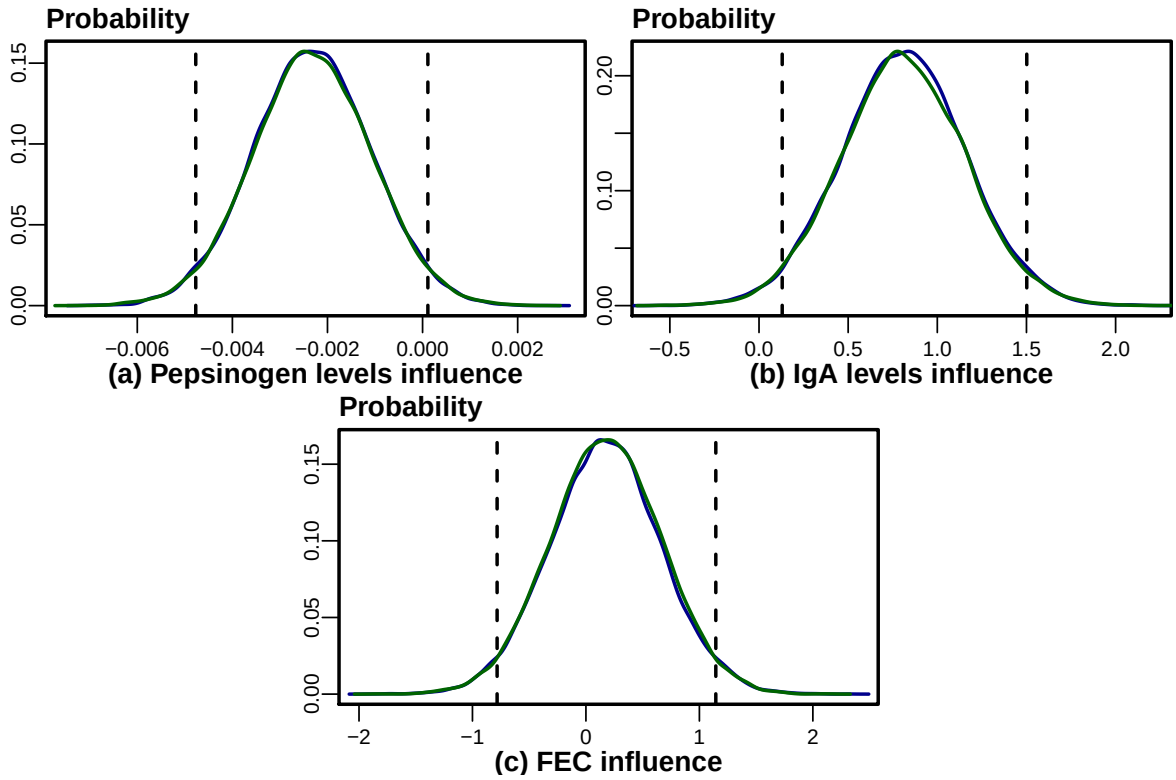

Figure 3.2: Posterior distributions for the influence of pepsinogen levels (a), plasma IgA levels (b) and faecal egg counts (c) on the percentage of fat in milk. Each colour represents one Monte Carlo chain; the chains are independent of each other. Vertical dashed lines indicate the confidence limits (95% credible interval).

3.3.3 Immunological and parasitological effects on milk yield

Milk yield is affected by gastrointestinal nematode infection. Higher plasma IgA levels are associated with reduced milk yield (Figure 3.3b) while higher pepsinogen levels (Figure 3.3c) are associated with increased milk yield. The effect of pepsinogen is statistically significant; the effect of plasma IgA is however not statistically significant.

This model however accounts for less than 1% of the variation in milk yield. The maximum reduction in milk yield due to plasma IgA is 7.8% while the maximum increase due to high pepsinogen levels is 15%.

The second model for this analysis yielded a posterior distribution for FEC that suggests that FEC is not associated with changes in milk yield (Figure 3.4b). Specifically, zero is close to the most probable value. In both models of this analysis the population mean is significantly different from zero and positive (Figure 3.3a and 3.4a).

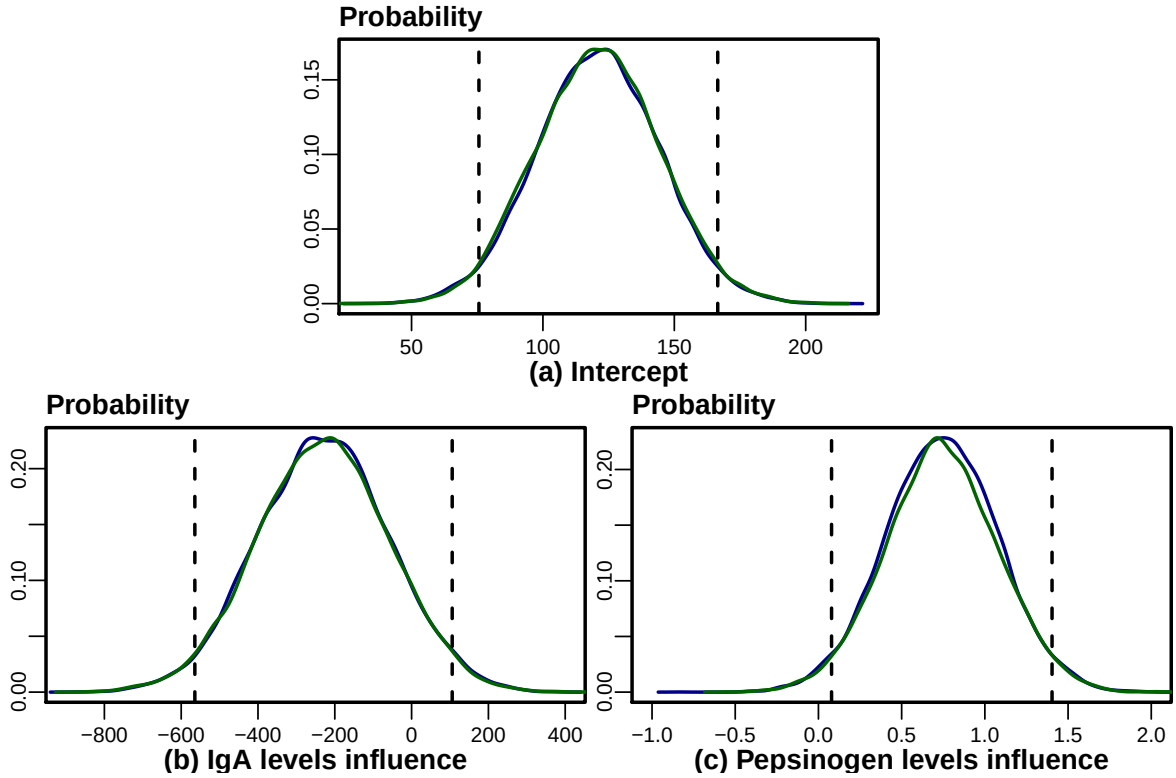

Figure 3.3: Posterior distributions for the influence of plasma IgA (b) and pepsinogen (c) on the milk yield. The posterior of the intercept, which is similar to the population mean, is also shown, (a). Each colour represents one Monte Carlo chain; the chains are independent of each other. Vertical dashed lines indicate the confidence limits (95% credible interval).

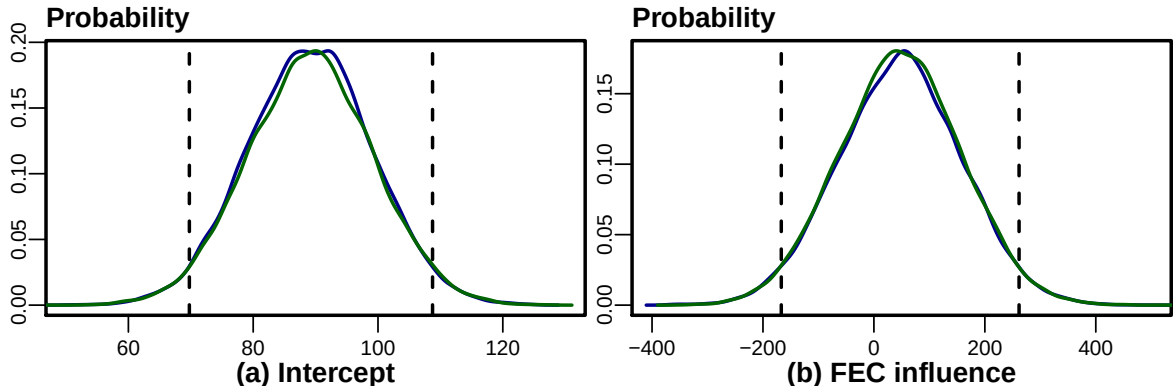

Figure 3.4: Posterior distributions for the influence of faecal egg counts (b) on the milk yield. The posterior of the intercept, which is similar to the population mean, is also shown, (a). Each colour represents one Monte Carlo chain; the chains are independent of each other. Vertical dashed lines indicate the confidence limits (95% credible interval).

3.4 Discussion

The amount of protein and fat in the milk is important, since it will increase the growth and success of the young lambs. Moreover, the Spanish dairy industry uses part of the production for cheese, which is highly dependent on the total solids in the milk. High quantities of liquid

milk production are associated with a lower percentage of protein in milk. The same applies to the fat percentage, which also decreases as milk production increases.

Pepsinogenaemia is a response to worm infection. The key mechanism appears to be a mast-cell mediated hypersensitivity reaction in the abomasum which can lead to a relative protein deficiency in the affected animal [8, 66]. The finding that pepsinogen is negatively related to percentage of protein in the milk is consistent with this hypothesis (Figure 3.1a). The prediction is therefore that animals with strong responses against nematodes will suffer a loss of protein and produce milk with a reduced protein concentration. Nematodes affect fat deposition [34, 146] and the finding that high levels of pepsinogen are also negatively associated with the fat percentage in milk is consistent with this view (Figure 3.2a).

The negative deviation caused by high levels of pepsinogen is similar to the one associated with high milk yield. For production purposes, an increase in milk yield is an attractive option. However, high yielding sheep may have a reduced quantity of milk solids and selection for high yielding ewes should involve both milk quantity and quality if possible. Moreover, if the animals are infected with nematodes and are protein deficient, the negative deviation from expected values in percentage protein and fat is going to increase.

Pepsinogen is also positively associated with milk yield (Figure 3.3b) while plasma IgA, although not statistically significantly, seems to be negatively related to milk yield, (Figure 3.3c). A potential explanation is that in adult sheep the prevention of worm establishment produces less parasite pressure, which positively influences the milk yield. However, the relationship between plasma IgA and infection is not linear as plasma IgA will be low for low levels of infection and also for animals with high levels of infection [66, 125]. A potential explanation for these results is that plasma IgA regulates worm fecundity but does not influence the hypersensitivity response, which causes protein loss. Plasma IgA also has a positive effect on fat percentage, which indicates that fat deposition is not compromised in animals that are infected but have a strong IgA response. This suggests that there are different benefits with each component of the immune response. Another explanation is that the relationship between the parasitological data and milk yield obscures the relationships with the other production traits. However, these results for plasma IgA agree with studies in cattle, which found a significant negative effect of milk antibodies on milk yield and protein percentage, as well as a non-significant positive effect on fat percentage, [147].

The size of the associations are however relatively small on average. This could be due to the fact that in this system the parasite pressure is relatively small compared to other systems. Nevertheless, the effects can sometimes be large, causing reductions in protein and fat percentages of almost 10%.

The effect of faecal egg counts on all production traits was non-significant, and moreover, there was no evidence of a potential relationship with milk yield (Figure 3.4b). The faecal

egg counts, like the other parasitological variables in the model, were corrected for effects such as sire, flock, month, etc. In Spain, faecal egg counts are typically low, and therefore exhaustive counting would be needed to provide precise estimates of the true number of eggs in the faeces. In addition, the relationship between faecal egg count and worm number is complex [141]. If parasitism has an influence on milk yield as the other results suggest, the expected effect of faecal egg counts, is a negative relationship with higher faecal egg counts being associated with reduced milk production.

The analysis was done using multiple models with a reduced number of explanatory variables each time to simplify the interpretation of the results. The intercept in the models for protein percentage and fat percentage is meaningless biologically, since in the absence of milk production there is no fat or protein available to measure. The equations still allow for values in the response variable different than zero even at zero milk yield due to the parasitological variables. The intercept in the models for milk yield was kept since it was statistically significant and biologically indicates the baseline milk production in absence of parasitism. The model for milk yield was split into two to allow for an easy interpretation of the results. Pepsinogen and IgA are related to two independent immune responses and faecal egg counts are and output that combines the effects of both immune responses. The models were also ranked via the R^2, with a full model including IgA, pepsinogen and FEC having a similar R^2 than the same model excluding FEC. In fact, faecal egg counts were not very informative in this dataset.

The results show that there is a relationship between parasitism and the quantity and quality of milk. Different markers for nematode infection were used, and the suggestion is that worm burden affects milk yield while having high anti-establishment responses will result in protein loss. In addition, these results add to the growing belief that faecal egg count is not the best indicator of the intensity of nematode infection.

3.5 Corollary

Although some results are statistically significant or borderline significant, a problem was identified with the data after the analysis had been finished. This was that the parasitological data and lactation data do not completely correspond. The parasitological data corresponds to sampling of animals between October 1999 and September 2003 at the beginning of the experiment and 2 months afterwards. The lactation data, however, was the average for all the lactations of each animal, rather than only for the year when the animal was sampled for the parasitological data. This mismatch between data means that these results are only valid under the assumption that the one-year (2 month) parasitological data in each animal is representative of parasitological data that could have been obtained across all the years of

lactation.

The data used had also been previously corrected and it is unclear the effects this corrections could have in our results. For example, the milk production data was corrected for the week of lactation, and this is important in ranking the animals for their genetic merit, however, it can obscure the interpretation or reduce the effect with parasitism due to the fact that a peri-parturient ewe will have a weaker immune response and could be more infected.

The presence of significant relationships suggests that such an assumption may be valid. Consequently the results may be conservative and stronger relationships may be observed in more closely corresponding data. Unfortunately, it was not possible to obtain the necessary data from my collaborators.

Chapter 4

Use of a zero-inflated model to measure low levels of infection

In this study, two traits related with the resistance to gastrointestinal nematodes (GIN) were measured in 529 adult sheep: faecal egg count (FEC) and serum levels of immunoglobulin A (IgA). Based on the observed high number of animals with zero FEC values (64%), a novel approach was developed to distinguish between not recently infected sheep and infected sheep at low levels. A zero inflated negative binomial model (ZINB) model was used to calculate the extent of zero inflation for the FEC trait and it was extended to include information from the IgA responses. In this dataset, the extended ZINB model suggested that 40% of the sampled sheep were not exposed to / had not been recently infected with GIN. For the animals considered as exposed to the infection, the correlations among the studied traits were calculated, and how they influence the discrimination between unexposed and infected animals. These correlations will be useful in the development of a reliable index of GIN resistance that could be of assistance for the study of host resistance in naturally infection-based studies and also the design of breeding programs aimed at achieving resistance to parasites. Further research is needed to extend the use of this model in other species and systems.

The work in this chapter is a collaboration with the Department of Animal Production of the university of León, Spain.

4.1 Introduction

Infection by gastrointestinal nematodes (GIN) is common in ruminants worldwide, causing major economic losses due to decreased growth and milk production. Grazing ruminants are infected by a variety of species of GIN with different pathogenicities and geographical

distributions [148].

The control of GIN in ruminants is largely based on the use of anthelmintics, combined with grazing management strategies. However, anthelmintic resistance has appeared worldwide [11, 149]. In Northwest (NW) Spain, a recent survey showed that the GIN in 63.6% of the sampled flocks were resistant to at least one of the most commonly used drugs [150]. The increasing prevalence of anthelmintic resistance has led to the search for alternative control methods, such as selective breeding for resistance to GIN. However, for this purpose, the identification of an appropriate method to measure resistance to infection is necessary, especially in conditions where the worm burden is low. Hence, a sensitive method for detecting infections is needed.

Faecal egg counts (FEC) have been the traditional indicator trait used to assess the level of infection, based on the number of eggs per gram (epg) of faeces, and it is related to both the worm burden and the fecundity of female adults in the host [122, 141]. Faecal egg counts have been used to measure genetic resistance to GINs, with zero FEC associated with resistance, although in natural infections they can be quite variable both within and between populations [151]. However, FEC are not particularly sensitive and should be interpreted in conjunction with information about the nutritional status, age and management of sheep flocks [152]. Moreover, FEC can be influenced by variation in the excretion of eggs by adult worms [153]. As adult grazing sheep are in general more resistant than naïve young animals because they have been exposed for longer, their FECs tend to be lower, adding an additional limitation to the sensitivity problem of the technique.

Other phenotypes related to GIN infections, such as the levels of IgA in serum may be taken into account with the goal of defining resistant animals under natural conditions. IgA is a secreted antibody that plays a major role in gut infections. Animals that display high IgA activity have been shown to present lower FEC and shorter adult female *Teladorsagia circumcincta* among experimentally infected sheep [66, 122, 123].

The distribution of FEC in naturally infected populations is characteristically over-dispersed within domestic and wild animals [154, 155], as well as in human populations [156]. The negative binomial distribution has been widely used to describe parasite eggs distribution. However, when there are more zero FEC values than expected, zero-inflated negative binomial (ZINB) models are more appropriate [154, 157]. A zero-inflated distribution is a mixture of two distributions and can arise if some animals with zero egg counts have been exposed and are resistant to the infection while other animals with zero egg counts have not been exposed or recently infected i.e. no established worms since the last anthelmintic treatment. Resistant animals tend to have small number of parasite eggs in their faeces. Due to the McMaster measurement technique, small egg numbers are difficult to detect and will be counted as zero, whether the animal has really zero eggs or just a small number of them. It

was hypothesized that by exploiting additional information, as that provided by other indicators of parasite resistance such as IgA, animals with low level of infection with zero egg counts could be discriminated from unexposed / recently uninfected animals. Therefore, the objective of the study was to determine the real prevalence of GIN infections in naturally infected adult sheep showing a low levels of infection by combining information from the two widely used indicator traits previously mentioned (FEC and IgA). For this purpose, we applied a ZINB model and extended it to include data from IgA responses with the aim of discriminating resistant animals, infected animals with zero FEC, and animals that have not been recently exposed to infection. For the subset of animals that were considered as exposed to the infection based on the ZINB model, we calculated the correlations among the two indicator traits related to the infection by GIN (FEC, IgA) and the hidden variable of animal status (i.e. the parameter that determines if the animal has been recently infected or not). The aim was to validate utility of the ZINB model in animals naturally infected at low levels of infection.

4.2 Materials and methods

4.2.1 Study area and animal sampling

The study was carried out in the region of Castilla y León, in the NW of Spain, and included 17 commercial dairy flocks distributed in seven out of the nine provinces of the region (Burgos, León, Palencia, Segovia, Valladolid, Salamanca and Zamora) (Figure 4.1). In the study area, the flocks are reared under a semi-extensive system in which sheep graze on natural pasture for six hours per day and are kept indoors the rest of the day. The average size of the sampled flocks was around 912, ranging from 302 to 2121 animals per flock.

The survey was conducted from December 2011 to June 2012. From a climatic point of view, this period was extremely dry, as can be observed in Table C.1 (Appendix C). Two conditions had to be met to include a flock in the study: first, the last anthelmintic treatment must have been administered at least two months before collecting the samples, and second, the sheep had to be grazing at the time of sampling. The animals included in this study were ewes obtained by artificial insemination from farms belonging to the Selection Nucleus of the National Association of Churra Breeders (ANCHE) and were a subset of those previous genotyped with the *Illumina* OvineSNP50 BeadChip which were still alive during the sampling period and for which for both phenotypes related to parasite resistance were available. Faecal samples were collected for each ewe directly from the rectum and blood samples were obtained by venipuncture of the jugular vein. Serum samples were stored at -20 °C until processing.

Figure 4.1: Map of the region of Castilla y León (Spain). The black dots indicate the location of the sampled flocks.

Therefore this study is based on 529 adult Churra sheep with faecal and blood serum samples available, with a mean of 31 animals sampled per flock (range: $11-60$ individuals). The age of the sheep included in the study varied between 4 and 11 years. All of the sheep were undergoing milking at the time of sampling and were experiencing at least their third lactation.

4.2.2 Parasitological measures

A modified McMaster technique using zinc sulphate as a flotation solution was used to determine the number of eggs in faeces. The minimum detection limit of this technique was 15 epg, i.e. faecal egg counts were determined by multiplying the raw egg counts (Neggs, numbers of eggs observed microscopically) by 15.

In each flock, pooled faeces were cultured to recover and identify third-stage larvae (L3) following standard parasitological techniques [158]. A total of 100 L3 were identified per flock to estimate the percentage of each species.

4.2.3 Titre of IgA

An indirect ELISA was carried out to determine the optical density (OD) of IgA in the serum. The preparation of somatic antigen from fourth-stage larvae (L4) of *T. circumcincta* was conducted as previously described by Strain and Stear [159]. Microtitre plates (Sigma)

were coated with 100 μl of PBS containing 2.5 μg/ml of *T. circumcincta* L4 somatic antigen, after which the plates were stored overnight at 4 °C. After discarding their contents, the plates were blocked with 250 μl of PT-Milk (4 g powdered milk + 100 ml PBS-Tween; PBS-Tween: 1 L PBS pH 7.4 + 1 ml Tween) for 30 min at 37 °C. Then, the blocking buffer was discarded, and 100 μl of serum was added, followed by incubation for 30 min at 37 °C. After washing the plates four times with PBS-Tween, 100 μl of a rabbit anti-sheep IgA antibody, conjugated to horseradish peroxidase (Serotec), at a dilution of $1/500$ in PT-Milk, was added, followed by incubation for 30 min at 37 °C. The plates were then washed again four times with PBS-Tween and subsequently incubated in a peroxidase substrate and tetramethylbenzidine solution to produce a colour reaction, which was stopped by the addition of 50 μl of 2 M H_2SO_4. Finally, the absorbance was measured at 450 nm in a microplate reader (Titertek Multiskan). Positive and negative controls were included in every plate. Positive control was obtained from a pool of serums of experimentally infected sheep with *T. circumcincta* and negative control of non-infected sheep that were placed indoors. The results were expressed as the optical density ratio (ODR):

$$\text{ODR} = \frac{\text{sample OD} - \text{negative OD}}{\text{positive OD} - \text{negative OD}} \tag{4.1}$$

4.2.4 Descriptive statistics

Descriptive statistical analysis for the two raw phenotype traits was conducted for the 529 sampled animals with the "`pastecs`" package in R [160]. The Shapiro-Wilk test was carried out to determine if the data for each trait was normally distributed. Due to the large number of zero counts in the FEC data and the fact that the animals graze during short periods of time and the low pasture contamination, we decided to use a zero-inflated negative binomial model to estimate the zero-inflation parameter and later on extended it to discriminate between exposed and unexposed animals. The zero inflated model with IgA data was compared to a simpler negative binomial model using a likelihood ratio test. Moreover, it is a biologically meaningful description of the system, with animals, that due to the low levels of infection in the field and low grazing periods, will not be infected at the time of sampling, and were not infected, at least since the last anthelmintic treatment. The zero inflated model also allows for a more natural extension into discriminating between infected and uninfected animals.

Estimation of zero-inflation

In the zero inflated model, positive FEC are derived from a negative binomial distribution, while a zero count can arise from either the negative binomial distribution or the zero dis-

tribution. The probability of belonging to the zero distribution is called the zero-inflation parameter. The animals that have zero counts arising from the zero distribution are assumed to not have been recently infected (i.e. have not been infected since the last anthelmintic treatment), so these animals can be excluded from further analysis. A Markov Chain Monte Carlo model similar to the one described in Denwood *et al.* [154] using the "`runjags`" package [161] was employed to estimate the zero-inflation parameter.

In this model, the negative binomial distribution arises from a gamma-Poisson mixture distribution. Uninformative priors were used for the parameters of the gamma distribution. The posterior distribution of the zero-inflation parameter is shown in Appendix A.

Extending the ZINB model

A zero-inflation model does not determine which animals are exposed and resistant (as opposed to unexposed). The classical ZINB model is therefore extended to accommodate IgA data as additional information for the animal status, i.e. infected or not recently infected. The animal status is calculated as,

$$\text{Status} = \begin{cases} 0; & \text{not recently infected} \quad \text{with probability } 1-P, \\ 1; & \text{infected} \quad \text{with probability } P \end{cases} \tag{4.2}$$

where P is the probability of being recently exposed and is equivalent to one minus the zero-inflation parameter. The raw egg counts (FEC$/15$) were used and it is assumed that for each animal *i*, the number of eggs counted arises from the following,

$$Neggs_i \sim \begin{cases} 0 & \text{if Status} = 0, \\ \text{Poisson}(\lambda_i) & \text{if Status} = 1 \end{cases} \tag{4.3}$$

where λ_i is the number of eggs arising from the gamma distribution (Equation 4.4).

$$\lambda_i \sim \text{gamma}\left(\text{shape}, \text{rate}\right) \tag{4.4}$$

with the *shape* and the *rate* parameters of the gamma being calculated by the model. Similarly the IgA data can be partitioned in 2 gamma distributions (Equation 4.5) based on the animal status.

$$\text{IgA}_i \sim \begin{cases} \text{gamma}\left(\text{sh}_1, \text{rt}_1\right) & \text{if Status} = 0, \\ \text{gamma}\left(\text{sh}_2, \text{rt}_2\right) & \text{if Status} = 1 \end{cases} \tag{4.5}$$

with sh_1,sh_2,rt_1 and rt_2 being the two shapes and two rates respectively that parametrize the two gamma distributions. In the model, samples are drawn for sh_1 and sh_2 as well as for mn_1

and mn_2, which are the two means of the two gamma distributions. The rates are calculated by rate $=$ shape$/$mean and the mean for the animals not recently infected (mn_1) is always smaller than then mean of the infected (mn_2). The fully commented R code of the model is given in Appendix B.

$50,000$ iterations were sampled, with the first $5,000$ being discarded (burn in), and assessed convergence with the Gelman-Rubin statistic from the "`coda`" package [162] being under 1.05.

Using the realisations of the animal status across the iterations (unexposed animals have status = 0, exposed and infected have status = 1), it is possible to calculate the probability for each animal to be in one status or the other, P_i^{exp}, animals without zero FEC will always be in the infected status. The animals that were estimated to be unexposed, i.e. the animals with status = 0, in each sample of the Markov Chain were excluded from further analyses, allowing the use of simple statistical tools to analyse the remaining dataset for each sample.

Correlations between phenotypes

Considering FEC, IgA and the realisations of animal status, P_i^{exp}, the Kendall's rank correlation coefficient was used to estimate the relationships among these three parameters. We used Kendall's rank because it is the most appropriate non-parametric hypothesis test to analyse our dataset. Correlations were calculated in R [131] for each sample of the Markov Chain and the average across the samples is reported below.

4.3 Results

4.3.1 Descriptive statistics of the raw phenotypic data

Faecal egg counts and larval identification

Faecal egg counts of GIN ranged from 0 to $1,290$ epg. In 64% of the faecal samples no eggs were detected. The FEC mean and total variance were 38.2 ($\pm$ 105.9) and $11,218.9$ respectively. Hence, the FEC distribution was heavily skewed to the right and showed a high level of over-dispersion (Figure 4.2a). The Shapiro-Wilk test for the raw FEC data indicated a clear deviation from normality ($P < 2.2 \times 10^{-16}$). Most of the eggs detected in positive samples were strongyle-type.

In the studied farms, apart from the GIN eggs that were described previously, other parasite eggs were detected in faeces: 13.3% of the sheep sampled had *D. dendriticum* eggs, with a

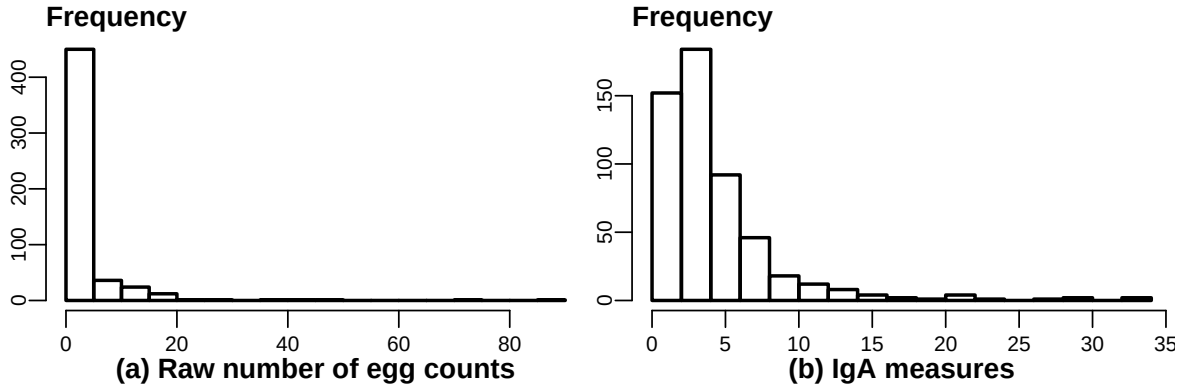

Figure 4.2: Distributions of (a) faecal egg counts and (b) plasma IgA of 529 Spanish adult Churra ewes.

range of $0-1,035$ epg, 2.9% had *Trichuris* eggs (range $0-30$ epg), 0.9% had *Moniezia spp*. eggs (range $0-1,035$ epg) and one single ewe had 15 *Capillaria spp* eggs.

After collecting L3 from coprocultures, the following genera of GIN were identified: *Trichostrongylus spp*. (49.3%), *Teladorsagia spp*. (48.6%), *Nematodirus spp*. (1.4%) and *Cooperia spp*. (0.7%). In all flocks, the presence of *T. circumcincta* was confirmed. A number of lungworm larvae were also observed, though they were not identified to the species level.

IgA activity in the serum samples

IgA activity against somatic antigen from *T. circumcincta* L4 was measured via an indirect ELISA and expressed as the ODR. For individual animals, the mean ODR was 4.2 ($\pm$ 4.3), showing a range between 0.09 and 32.9. The ODR activity total variance was 18.4. The distribution of IgA activities was positively skewed (Figure 4.2b) with most of the sheep displaying relatively low IgA values, and only a few sheep presenting particularly high levels of IgA. The Shapiro-Wilk test indicated a clear deviation from the normality (p-value $<$ 2.2×10^{-16}). Kolmogorov-Smirnov test indicated that the IgA was not gamma distributed (p-value $= 0.0088$), however this is due to the long tail of high IgA values, if the analysis is done with 10 animals less (effectively cutting the max IgA values to 20), the test indicates that the data is indeed gamma distributed (p-value < 0.21).

4.3.2 Zero-inflation parameter and extension of the ZINB model for FEC data

To verify that the data is zero inflated, a likelihood ratio test was performed comparing the ZINB model to a simpler NB model, with a p-value of the likelihood ratio test $= 6.62 \cdot 10^{-5}$,

which indicates that the zero-inflated model is a better fit to the data. The mean of the zero-inflation parameter was 0.38, this indicates that on average, 38% of all the animals were not exposed and infected since the last anthelmintic treatment (1 month before the samples were taken), therefore it was estimated that 328 ewes were infected at sampling, even though only 190 had non-zero FEC. The zero-inflation parameter credible interval was much narrower when using the extended ZINB model as opposed to the ZINB model using FEC data only (from $0.013-0.46$ to $0.25-0.49$). The distribution of the probability of being exposed across all the animals in the data can be seen in Figure 4.3.

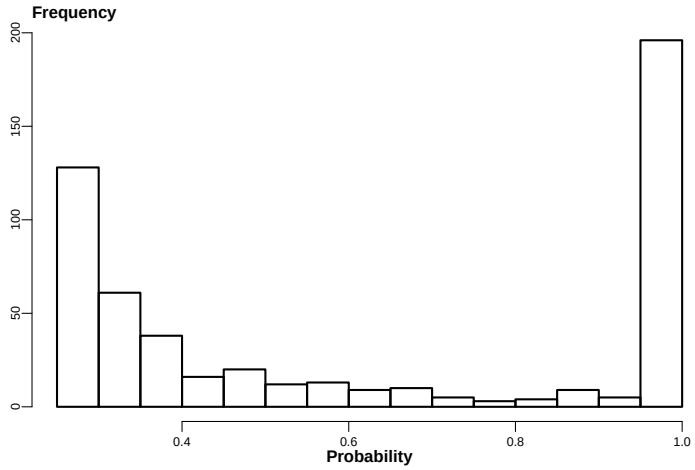

Figure 4.3: Histogram of the probability of being exposed, P_i^{exp}, for the 529 animals sampled, which is calculated from the realisations of animal status (unexposed vs exposed).

4.3.3 Associations between phenotypes

The associations between phenotypes was calculated for the subset of animals that were considered exposed to the infection based on the implementation of the ZINB model (status $= 1$) in each sample of the Markov Chain. The correlations between Neggs, IgA and the estimated probability of being exposed to infection (P_i^{exp}) are shown in Table 4.1. The phenotypic correlation between plasma IgA and number of eggs was close to zero and not statistically significant, while animal status was positively correlated to the number of eggs and IgA.

4.4 Discussion

Adult female sheep play a key role in the epidemiology of gastrointestinal nematode infections because eggs deposited during the periparturient period influence the severity of the infection during the grazing season. However, outside the periparturient period, egg counts

Table 4.1: Correlations estimated between number of eggs in the faeces, Neggs, IgA activity and the estimated probability of being exposed (P_i^{exp}) in the Churra sheep population studied in this work.

	Neggs	IgA	P_i^{exp}
Neggs	1	0.012	0.67**
IgA		1	0.18**
P_i^{exp}			1

$^{**}P < 0.001$; $^{*}P < 0.015$

in adult sheep are typically low [163]. In general, GIN populations in naturally infected sheep are usually over-dispersed, with the majority of sheep showing low epg values and only a few sheep presenting a high level of infection [164]. Therefore, supplementary information is needed to the egg counts to evaluate the prevalence of infection in adult sheep flocks.

In this study, the GIN infection level observed, based on the mean FEC per flock was quite low (38.2 epg) compared with other studies carried out in the same area (NW of Spain). Gutiérrez Gil *et al.* [142] reported that the mean FEC was 260 epg between the years 1999 and 2003. Similar records were described by Martínez-Valladares *et al.* [165], who showed that the prevalence of GINs, based solely on the presence or absence of FEC, in sheep flocks was 100%, and the mean epg was 237.2 ($\pm$ 375.9) between the years 2006 and 2011. In the current study, the low levels of infection are likely a consequence of the exceptional climatic conditions during this study since the longevity of infective L3 of *trichostrongylid* nematode parasites of ruminants is related to temperature and humidity [166, 165]. Table C.1 in Appendix C displays the mean temperature and precipitation for the period between December-June of the last five years ($2007/2008 - 2011/2012$) in the region of Castilla y León, highlighting the fact that the year $2011/2012$ was extremely dry. According to Martínez-Valladares *et al.* [165], there is a direct relationship between GIN infection levels and the humidity of ambient air.

Faecal egg count, which has been for many years the traditional diagnostic tool for assessing GIN infection, has a low sensitivity, especially for very low counts as it is the case of this study [141]. Therefore, when the excretion of eggs in faeces is low, it is necessary to use other, more sensitive, diagnostic methods that might provide an efficient indicator measure of infection.

IgA levels in the current study are moderately high, and this is presumed to be due to the fact that the antibodies are maintained at high levels for some time after GIN infections. The experimental studies of different breeds of sheep infected with GIN showed a sustained higher IgA level post infection for prolonged periods of time. In an experiment carried out by

Henderson and Stear [124], the peak of IgA was at $6-10$ days after a deliberate infection with *T. circumcincta* in sheep although detectable IgA was evident six weeks later. Furthermore in an experiment with Churra sheep, Martínez-Valladares *et al*. [167] also showed that the elevated level of IgA in blood and nasal secretions was maintained four weeks post infection with this same parasite species.

In this study, a ZINB model is used to calculate the extent of zero inflation. This approach has been applied to several parasitic infections [154, 156, 157]. This model was then extended to identify the animals that are likely to be uninfected. This was done by adding the IgA information to the model. In a ZINB model using only FEC data, all animals with zero FEC have very similar probability of being in the "infected" status, which is the same as the zero-inflation parameter (Appendix D), as opposed to the distribution shown in Figure 4.3. To my knowledge, this is the first description of using a ZINB model for the analysis of these traits with the aim of discerning which animals are infected and which have not been recently exposed. This procedure is relatively straightforward and allows the study of nematode infections in adult animals and in flocks with low prevalence of infection, such as in Mediterranean dairy farms where animals are under a semi-extensive management system. The approach improves our ability to identify resistant animals, those that have been infected but show very low FEC, which is needed for the breeding of resistant sheep and the study of host resistance in naturally infected individuals.

Because the over-dispersion pattern of GIN (number of eggs and adult worms found in the host) is also observed in other hosts such as cattle, free-range pigs, chickens, humans and wild animals, the approach described here could also be useful in other systems.

The correlations between the number of eggs and IgA and animal status were calculated using the non-parametric Kendall's test. Although the number of eggs has been found negatively correlated with IgA in young lambs [168, 169], in the case of adult sheep, this correlation is not as clear and both Coltman *et al*. [168] and Gutiérrez Gil *et al*. [142] reported positive but non-significant correlations in naturally infected adult sheep after comparing logFEC and IgA against somatic antigen from *T. circumcinta* L4. The results of this chapter are similar and suggest that this correlation is indeed close to zero in adult sheep, although if the data were analysed without the ZINB model, this correlation would not have been found (Appendix E).

According to the results of all of these studies, the correlations among the phenotypes studied in this work appear to depend on the level of infection, the time of sampling and the age of animals. Mugambi *et al*. [170] observed that sheep of the Red Maasai breed, which is resistant to *Haemonchus contortus* infections, did not respond to an experimental challenge in the same way as they responded to natural infections. Moreover, other factors such as nutrition can influence on immune response. In this sense, Martínez-Valladares *et al*. [167]

reported significant differences in IgA titre between resistant and susceptible Churra sheep to the infection by *T. circumcincta* although these differences were not maintained when sheep were fed a low protein diet. Consequently, increasing the knowledge of the host immune response under field conditions is an essential issue for the development of strategies to control GIN infections. Hence, the present study provides a valuable survey of indicator traits for GIN infections measured in naturally infected adult sheep and under extreme dry autumn-winter climate conditions, which may be a quite frequent situation in Southern regions of Europe.

The extension of the ZINB model has allowed the combination of information from two different traits that indicate resistance or susceptibility to GINs. The IgA response was added to the model to help discriminate between unexposed and infected animals zero FEC. Further research is necessary to create a reliable index of the intensity of nematode infection, which will take into account the observed correlations among the parasitological variables. As mentioned previously, the use of a reliable indicator trait may be of interest not only for the management of parasite infections but also for the design of breeding programs aimed at achieving resistance to parasites.

4.5 Conclusions

In the current study, two different phenotypes related to GIN infection (FEC and IgA against somatic antigen from L4 of *T. circumcincta*) were analysed. There was a high percentage of sheep without eggs in faeces (64%) and a zero inflated model was used to detect the amount of zero inflation in the data. It was found that 38% of sampled sheep had not been exposed to nematode infection in the last month, since the last anthelmintic treatment. In each sample of the Markov Chain, the animals that had a non-recently exposed status were removed from the subsequent data analysis. A correlation close to zero was obtained between FEC and IgA, and a positive correlation was obtained between IgA and P_i^{exp}. Therefore, in addition to FEC data, the evaluation of the level of IgA in serum may be a useful method in the context of the control of GIN infections in flocks of adult animals with low level of infection and for selective breeding aimed at achieving resistance to GINs.

Chapter 5

An explicit immunogenetic model of worm infection

Gastrointestinal nematodes (GIN) are a global cause of disease and death in humans, wildlife and livestock. Livestock infections have been historically controlled with anthelmintic drugs, but the emergence of resistance requires the use of alternative control methods. The most promising alternatives are vaccination, nutritional supplementation and selective breeding, all of which act by enhancing the immune response. Currently, control planning is hampered by reliance on the faecal egg count (FEC), which suffers from low accuracy and a nonlinear and indirect relationship with infection intensity and host immune responses. This gap is addressed by using extensive parasitological, immunological and genetic data on the sheep − *T. circumcincta* interaction to create an immunologically explicit model of infection dynamics in a sheep flock that links host genetic variation with variation in the two key immune responses to predict the observed parasitological measures. Using the model, it is shown that the immune responses are highly heritable and by comparing selective breeding based on low faecal egg counts versus high plasma IgA responses it can be concluded that the immune markers are a much-improved measure of host resistance. In summary, a model of host-parasite infections has been created, that explicitly captures the development of the adaptive immune response, and by integrating genetic, immunological and parasitological understanding new immune-based markers for diagnosis and control can be identified.

5.1 Introduction

Gastrointestinal nematode infection is one of the major diseases affecting small ruminants [7, 171]. Different nematodes cause different pathologies. In cool temperate climates such as the UK, the predominant nematode in sheep is *Teladorsagia circumcincta*. This nematode causes

a relative protein deficiency [8] which affects growth and production and, in extreme cases, can kill the host. Economically efficient and welfare friendly sheep husbandry therefore requires the control of these parasites. Historically, nematode infections have been controlled at least partly by anthelmintic treatment, but the evolution of resistance to drug treatment [11, 12] means that alternative methods of parasite control are urgently needed.

Mathematical models have been extensively used to gain insight into the dynamics of host-parasite interactions in humans, wildlife and livestock, and to help identify effective control measures [94, 95, 96, 97]. Since the review by Smith and Grenfell [77], the dynamics of gastrointestinal parasites of ruminants have received considerable modelling attention. The models developed, which have been reviewed elsewhere [80, 82], range in complexity from relatively simple phenomenological models [89, 98, 99] to detailed models which capture the multiple stages of the parasite life cycle within and outwith the host, allowing effects such as temperature, climate, grazing behaviour, nutrition and management to be incorporated [77, 92, 100, 101, 102]. The focus of these models has encompassed the use of grazing management as a control measure [103, 104], the impact of drenching regimes [101], the generation and spread of anthelminthic resistance [92, 105, 106, 107, 108, 109], selective breeding for disease resistance [112] and the implementation of targeted or strategic treatments [110] including the unexpected prediction that estimated breeding values for faecal egg counts derived from pedigrees were less effective tools than the original data [172].

Two important themes that recur in these modelling studies are the aggregation of infection loads and the acquisition of immunity by the host. Some studies have characterised the observed aggregation of parasite burdens across numerous host-parasite systems [113, 114, 115, 116], whilst mathematical modelling has been used to investigate the mechanisms and consequences of aggregation [85, 116, 118, 119, 120, 173]. The studies by Cornell *et al.* [87] and Grenfell *et al.* [118] suggest that much of the observed variation in parasite burden between hosts is attributable to some form of host heterogeneity.

Although the importance of acquired immunity has long been recognized, there are few models addressing in detail the immunoepidemiology of farmed ruminants. Host immunity has been assumed to increase over time following exposure to infective larvae, and to reduce the establishment, fecundity and survival of adult parasites [89, 174]. However, Roberts [88] identifies a need to move beyond the common phenomenological approaches to host immunity in host-nematode models in order to facilitate the integration of epidemiological models with data from immunological studies. Hellriegel [81] and Stear *et al.* [163] also issued calls for the greater integration of immunology, parasitology, genetics, epidemiology, mathematical modelling and statistics in host-parasite models.

T. circumcincta infection in sheep is one of the best understood of all host-parasite interactions, where detailed investigations have led to a much clearer understanding of the de-

velopment of acquired immunity and the mechanisms involved in within-host regulation of parasite burden, length and fecundity [66, 175, 176]. Previous analyses show that there are two components to the host response in sheep. Immunity is acquired in response to exposure and develops in two stages, with lambs initially regulating worm growth and fecundity, and then worm number [67]. Immunoglobulin A (IgA) regulates worm growth and consequently fecundity as well as the numbers of eggs in utero [123, 124, 177]. The IgE (immunoglobulin E) response regulates larval establishment and therefore the number of worms in the host [66]. In addition, there is detailed understanding of the genetic basis for variation in resistance to *T. circumcincta* infection, ranging from quantification of heritabilities, to the identification of particular genes associated with resistance [163, 178]. This detailed understanding of the epidemiology, immunology and genetics underpinning the sheep – *T. circumcincta* interaction make it an ideal model system for the development of data-driven models, which capture and integrate information from these disciplines.

Here, an immunologically explicit model of infection dynamics in a sheep flock is created, that links host genetic variation with variation in the two key immune responses described above to predict observed parasitological measures. One important advantage of this model is that by capturing the mechanistic link between the immune response and parasitological variables, the model allows for identification of improved markers for diagnosis and control. First, the model is fitted to genetic, immunological and parasitological data using Approximate Bayesian Computation (ABC). Second, using the fitted model, faecal egg count are contrasted with an immune marker (plasma IgA) as a measure of host resistance by comparing selective breeding in which selection is based either on low faecal egg counts or on high plasma IgA activity.

5.2 Model outline

5.2.1 Overview of the sheep – *T. circumcinta* system

Briefly, *T. circumcinta* is a parasitic nematode that lives and reproduces as an adult in the abomasum (fourth stomach) of sheep. The worms lay eggs that are excreted with faeces onto pasture. The eggs hatch and after two larval stages (L1 and L2), they develop into infective L3 (stage 3 larvae). The L3 cannot develop further unless ingested by a potential host. Once inside the host, if they successfully establish, they moult to become L4 (stage 4 larvae) and subsequently progress to the adult stage (Figure 5.1).

The life cycle of the nematode inside and outside the host is reproduced in the model, which is based on a published model of immunity to *T. circumcinta* infection in lambs [93]. Here, this model is modified and extended to capture infection dynamics in a flock of sheep in

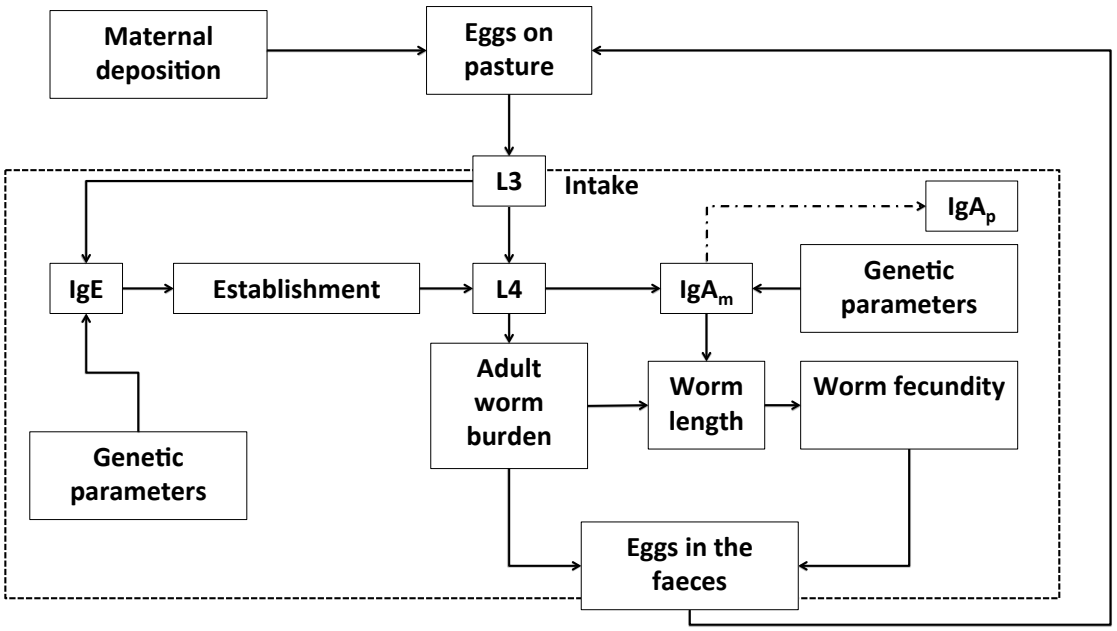

Figure 5.1: Model schematic. The region inside the dotted line represents the lifecycle within the host. Worms develop from egg to adults with the larval stages L3 and L4 being explicitly included in the model. The L3 and L4 larval stage each influence a different component of the immune response of the host and, at the same time, different genetic parameters control the intensity of the immune response resulting from exposure to L3 and L4. The number of adults, as well as IgA, affects the average worm length, which is the major determinant of worm fecundity. The number of worms and the average fecundity determines the number of eggs excreted in the faeces each day. This deposition adds to the current pasture contamination. Arrows indicate the direction of the effect.

which genetic variation between individuals underpins heterogeneous immune responses to infection. A complete description of this individual-based discrete time (daily time step) model is given in Appendix F, but the key details that define the integration of the immunology and genetics are outlined below.

An important feature that distinguishes this model from previous models is the explicit modelling of the protective mechanisms. Sheep control *T. circumcinta* infection through two key protective immune responses, which vary between hosts according to their genetic predisposition (Figure 5.1). Antibodies including IgGI, IgA and IgE are produced against all parasitic stages of gastrointestinal nematodes but protection is most strongly associated with IgE activity against L3 and IgA activity against L4. Larval establishment is controlled by the local IgE response, whilst worm fecundity is controlled by local IgA. As these two immune responses act upon different stages of the nematode lifecycle, the L3 and L4 stages as well as the adults are modelled explicitly. The ingestion of L3 triggers mast cell degranulation that prevents worms from establishing [66]. L3 that establish develop into L4 and IgA responses to L4, possibly in conjunction with eosinophils [124], influence worm size and consequently

worm fecundity [179]. IgA is modelled at two sites in the host: mucosal IgA (IgA_m), which is unobserved (other than at post-mortem) and represents local IgA at the site of infection and affects worm length; and plasma IgA (IgA_p), which represents the IgA that has migrated to the plasma and can be routinely measured in the bloodstream of live animals.

Worm fecundity is strongly correlated with the size of the worm; at the same time, worm size depends on the strength of the IgA response (specifically, IgA_m) and a density-dependent effect of the number of worms in the animal [66]. Worm number and worm fecundity determine the egg deposition onto pasture and subsequently the number of infective L3 larvae available to be ingested.

Infective larvae are ingested during grazing. The amount of herbage consumed depends upon the size of the animal. The growth of an animal during the course of the grazing season in the flock has been described in a standard manner by the Gompertz equation [93]. Rather than model herbage intake and larval intake separately, daily variation in larval intake among animals is modelled as a Poisson distribution with its parameter equal to the mean daily number of ingested L3 larvae. The mean number of ingested larvae increased concomitantly with lamb growth.

5.2.2 Genetic variation among lambs in immune responsiveness

Lambs differ in their capacity to mount the anti-establishment and anti-fecundity immune responses. This is captured by allowing parameters ρ_A and ρ_E, which determine the rates at which IgA_m and IgE respond to parasite exposure of the lamb, to vary across the population. These parameters are assumed to be normally distributed across the flock and comprise an additive genetic component and an environmental component, as follows (with i representing each of the two immune responses)

$$\rho_i = \rho_{\text{gen}_i} + \rho_{\text{env}_i} \tag{5.1}$$

Total phenotypic variation is conventionally divided into additive genetic, non-additive genetic and environmental components [180]. As the non-additive component does not affect the response to selection, it was subsumed into the environmental component. The additive genetic and environmental components are sampled from normal distributions, which for immune response can be written in the general form,

$$\begin{aligned} \rho_{\text{gen}_i} &\sim N\left(\mu_{\rho_i}, h^2_{\rho_i} \cdot \tau^2_{\rho_i}\right) \\ \rho_{\text{env}_i} &\sim N\left(\mu_{\rho_i}, (1 - h^2_{\rho_i}) \cdot \tau^2_{\rho_i}\right) \end{aligned} \tag{5.2}$$

such that the additive genetic component has mean μ_{ρ_i} and the variance is partitioned between the genetic and the environmental component; $h^2_{\rho_i}$ denotes the heritability of the trait, i.e. the proportion of the variance attributable to additive genetic effects [180]. These six parameters (μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$, $h^2_{\rho_E}$) parameterising the IgE and IgA_m mediated immune responses are the free parameters used to fit the model to the field data. The other parameters in the model have been described in the literature and are assigned appropriate values (see Appendix F for details).

These parameters link host genetic variation with variation across the population in response to infection and ultimately determine the observed parasitological variables. As discussed above, one component of the immune response is related to the generation of mucosal IgA (IgA_m), and is assumed to increase with rate ρ_A in proportion to the number of established L4 larvae, with a delay between exposure and initiation of an immune response of z days, and a half-life of τ days:

$$\text{IgA}_{m_t} = 0.5^{1/\tau} \cdot \text{IgA}_{m_{t-1}} + \rho_A \cdot L4_{t-z} \tag{5.3}$$

The fecundity of worms is defined as the number of eggs produced per adult female worm per day. It depends on worm length, which is determined by both worm burden (number) and IgA activity [66] as follows:

$$WL_t = \alpha - \beta \cdot \log_{10}(\text{IgA}_{m_t} + 1) - \gamma \cdot WB_t \tag{5.4}$$

where α is the intercept term in the regression model, giving the expected mean length of adult worms in absence of the immune response and density dependent effects. β and γ are the coefficients for the effect of the immune response and worm number respectively [66].

The numbers of eggs per worm on day t, Wf_t, as a function of worm length, was adapted from the published relationship [179]:

$$Wf_t = (\epsilon \cdot WL_t^{\omega} - 1) \cdot 500 \tag{5.5}$$

where the scaling by 500 accounts for the average weight of faeces (in grams) produced by lambs in this experiment to produce a fecundity in terms of eggs per worm per day.

The second component of the immune response controls the establishment of adult nematodes, which is strongly associated with mast cell degranulation and IgE activity [66]. The combined effect of these two responses are refered to as the establishment control factor (ECF). This is assumed to increase with rate ρ_E in proportion to the daily number of ingested L3 larvae, with a delay between exposure and initiation of an immune response of z days, and a half-life τ (measured in days).

$$ECF_t = 0.5^{1/\tau} \cdot ECF_{t-1} + \rho_E \cdot I_{t-z} \tag{5.6}$$

Under the assumption that establishment decreases over the grazing season as the immune system develops, an establishment equation is specified that reproduces field observations [93] summarised in a meta-analysis [181]. Establishment at time t is given by

$$E_t = (E_{\text{early}} - E_{\text{late}}) \cdot e^{-ECF_t} + E_{\text{late}} \tag{5.7}$$

where E_{early} is the establishment in naïve lambs while E_{late} is the minimum establishment.

5.2.3 Observation processes

The faecal egg count is a measure of the number of eggs in one gram of faeces. The McMaster technique counts the number of eggs in $1/50^{th}$ of a gram of faeces and is multipled by 50. Measurement error was simulated by assuming Poisson counting error and accounting for the scaling up by a factor of 50. The model could therefore be used to output both the true faecal egg count (i.e. without measurement error) and the predicted count with measurement error. Model fitting and selection were based on the measured, rather than the true faecal egg count.

Plasma IgA, denoted IgA_p, has been previously shown to depend on IgA_m and the worm burden, WB, which is the number of worms, at the site of infection [125]. A slightly improved fit to the data was found (Chapter 2) with the following function relating IgA_m and worm biomass (WM, the product of the worm number and the mean worm length), with plasma IgA:

$$\text{IgA}_p = \lambda_1 \cdot \text{IgA}_m + \lambda_2 \cdot \text{IgA}_m \cdot \log_{10}(WM) \tag{5.8}$$

The IgA in the mucus is acting against the parasites, while the IgA in the plasma is a spillover (Chapter 2). The advantage of modeling them separately is that IgA_p can be measured in live animals. IgE is modeled based on the meta-analysis of Gaba *et al.* [181]. Most local IgE is bound on the surface of mast cells and the relationship between plasma IgE and local IgE is not known. Therefore the modelling of plasma IgE was not attempted.

5.3 Selective breeding with alternative markers

The model can be used to compare different methods of parasite control such as grazing management, vaccination, nutritional supplementation and selective breeding, but here the

chosen focus is on selective breeding [15, 182]. Currently, faecal egg counts are the marker most widely used to assess the intensity and severity of gastrointestinal nematode infection, and these are also used in selective breeding schemes. However, they are not particularly useful for *T. circumcincta* infections, because density-dependent constraints on fecundity mean that heavily infected animals produce few eggs [141]. The use of faecal egg counts is therefore hampered by its nonlinear and indirect relationship with host immune responses, and compounded by difficulties in obtaining accurate measurements of them. A possible alternative is IgA, which affects worm size and fecundity [144, 169]. Here, the focus is on a selection scheme for reduced faecal egg counts and compare it with selection for high plasma IgA activity to see which of these markers gives a better overall reduction in the intensity of infection.

5.3.1 Reference scenario

The reference scenario is two selection schemes (selection on low faecal egg counts versus selection on high plasma IgA activity) run for 10 successive generations. For each year of selection, the model was used to simulate infection dynamics over the course of the grazing season, which started in early May and ended in September. The simulations ran for 140 days, updating daily, with simulated anthelminthic treatment every 28 days to match the timing of treatments that were administered to the animals in the field. A 100% effectiveness of the treatment is assumed, i.e. all adult and larval stages in the host of all gastrointestinal nematode species were killed. The model was based on data from a naturally infected flock [115]. This flock was treated with albendazole sulphoxide every 28 days from 4 to 24 weeks of age. Faecal egg count reduction tests were used every year to test drug efficacy and there was no evidence for resistance during the trial. As the model is stochastic, 100 repeats were run and the model outputs are taken to be the arithmetic means of the 100 repeats.

The initial flock in each repeat run of the model (i.e. generation 0) comprised 500 male and 500 female sheep with ages uniformly distributed between 1 and 3 years of age. The 500 female sheep were used to breed the next generation of 1000 lambs (500 male and 500 female), and were kept as a breeding flock of ewes that was updated every generation. As is common practice in sheep breeding, these ewes were not selected on performance. Each year, around one third of the ewes are assumed to leave the flock due to sale or mortality and replacement female sheep were picked at random from the flock of young sheep in that generation (one year of age).

Each year, 25 males were used for breeding. To avoid inbreeding, rams are often bought in from outside and are chosen to improve the flock. It is therefore assumed that each year the rams were unrelated to the ewes and conservatively assumed they had a distribution of resistance to infection similar to the current flock. In practice, in selective breeding, farmers

would buy rams from more resistant flocks. These rams were used to breed the first generation. In subsequent years of selective breeding, the rams used mimicked the distribution of resistance among the best male lambs in the existing flock. Rams were selected for either low faecal egg counts or for high plasma IgA responses, with the 25 best rams selected for breeding. If more than 25 rams had a zero faecal egg count, then 25 rams were chosen at random from this group. Each ram was mated to 20 ewes, resulting in each case in a twin male-female birth (1000 lambs in total).

5.3.2 Defining offspring parameters

To create a new generation of lambs, values for ρ_A (used in Equation 5.3) and ρ_E (used in Equation 5.6) for each new lamb were calculated. The additive genetic component (or breeding value) for each offspring is given by

$$\rho_{gen}^{\text{offspring}} = (\rho_{gen}^{\text{ram}} + \rho_{gen}^{\text{dam}})/2 + N(0, 0.5 \cdot h_\rho^2 \cdot \sigma_\rho^2) \tag{5.9}$$

i.e. it is simply the mean of the parental values plus a Mendelian sampling term [183]. The environmental component is as given by Equation 5.2.

5.3.3 Heritabilities

The heritabilities in the model were obtained by breeding one unselected generation of lambs and recording the parental values for each lamb. The heritability for a particular trait was then calculated by taking the ratio of the covariance of the parental mean and offspring values to the variance in the parental values.

$$h^2 = \frac{cov(\text{offspring}, \mu_{\text{parental}})}{var(\mu_{\text{parental}})} \tag{5.10}$$

As heritability calculations are typically based on normal distributions [183], simulated values such as plasma IgA and worm length, were normalized using a Box-Cox transformation [184] before calculating the covariances and variances.

5.3.4 The carryover effect

At the start of the season, the number of infective larvae on the pasture is largely determined by the deposition of worm eggs onto pasture by the ewes. As the flock improves through successive generations of selective breeding, the deposition by the replacement ewes will be less due to their increased resistance. This relative reduction in start of season deposition is

assumed to reflect the relative reduction in average faecal egg count. The deposition, S_{y+1}, for the following year therefore depends on the previous years deposition, S_y, via

$$S_{y+1} = \frac{n_{\text{ewes}} - r_{\text{ewes}_y}}{n_{\text{ewes}}} \cdot S_y + \frac{r_{\text{ewes}_y}}{n_{\text{ewes}}} \cdot S_r \tag{5.11}$$

where n_{ewes} and r_{ewes} are the total and the replaced number of ewes respectively, and S_r is the deposition of the replaced ewes which is calculated by scaling the initial deposition S_0 with the reduction seen in the faecal egg counts ($S_r = \frac{\text{FEC}_y}{\text{FEC}_0} \cdot S_0$), with FEC_y being the mean egg deposition in year y and FEC_0 is the mean egg deposition in the original generation.

This carryover effect captures the expected reduction in the initial larval availability in subsequent generations as the flock becomes more resistant. Simulations were run with and without this effect but unless stated otherwise, the results shown are for simulations with the carryover effect.

5.4 Field data and ABC model fitting

The field data used to fit the model are generated from a study based on five cohorts, each of 200 lambs, from a naturally infected commercial flock in southwest Strathclyde [115, 144]. The lambs were monitored monthly during their first grazing season (from mid April to late September) for plasma IgA and faecal egg counts, post-mortem analyses were performed late September and early October to obtain worm number and length. The parameters (μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$, $h^2_{\rho_E}$) namely the means, variances and heritabilities of the immune response factors are fitted to the field data. The values of all other model parameters have been extensively researched previously and were therefore determined from the literature as described in the Appendix F. Six summary statistics from the field data were used as target values for the fitting (Table 5.1), these values correspond to the average between the five years at the end of the grazing season (or post mortem) and have been extensively analyzed elsewhere [115]. The remaining field data were used to provide independent checks on the model fit (mean and variance of worm number).

An Approximate Bayesian Computation (ABC) Regression-Based Conditional Density Estimation algorithm [185, 186, 187] was used to fit the model. This assumes that the inference is conducted in a Bayesian framework, where given a set of data y (i.e. the summary statistics in Table 5.1), the objective is to determine the posterior distribution $p(\theta|y)$ of the parameter vector θ. In Bayesian inference, the posterior summarises all information about the parameters conditional on the data and the specification of the model (including any fixed parameters) and the prior distribution of unknown parameters $p(\theta)$. A common approach adopted here is that the prior assumes that the parameters are drawn from independent uniform dis-

Table 5.1: Summary statistics to be used as target model outputs taken from the 5th month of the grazing season for plasma IgA (IgA_p) and faecal egg count (FEC), and at post-mortem (6^{th} month) for worm length (WL).

Mean IgA_p	0.2
Mean $\log_{10}(\text{FEC}+1)$	1.85
Variance of IgA_p	0.027
Variance of $\log_{10}(\text{FEC}+1)$	0.88
Heritability (h^2) of IgA_p	0.027
Heritability (h^2) of WL	0.88

tributions whose ranges are given in Table 5.2. The priors are chosen to allow a range wider than that of the posterior.

Table 5.2: Ranges for the uniform prior distribution of the six parameters used to fit the model.

	μ $(\times 10^{-5})$	σ^2 $(\times 10^{-11})$	h^2
ρ_A	$1.5-1.9$	$6-20$	$0.5-1$
ρ_A	$1.2-1.6$	$1-6$	$0.4-1$

In the ABC algorithm, a so-called particle is defined as a set of values, one per parameter being fitted, so that each particle corresponds to a different value of the parameter vector θ. In this case, it contains the means and variances and heritabilities of the immune response factors $\theta = (\mu_{\rho_A}, \mu_{\rho_E}, \sigma^2_{\rho_A}, \sigma^2_{\rho_E}, h^2_{\rho_A}, h^2_{\rho_E})$. A different value for any of the parameters in θ corresponds to a distinct particle. The steps are as follows:

1. Given the unknown parameters θ and their prior distributions $p(\theta)$, M particles ($M = 100,000$ in this case) are generated by
   (a) Drawing the parameter values randomly from the prior $p(\theta)$ for each particle (range of the uniform distributions in Table 5.2).
   (b) Running the model for each particle.
2. Compute the empirical standard deviation, across the M particles, for each of the simulated model outputs.
3. Calculate the distance for each particle between the model and target outputs using the distance kernel as in Beaumont *et al.* [185].
4. Choose a tolerance or proportion of points accepted; in this case, 1000 accepted (1%) with the lowest distance.

5. Weight the accepted particles as in Beaumont *et al.* [185].

6. Correct the particles (i.e. adjust their position in parameter space) with the results from a weighted linear regression applied to the accepted particles as in Beaumont *et al.* [185].

7. These corrected particles with the weights obtained in step 5 are taken to be random draws from an approximation to the posterior distribution $p(\theta|y)$.

This ABC algorithm yields an approximate posterior distribution for each of the fitted parameters, (μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$, $h^2_{\rho_E}$) (Figure 5.2). The heritabilities and the variances are correlated, and therefore after the correction (step 6), some corrected values of the posterior might fall outside of the distribution of the prior. This happens because the correction makes linear assumptions about the effect of parameters, which are not valid very close to the boundaries set by the prior. This has to be minimized as much as possible, i.e. by decreasing the tolerance or using a wider range for the prior. However, for the heritabilities, the upper bound of the prior has to be 1. When the value of the draw of $\sigma^2_{\rho_A}$ and $h^2_{\rho_A}$ is high and the value of $h^2_{\rho_E}$ is small, the correction of $h^2_{\rho_A}$ could increase it to a value higher than 1. However, this is rare in the simulations and the effect of the values higher than 1 is very small. The values were checked, and the highest was 1.05. The mean of the heritabilities of ρ_A and ρ_E remains almost unchanged, independently of whether the values over 1 are kept (0.867 and 0.74), dropped (0.859 and 0.743) or are set to equal 1 (0.866 and 0.74).

Random draws from the corrected particles previously obtained are used in the model simulation. One draw is used for each of the repeats. The results presented in this chapter are the average of 100 repeats.

5.4.1 Assessing model fit

To assess the fit of the model and observe the distribution of faecal egg count and plasma IgA across the flock, the results of each repeat are pooled and averaged across the 100 repeats.

The model, as expected after the fitting, successfully reproduces the mean and the variance of the faecal egg count and plasma IgA. Moreover, the distributions for each of these observed quantities are also successfully reproduced (Figure 5.3a, 5.3b). The model fit was also investigated in variables the model was deliberately not fitted to. For the worm number, the estimated mean, variance and distribution obtained in the model are also similar to the field observations (Figure 5.3c). The heritability of the faecal egg counts was also calculated (as in Equation 5.10; value obtained 0.22) and although the model was not fitted to it, is in accordance with the field observation (0.2-0.3). These outcomes provide additional independent validation of the model.

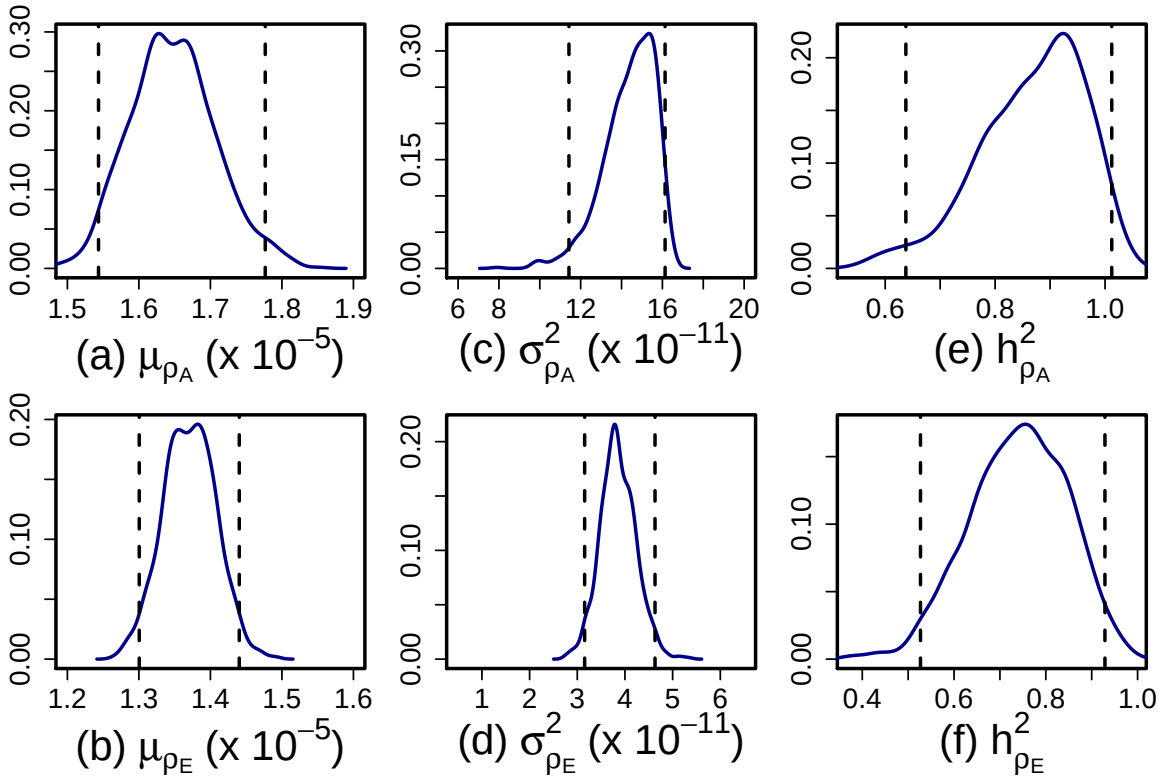

Figure 5.2: Approximate posterior distributions for the six fitted parameters, mean of ρ_A (a), and ρ_E (b), variance of ρ_A (c) and ρ_E (d) and heritability of ρ_A (e) and ρ_E (f). Vertical dashed lines indicate the 95% credible interval.

5.5 Results

In the reference scenario, model predictions are compared for selective breeding based on low faecal egg counts versus high plasma IgA. The breeders equation prediction is also included (Figure 5.4a), which is the expected response to selection estimated from the average difference between the whole parental generation and the subset of selected parents [180], Appendix G. In this case, it is the difference in average faecal egg count breeding values between all the male lambs and the subset of 25 selected rams.

Under each selection scenario, the mean faecal egg count across the flock is calculated at the end of each grazing season. The reduction in faecal egg count at the end of each grazing season based on selection for low faecal egg count was 1.7 times faster than is estimated by the breeders equation over 10 generations (Figure 5.4a, dotted line). A more rapid decrease in the mean flock faecal egg count is observed under selection for high plasma IgA responses (Figure 5.4a, light solid line). By the seventh generation, selection on plasma IgA achieved a drop in faecal egg count of almost 85% while selection based on faecal egg count achieved a reduction of approximately 50%.

The worm biomass is defined to be the product of worm number and average worm length

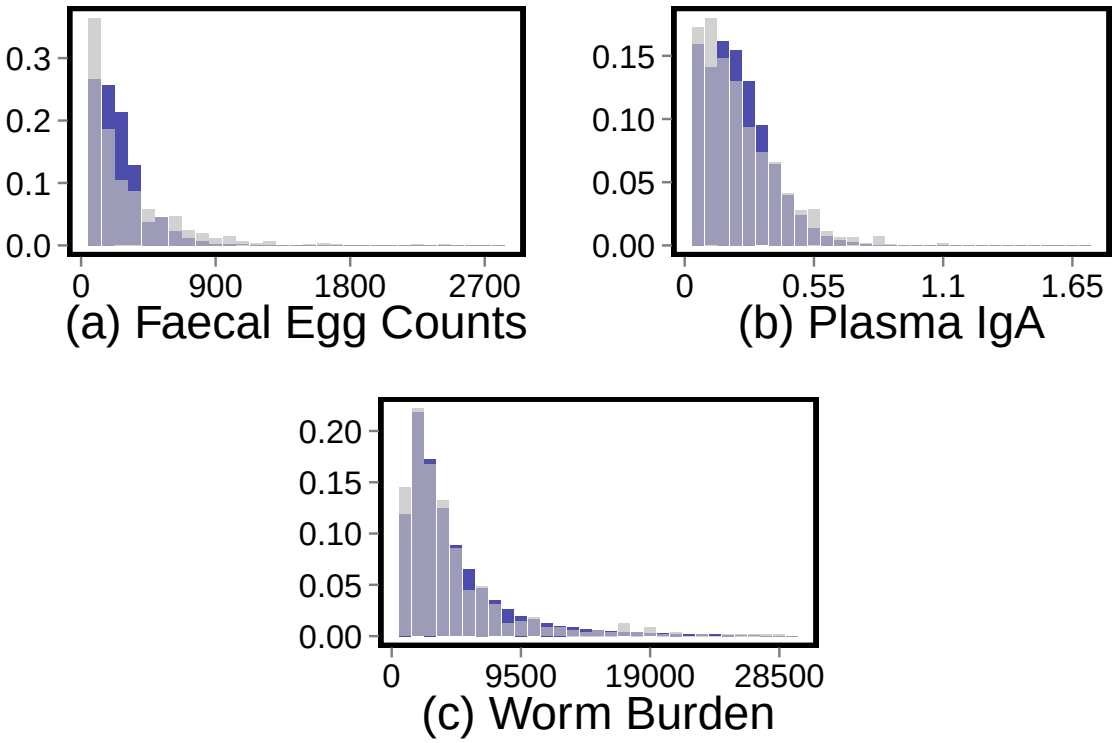

Figure 5.3: Comparison between field observations (light) and simulated values (dark) of (a) faecal egg counts, (b) plasma IgA, (c) worm burden (number). Intermediate colour is the overlap. To generate these distributions across the flock, the results of each repeat are pooled and averaged across the 100 repeats.

(Chapter 2). As this quantity accounts for the reported decrease in worm activity and fecundity in shorter worms [66], this is used as a measure of the intensity and pathology of infection. Worm biomass decreases by almost half after 10 generations of selection based on high plasma IgA activity whilst, when selecting on low faecal egg counts, the worm biomass slightly increases before starting to decrease (Figure 5.4b).

Under selection for low faecal egg counts, after the initial increase in worm biomass, running simulations for more than 10 generations show that values for worm biomass similar to those prior to selection are obtained after 15 generations of selection. However, it takes 50 generations of selection based on low faecal egg count to obtain similar values of worm biomass to the ones obtained after only 10 generations of selection on plasma IgA (Figure 5.4c in comparison with Figure 5.4b, light solid line).

5.6 Discussion

This chapter presents an immunologically explicit model of an important host-parasite system and links host genetic variation with variation in the two key immune responses, accu-

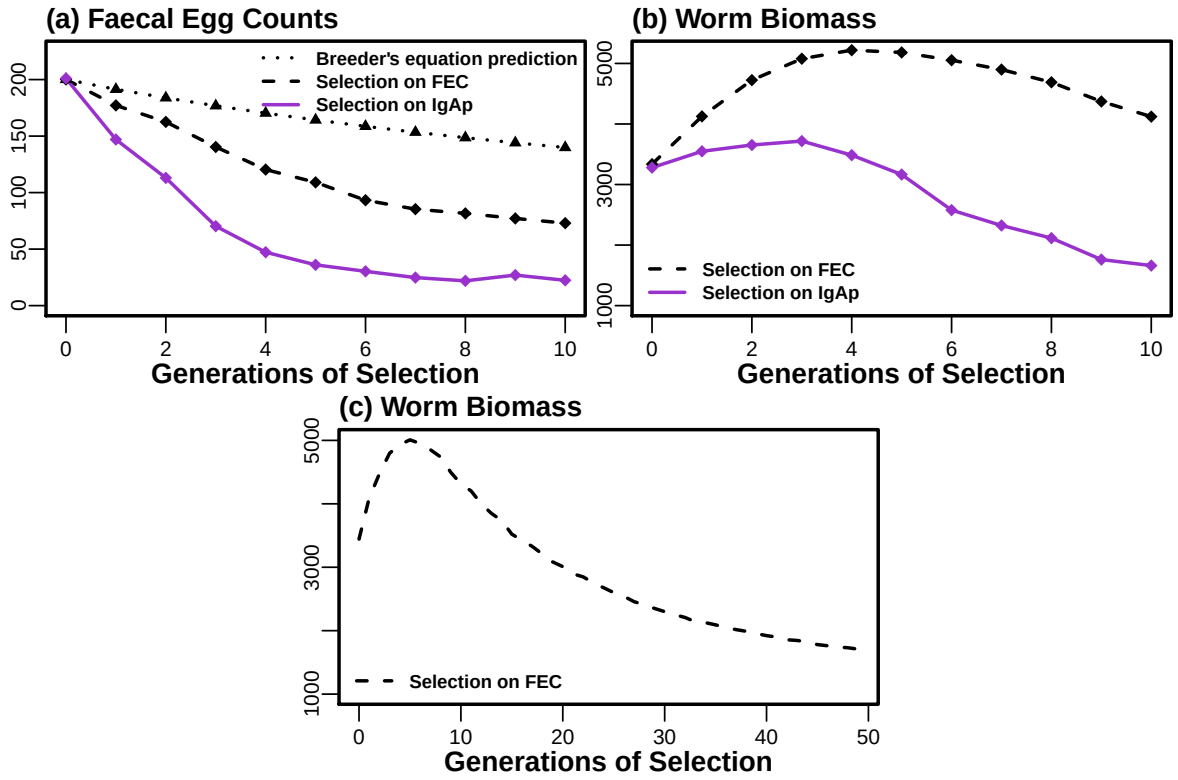

Figure 5.4: (a) Mean flock faecal egg count at the end of the grazing season over 10 generations of selection. The dotted line is the predicted response using the breeders equation; the dashed dark line shows the response to selection based on low faecal egg counts; the solid light line shows the response to selection based on high plasma IgA activity. (b) Mean flock worm biomass at the end of the grazing season over 10 generations of selection: selection based on low faecal egg count in dashed dark; selection based on high plasma IgA activity in solid light. (c) Average flock worm biomass at the end of the season for 50 generations of selection on low faecal egg count.

rately reproducing the means and distributions of parasitological and immunological observations. To my knowledge, this is the first data-driven model of the host-nematode interaction that combines the epidemiology, the genetics and the explicit development of the adaptive immune response. This model therefore represents an important step forward in host-parasite modelling and, moreover, provides a base tool for modelling GIN infections. In this chapter, the focus was on selective breeding schemes as a means of parasite control and tested novel markers for the efficient identification of resistant animals.

An important advantage of this model is connecting the underlying genetic variation with variation in the two protective components of the immune response across the host population to predict observed parasitological variables. By capturing the mechanistic link between the immune response and parasitological variables, the model allows the identification of alternative markers for diagnosis and control. Novel markers could offer substantial improvements over the widely used faecal egg count, which suffers from substantial measurement error, and is only indirectly and nonlinearly related to the host immune response. Specif-

ically, the hypothesis was that the IgA response would provide a better marker than faecal egg count, because IgA activity directly affects worm length and fecundity and therefore faecal egg count, but is subject to less observation error than the faecal egg count. To test this hypothesis, the outcome of selection schemes based on selection for low faecal egg count versus selection for high plasma IgA activity were compared.

The estimated response to selection based on low faecal egg counts is much faster than that predicted by the breeders equation, which is typically used to predict the response to selection for quantitative traits when there is no change in the environment during selection. These results are in accordance to the ones presented in Bishop and Stear [112], although their predicted end of season average faecal egg count was much higher as the flock was initiated with a higher mean infection load. The model predictions are consistent with independent field observations: Karlsson and Greeff [54] calculated in their Rylington Merino flock a genetic reduction of 2.7% in faecal egg counts per year in their selection scheme based on both production traits and faecal egg counts. In this model, the predicted response rate was a comparable average reduction in faecal egg counts of 4.2% per year for selection based solely on faecal egg count.

It has been shown that an immune marker, plasma IgA, which can be sampled in live animals, provides a potentially valuable alternative to faecal egg counts. However, the ultimate objective of a selection scheme is to reduce the pathology associated with infection. To this end, worm biomass was defined as the product of worm length and worm number; since small worms are thought to be less damaging than large ones [144], this measure provides a better measure of the pathology associated with infection than worm number alone. Thus, the outcome of the selection scheme should be assessed not only in terms of faecal egg count, but also in terms of the predicted reduction in worm biomass.

The comparison between selection schemes based on low faecal egg count versus high plasma IgA activity show that after a few years of stabilization, the worm mass decreases in both selection scenarios. Selection on low FEC will indirectly act on both components of the immune response, reducing the establishment, and thus the worm number, and the fecundity. With a lower establishment, the number of L4 will also be smaller, which in turn causes animals to have a weaker anti-fecundity response. During the early years of selection, the reduction of adult worms (due to the reduction in establishment) is not enough to compensate for a slightly higher mean worm length (due to the weaker anti-fecundity response), which causes the overall worm biomass to be higher.

Although both selection schemes successfully reduce worm mass in the long run, selection for high plasma IgA reduces worm biomass substantially more quickly, with a decrease of around 50% in 10 generations. Although it is commonly assumed that selection directly on a trait is the most effective way to alter it, this system is different because plasma IgA has

a higher heritability than faecal egg counts and high levels of IgA reduce worm growth and fecundity. Hence selection on this trait reduces both the egg output and worm biomass more quickly than direct selection on faecal egg count.

The model uses monthly anthelmintic treatment. This is a widely used method of parasite control particularly when pasture contamination is high. The model was validated by testing it against field data from a farm that treated lambs every 28 days. Other farmers treat less frequently or use anthelmintics that are less efficacious because of drug resistance in the parasite population. These scenarios could lead to higher levels of infection and stronger immune responses depending on the initial pasture contamination. However, there are too few detailed field studies to predict the consequences with confidence.

Future models will examine the impact of selection on growth as a production trait (Chapter 7). This will allow the evaluation of IgE as a marker of resistance. The IgE mediated hypersensitivity response is associated with reduced larval establishment [66] but is weakly associated with reduced growth [130]. Binding of parasite molecules to IgE induces mast cell degranulation which breaks down the tight junction between epithelial cells and induces a relative protein deficiency [8]. Therefore IgE is less attractive as a marker than IgA, which is not associated with reduced growth rate [8]. In future work, the model could be extended to allow growth to depend on worm number and IgE activity (Chapter 7). It will then be possible to contrast selection schemes that use growth, IgA and IgE to identify the optimal combination of markers.

This model addresses long-standing gaps and issues in host parasite models, simultaneously capturing aggregation of infection burdens, explicitly modelling the development of the adaptive immune response and the role of host heterogeneity. This step forward has been facilitated by the understanding of immunological mechanisms of control, extensive parasitological and immunological observations, and the availability of pedigree data to determine the heritability of these traits. Fitting these data to a mechanistic model has enabled the characterisation of the variation and heritability of the underlying immune responsiveness, providing new insights into the role of host heterogeneity in the host-parasite interaction. The most promising methods of control in parasite infections of livestock − selective breeding, improved nutrition, vaccination − all involve improving the immune response. This model provides not only a deeper understanding of the role of host heterogeneity and adaptive immunity, but also a valuable tool for improved understanding, analysis and prediction of the impacts of a wide range of control measures.

In conclusion, this chapter presents a model of developing immunity through the grazing season and has been applied, as an example, to the comparison of selection schemes that use different indicators of resistance. The model is immunologically and genetically explicit, and it was fitted to field observations. The results show that IgA can be a better indicator of

resistance to infection than faecal egg count and that selection schemes based on parasite-specific IgA activity are likely to be more effective than selection based on faecal egg count.

Chapter 6

Quantifying the sources of aggregation in host − parasite systems

As said previously, gastrointestinal nematode infections cause disease and mortality in humans, wildlife and livestock. A key feature of these parasite infections is the aggregation of infection loads across the population. Explaining and identifying the sources of this aggregation has been a long standing question. Here, an immunologically and genetically explicit model of infection is used, in a population of sheep (Chaper 5), to quantify the variation in parasite intake that best fits the observed parasitological and immunological data, and simultaneously quantify the genetic variation in host immune responsiveness. Variation in intake is shown to contribute a relatively small proportion of the observed variation in parasitological variables, and the genetic variation in host immune responsiveness dominates. However, the interaction between the immune responses means that a large proportion of the variation in infection burdens appears unexplained. Thus, despite the very high heritabilities (around 0.9 and 0.7 respectively) of the host immune-responsiveness, the faecal egg count, appears to be under only moderate genetic control. These findings have important implications for the use of immunological markers for selective breeding.

6.1 Introduction

Parasitic infections of humans and livestock are important diseases with a substantial health, welfare and economic costs. Parasite dynamics have been studied with the help of mathematical models for the last three decades, with one of the first models on gastrointestinal nematodes of ruminants published in 1985 [77]. Since that review, multiple models have

been developed, and have been reviewed elsewhere [80, 82]. A key feature that has still not been fully explained is the ubiquitous aggregation of parasite burdens among hosts. The main effects thought to contribute to this aggregation are heterogeneities in host resistance / susceptibility and variation in exposure, i.e. heterogeneities in parasite intake [85, 188]. Patterns of aggregation in wildlife [114] and livestock [115, 118] have been described, but the quantification of the sources of this aggregation has been a recurrent concern [84, 87, 118]. Recently, parasite aggregation was partitioned between intrinsic and extrinsic components in a tick − cattle system [189] using a Poisson-gamma mixture model, which is not clearly mechanistic, and combining it with a model of parasite accumulation. They concluded that extrinsic components were more important.

The sheep − *T. circumcincta* is one of the best understood host-parasite systems, for which we have a detailed understanding of the development of immunity and within-host regulation of parasite burden, length and fecundity [66, 190]. In this chapter, a genetically and immunologically explicit model of gastrointestinal nematode infection in sheep (Chapter 5) is used to quantify the variation in intake consistent with observed data, and the relative contributions of host variation in susceptibility and variation in exposure to the observed variation in parasite burdens is examined.

An issue closely related to the sources of aggregation is that of the heritabilities of disease resistance traits. It is often assumed that they will be selected against [191] and therefore those heritabilities are expected to be low. Some disease related traits have been quantified in the past and in this system, the heritabilities for Immunoglobulin A (IgA), which regulates worm growth and fecundity [123, 124], and Immunoglobulin E (IgE), which regulates larval establishment [66], are moderately high at 0.56 and 0.39 respectively, while the heritabilities of faecal egg counts are in the range $0.2-0.4$ (Mair *et al.*, unpublished). In this chapter, estimates for new parameters are provided, specifically the potential to mount an immune response, referred to as immune responsiveness hereafter, that have not previously been calculated. The potential immune responsiveness is a very important concept, since it is independent of exposure, and it captures the host's innate ability to trigger an immune response against infection.

By using the model to partition the sources of variation, the heritabilities of the host immune responsiveness − the potential to mount an immune response can also be determined, quantifying their impact on the observed variation in parasite burdens and assessing the implications for selective breeding.

6.2 Materials and methods

6.2.1 Model summary

The model of nematode infection in sheep presented in Chapter 5 is used. Briefly, it is a data driven, immunologically and genetically explicit, individual-based mathematical model of sheep (Scottish Blackface) naturally infected with the gastrointestinal nematode *T. circumcincta*. The model reproduces the life cycle of the worm, from egg to adult stage, including the L3 and L4 stage, which are the 2 stages that drive the IgE mediated anti-establishment and IgA mediated anti-fecundity immune response respectively, and are explicitly modelled (Figure 6.1). The term ECF (Establishment Control Factor) is used as a proxy for the IgE mediated immune response, because the response also includes mast cell degranulation. The time course of ECF and mucosal IgA (IgA_m) are assumed to be governed by the following discrete time equations:

$$\begin{aligned} ECF_t &= 0.5^{1/\tau} \cdot ECF_{t-1} + \rho_E \cdot I_{t-z} \\ \text{IgA}_{m_t} &= 0.5^{1/\tau} \cdot \text{IgA}_{m_{t-1}} + \rho_A \cdot L4_{t-z} \end{aligned} \tag{6.1}$$

Both immune responses decrease at a rate determined by the half-life, τ. The development of the immune response is governed by two parameters, ρ_A and ρ_E, which determine the potential to mount an anti-fecundity or anti-establishment response following exposure to L4 or L3 larvae respectively. These parameters comprise an additive genetic (ρ_{gen}) and an environmental component (ρ_{env}) and are assumed to be normally distributed across the flock as follows

$$\begin{aligned} \rho_{\text{gen}_i} &\sim N\left(\mu_{\rho_i}, h^2_{\rho_i} \cdot \tau^2_{\rho_i}\right) \\ \rho_{\text{env}_i} &\sim N\left(\mu_{\rho_i}, (1 - h^2_{\rho_i}) \cdot \tau^2_{\rho_i}\right) \\ \rho_i &= \rho_{\text{gen}_i} + \rho_{\text{env}_i} \end{aligned} \tag{6.2}$$

where μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$ and $h^2_{\rho_E}$, are the means, variances and heritabilitites of the immune responsiveness parameters ρ_A and ρ_E. This gives six unknown model parameters to be estimated. These were estimated by fitting the model to the same data as in Chapter 5 using an Approximate Bayesian Computation (ABC) Regression-Based Conditional Density Estimation algorithm [185, 186], as described in Chapter 5. The model was fitted to the end of season means and variances in plasma IgA and faecal egg count and the heritabilities of plasma IgA and worm length (Chapter 5).

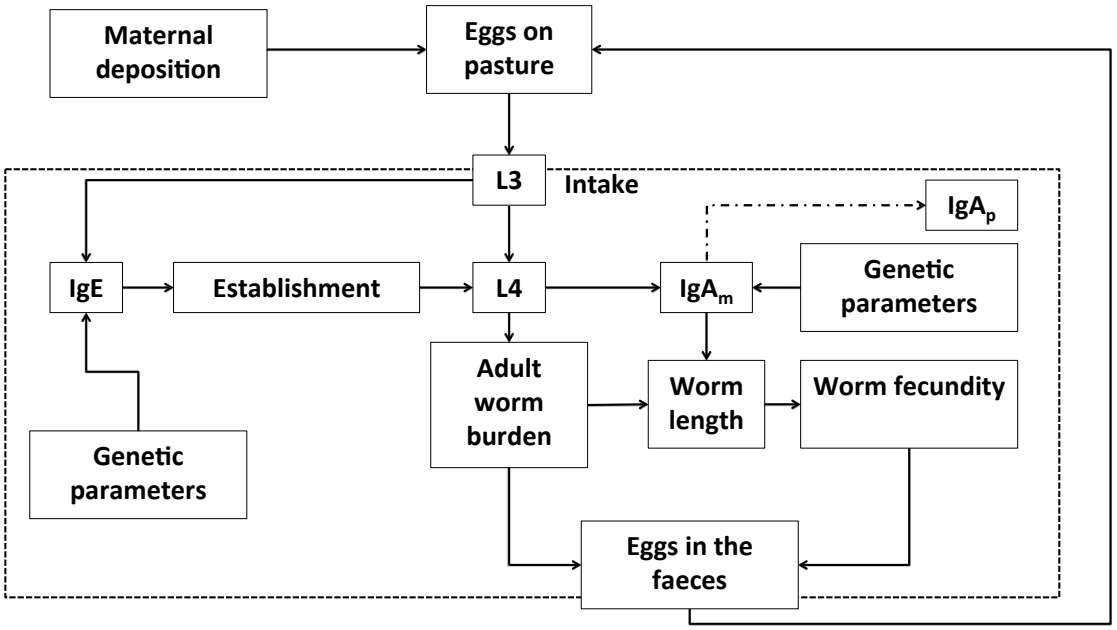

Figure 6.1: Model schematic taken from Chapter 5. The region inside the dotted line represents the life-cycle within the host. Arrows indicate the direction of the effects. The genetic parameters refer to the immune responsiveness parameters, ρ_A and ρ_E, which determine the potential to mount anti-establishment and anti-fecundity immune responses.

6.2.2 Modelling variation in larval intake

In the previously described model (Chapter 5), the daily variation in intake of L3 larvae was assumed Poisson distributed around the mean herbage intake across the flock. The Poisson distribution can be characterised by a single parameter, λ, equal to the mean and the variance of the distribution. A heterogenous Poisson can be generated adding variation to the parameter λ, creating an over-dispersed distribution with a variance greater than the mean. For example, the gamma-Poisson, equivalent to the negative binomial distribution, is generated by allowing λ to be gamma distributed.

To quantify the extent of variation in larval intake, the model fit was tested for biologically plausible parametrizations of the heterogeneous Poisson. A similar approach to previous models was adopted, which have used negative binomial distributions characterised by the dispersion parameter, k, either to describe the parasite burdens, or directly model clumped variation in intake [85, 87, 118].

To proceed, the definition of an appropriate relationship between the mean and the variance is needed. The mean of the distribution (E) is the expected mean of ingested infective L3

larvae (I), i.e. $E = I$, and following the above work [85] which assumes a fixed dispersion parameter, k, the variance (V) is assumed to scale with the square of the mean larval intake, $V = \omega \cdot I^2$. The scaling factor ω is referred to as the noise parameter.

The heterogeneous Poisson was generated using a log−normal distribution, since the samples from this distribution can be generated quickly, allowing the fitting algorithm to run within an acceptable time frame. The log−normal distribution is defined in terms of the mean, μ, and variance, σ^2, of the associated normal distribution. Requiring that the variance scale with the square of the mean, as above, gives the following parametrization:

$$\begin{aligned} \mu &= \ln(I) - 1/2 \cdot \ln(1+\omega) \\ \sigma^2 &= \ln(1+\omega) \end{aligned} \tag{6.3}$$

6.2.3 Sensitivity analysis

Values of the noise parameter ω ranging from zero to 50 were explored. A noise parameter of zero implies that the variation in intake is Poisson distributed; all other values generate over-dispersion in the intake of L3 larvae. The upper limit of 50 was set to reflect unrealistically high values of variation in intake, which would correspond to occasional very high values of daily larval intake (Figure 6.2).

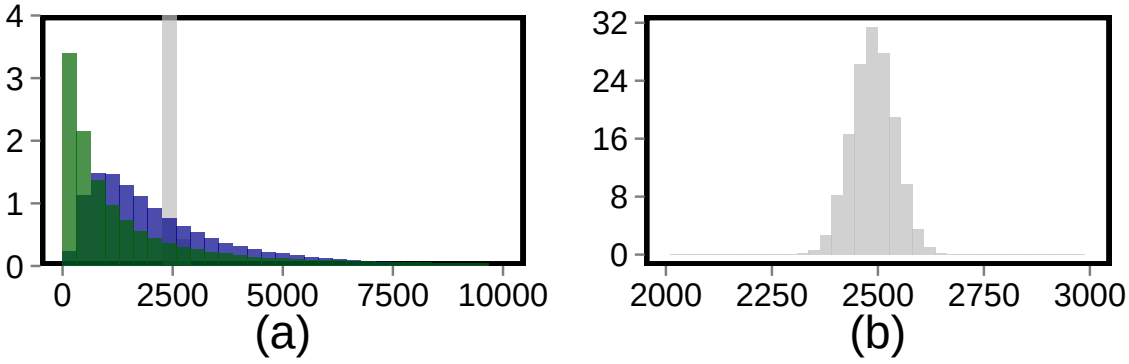

Figure 6.2: (a) Histograms of the larval intake across the flock for a noise parameter $\omega = 0$ (grey), $\omega = 1$ (blue), and $\omega = 10$ (green). (b) Histogram with $\omega = 0$ with a finer scale x-axis for greater resolution. The x-axis in (a) has been truncated at $10,000$, but note that the distributions in green and blue have a longer tail, with respectively 6% and 2% of the values over the $10,000$ truncation point. The y axis in (a) was also truncated, with the full Poisson distribution shown in (b).

To determine plausible values of the noise parameter, it was included as a free parameter to be estimated by the model fitting procedure. The model is fitted using an Approximate Bayesian Computation (ABC) Regression-Based Conditional Density Estimation algorithm presented in [186] and used in Chapter 5. Briefly, a particle is created by drawing a value for

each of the model parameters (μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$, $h^2_{\rho_E}$) from their prior distributions and an ω value uniformly between 0 and 50. For each particle, the model is run and the distance, or tolerance, between the model outputs and target outputs (mean of FEC and IgAp, variance of FEC and IgAp and heritabilities of IgAp and worm length at the end of the grazing season) is calculated. A fraction of particles with the lowest distance is then retained and corrected with the regression component of the algorithm [186]. Those corrected kept draws are assumed to provide approximate posteriors of the distribution of parameters [186]. $100,000$ draws are sampled and run with the model and $1,000$ are kept.

6.2.4 Underlying heritabilities

The noise parameter ω was fixed to the value that best fits the data and extracted from the simulations above. Another ABC algorithm is then run to fit the remaining 6 model parameters (μ_{ρ_A}, μ_{ρ_E}, $\sigma^2_{\rho_A}$, $\sigma^2_{\rho_E}$, $h^2_{\rho_A}$, $h^2_{\rho_E}$) again. With the corrected particles, multiple repeats (100) of the model were ran using one particle at random in each repeat. Is this section, a model run is running the model for 2 generations, with no selection scheme, for a flock of 1000 animals. The average heritabilities for the immunological and parasitological variables (worm number, worm length, plasma and mucosal IgA, ECF and FEC) across the 100 repeats were calculated. Independently from the parameters being fitted, the worm burden distribution across the flock can also be verified as an independent check (as was done in Chapter 5).

6.2.5 Partitioning the variance

To partition the variance, the same setup as in the previous section is used, which is done to more easily interpret the results. In this section, a simulation is defined as a model run for 1 generation, for a flock of 1000 animals, and doing 100 repeats. The variation was partitioned for the number of adult worms, the worm length and the faecal egg count. The three variables used to partition the variance are the intake and the two components of immune responsiveness, ρ_A and ρ_E, which respectively represent the potential to mount the anti-fecundity and anti-establishment responses. In addition, because the heritabilities of ρ_A and ρ_E were also estimated, the variation in the potential to mount an immune response can be divided between the additive genetic component, which is inherited, and the environmental component. The total variation in the full model, with all the variables present, is known from the previous section (now named Var_{full}). 3 simulations are run setting the variance of two out of the three variables (ω, ρ_A and ρ_E) to zero in each simulation; this allows for the calculation of the variation that each of the effects individually is contributing (Var_{ω}, Var_{ρ_A} and Var_{ρ_E}). A simulation with the variance of all three variables set to zero is also run as the null model (Var_{null}; i.e. to calculate the variation due to stochasticity or measurement

error, but that is otherwise unexplained by the three studied variables). The percentage of the contribution of parameter x is calculated as $(Var_x - Var_{\text{null}}) \ / \ Var_{\text{full}}$, where x is ω, ρ_A or ρ_E. The percentage of unexplained variation is $Var_{\text{null}} \ / \ Var_{\text{full}}$. The interactions between two variables cannot be disentangled and a generic overlap effect that accounts for all interactions is calculated as $1-$ the contribution of the individual effects and the stochastic effect.

6.3 Results

6.3.1 Model fitting using the ABC algorithm

The approximate posterior distribution of the noise parameter among the $1,000$ accepted particles is shown in Figure 6.3 and peaks at low values of ω showing that the best fit to the data is for relatively low variation in intake. No particle with a noise parameter higher than 8 was among the best-fitting particles. The best fitting value of the noise parameter ω is approximately 1.

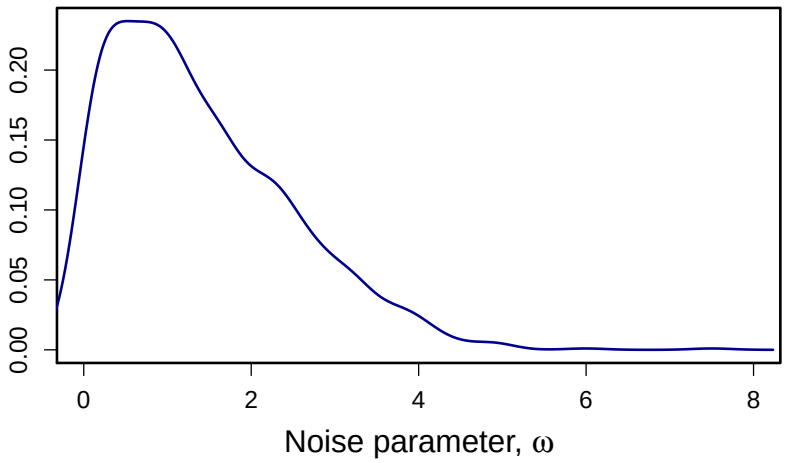

Figure 6.3: Posterior distribution of the noise parameter across the best-fit particles obtained through the ABC fitting algorithm. No particle with a noise parameter higher than 8 was among the best-fitting particles.

The posterior distribution peaks at noise parameters slightly lower than 1, while the median is higher than 1. To examine the impact of higher values of the noise parameter on other parameter estimates, the noise parameter was fixed at 0, 10, 20 and 30 and the ABC fitting for each value was rerun. The range of values of the distance parameter, the measure of goodness of fit, for each noise parameter are given in Table 6.1.

Table 6.1: Summary of the distance values from the subset of kept particles to the fitting target. Each set of simulations has a fixed number for the noise parameter (0, 10, 20, 30). The minimum value of the distance would correspond to the best fitting particle in each set of simulations.

	$\omega = 0$	$\omega = 10$	$\omega = 20$	$\omega = 30$
Min	0.29	2.12	4.81	7.40
Median	0.80	3.81	7.10	9.44
Max	0.91	4.07	7.39	9.76

Specifically, the heritabilities of the immune responsiveness parameters (ρ_A and ρ_E) were examined for each scenario. As expected, at least one of the two values increases as the noise parameter increases (Figure 6.4). Moreover, these results show that for some models to fit, the heritabilities would need to be greater than 1 (Figure 6.4c, 6.4d, 6.4f, 6.4g and 6.4h).

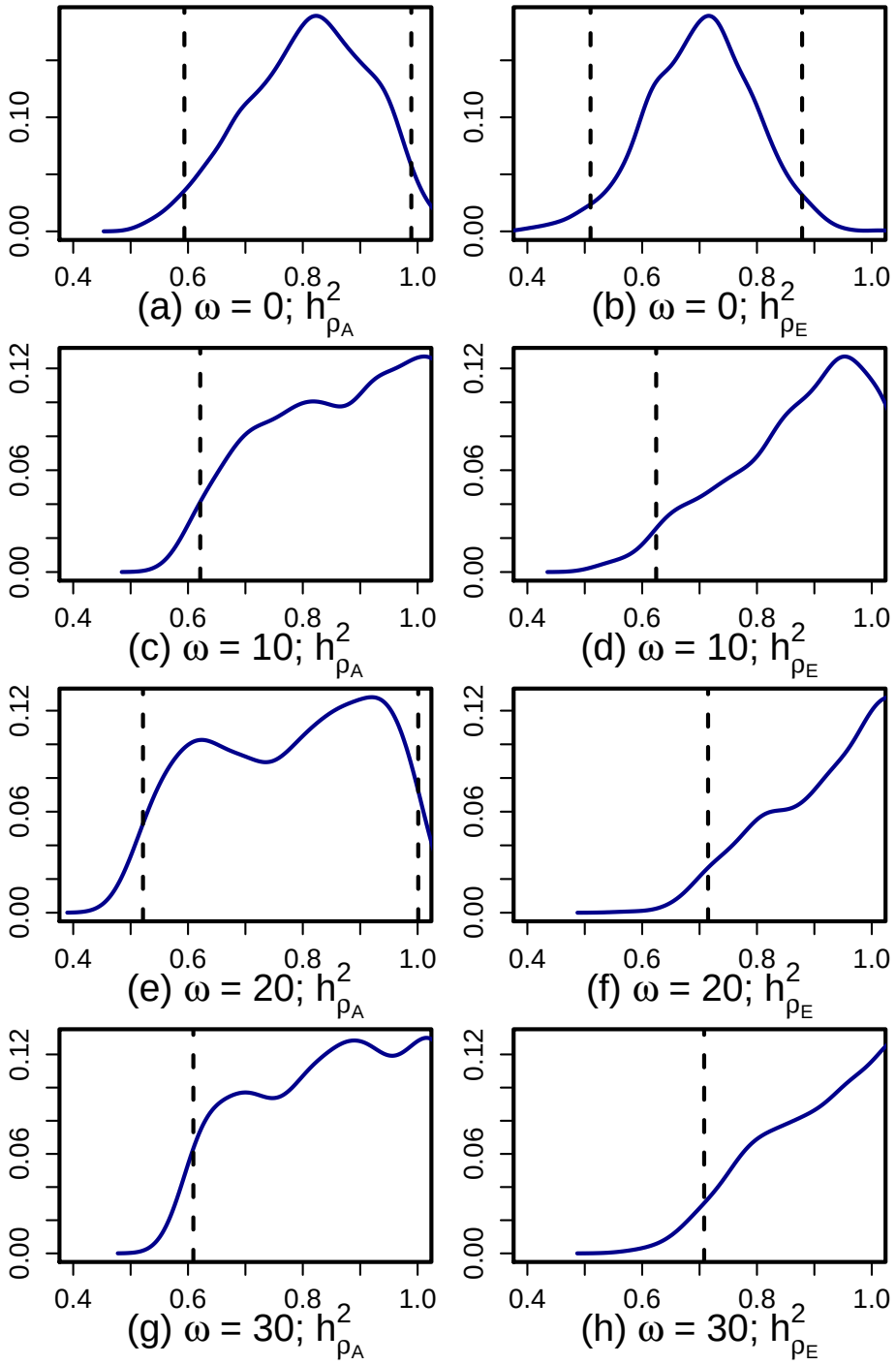

Figure 6.4: The marginal posterior distributions for the corrected heritabilities (see ABC fitting in materials and methods) of the immune responsiveness parameters for values for the noise parameter 0, 10, 20 and 30. Vertical dashed lines show the 95% credible interval; when only one line is shown, is because the credible interval is out of bounds (i.e. the model fitting suggests a heritability higher than 1).

$\omega = 1$ was selected as the noise parameter that best fits the data and was used in the subsequent simulations. The marginal posterior distributions for the heritabilities of ρ_A and ρ_E are shown in Figure 6.5. It was also verified that the worm number distribution, which is not fitted in the model, is reproduced correctly by the model (Figure 6.6).

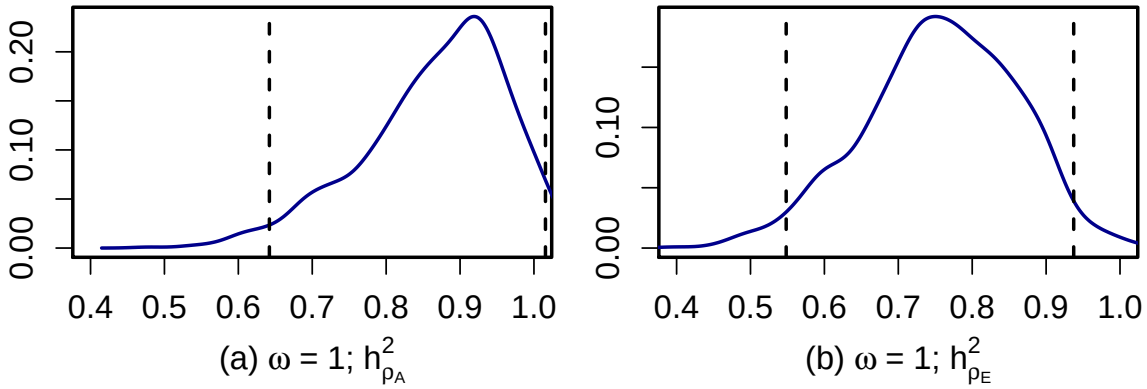

Figure 6.5: Marginal posterior distribution for the heritabilities of ρ_A (a) and ρ_A (b) using the noise parameter $\omega = 1$

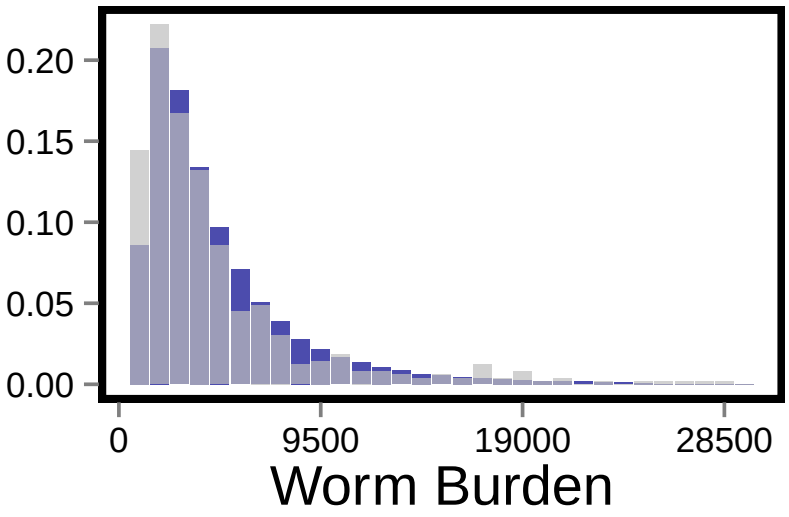

Figure 6.6: Worm burden (number) comparison between field observations (light) and simulated values (dark) with noise parameter $\omega = 1$. Intermediate colour is the overlap. To generate these distributions across the flock, the results of each repeat are pooled and averaged across the 100 repeats.

6.3.2 Underlying heritabilities

The marginal posterior heritabilities for the ρ_A and ρ_E have a credible interval of $(0.64 - 1)$ and $(0.55 - 0.94)$ respectively. The simulations also allow the calculation of the heritabilities of immunological and parasitological parameters, shown in Table 6.2. These are calculated

as the mean of the flock across the 100 repeats. The heritabilities of plasma IgA and worm length are close to 0.56 and 0.6 respectively as expected, since the model is fitted to those two heritabilities. There are other heritabilities, ECF (Establishment Control Factor), worm number and mucosal IgA also with relatively high values (Table 6.2). Moreover, because the measurement error of the counting process of the FEC is simulated, it is possible to calculate the heritability of the true FEC (0.33), which is higher than the value obtained when the counting process is also simulated (0.25).

Table 6.2: Heritabilities for immunological and parasitological parameters at the end of the grazing season averaged over 100 repeats using a noise parameter $\omega = 1$.

Heritability (h^2) of mucosal IgA	0.66
Heritability (h^2) of plasma IgA	0.57
Heritability (h^2) of worm number	0.57
Heritability (h^2) of worm length	0.59
Heritability (h^2) of ECF	0.65
Heritability (h^2) of true FEC	0.33
Heritability (h^2) of measured FEC	0.25

6.3.3 Partitioning the variance

For a noise parameter of $\omega = 1$ and the best fitting particle for the means, variances and heritabilities of ρ_A and ρ_E each simulation was run with 1000 animals and 100 repeats. By running the different models and dropping the different effects, the influence that the intake, ρ_A and ρ_E have on worm number, worm length and true and measured faecal egg count at the end of the season was quantified (Figure 6.7).

Intake accounts for approximately 14% of the variation in worm number while ρ_A has no effect ($< 0.1\%$) and ρ_E explains approximately 67% of the variation. There is 18% of the variation that is due to overlaps, i.e. the effect of multiple variables, and there is no unexplained variation ($< 0.1\%$). Using the estimates of the heritability of ρ_E, the 67% of the variation explained by the immune response can be divided into the approximately 46% that is additive genetic and 21% which is the environmental variation in the immune response (Figure 6.7a).

Most of the variation in average worm length is also explained by the immune responsiveness, around 77% (Figure 6.7b), while variation in intake during the last month accounts only for 10% of the variation in worm length. The variation explained by the immune responsiveness is split between the anti-establishment and anti-fecundity immune response with 61% and 16% respectively, with 42% and 15% being additive genetic variation. 7.5% of the

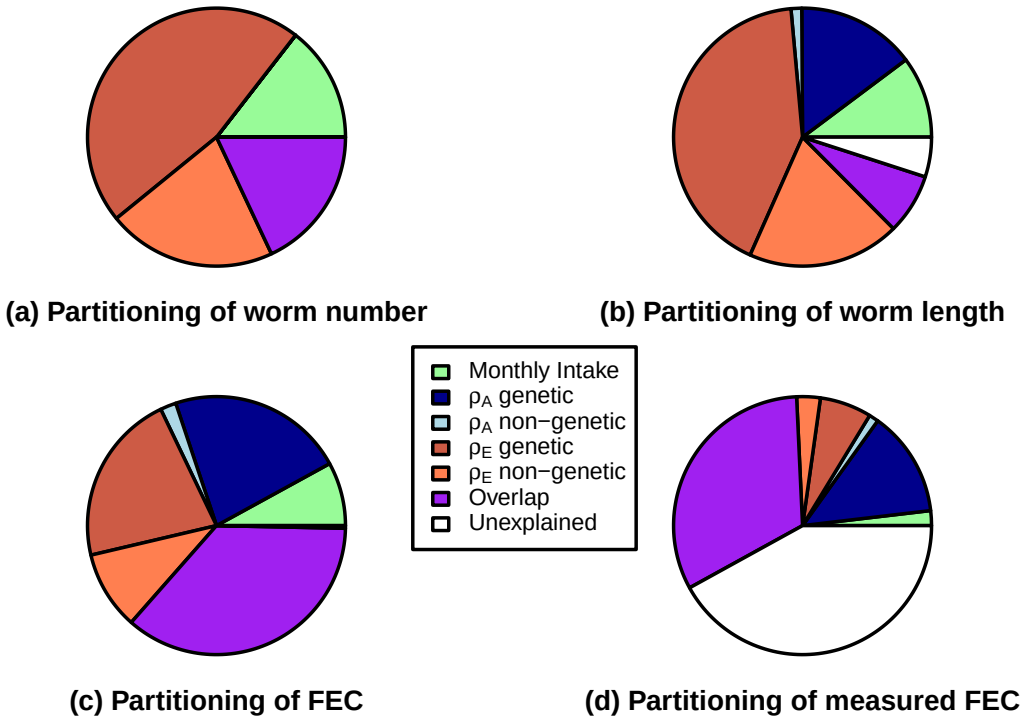

Figure 6.7: Variance components for model outputs for the best-fit estimates of the noise parameter, and the means, variances and heritabilities of the imune-responsiveness parameters, ρ_A and ρ_E. The variance was partitioned for (a) adult worm number, (b) mean worm length, (c) faecal egg counts assuming there is no error in the measuring technique, (d) faecal egg counts including the effect of the counting process.

variation is due to Overlap of multiple effects while 5% is unexplained. This unexplained variation arises from stochasticity in the simulations.

When the faecal egg counts, which are entirely determined by worm length and number, are partitioned however, less variation appears to be explained purely by the immune responsiveness parameters, ρ_A (24%) and ρ_E (31%), and by the variation in intake (8%). Over $1/3$ of the variation (36%) is due to combined effects while the variation unexplained is negligible $(< 0.1\%)$. While almost 100% of the variation in the model is explained by the immune responsiveness and intake and their combined effects when using the faecal egg counts with no measurement error (Figure 6.7c) when the counting process is simulated, the variation explained by those three parameters is only 58%. Thus, around 42% of the variation in the faecal egg counts is due to the counting process and the stochasticity that arises from simulating the counting process.

Although there is relatively large variation explained by the overlap between the three components, especially in the true and measured faecal egg counts (36%) and (32%), the maximum of that overlap that is accounted for by the intake combined with any of the 2 immune responsiveness parameters is (2.6%) and (5.5%). Similarly, in the overlap component in the

partition of the variation of worm burden, only (3.3%) is overlap that could include intake as one of the components. This suggests that most of the overlap is between the 2 immune responsiveness parameters.

6.4 Discussion

In this chapter, the distribution of larval intake that best fits the data was estimated, and then the partition of the variation in adult worm number, worm length and faecal egg count at the end of the season between heterogeneity in larval intake and heterogeneity in the host immune response was calculated. The model of gastrointestinal nematode infection in sheep presented in Chapter 5 was used to estimate the variation in intake that best fits the field data and conclude that in this system, and with the current understanding of the biology, the infection burdens are under strong control by the immune response. Specifically, the results show that variation in larval intake has a relatively small impact on the over-dispersion in infection burden, with the main driver being the host heterogeneity in the response to infection. Even with a tenfold increase in the noise parameter, which translates into a 15 fold increase in the variation in larval intake the last month of the grazing season, the contribution is still relatively small compared to the contribution of the host immune response.

Previous analyses of sources of variation in faecal egg counts in six month-old lambs showed that additive genetic effects accounted for 20% of the variation [175]. In the current analysis, the variation explained by the immune responsiveness was estimated to be 24% (Figure 6.7d). However, faecal egg counts, the number of nematode eggs recovered from the animals' faeces, is just a marker of the infection that is partly obscured by measurement error in the counting process. A substantial proportion of the variation, 42%, appears unexplained, and is attributed to the measurement error and the stochasticity of the model. The 32% overlap corresponds to feedbacks and interactions between the immune response, as well as variation in its development through the grazing season. The results suggest that the contribution of the additive genetic effects to the variation in worm number would exceed 50% (Figure 6.7a), 46% purely explained by the immune responses plus some interactions that would be contained in variation explained by the overlap of more than one response. Similarly, the mean worm length is under strong genetic control, with the proportion attributed to additive genetic variation being most like well over 60% (Figure 6.7b), 57% of the variation explained by individual effects plus some variation explained by a combination of both, and hence contained in the overlap component.

The heritabilities of the immune markers related to the anti-fecundity and anti-establishment immune responses against the appropriate larval stage are moderately high, 0.56 for plasma IgA against L4 larvae [123] and 0.39 for plasma IgE against third stage larvae [130] respec-

tively. The strength of the immune response mounted however, also depends on the level of exposure. This model captures the capacity to respond to exposure (the immune responsiveness) in terms of two parameters, ρ_A and ρ_E, and the result in this chapter suggest that this responsiveness has a much higher heritability, close to 0.8 or 0.9 for the potential to mount an anti-fecundity response, ρ_A (Figure 6.5a), and around 0.7 for the potential to mount an anti-establishment response, ρ_E (Figure 6.5b), which again favours the idea of breeding for disease resistance in livestock.

There are a number of implications in favour of selective breeding for parasite resistance, since it seems clear that selecting on animals resistant to infection is plausible and needed [15]. Breeding for resistance to nematode infections in the field has been successful in the last decades and there seems to be no negative effects on production traits either [54]. The heritability of a downstream trait does not necessarily give a clear picture of the extent to which that trait is under genetic control, and identifying the drivers of the observed traits and stating their heritabilities provides a clearer picture and opens the route to more powerful selective breeding schemes.

This chapter has calculated the most likely distribution of daily larval intake in a flock of sheep and used this information, in a mathematical model fitted to field data, to answer a longstanding question in the field, which is what is the relative contribution of larval intake and host genetics to the variation in the infection burden between hosts. This question has not been answered thus far in a sheep-nematode system [82, 84, 118]. It can be addressed now because there is a better understanding of the system, with genetic, immunological and parasitological data, and a powerful mathematical model to help describe the mechanisms of the host-parasite interactions.

Chapter 7

Future directions and general conclusions

This last chapter is split in two sections: future directions in the model, where adding animal growth and selection on multiple markers of disease is discussed and the second section with the general conclusions from the thesis.

7.1 Future directions

This section extends Chapter 5 to include animal growth and allow new predictions to be made. Two particular areas of interest that more closely reproduce the practical application of selective breeding are the use of breeding values, rather than just phenotypic measures, and also the combination of both components of the immune response in a selection index, as was mentioned in the discussion section of Chapter 5. Here, the model is adapted to allow selection using breeding values, and the impact of an index using IgA and IgE for a range of weightings between those two components is also examined. Selection indices would usually include production traits; in this system, the production trait of interest is growth. Taking a first step toward this goal, it was considered how growth could be added to the model explicitly. However, due to a lack of data on the genetic relationship between IgE and growth, a simplified approach was adopted that does not dynamically track growth, but which exploits the known genetic correlation between weight and faecal egg count [190] at the end of the grazing season. This allows animal weight at the end of each grazing season to be estimated.

7.1.1 Introduction

Parasitic infections of humans and livestock are important diseases with substantial health, welfare and economic costs [7]. As discussed in detail in the introduction of this thesis, the most commonly used nematode control method, chemical drenching, is not viable as a stand-alone option in the long term due to the development of parasite resistance to the most common anthelmintic classes [13, 14]. One of the alternatives is selective breeding, and in particular, selection on immunological markers instead of faecal egg counts which was identified as a promising and more powerful option in Chapter 5.

Genetic improvement programs are based on the selection of animals of higher genetic merit than average. Whilst phenotypic values can be used in selection schemes these suffer from the disadvantage that some individuals will have high values due to favourable environmental deviations, rather than as a consequence of high genetic merit. To counter this problem, selection schemes in practice use estimated breeding values (EBVs) rather than phenotypic values. An extension to the analysis in Chapter 5, would be to simulate the selection schemes using the estimated breeding value (EBV) of the traits, faecal egg counts and plasma IgA, instead of the phenotypic values.

In Chapter 5, a selection scheme was run using plasma IgA (in this chapter called simply IgA) as a marker of resistance to parasite infections, and this selection scheme reduces faecal egg counts faster than selection on faecal egg counts themselves (Figure 5.4a). Selection for a stronger IgA response reduces egg deposition in the field because it reduces parasite growth and fecundity [66]. The epidemiological benefit of lower egg deposition is a reduction in the numbers of established adult worms due to decreased exposure to infective larvae (Chapter 5). However, IgA has no direct effect on parasite establishment in the host. IgE, on the other hand, is involved in the hypersensitivity reaction that prevents larval establishment [62, 66].

Therefore, it is of interest to predict the outcome of selection on a combined index that selects on both IgA and IgE. However, a complete analysis of any selection scheme requires investigation of whether there are any important trade-offs with production traits. This is particularly relevant for IgE, which is known to be associated with reduced growth [130]. Therefore, to fully explore a selection scheme on high IgE levels or use it in an index (that aims to control both worm number and fecundity), it is necessary to model growth and its interaction with the immune response.

There are models of sheep-nematode systems in the literature that include growth [102, 192]. However, these models have not directly modelled the negative effect of the anti-establishment response on growth. Alternatively, the effect of reduced faecal egg counts on growth, can be predicted using the strong negative genetic correlation (mean$= -0.85$) between weight and faecal egg count at the end of the grazing season [190] $-$ equivalently, this means there is a strong negative correlation between the breeding value for weight and the

breeding value for faecal egg count. The analysis in Chapter 5 can be repeated using breeding values instead of phenotypic values in the selection scheme and the changes in animal weight after 10 generations of selection can be predicted.

7.1.2 Calculating breeding values

To estimate the breeding value when the only available information is a single measurement on each individual and no individuals are related then,

$$\hat{A} = h^2 \cdot \left(P - \bar{P}_{Pop}\right) \tag{7.1}$$

Where $\hat{A}$ is the estimated breeding value (predicted additive genetic merit) of the individual, h^2 is the heritability and P and $\bar{P}_{Pop}$ are the phenotypic values for the individual and the mean of the population respectively. However, this equation can be extended to include measures from relatives, as follows [193],

$$\hat{Y} = b_1 \cdot Y_A + b_2 \cdot Y_{FS} + b_3 \cdot \bar{Y}_{HS} \tag{7.2}$$

where Y_A, Y_{FS} and $\bar{Y}_{HS}$ are the phenotypic values of the animal, its full sibling (1 individual in the model) and the mean of its half siblings (38 individuals in the model) respectively (bar represents that the value is a mean). The heritability of the trait is h^2 while $b = [b_1, b_2, b_3]^T$ is the vector of coefficients. $b = P^{-1} \cdot G \cdot a$, with P and G being respectively the phenotypic and genetic variance-covariance matrices, a is the vector of economics weights (in this case assumed $= 1$) [193], see Appendix H.

7.1.3 Implicitly estimating growth

The model used samples from a bivariate conditional distribution to predict the breeding values for weights using the model predictions of breeding values for the faecal egg counts. Note that this methodology assumes that the breeding values for the faecal egg counts and weight are normally distributed. Therefore faecal egg counts were log-transformed, $\log_{10}(\text{FEC} + 1)$, before the breeding values were calculated.

To estimate the breeding value for weight, based on the breeding value of the faecal egg count, the equation for a conditional distribution is used. The distribution of the weight breeding value (X_w) given a known value of faecal egg count (x_F) from the distribution X_F is drawn from a normal distribution as follows:

$$[X_w | X_F = x_F] \sim N\left(\mu_w + \frac{\sigma_w}{\sigma_F} \cdot \rho \cdot (x_F - \mu_F), (1 - \rho^2) \cdot \sigma_w^2\right) \tag{7.3}$$

where μ_w and σ_w are respectively the mean and the standard deviation of the breeding values for weight across the flock, while μ_F and σ_F are the mean and the standard deviation of the breeding values of the log transformed faecal egg counts. x_F is the breeding value of the log faecal egg count of an animal at the end of the grazing season, while ρ is the correlation between both distributions.

To inform Equation 7.3, the means and variances of the breeding values and the correlation ρ are needed. These can be calculated from the data using a bivariate animal model in R [131], with the "`MCMCglmm`" package [194]. All known pedigree relationships between individuals were considered and the model fitted was the following:

$$Y = \mu + \text{Sex} + \text{Year} + \text{Animal} + \text{Dam} + \epsilon \tag{7.4}$$

Where Y is the dependent variable (faecal egg count and weight in this bivariate model), μ is the population mean, *Sex* and *Year* are fixed effects, *Animal* and *Dam* are the random effects that account for the additive genetic component and the maternal effect, while ϵ is the random residual effect. The MCMC chain was run for $100,000$ iterations with $10,000$ iterations as burnin period (and hence discarded) and a thinning of 10 (one in every 10 iterations was taken as a sample). Uninformative priors were used. In this analysis the correlation obtained was -0.72, which is similar to the values previously reported [190].

Breeding values that have been calculated using different methods and in different datasets cannot be compared, to apply the results from the real data into the model data, the assumption is made that the faecal egg count breeding values in the model at the end of the season in generation 0 (before any selection has taken place) should match the breeding values obtained from the field data (i.e. same mean and same variance). To match them, the faecal egg count breeding values in the model and the field data were standardized (scaled to the same mean $= 0$ and variance $= 1$), this is possible since breeding values in a population are only relevant with respect to animals in that population but not in absolute values (i.e. the breeding value of one animal is relevant only in how much higher/lower it is from the population mean). At the same time, it is important to conserve the relationship between the breeding values of the faecal egg counts and the weight from the field data, and therefore the weight breeding values are scaled in the way as the field faecal egg counts (i.e. substracted the mean of the field FEC and divided by the standard deviation of the field FEC).

The estimated improvement of the flock in weight through the generations of selection in both selection schemes was calculated in a run with a flock of 1000 lambs, for 10 generations of selection and with 100 repeats.

7.1.4 Selection using an immunological index

The immunologically explicit mathematical model described in Chapter 5 (full model description available in Appendix F) was used. Briefly, the two components of the immune response, the anti-establishment immune response (mainly driven by IgE against L3 larvae) and the anti-fecundity immune response (mainly driven by IgA against L4 larvae), are assumed to be governed by the following discrete time equations. With ECF (Establishment Control Factor) used as a proxy for the IgE mediated immune response:

$$\begin{aligned} \text{ECF}_t &= 0.5^{1/\tau} \cdot \text{ECF}_{t-1} + \rho_E \cdot I_{t-z} \\ \text{IgA}_{m_t} &= 0.5^{1/\tau} \cdot \text{IgA}_{m_{t-1}} + \rho_A \cdot L4_{t-z} \end{aligned} \tag{7.5}$$

Both immune responses decrease at a rate determined by the half-life τ. The immune responses are triggered by the ingestion of L3, I_{t-z}, and presence of L4, $L4_{t-z}$, respectively and allowing for a delay of z days for their activation. The ρ_E and ρ_A parameters are the potential to mount an immune response and are referred to as the immune responsiveness parameters. High ρ_i values indicate that the animal will mount a higher immune response with lower exposure.

The genetic or phenotypic correlations between the IgA and IgE immune responses are unknown. There is a small phenotypic correlation between plasma IgA and ECF of 0.1 in the model, which appears because of the feedback loops in the triggering of both immune responses, i.e. the ECF immune response prevents L3 establishment, which in turn means there will be fewer L4 to trigger the anti-fecundity mucosal IgA immune response, but at the same time, plasma IgA is regulated by the interaction between worm number and mucosal IgA (Chapter 2) with more IgA transferring to the blood (plasma) with a lower worm number (for a fixed amount of mucosal IgA).

The methodology of selection based on an index requires the definition of the selection objective, which is the sum of the traits to be improved, multiplied by their economic value, and the definition of the selection criterion, which is the sum of the traits actually measured to predict the animals breeding value, multiplied by the selection coefficients [193].

In this system however, there is not enough data available to calculate the selection objective, which requires the relative economic value of each trait. It was then decided to allow the ratio, or weighting, between IgA and ECF in the index to vary from 0 to 1, with 0 being selection only on plasma IgA and 1 being selection purely on ECF. Prior to applying the weighting, both IgA and ECF parameters were scaled to lie between 0 and 1. Each simulation was run for a flock of 1000 lambs, with ten generations of selection and with 100 repeats. The values shown below are the average across the repeats.

7.1.5 Results

Response to selection using breeding values

Selection on faecal egg counts reduces faecal egg counts faster when the breeding values are used instead of the phenotypic values (Figure 7.1a), while selection on plasma IgA decreases faecal egg counts at a similar rate irrespective of whether the breeding values or the phenotypic values are used (Figure 7.1a). A similar result can be observed in the response to selection in worm mass, with selection on the faecal egg count breeding value reducing worm mass faster than selection on the phenotypic value (Figure 7.1b).

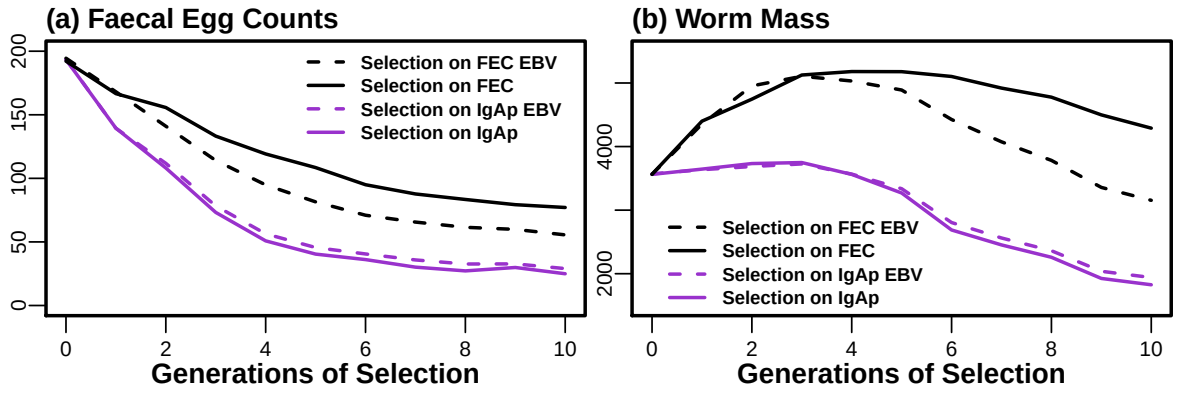

Figure 7.1: Response in faecal egg counts (a) and worm mass (b) to selection on faecal egg counts (black) or plasma IgA (purple) using the phenotypic values (solid lines) or the estimated breeding values (EBV, dashed lines).

Implicitly estimating growth

The weight changes through 10 generations of selection are shown in Figure 7.2. The mean flock estimated breeding value for weight increases in both selection schemes, with improvements of around 1.5 and 2.25 Kg for selection on low faecal egg counts and selection on plasma IgA respectively.

Selection using an immunological index

The reduction in faecal egg counts varies with the ratio between IgA and ECF in a mixed selection scenario (Figure 7.3a). The fastest reduction in FEC is obtained with a selection index dominated by plasma IgA, with a maximum reduction in FEC of around 88% for a ratio of 0. The slowest response to selection is observed with ratios around 0.6, with a reduction in FEC of around 60%. A relatively fast response to selection is obtained with an index dominated by the ECF immune response, with a reduction in the faecal egg count of almost 80% (Figure 7.3a).

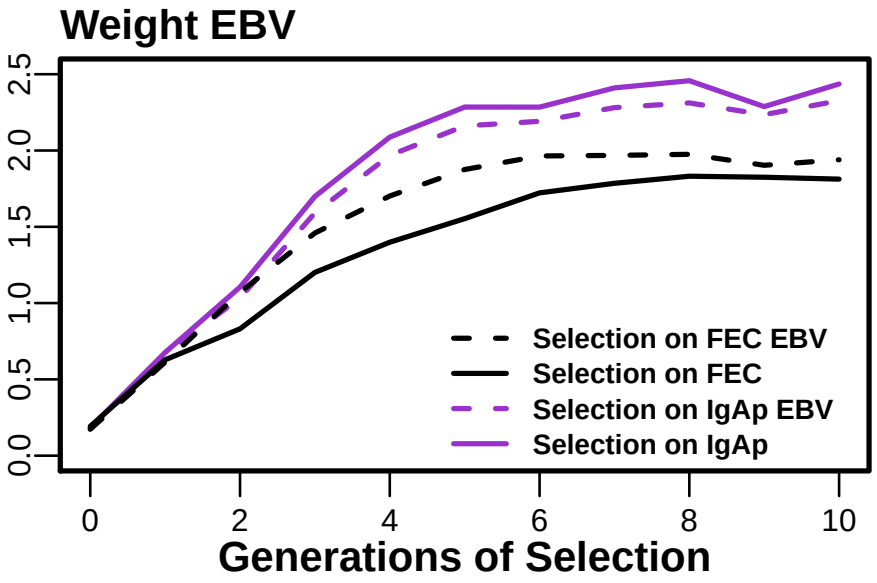

Figure 7.2: Mean flock estimated breeding value for weight at the end of the grazing season over 10 generations of selection on faecal egg counts (black) or plasma IgA (purple) using the phenotypic values (solid lines) or the estimated breeding values (EBV, dashed lines).

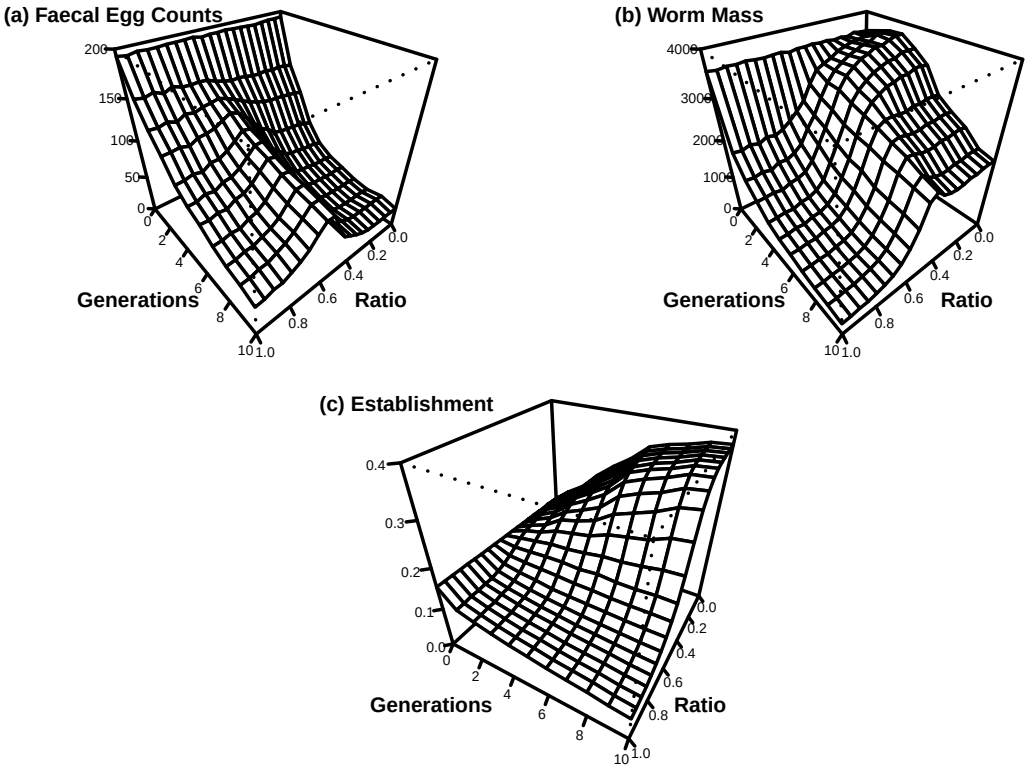

Figure 7.3: Response in (a) faecal egg count, (b) worm mass and (c) establishment to a selection using an index that combines IgA and ECF, over 10 generations. The weight of the two components in the index is given by the ratio (a ratio of 0 implies selection only on IgA; a ratio of 1 implies selection only on ECF).

Worm mass decreases in response to selection, but is nonlinearly related to the ratio in the index. Although the highest worm mass after 10 generations of selection is obtained with the index that has a ratio of 0.45, the other lower ratios yield very similar worm mass values (Figure 7.3b), with reductions of approximately 50%. On the other hand, selection on an index dominated by the ECF response has reductions in worm mass of almost 90%.

Another interesting feature is the trend through the 10 generations. When the selection index is dominated by IgA, the worm mass increases / plateaus during the first few generations and then it decreases. With the selection index dominated by ECF, there is a large drop in worm mass after the first generation of selection (Figure 7.3b).

The establishment decreases, as expected, as the ratio in the selection index increases, i.e. selection dominated by ECF (Figure 7.3c). When the ratio in the selection index is around 0.6, the mean establishment remains unchanged through the generations. Selection mainly on ECF reduces establishment to around 5%, while selection mainly on IgA increases establishment to 40%.

7.1.6 Discussion

In this section, the impact of selection using breeding values, using an index of immunological traits and its effect on growth were explored.

The use of breeding values for selection on low faecal egg counts clearly improves the response to selection compared to selecting on the phenotypic values. This however is not apparent in the IgA selection scheme, where the response to selection using breeding values is similar to using phenotypic values. The advantage of selection using breeding values is that the amount of environmental noise is reduced. For IgA, higher values reduce environmental contamination even if they are not due to genetic merit.

Because of an absence of explicit genetic correlations between the anti-establishment response and the reduction in growth, it was decided to exploit the genetic correlation between faecal egg count and weight to predict the impact of selection on weight. The mean weight gain expected after 10 generations of selection on resistance to parasites was estimated, using selection schemes based on either faecal egg count or IgA. Neither scheme explicitly included growth, although it is a common approach in the field [54], there was an improvement in animal size of 1.5 to 2 Kg due to the reduction in parasitism.

When using an immunological index in the selection scheme, each of the components of the immune response was found to independently reduce the faecal output more than a mixed selection (Figure 7.3a), which is due to negative feedbacks between different components of the immune response (i.e. increasing one decreases the efficacy of the other one). Worm mass is also substantially reduced after a few years of a selection index dominated by ECF

(Figure 7.3b). However, because the anti-establishment response is considered pathogenic and there is a negative correlation between IgE and growth [130], selection on IgE only has been discouraged.

Selection on plasma IgA lowers faecal egg count rapidly and hence controls the infection levels in the field. The animals selected tend to have weaker anti-establishment responses because the selection process inadvertently selects animals that have a high IgA response due to the presence of many L4 stage larvae. This also means that the autoimmune pathology and protein loss caused by the IgE driven sensitivity reaction and the tissue damage will be reduced. The implication is then that in the case of selection on plasma IgA, the estimate of improvement in growth (Figure 7.2) is conservative, since animals with less protein loss will grow faster.

Moreover, because the available number of infective L3 has been reduced, the selected animals with high plasma IgA will have relatively low worm numbers, even without a strong anti-establishment response. Such animals will be very successful if kept together and allowed to create low contamination pasture, but could suffer high levels of infection if moved to other more contaminated paddocks. Success of selection on plasma IgA therefore revolves around decreasing egg deposition through the years and slowly "cleaning" the field of nematodes. If the sheep are rotated to other, more infected, fields, it is unclear how they would deal with larval ingestion, since they would have a slightly weaker anti-establishment response.

Because of the negative correlation between growth and the IgE response, the selection index of interest should have a ratio that does not significantly lower establishment, because that would be at the price of tissue damage and a protein deficiency [8]. In a mixed selection scheme with a ratio between the immune responses of 0.55 (slightly favouring selection on IgE), the establishment will stay almost constant throughout the 10 generations (Figure 7.3c). Hence, there should be no negative impact on production traits. This selection scheme, although having the highest faecal egg count deposition, produces a lower worm biomass than selection purely on IgA, so animals are less infected. However, because the FEC are not as well controlled, pasture will not be "cleaned" as with a selection purely on plasma IgA.

The drawback of using the estimated animal weight at the end of the season is that it is based on the faecal egg counts; therefore, as long as there is a reduction in faecal egg counts, the model would predict an increase in animal size. There is good evidence of a relationship between tissue damage, protein deficiency, reduced growth and IgE [130], so predicting the production benefits of selecting on IgE requires its effect on growth to be accounted for. Therefore the results of using a combined IgA / IgE index have at this stage uncertain implications for growth. The best approach would be to generate the appropriate data, which would include the relationship between IgE and mast cells in the abomasal mucus, with IgE

in the plasma and animals size. With this data the model could be expanded to explicitly include growth and its relation with the immune response. It is therefore unfortunate that there is insufficient data to do this at this point, since being able to capture the trade offs between IgE and growth would allow the impact on growth of any selection schemes to be predicted, and also allow the development of a selection index including IgA and IgE and growth.

These preliminary results have shown that without explicitly including growth as a selection objective, mean animal size will increase when lambs are selected for resistance to nematode infections. Moreover, selection on each of the components of the immune response individually, reduces faecal egg counts faster the selection on a combined index.

7.2 General conclusions

One of the main ideas that this thesis tries to transmit is that it is very important to adequately and appropriately analyse biological data. Parasitology in particular requires sophisticated data analysis because the distribution of parasites among hosts often follows non-normal distributions. Inappropriate data analysis can lead to erroneous conclusions and misleading recommendations for disease control.

In Chapter 2, the focus was on reanalysing a published relationship [125] and the transfer of antibodies (IgA) from the site of infection to the blood was quantified. This work, other than to provide biological insight, helps to develop mathematical models. More specifically, this relationship was used in the mathematical model described in Chapters 5 7. Similar equations could be obtained in different hosts with antibodies that are mechanistically similar so long as the necessary data is available. Most of the variation (88%) in plasma IgA is accounted for which leaves little unexplained variation. Inter-assay variation in the ELISA and the fact that the worm mass measure could be improved are probably the two major effects that account for the unexplained variation. Another interesting feature of this analysis is that it can be extrapolated to other host-parasite systems, as shown in Appendix J, the transfer mechanisms in Spanish Churra sheep and Scottish Blackface sheep seems not to differ, which indicates it is a bio-chemical mechanism that is not breed dependant.

Chapter 3 focused on the relationship between parasitological and production traits in adult ewes in Spain. In both Chapters 3 and 4 of this thesis, the animals analysed are adults, which will generally have lower levels of infection, due to their higher resistance [139]. Moreover, the relationship between production traits and low levels of infection is not quite clear. While is it generally assumed that the effect is very small, it has never been quantified, and hence the work in Chapter 3 was of crucial importance. Even a relatively small effect in each animal, given the large number of animals in each flock and at a country level, translates

into large sums of money being lost due to poor animal performance. This chapter also shows the importance of the nutritional value in milk, which is compromised by nematode infections. This is not only important in livestock (sheep, cattle), but in human populations heavily infected with nematodes; deworming mothers will improve the nutritional value in the milk they feed to their babies.

Both analysis done in Chapter 2 and Chapter 3 were done in a Bayesian framework. Without comparing frequentist and Bayesian frameworks in depth, Bayesian statistics were used because the results can be then used in future work more naturally. One of the properties of having prior and posterior distributions is that a similar model could be run with different or new data using as a prior distribution the posterior that was obtained with the data used in this thesis.

In Chapter 4, another statistical tool was used, namely, a zero-inflated model, to help analyse the data from my Spanish colleagues in León. Zero inflated models are named a few times in the literature [154, 157], but their use is by no means extensive. In this chapter, the use of zero-inflated models is extended, not only to calculate the zero-inflation parameter (i.e. what percentages of zeroes are not "real" zeroes), but also to differentiate between animals that are infected and have zero faecal egg counts with animals that were not exposed and infected in the first place. If this difference is not made and the animals that were not exposed are taken into account, especially in this data that with a zero inflation of around 40%, the correlations would be misleading since low levels of IgA, arising from animals that are not infected and hence dont have an L4 specific IgA response, would appear to be correlated with zero FEC.

Furthermore, this new way of analysing zero inflated data is going to help understand the host-parasite relationship [154]. Moreover, serological data is routinely sampled from these animals, which can help support similar zero-inflated model setups like the one presented in Chapter 4.

Most of the work in Chapters 3 and 4 integrates as much information as possible from immunology and parasitology to find an appropriate way to distinguish the intensity of infection. This is very important since the days when all animals are treated in a generic way are long gone, mainly due to the rise of anthelmintic resistance by the worms [11, 12, 14]. This thesis summarizes some of the efforts on merging all the information available (Chapters $5-7$), with the clear result that the use of immunological markers better quantifies the infection in a flock.

In Chapter 5, a model is presented, which has been developed and extended during the last three years. This model tries to address the gaps in our understanding of parasite infections, by adding in a simple, yet biologically meaningful way, the two main components of the immune response. This allows us to create a model that integrates immunology, parasitology, genetics and epidemiology. Such a model has long been requested [81, 88, 163]. Chapter

5 focuses mainly on how effective is breeding for resistance, and the importance of using alternative markers instead of the faecal egg counts.

Although there are multiple models of nematode infection in sheep, this new model was necessary. The main reason for this is that although most published models are in essence data-driven [77, 92, 100, 101, 102], this model is immunologically explicit and data driven. Moreover, the model was rigorously fitted using an ABC fitting algorithm to reproduce means, variances and heritabilities of the field data. The model also reproduces the distributions seen in the field and it can be independently assessed to other field data (Figure 5.3) that was not used in the fitting procedure.

The use of the model demonstrates that plasma IgA is a better marker for selective breeding since it reduces faecal egg counts and worm mass (worm numbers and worm length) much faster than selection on reduced faecal egg counts. Part of the reason for this success is the higher heritability of plasma IgA compared to faecal egg counts, but also the fact that plasma IgA as a proxy for IgA activity at the site of infection, the sheeps abomasal mucosa, controls the infection upstream from the faecal egg counts (Chapter 2).

One of the implications for selection on plasma IgA alone is that the establishment increases. This is due mainly to the fact that high IgA only appears in animals with L4 larvae to trigger the response. However, the worm burdens do not increase, and this is because the overall quantity of larvae available on pasture is smaller. This means that to get the most out of this type of selection, the animals would have to stay in the same field and no introductions of susceptible animals would be allowed, since it could compromise the epidemiological benefit of this type of resilient flock.

It is therefore important to assess the effect of combining the selective breeding scheme presented here with other control methods. Biological control would reduce pasture contamination, which is the same benefit obtained when selecting for high IgA responses, while being beneficial, it is likely it will reduce the response to selection due to a reduction in exposure. Supplementary feeding, on the other hand, will increase the ability of the animals to mount a strong immune response and should therefore not compromise the benefits of selective breeding. Rotating fields has an unclear effect on the selective breeding scheme. Rotating animals to a clean pasture will reduce the response to selection, although the animals will be healthier. However, rotating animals to a heavily infected pasture to “clean” it will have unclear effects since the animals selected for strong IgA responses control worm infections mainly by reducing larval prevalence in the field, but have a relatively weak anti-establishment response.

The suggestion is not that plasma IgA is the new “gold” standard, but rather an example of what can be done when looking at the immune response of animals infected with parasites. Selection purely on plasma IgA shows very good results, but has many specific requirements

and makes a few assumptions. One of the unknowns is the genetic correlation of the immune responses. And this is of key importance in the predictions that can be made with the model and the likelihood of those predictions. As the selection on plasma IgA shows, the establishment increases. This is, as stated before, because animals with high IgA values must be infected with L4 stage. However, IgE (or the ECF − establishment control factor) is negatively correlated to mucosal IgA due to the feedback loops, but positively correlated to plasma IgA in the model (Chapter 7). If the worms are not established, there are no L4 larvae to trigger the IgA response. This however does not tell us the correlation between the ρ_A and ρ_E, which is the immune responsiveness. Because in the model these variables are independent, the establishment of worms increases relatively quickly, and so does plasma IgA. Nevertheless, what is really necessary are animals with a strong IgA response at the site of infection, not just in the plasma. As it was shown in Chapter 2, there is a fine balance between mucosal and plasma IgA. The model results suggest that it is not an issue since the local IgA response in the abomasal mucus increases also steadily through the generations.

One area that has not been developed in the model is L4 inhibition. Although it has been included in some other models [102], there is clearly not enough knowledge of the biology. There is poor understanding of the density dependent effects between infective L3, inhibited L4 and adults. The effects of the hosts immune system and parasite genetics (i.e. some parasite strains are more prone to inhibition that others) have to be also accounted for. There is also a seasonality effect with more larval inhibition at the end of the grazing season. In the model in this thesis, the strategy was to include the areas which are clearly understood. Building complex models on poorly understood data only leads to errors.

In Chapter 6,larval intake was modelled around food intake. Variation around the mean larval intake was used, which increases concomitantly with food intake. The variation that better fits the field observations was calculated and it followed an over-dispersed Poisson distribution. The variation in larval intake however, has little effect on worm numbers and most of the variation in adult worms is explained by variation in immune responsiveness, i.e. the potential to mount a strong immune response.

There are two main conclusions from Chapter 7: first, selection on any of the two components of the immune response independently, reduces egg deposition on pasture faster than selection on a combined index. This is due to the two components of the immune response negatively interacting with each other. The second conclusion is that genetic selection for resistance to parasites, improves animal size due to a reduction in parasite induced protein loss.

One of the main values in modelling is that it can identify areas where more research is needed. This study has found several areas still need more research. The first is IgE activity, which has clear importance, especially for its negative correlation with animal growth [130].

Ideally, IgE activity should be understood in as much detail as the IgA response. However, there are biological difficulties: most IgE is bound to the surface of mast cells and concentrations cannot be easily measured. In addition, there are inescapable antagonistic relationships between IgA and IgE activity and it will be difficult to disentangle genetic and phenotypic relationships. Increased IgE activity reduces the number of larvae that generate IgA responses. Another area is larval inhibition, which is still poorly understood, although in this particular system, the importance is relatively low, due to frequent anthelmintic treatments. The third area in which more detail is needed is food intake and anorexia in natural infections. Although in a controlled environment and with a deliberate infection, most of these parameters can be quantified, natural infections may differ and there is a lack of understanding on the mechanisms that drive intake and anorexia.

Last, but not least, it is important to mention that the work in this thesis has been a constant exchange between the wet-lab and modelling group. This provides a strong case for multidisciplinary projects in which field data informs the modelling, but also the models can inform areas where the biology is not clear and where more research is needed.

Appendix A

Zero-inflation posterior distribution

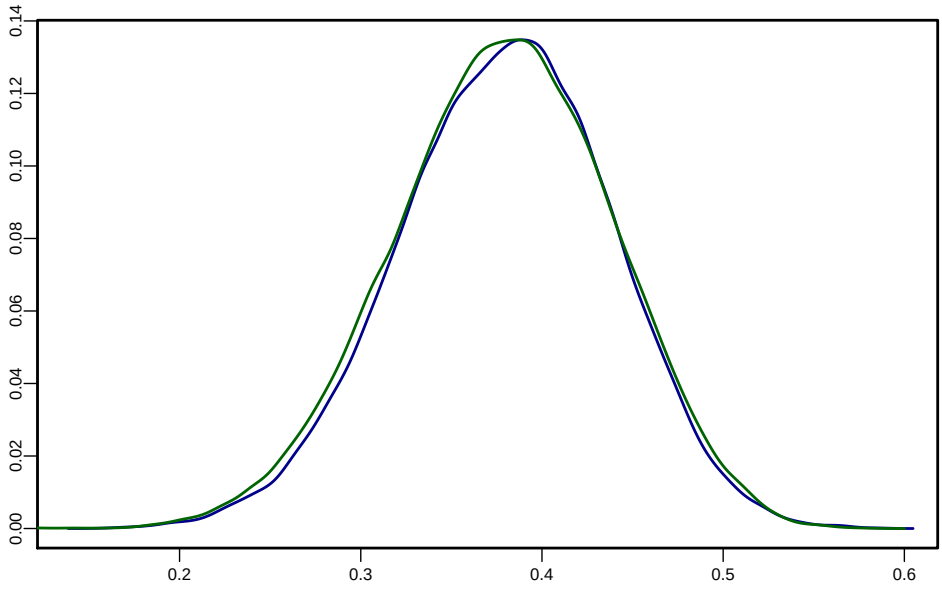

Figure A.1: Posterior distribution of the zero-inflation parameter obtained from the extended ZINB model. Each colour represents a different chain. Both chains have a mean around 0.38 and no sample was recovered from either of the chains with a zero inflation parameter equal to zero (minimum value recovered $= 0.12$).

Appendix B

Extended ZINB model code in R

m $<-$ "model {
for (i in 1 : nsheep) {
Neggs$[i]$ $\sim$ dpois(lambda$[i]$)
lambda$[i]$ $<-$ lamb$\big[i,$ status$[i]+1\big]$
status$[i]$ $\sim$ dbern(P) #animals status: 0 not recently infected, 1 infected
lamb$[i,1]$ $<-$ 0 #zero distribution for not recently infected
lamb$[i,2]$ $\sim$ dgamma(shape, rate) #gamma-poisson for infected

IgA$[i]$ $\sim$ dgamma$\big($sh$[i]$, rt$[i]\big)$ #IgA is gamma distributed
#vector of means: position 2 exposed, 1 for non-exposed
mn$[i]$ $<-$ vmn$\Big[\big($status$[i]\big)\cdot 2+\big(1-$status$[i]\big)\cdot 1\Big]$
#vector of shapes: position 2 exposed, 1 for non-exposed
sh$[i]$ $<-$ vsh$\Big[\big($status$[i]\big)\cdot 2+\big(1-$status$[i]\big)\cdot 1\Big]$
rt$[i]$ $<-$ sh$[i]/$mn$[i]$ #rate $=$ shape $/$ mean
}

Prior zero-inflation
P $\sim$ dbeta$(1,1)$

#Priors Egg counts
shape $\sim$ dgamma$(0.001, 0.001)$
$p \sim$ dbeta$(1,1)$
rate $<-\ p/(1-p)$

Priors IgA

for(i in $1:2$){
unorderedmeans$[i] \sim$ dgamma$(0.001, 0.001)$ #uninformative means
}
vmn $<-$ sort(unorderedmeans) #make sure uninfected mean is lower
vsh$[1] \sim$ dgamma$(0.001, 0.001)$
vsh$[2] \sim$ dgamma$(0.001, 0.001)$

#To avoid problems finding initial values
#inits# status, .RNG.seed, .RNG.name #initial values (animals status and RNG)
#data# FEC, IgA, nsheep #data used
#monitor# shape, rate, status, P, vmn, vsh # Outputs of the model
}"

Appendix C

Historical weather observations for the Spanish Churra sheep dataset

Table C.1: Mean temperatures (°C) and precipitation (mm) from December to June during the sampling period (highlighted in gray), and during the four previous years.

Month	2007/2008		2008/2009		2009/2010		2010/2011		2011/2012	
	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
December	1.9	11.4	2.9	50.3	3.7	110.4	3.1	92.5	3.8	14.0
January	4.5	33.2	2.7	38.1	3.4	62.1	3.9	42.4	2.4	12.2
February	6.3	32.5	4.3	21.2	3.6	59.3	4.5	28.5	1.9	5.8
March	6.5	16.6	7.5	12.5	6.2	55.3	7.3	41.1	8.0	9.8
April	9.5	68.4	8.6	28.4	11.0	37.6	12.2	43.2	7.7	72.7
May	12.2	100.3	14.9	22.6	12.3	36.0	15.3	36.7	14.8	35.8
June	16.9	34.1	18.7	30.5	16.9	68.5	17.6	22.6	18.8	14.8

Appendix D

Probability of exposed in a classic ZINB model

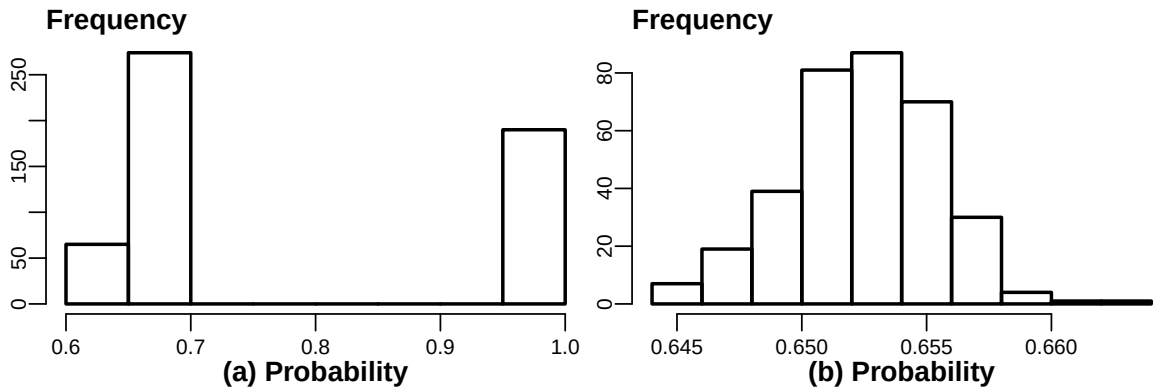

Figure D.1: Histogram of Probabilities of being exposed for the dataset (a) and zoom of only the zero FEC (b) using only FEC data in the ZINB model. Animals with non-zero FEC will always have an ”infected” status in the model ($= 1$) while animals with zero FEC can be exposed or unexposed. If only the FEC data is used, each animal with zero FEC will have a probability of being infected similar to one minus the zero-inflation parameter (b).

Appendix E

Correlations in the Churra sheep full dataset

Table E.1: Correlation estimated between the number of eggs in the faeces and IgA activity for the whole dataset (529 animals), without using the ZINB model to discard unexposed animals from the analysis.

	Neggs	IgA
Neggs	1	0.141^{**}
IgA		1

$^{**}P < 0.001$; $^{*}P < 0.015$

Appendix F

Full Model description

The model is based on a previous model of immunity to *T. circumcincta* infection in lambs [93], which has been extended here to capture individual variation in response to infection across a flock of sheep. First, I present the model equations, and then discuss model parameterisation.

F.1 Model equations

F.1.1 Nematode life cycle

This component of the model is based, with certain modifications detailed below, on Bishop and Stear [112] which captured the nematode life cycle with two equations, one for the worm burden and one for infective larvae in the field (L3 larvae). We have modified these equations to additionally explicitly include the L4 larvae, which trigger the anti-fecundity response.

The number of adult worms (WB) per lamb on day t, WB_t, depends on the worm burden on day $t-1$; the mortality rate of the L4 larvae and adult worms, m_L4 and m_A, respectively; the number of larvae ingested j days ago, I_{t-j}; and the proportion of ingested L3 larvae that establish in the host and progress to become L4 larvae, $E_{t-j_{L4}}$ (Equation F.1). The pre-patent period, $j = j_{L3} + j_{L4}$, is the time taken for ingested L3 larvae to develop to a fully-grown adult worm, where j_{L3} and j_{L4} are the times, post ingestion, spent in the L3 and L4 stages respectively.

$$WB_t = WB_{t-1} \cdot (1 - m_A) + I_{t-j} \cdot E_{t-j_{L4}} \cdot (1 - m_{L4})^{j_{L4}} \tag{F.1}$$

The infective larvae on pasture on day t, L_t , depends on the infective larvae present on day $t-1$; the number of ingested larvae, I_{t-1}; the mortality rate of infective larvae, m_{L3}; the

number of eggs deposited on pasture by ewes, S_{t-u}; the fecundity Wf_{t-u} and worm burden, WB_{t-u}, each u days earlier, where u_t is the number of days taken to develop from egg to infective stage (here, assumed to vary monthly); and the proportion of eggs reaching the infective L3 stage (Equation F.2) at day t, e_t, as follows:

$$L_t = (L_{t-1} - I_{t-1}) \cdot (1 - m_{L3}) + (S_{t-u_t} + WB_{t-u_t} \cdot Wf_{t-u_t}) \cdot e_t \tag{F.2}$$

The number of L4 in a given animal on day t, $L4_t$, depends on the rate at which L4 die, the rate at which ingested larvae (L3) become L4 and the rate at L4 become adults and is given by,

$$L4_t = L4_{t-1} \cdot (1 - m_{L4}) + I_{t-j_{L3}} \cdot E_t - I_{t-j} \cdot E_{t-j_{L4}} \cdot (1 - m_{L4})^{j_{L4}} \tag{F.3}$$

where m_{L4} is the mortality rate for the L4 stage, $I_{t-j_{L3}} \cdot E_t$ is the number of larvae ingested j_{L3} days previously establishing to become L4, and $I_{t-j} \cdot E_{t-j_{L4}}$ scaled by the mortality term gives the number of ingested and established larvae that are still alive and ready to leave the L4 stage to become adults.

F.1.2 Immune Responses

IgA activity against L4 is strongly associated with reduced parasite fecundity [66]. We have modelled both mucosal and plasma IgA because mucosal IgA acts at the site of infection, whereas the related quantity, plasma IgA, is the quantity measured in the blood stream. Mucosal IgA, denoted IgA_m, is produced in response to the L4 population, and is assumed to increase with rate ρ_A in proportion to the number of L4, with a delay from exposure to initiation of an immune response of z days and a half-life of τ days, as follows:

$$IgA_{m_t} = 0.5^{1/\tau} \cdot IgA_{m_{t-1}} + \rho_A \cdot L4_{t-z} \tag{F.4}$$

Plasma IgA, denoted IgA_p, has been previously shown to depend on IgA_m and the worm burden, WB, at the site of infection [125]. I found an improved fit to the data (Chapter 2) to be given by a relationship between worm biomass, WM, which is the product of the worm burden and the mean worm length, IgA_m and IgA_p as follows:

$$IgA_p = \lambda_1 \cdot IgA_m + \lambda_2 \cdot IgA_m \cdot log_{10}(WM) \tag{F.5}$$

The establishment of adult nematodes is strongly associated with mast cell degranulation and IgE activity [66], whose effects are captured jointly via establishment control factor (ECF).

This is assumed to increase with rate ρ_E in proportion to the number of ingested L3 larvae and is assumed to decay with a half-life of τ days, as follows:

$$ECF_t = 0.5^{1/\tau} \cdot ECF_{t-1} + \rho_E \cdot I_{t-z} \tag{F.6}$$

To capture the decrease in establishment over the grazing season as the immune system develops, an establishment equation was created that reproduces field observations that were summarised in a meta-analysis [181]. The establishment, E_t, at time, t, is expressed in terms of E_{early}, the parasite establishment for naïve lambs, and E_{late} which is the minimum long term establishment, as follows:

$$E_t = (E_{early} - E_{late}) \cdot e^{-ECF_t} + E_{late} \tag{F.7}$$

F.1.3 Nematode fecundity

The fecundity of worms depends on worm length, WL which is known to be influenced by both worm burden and IgA activity [66]. A regression model fitted to the data gave the following relationship (Equation F.8) between worm length, mucosal IgA activity and worm burden,

$$WL_t = \alpha - \beta \cdot log_{10}(IgA_{m_t} + 1) - \gamma \cdot WB_t \tag{F.8}$$

where α is the intercept term in the regression model, giving the expected mean length of adult worms in absence of the immune response and density dependent effects. β and γ are the coefficients for the effect of the immune response and worm burden respectively [66].

The numbers of eggs per worm on day t, Wf_t, was taken from the published relationship [179]. Adult size ranged from 0.7 cm to 1.2 cm [66] and worms smaller than the threshold size (estimated to be 0.7 cm) are assumed to not reach maturity and therefore produce no eggs. The fitted relationship is given by,

$$Wf_t = (\epsilon \cdot WL_t^{\omega} - 1) \cdot 500 \tag{F.9}$$

where the scaling by 500 accounts for the average weight of faeces (in grams) produced by lambs in this experiment to produce a fecundity in terms of eggs per worm per day.

F.1.4 Ingestion and egg deposition in the field

We assume that the number of larvae ingested by lambs and their faecal deposition onto pasture depends on the food consumption and therefore, the weight of the animal. Lamb weights were assumed to follow a Gompertz equation [93]:

$$weight_t = \theta \cdot exp[\mu(1 - e^{-\kappa t})/\kappa] + \phi \tag{F.10}$$

where ϕ is the weight at birth and the parameters θ, μ, κ were estimated by fitted the expression to observed lamb weights [93].

The herbage consumed per lamb per day, Q_t, is assumed to be proportional to the weight gain in the lamb since birth [93]:

$$Q_t = \nu \cdot (weight_t - \phi) \tag{F.11}$$

The number of infective larvae ingested per lamb depends on the larvae available on pasture, L_t, and the herbage consumed, Q_t, is proportional to the stocking density of lambs on pasture, D, and inversely proportional to herbage density, H, and is given by,

$$I_t = L_t \cdot Q_t \cdot \frac{D}{H} \tag{F.12}$$

Using the growth curve given by Equation F.10, we can calculate the faecal deposition in pasture and the quantity of egg deposited as follows:

$$FEC_t = \frac{WB_t \cdot Wf_t}{weight_t \cdot f} \tag{F.13}$$

where the numerator is the total number of eggs in faeces per day whilst the denominator is the mass of faeces produced in a day, assumed to be proportional to the size of the animal.

F.2 Model parameterisation

Table F.1 gives the variables used along with the equations that govern their dynamics.

Table F.2 gives the parameter values used in the model either with a reference from the literature, or with a justification for the selected values for parameters not estimated in the literature, or where estimates vary.

Table F.1: Model variables and the respective equations that govern their dynamics.

	Variable	Equation
WB	Worm Burden	F.1
L	L3 population on pasture	F.2
$L4$	L4 burden	F.3
IgA_m	Immune response − Mucosal IgA (site of infection)	F.4
IgA_p	Immune response − Plasma IgA (blood)	F.5
ECF	Immune response − Establishment control factor	F.6
E	Worm establishment	F.7
WL	Worm length	F.8
Wf	Worm fecundity	F.9
$weight$	Lamb weight	F.10
Q	Daily herbage intake	F.11
I	Daily ingestion of infective larvae	F.12
FEC	Faecal egg counts	F.13

Mortality rates depend on the larval stage. The pre-infective larval stages are the most vulnerable to weather conditions and predation and consequently have the highest mortality rate; this mortality is captured in the larval development success term, e_t. The mortality of L4 is not available in the literature, presumably because it would be difficult to measure experimentally. As the L4s are shielded in areas of the intestine known as gastric pits while they develop, the mortality, m_{L4}, is believed to be low relatively to the adult mortality rate; therefore it is set at 0.01 per day.

The time taken to develop from egg to infective stage (L3), u, ranges from 6 days to 4 weeks in the literature [92, 10, 100], varying in response to environmental factors such as humidity and temperature and by nematode species [10]. Salih and Grainger [200] proposed the following equation for the development time of *T. circumcincta* in the UK as a function of temperature:

$$u_t = 132 \cdot T^{-1.1018} \tag{F.14}$$

Historic weather records for years 1992 to 1996 (years when our data were collected) were used to provide the average monthly temperatures for May to September, which is the period simulated by the model (Appendix I). Using Equation F.14, the mean monthly development times, u_t, was calculated and ranged from 6 to 12 days. This variation in development time from egg to L3 coupled with the mortality in this pre-infective stage influence the success, e_t, which is calculated monthly as follows:

Table F.2: Model parameters with the value used in the model and the reference (where available).

	Parameter	Value	Reference
e_t	Larval development success in the field (per day)	$0.09(1 + sin(\pi t/140))$	(see below for justification)
m_{L3}	Mortality rate for L3 in the field (per day)	0.008	[195]
m_{L4}	Mortality rate for L4 (per day)	0.01	(see below for justification)
m_A	Mortality rate for adults (per day)	0.0307	[196]
u_t	Development time from egg to L3 (days)	$6 - 12$	(see below for justification)
j_{L3}	Time from ingestion to L4 stage (days)	2	[197]
j_{L4}	Time from L4 to adult stage (days)	14	[197]
L_0	Initial larval availability (larvae per lamb)	$10,000$	[93]
S_t	Ewes egg deposition (eggs per lamb)	Initially $250,000$ with linear decrease to 0 at day 84	[93]
f	Faeces per Kg of bodyweight (g)	20	[93]
D	Stocking density (lambs/ha)	35	[198]
H	Herbage density (Kg DM/ha)	1200	[198]
τ	Half-life of antibodies (days)	8.1	[199]
z	Lag in acquisition of immunity (days)	7	[93]
ρ_A	IgA response factor	See Equation F.1-F.2	
ρ_E	Establishment response factor	See Equation F.1-F.2	

$$e_t = (1 - m_p)^{u_t} \tag{F.15}$$

where m_p is the mortality rate of the pre-infective stage (0.23, [92]) and u_t is the development time. To these monthly data (red dots, Figure F.1), using maximum likelihood, we fitted a sinusoidal curve to represent the daily change in larval success over the season (black dots, Figure F.1).

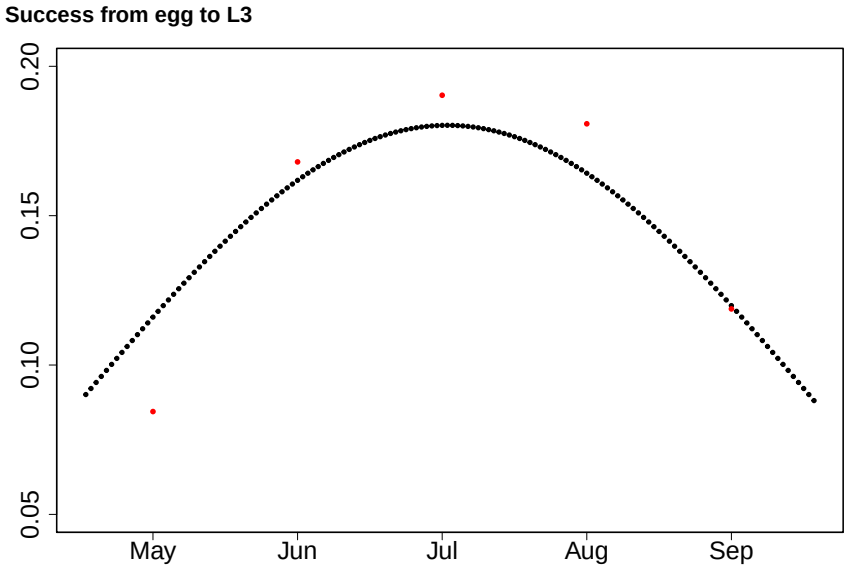

Figure F.1: Proportion of eggs successfully developing into L3 larvae. The red dots are the data points (one for each month). Black dots are the fitted sinusoidal success curve used in the model for parameter e_t.

The time post ingestion taken to develop to the L4 stage, j_{L3}, lies between 1 to 3 days [197], which is the time taken to ex-sheath, travel to the abomasum and settle in a gastric pit and moult; the mean value of 2 days was chosen for our simulations.

The next development interval, the time taken by the L4 stage to develop into adults is particularly important because it is L4 antigens that trigger the IgA immune response. The first adults appear 12 days post ingestion while the last L4s become adults 20 days post ingestion [197]. A mean value of 16 days was chosen for the pre-patent period, with 14 days assigned to the development from L4 to adult, j_{L4}, (since we assigned 2 days to j_{L3}).

Model coefficients are listed in Table F.3.

Table F.3: Coefficients used in the model equations with the relevant parameter.

Coefficient	Value	Related parameter
α	1.071	Worm length, WL; [66]
β	0.65	
γ	5.2×10^{-6}	
ϵ	1.12	Worm fecundity, Wf; [66]
ω	0.41	
θ	3.6×10^{-5}	Live weight; [93]
μ	0.614	
κ	0.0471	
ϕ	10.18	Live weight and herbage consumed, Q; [93]
ν	0.109	Herbage consumed, Q; [93]
E_{early}	0.4	Establishment, E; [93]
E_{late}	0.0	
λ_1	3.98	Transfer equation, IgA_p;
λ_2	1.02	(Chapter 2)

Appendix G

Breeder's equation

In selective breeding of animals, the expected response to selection, R, can be estimated from the average difference between the parent generation and the selected parents, S. This equation is commonly referred to as breeder's equation:

$$R = h^2 \cdot S \tag{G.1}$$

R is the response to selection, or the improvement expected in the offspring generation compared to the parental generation. S is the selection differential.

Over more than one generation however, this equation doesnt account for the effects of selection in the variance. After a selection process, the variance of the trait selected for is reduced; this effect is known as Bulmer effect, Bulmer was the first to publish an examination of this phenomenon [201].

Since the group of selected parents are in one of the tails of the phenotypic distribution, their variance is smaller than the variance of the whole parental population. The variance after selection, V_p', is hence the variance before selection, V_p, minus the reduction in variance. k is the reduction factor.

$$V_p' = (1 - k) \cdot V_p \tag{G.2}$$

k depends on the intensity of selection, assuming a normal distribution of the trait, then

$$k = i \cdot (i - x) \tag{G.3}$$

where i is the intensity of selection and x is the deviation of the truncation point from the parental population mean. Assuming the normal distribution, the parameters i and x were

calculated by Falconer and MacKay [180] and are shown in the following table (assumption of truncation of a normal distribution):

Table G.1: Parameters to calculate the reduction factor k

Proportion of population selected	Intensity of selection (i)	Truncation point (x)
5%	2.063	1.645

The Response to selection in year y can be calculated from the previous year $y-1$ with the following correction:

$$R_y = \frac{1 - 1/2 \cdot h^2 \cdot k}{\sqrt{1 - 1/2 \cdot h^4 \cdot k}} \cdot R_{y-1} \tag{G.4}$$

The equation above was used to calculate the dotted line shown in Chapter 5 as the expected response to selection for FEC from traditional genetic theory. If the Bulmer effect were not included, the dotted line would be straight instead of curved. The response was calculated in the model for the first year of selection and corrected for all the following years with the equation above. Heritabilities were calculated in the model and the other parameters were taken from the table above. Note here that the breeders equation was designed for a normally distributed variable, in our case, we have to log transform the FEC to use the breeders equation.

Appendix H

Selection index coefficient calculation

The breeding value for an individual can be estimated from phenotypic measures of the individual and its relatives, using equation 7.2.

$$\hat{Y} = b_1 \cdot Y_A + b_2 \cdot Y_{FS} + b_3 \cdot \bar{Y}_{HS} \tag{H.1}$$

where Y_A, Y_{FS} and $\bar{Y}_{HS}$ are the phenotypic values of the animal, its full sibling (1 individual in the model) and the mean of its half siblings (38 individuals in the model) respectively (bar represents that the value is a mean), while $b = [b_1, b_2, b_3]^T$ is the vector of coefficients. The value of these coefficients can be calculated using the following relationship:

$$P \cdot b = G \cdot a \tag{H.2}$$

where P is the phenotypic variance-covariance matrix, b is the vector of coefficients, G is the genetic variance-covariance matrix and a is the vector of economic weights. The vector of coefficients can therefore be calculated from the previous equation,

$$b = P^{-1} \cdot G \cdot a \tag{H.3}$$

The phenotypic and genetic matrices are defined below, and are calculated based on the phenotypic / genetic distance, which is $1/2$ for full siblings and $1/4$ for half siblings. The economic weight is assumed to be 1 for all measures (i.e. equivalent value to all measures), more details in *N. D. Cameron*'s book [193].

$$P = \begin{pmatrix} 1 & \frac{1}{2} \cdot h^2 & \frac{1}{4} \cdot h^2 \\ \frac{1}{2} \cdot h^2 & 1 & \frac{1}{4} \cdot h^2 \\ \frac{1}{4} \cdot h^2 & \frac{1}{4} \cdot h^2 & \frac{1+(n-1) \cdot 1/4 \cdot h^2}{n} \end{pmatrix}$$

with $n = 38$, which is the number of half siblings in the simulation.

$$G = \begin{pmatrix} h^2 \\ \frac{1}{2} \cdot h^2 \\ \frac{1}{4} \cdot h^2 \end{pmatrix}$$

$$a = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$$

Appendix I

Historical weather observations for the Scottish Black-Faced sheep dataset

Table I.1: Mean temperatures (°C) and precipitation (mm) from April to October during the grazing season of the five consecutive years where the data was collected.

Month	1992		1993		1994		1995		1996	
	°C	mm	°C	mm	°C	mm	°C	mm	°C	mm
April	8.1	73.5	8.5	118.6	7.9	91.1	9.2	36.4	8.6	83
May	12.7	84.4	10.5	96.5	10.7	12.7	11.4	63.1	9.5	50.1
June	15.5	29.1	16.2	42.6	13	89.8	14.8	26.2	14.2	34.2
July	14.9	72.9	15.7	62.9	16.1	68.7	16.7	106	15.3	59
August	14.1	171.5	13.6	61.8	14.6	124.2	17.9	22.1	16.4	41.9
September	11.9	161.8	11.9	73.9	11.9	55.2	13.1	128.3	13.9	49.8
October	6.65	78.5	7.4	72.3	9.2	71.7	11.8	279.5	11	194.4

Appendix J

Comparison between Scottish and Spanish data

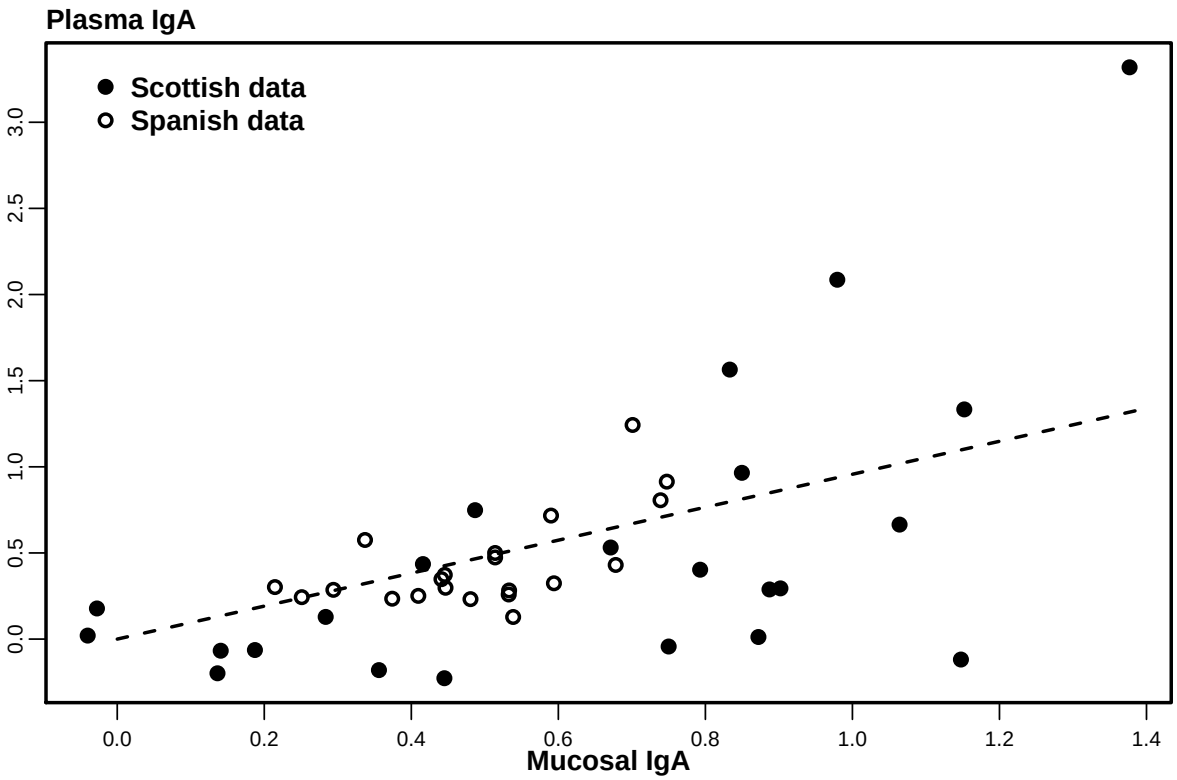

Figure J.1: Relationship between plasma IgA (IgA_p) and mucosal IgA (IgA_m). Black dots are the Scottish data, Black-Faced sheep trickle infected with *T. circumcincta*; white dots are the Spanish data, Churra sheep trickle infected with *T. circumcincta*.

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