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Genomic solutions for selective breeding towards increased disease resistance in sheep

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“Forsaken all inhibitions, pursue thy dreams. Well, every man has a religion; has something in heaven or earth which he will give up everything else for – something which absorbs him – which may be regarded by others as being useless – yet it is his dream, it is his lodestar, it is his master.”

- Walt Whitman

Declaration

I hereby declare that the research presented in this thesis and any included publications are my own, unless otherwise stated. The work described has been carried out with guidance from my supervisors and has not been submitted for any other degree or professional qualification.

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Peer-reviewed Publications

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Abstract

Gastrointestinal parasitism is a global problem for grazing ruminants which can be addressed sustainably by breeding animals to be more resistant to disease caused by gastrointestinal parasites. This thesis sets out to estimate the genetic parameters of parasitic infection associated with natural nematode and coccidian infections, productivity and immunological phenotypes associated with immune responses including various cytokines and immunoglobulin A (IgA). The thesis sheds light on the genetic architecture of these traits and uses animal genomic and phenotypic data to identify candidate genes associated with resistance to disease.

Individual animal phenotypic data on faecal egg counts from different nematode species (Strongyles (FEC_S), *Nematodirus* (FEC_N) and faecal oocyst counts (FOC) from coccidian parasites were collected on Scottish Blackface lambs together with a faecal soiling score in the breach area 'dag' score (DAG) and live weight (LWT). Data from 3,731 Scottish Blackface sheep lambs reared on one experimental farm at SRUC (Castlelaw) were used from 2011 to 2017. Parasitic infection traits (FEC, FOC and DAG) were shown to be heritable (0.09±0.02 to 0.17±0.03) exhibiting significant genetic variation among individuals to underpin a selective breeding programme with the goal of enhancing animal resistance. FEC_S was shown to be positively (genetically) correlated with FEC_N (0.74±0.09) and FOC (0.39±0.15). Additionally, DAG was negatively (genetically) correlated with LWT (-0.33±0.15). Significant and positive associations between FEC_S and FEC_N, and FEC_S and FOC at around 3 months of age show that co-selection for increased resistance to different parasites is feasible. Furthermore, selection for increased resistance is unlikely to

adversely affect LWT, as no significant antagonistic relationship was found between faecal counts and LWT. Significant antagonistic phenotypic correlations between LWT and DAG, and LWT and FEC_S/FEC_N indicate that the expression of manifestation of disease in lambs via the DAG score may be a meaningful indicator of the impact of parasitic burden on productivity.

Additionally, whole blood stimulation assays were used to characterise the adaptive immune response of 1,040 lambs measured in 2016-2017, with either pokeweed mitogen (PWM, a lectin that non-specifically activates lymphocytes irrespectively of their antigen specificity), and *Teladorsagia circumcincta* (T-ci) larval antigen to activate parasite-specific T lymphocytes. The type of adaptive immune response was determined by quantifying the cytokines interferon-gamma (IFN-γ), interleukin (IL)-4, and IL-10, which relate to T-helper type 1 (Th1), Th2 and regulatory T cell (Treg) responses, respectively. T-ci specific IgA within serum was also quantified. Heritability estimates for each immune trait, and genetic and phenotypic correlations with parasitic infection and productivity phenotypes were estimated. Heritabilities of cytokine production varied from low to high (0.14±0.06 to 0.77±0.09), while IgA heritability was found to be moderate (0.41±0.09). A positive genetic correlation was found between FOC and PWM-induced IFN-γ ($IFN\text{-}\gamma_{(PWM)}$) production (0.67±0.30) while a negative correlation was found between FOC and T-ci induced IL-10 ($IL\text{-}10_{(T\text{-}ci)}$) (-0.84±0.31). Live weight was negatively, genetically correlated with IFN-γ responses (-0.54±0.18 and -0.51±0.20). Overall, IFN-γ and IL-4 responses were positively correlated (from 0.50±0.15 to 0.74±0.21), providing little evidence of cross-regulation of Th1 and Th2 immunity within individual sheep. The results show

a negative correlation between IL-$10_{(PWM)}$ and IL-$4_{(T.ci)}$, which might indicative of a regulatory function of IL-10 over IL-4. Furthermore, Immunoglobulin A was shown to be genetically correlated with IL-$10_{(PWM)}$ and IL-$4_{(T\text{-}ci)}$ (0.85±0.17 and 0.32±0.17, respectively). The results suggest that while selection for high IFN-γ responsiveness may be beneficial for coccidian parasite control, selection for this trait may negatively affect productivity, which will need to be considered in genetic improvement programmes.

DNA samples from a subset of 1,766 animals in the study were collected and whole genome sequenced. The genotypic effects on each one of the traits described above were quantified, including the additive and dominance effects as well as the proportion of additive genetic variance due to each SNP locus. A total of 15 SNPs were associated at least at a suggestive level with FEC_S, FEC_N, DAG, IgA, PWM-induced IFN-γ and IL-4, and T-ci-induced IL-10. A total of 52 genes closely related to immune function were found to be in close proximity to these SNPs. While most of the SNPs were not significant beyond a suggestive level, this study was able to confirm the polygenic nature of both parasitic infection and immunological traits such as FEC and IgA. The results highlight several C-type lectins and killer cell lectin-like family members close to a SNP associated with $FEC_{N,}$ and several genes encoding IL-1 cytokine family members associated with a SNP associated with IgA. There were also several potential candidate genes belonging to, or in close proximity to, the Major Histocompatibility Complex (MHC) which, due to its importance in the control of immune responses, could play important roles in resistance to such

parasitic infections. These include HFE and butyrophilin coding genes, associated with IFN-$\gamma_{(PWM)}$, and IL-17 coding genes associated with IgA.

The results reveal a largely complex and polygenic genetic control on resistance to parasitic infection and immunological traits in this Scottish Blackface sheep population. Lastly, these results also suggested that the studied animal traits are amenable to improvement with genomic selection.

Lay Summary

Gastrointestinal parasitism is a pervasive problem affecting ruminants globally that can be addressed sustainably by breeding animals for increased resistance to disease. Selecting animals that are more resistant to infection is advantageous since the effect is cumulative over time and reduces infestation levels over consecutive generations, thereby reducing the economic losses in production due to disease. The present project's aims were to, i) investigate the genetics underlying sheep that were co-infected with distinct parasites and their genetic relationships with productivity, ii) investigate the immune profile of lambs infected and assessing the viability of using immunological traits to select more resistant lambs to disease and, iii) perform genomic studies to identify potential candidate genes linked to immune response and disease, that could potentially be used to select more resistant animals. This study found that there is enough genetic variability underlying parasitic infection, highlighting the feasibility of including these traits in breeding programmes with the objective of enhancing resistance to infectious diseases in sheep. Furthermore, selection for enhanced resistance to the parasites in this study would have no impact on lamb growth. Results indicate that production is likely to be improved with lower levels of infection. The investigation of immunological traits also revealed significant variation and confirmed the lack of cross-regulation of the distinct immune response types. Substituting immunological data as an alternative to recording actual disease traits is not recommended due to the lack of confirmed, significant relationships amongst these and disease traits. It is also possible that selection for specific immune response might negatively impact productivity.

Including all traits in a breeding programme is likely to be the best solution which can be achieved with a selection index, whereby the information regarding all traits can be used to derive breeding values. Finally, genomic studies attempted at identifying potential chromosomal regions and candidate genes involved in immune function and confirmed the complex nature of the genetic control of the traits analysed in this study. This thesis was successful in identifying potential genomic regions holding significant roles related to immune responses. New insights into these regions can prove useful and increase the accuracy of the genomic evaluation and selection of animals with increased disease resistance. The study revealed multiple potential candidate genes with known biological functions related to parasitic infection and immunological traits and may prove useful in future breeding programmes.

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List of Abbreviations

a	Additive genetic effect
BSA	Bovine Serum Albumin
CD4+	Cluster of differentiation molecule 4 positive
CD8+	Cluster of differentiation molecule 8 positive
CTL	C-type lectin
d	Dominance genetic effect
DAG	Faecal soiling scores or dagginess
DAVID	Database for Annotation, Visualisation and Integrated Discovery
ELISA	Enzyme-linked Immunoassay
FCS	Faecal Consistency Score
FEC	Faecal egg counts
FEC_N	Faecal *Nematodirus* egg counts
FEC_S	Faecal *Strongyle* egg counts
FOC	Faecal oocyst counts (*Coccidia*)
GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GIT	Gastrointestinal tract
GO	Gene ontology
GWAS	Genome-wide association study
h^2	Heritability
IFN-γ	Interferon-gamma
IFN-$\gamma_{(PWM)}$	PWM-induced IFN-γ
IFN-$\gamma_{(T\text{-}ci)}$	T-ci-induced IFN-γ
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL-1	Interleukin-1

IL-10	Interleukin-10
$IL\text{-}10_{(PWM)}$	PWM-induced IL-10
$IL\text{-}10_{(T\text{-}ci)}$	T-ci-induced IL-10
IL-17	Interleukin-17
IL-36	Interleukin-36
IL-37	Interleukin-37
IL-38	Interleukin-38
IL-4	Interleukin-4
$IL\text{-}4_{(PWM)}$	PWM-induced IL-4
$IL\text{-}4_{(T\text{-}ci)}$	T-ci-induced IL-4
Kbp	Kilo-base pair
KEGG	Kyoto Encyclopaedia of Genes and Genomes
Kg	Kilogram
L1	First larval stage
L2	Second larval stage
L3	Third (infective) larval stage
L4	Fourth larval stage
L5	Fifth larval stage
LD	Linkage Disequilibrium
LWT	Live weight
MAF	Minor allele frequency
Mbp	Mega-base pair
MHC	Major Histocompatibility complex
NCBI	National Centre for Biotechnology Information
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural Killer
O.D.	Optical density
OAR	Sheep chromosome

Oar v3.1	Version 3.1 of sheep genome
PBS	Phosphate Buffered Saline
PCA	Principal component analysis
PV_A	Proportion of genetic variance explained by SNP
PWM	Pokeweed mitogen
Q-Q plot	Quantile-Quantile plot
QTL	Quantitative Trait Loci
r_G	Genetic correlation
r_P	Phenotypic correlation
RT-PCR	Reverse transcription polymerase chain reaction
SBF	Scottish Black Face
SD	Standard deviation
SE	Standard error
SNP	Single Nucleotide Polymorphism
spp.	Species
T-ci	*T. circumcincta* specific antigen
TGF-β	Tumour growth factor beta
Th0	Naïve T cell
Th1	Type 1 T helper cell
Th17	Type 17 T helper cell
Th2	Type 2 T helper cell
Treg	Regulatory T cell
V_A	Genetic Variance

Chapter 1 – General Introduction

1.1 Background

Animal infection by gastrointestinal (GI) parasites represents one of the most important contributors to important economic losses worldwide. Ranging from subclinical weight loss to lethal pathologies, GI infections result in sub-optimal performance which has been estimated to cost the British farming industry around £84 million, making it the most costly disease in ruminant farming (Charlier *et al.*, 2014; Chartier and Paraud, 2012; Mavrot *et al.*, 2015; Nieuwhof and Bishop, 2005). In their recent assessment of the economic burden of major helminth infections to ruminant livestock in several European countries, Charlier *et al*., (2020) estimated that the cost to the sheep industry is around €47 million, or around half the estimates reported by Nieuwhof and Bishop (2005) (roughly £40 million). Charlier *et al*., (2020) model provides more conservative overall estimates and these authors note that the previously published methodologies lack detail to allow full replication. In sheep, the highest susceptibility is mainly observed in weaned lambs during their first grazing season (Gossner *et al.*, 2012), due to their lower resistance when compared to older sheep, with faecal egg counts (FEC) usually peaking at the end of the first grazing season (Stear *et al*., 1999b). The delay in acquisition of immunity may partly reflect age-dependent effects on the anti-parasite immune responses as well as parasite-induced immune suppression (McNeilly *et al*., 2013).

The development of breeds selected for resistance to GI parasites is probably one of the most promising alternative method to control infections (Venturina, 2012) in addition to nutrition, mixed species/rotational grazing and refugia. There is evidence of genetic variation among individual sheep in resistance to parasites, which has

been documented in different breeds (Sechi *et al.,* 2009). The genetic control methods used to select individuals more resistant to disease rely on the existence of genetic variation (Falconer, 1965). Traditionally, breeding strategies for enhanced resistance are based on indicator traits like FEC, for which genetic variation among animals may be manifested even with more moderate levels of infection (Zvinorova *et al.,* 2016). In the UK, selection of sheep for enhanced resistance to GI parasites is considered feasible under normal commercial conditions in which sheep face natural parasitic challenge (Bishop *et al.,* 2004). Resistance to GI parasites should integrate broader control programmes (Nieuwoudt *et al.,* 2002) as discussed below, which may benefit sheep production enterprises (Bishop *et al.,* 2004).

1.2 UK sheep production

The UK sheep flock is characterised by its three-tier stratified breeding structure, which employs systematic crossbreeding which sheep to be farmed in a wide range of climates, environments and management systems, allowing hill breeds to be kept in generally inhospitable environments (Sargison, 2008). In 2012, the UK had the largest sheep flock out of all the EU member states accounting for more than a quarter of all the sheep within the EU (32.2 million sheep, including 15.2 million breeding females). In the UK, the sheep industry is primarily focused on meat production (Sargison, 2014). In terms of the global lamb trade, the UK accounted for almost 10% of the sheep meat exports, also in 2012 (National Sheep Association and National Farmers Union, 2014).

1.2.1 Scottish Blackface sheep

The Scottish Blackface (SBF) sheep breed was established in the 16th century. These hardy, medium-sized sheep are characterised by their short tail and long white wool. Their face is mainly black with some white spots, and both sexes have horns. As hill sheep, SBF sheep make ideal mothers to breed crossbreds, which can subsequently breed fat lambs on lowland areas (Duncanson, 2012).

1.3 GI parasitism and its impact on sheep production

The intensification of farming practices has brought about an increased risk of animal disease outbreaks, which impacts both animal welfare and production (Sweeney *et al*., 2016). Gastrointestinal parasitic infections pose a serious constraint in small ruminant production (Benavides *et al*., 2015). Suboptimal productivity due to parasitic gastroenteritis has become a common problem in UK's flocks, in recent years, despite the adoption of successful parasitic control programmes that involve drugs (Sargison, 2014). Total costs associated with disease can account for 20% of turnover in developed countries and up to 50% of turnover in the livestock sector in developing countries (Bishop and Woolliams, 2014). In the UK, economic losses have been estimated to average at around £84 million in 2005 (Nieuwhof and Bishop, 2005) with more than 2/3 corresponding to losses in the rate of growth.

Gastrointestinal nematodes (GIN) represent a majorly serious health limitation in sheep industry worldwide, being a significant source of economic losses (Estrada-Reyes *et al*., 2019b; Hayward, 2022). The losses associated with these parasites come from decreased productivity and significant treatment costs (Charlier et al.,

2020). Major nematode species affecting sheep include *Nematodirus battus* and *Teladorsagia circumcincta* (Van Dijk *et al*., 2010), the latter of which is significantly prevalent and economically important in northern Europe, dominating the parasitic fauna affecting sheep (Bishop *et al*., 1996). Practically all sheep will be exposed to parasitic nematodes during their lifetime (Davies *et al*., 2006). Similar to GIN, coccidian parasites, causing agents of coccidiosis in lambs, can also have an important impact in small ruminants production which has been shown in housed flocks both after experimental and natural infections (Reeg *et al*., 2005). Coccidia represents a protozoan parasite (Jawasreh *et al*., 2013), and almost young sheep will also become infected coccidiosis (Andrews, 2013), although in most cases, these parasites cause little to no effect, with disease only manifesting when animals are subjected to heavy infections or if their resistance is lowered (Taylor, 1995). Coccidiosis becomes important economically as a consequence of losses due to clinical disease, which usually translates in diarrhoea, and subclinical infections devoid of clinical signs, but still characterised reduced production due poor weight gain (Chartier and Paraud, 2012).

1.3.1 Nematode life cycle

The life cycle of nematode parasites, such as *Teladorsagia circumcincta*, starts when eggs are passed out of the sheep with faeces: once hatched, parasitic worms develop into free-living larval stage 1 (L1) and L2 using bacteria in the faeces as sustenance, subsequently developing into infective L3 (Mackinnon, 2007). If moisture is adequate, the surviving L3 larvae will migrate out of the faeces and onto to the grass (MacKinnon, 2007). Within 1-3 days after ingestion, L3 enter the gastric glands

where a further moult is undergone from which L4 larvae emerge and will often penetrate the wall of the abomasum where they can enter a dormant stage and arrest their development within the gastric gland until external conditions improve, if necessary (Wilkie, 2016). L5 larvae finally leave the abomasal gland approximately 10 days post-infection and become sexually mature on the mucosal surface with adult females producing eggs that are subsequently excreted in the faeces, thus completing the life cycle (McRae, 2015).

The entire life cycle will usually be completed in approximately 4 to 5 weeks, although this is dependent on temperature and immune status of the host. Egg production can be rapid but resistant hosts are capable of delaying larval maturation for at least 8 more weeks (McNeilly *et al.,* 2009). Differences in susceptibility observed in lambs is one reason for the aggregation of parasites in the host population, meaning a large number a large number of animals harbour a few parasites, while a few animals are highly infected (Guillaume, 2012).

1.3.1.1 Pathology of Nematode infection

Infection is responsible for a relative protein deficiency that translates to poor growth. This deficiency has four causes: infected animals eat less, protein is digested less efficiently, protein is leaked into the gastrointestinal tract and infection results in an increase of protein demand as proteins is diverted into repair processes and immune and inflammatory responses (Stear *et al.,* 2009).

Gastrointestinal nematodes are responsible for a range of clinical signs in host, particularly young or nutritionally stressed or infected animals (Bartley, 2008;

Wilkie, 2016). Common clinical signs of a GIN infection include anorexia, emaciation and, in extreme cases, infections with nematodes may lead to the death of infected animals (Idris *et al.,* 2012). Diarrhoea is a major factor for loss of bodyweight and condition, and causes soiling of the wool around the breech of the animal consequently resulting in devalued wool and contaminated meat carcasses in the abattoir (Williams and Palmer, 2012).

While immunity may reduce the impact of infection, it comes at a cost since the activation of immune responses involves the recruitment and proliferation of a large number of cells and the various components involved in the response to infection are expected to carry a substantial nutritional penalty (Greer, 2008).

1.3.2 *Coccidia* life cycle

Coccidiosis is a disease caused by protozoan parasites that most commonly affects young animals, with lambs being more susceptible to infection (Jawasreh *et al.,* 2013). The most common causal agent of lamb coccidiosis is *Eimeria spp.* (Odden *et al.,* 2017) with outbreaks majorly impacting economy and animal welfare (Ozmen *et al.,* 2012). Because the life cycle of *Eimeria* comprises intracellular and extracellular, asexual and sexual stages, immune responses elicited are highly complex, involving aspects of both specific and non-specific immune responses, including cellular and humoral mechanisms (Lillehoj *et al.,* 2004).

Eimeria enters the host by penetrating the epithelial cell lining of the intestinal mucosa, often causing serious damage to the physical integrity of the gut (Yun *et al.,* 2000). Following ingestion of *Eimeria* oocyst, mechanical disruption and digestive

processes release spoorocysts and subsequently their sporozoites which attach and invade the most susceptible region of the intestine (Broom and Kogut, 2019). The sporozoite goes through a series of developmental stages inside the host leading to the formation of unsporolated oocysts that are ultimately excreted into the environment, eventually sporulating (Broom and Kogut, 2019). Once excreted, oocysts become infective, remaining viable for prolonged periods of time until they are ingested again and thus restarting the cycle (Yun *et al.*, 2000).

1.3.2.1 Pathology of coccidian infection

Clinical signs of coccidian infection include diarrhoea, which can be haemorrhagic, abdominal pain, weight loss and/or reduced weight gain (Odden *et al.*, 2017). Severe diarrhoea can lead to damage in the intestinal lining, which in extreme cases can result in death (Ozmen *et al.*, 2012). Clinical coccidiosis is a self-limiting and severe disease is generally related to high infection pressure (Chartier and Paraud, 2012). Due to the fact that ovine coccidiosis can have major economic impact caused by a reduction in weight gain and increased mortality, controlling the infection is important (Odden *et al.*, 2017). Diagnosis is frequently based on oocysts counts but a definite diagnosis must involve factors such as epidemiology and observable clinical signs (Andrews, 2013).

1.4 Methods of parasitic control

Control programmes are based on the knowledge of epidemiology and in that sense, the manner in which host-parasite relationship has changed due to animal and pasture management changes, the evolution of immune mechanisms in response to

larval challenge, among other factors, has led to the rise production associated losses (Sargison, 2014). According to Sargison (2014), heavy dependency on the use of pharmaceutical treatments to suppress infective helminth challenge could lead to the UK sheep becoming uneconomical. Worm management tools include pasture management, guaranteeing it meets the nutritional requirements and that pasture contains low levels of eggs and larvae, and stocking management: density of stocking rate represents an important factor in limiting the problems of worms (Wormwise Technical Advisory Group, 2019).

1.4.1 Anthelmintics

Animals are regularly subjected to anthelmintic drenching throughout the year in order to reduce their worm burden (Wilkie, 2016). In fact, since the launch of the first anthelmintic drug more than half a century ago, the use of these compounds has become the cornerstone for the control of GI nematode infections (Hoste and Torres-Acosta, 2011; Jack *et al*., 2017) with the majority of farmers in the UK having become dependent on the use of these pharmaceutical control methods aiming at finding a balance between levels of larval challenge that can potentially impact performance negatively and those required for the development of protective immunity (Sargison, 2014). In the UK, there are five classes of commercially available anthelmintics licensed for administration to small ruminants: benzimidazoles, the first class of anthelmintic to be introduced, imidothiazoles, macrocyclic lactones, amino-acetonitrile derivatives (AADs) and spiroindole compounds (Ellis, 2014). The widespread and conventional use of anthelmintics may mean that the sheep industry could not exist in its current form without anthelmintic

drugs (Sayers and Sweeney, 2005). Several studies have demonstrated that the administration of anthelminthics led to a significant reduction of the appearance of diarrhoea and subsequent breech soiling (Allerton *et al*., 1998; Larsen *et al*., 1994; Watts *et al*., 1978), supporting the notion that nematodes represent a major cause of diarrhoea in sheep (Williams and Palmer, 2012). Parasitism may be suspected as a primary cause of diarrhoea and an immediate response to an effective anthelmintic treatment usually confirms the diagnosis (Jacobson *et al*., 2020). While it is common to see nematodes and coccidia affecting lambs concurrently, in most cases diarrhoea stops following anthelmintic treatment (Sargison, 2004).

1.4.1.1 Anthelmintic resistance

While the use of anthelmintics is often the preferred choice of GI nematodes control, mainly because their availability, cost-effectiveness and convenience (Venturina, 2012), the continuous use of these drugs has led to the emergence of strains resistant to commonly used forms of control (Benavides *et al.*, 2015; Ellis, 2014). Continuous treatment with anthelmintics can lead to the survival of parasites resistant to drugs, thus resulting in a drug-resistant subpopulation capable of proliferating freely (Davies, 2006). Surviving worms will go on to contaminate pasture with resistant larvae, leading to gradual selection pressure and the emergence of anthelmintic resistance (Papadopoulos, 2008). The increasingly global problem of anthelmintic resistance in sheep nematodes has led to the development of sustainable methodologies for parasitic control (Venturina, 2012) in order to reduce the reliance on these compounds.

The development of drug resistance is the key constraint for the use of anthelmintics, which can be considered a consequence of host-pathogen co-evolution, with parasites surviving exposure to the recommended doses of anthelmintics (Zvinorova *et al.*, 2016). The increasing prevalence of anthelmintic resistance suggests the reliance on these chemical compounds is unsustainable (McRae, 2015), with pressure falling onto breeders to reduce reliance on the usage of these compounds as a form of control (Bishop and Woolliams, 2014). It takes longer to discover, test and commercialise new anthelmintics than to maintain efficacy of these compounds (Venturina, 2012). The maintenance of the efficacy of currently available anthelmintics is thus crucial (Papadopoulos *et al.*, 2012). For these reasons, the use of worm management tools other than anthelmintics can help with reducing the use of these compounds and the development of anthelmintic resistance.

1.4.2 *Refugia*

Refugia refer to larvae on pasture, or worms at stages in animals not affected by treatment, or simply worms in untreated animals. It has been widely accepted that the sustainability of chemically based strategies of worm control depends on guaranteeing that a significant proportion of animals remain unexposed to treatment, which can be achieved through targeted treatment plans that vary with the level of *refugia* (Jackson *et al*., 2009; Sargison *et al*., 2007). The larger the population in *refugia*, the slower the rate of anthelmintic resistance development will be (Duncanson, 2012; Traversa and von Samson-Himmelstjerna, 2016). The number of worms in *refugia* is expected to increase if a portion of the flock is not subjected to treatment (Ellis, 2014). Worms in *refugia* will be responsible for the next generation

of parasites and the ones surviving treatment must contribute as little as possible to the next generation (Papadopoulos, 2008). The preservation of susceptible worms within an in *refugia* population is important and increasing the size of this population in order to dilute resistant alleles in the population is a crucial concept in delaying the development of anthelmintic resistance (Stubbings *et al*., 2020). The rate of development of anthelmintic resistance varies inversely with the proportion of nematode population that is in *refugia* at the time of treatment: if the proportion of nematodes in *refugia* is large at the time of treatment, the offspring of resistant nematodes will be diluted, while small nematode populations in *refugia* will result in a larger proportion of resistant parasites in the next generation (Sargison *et al*., 2007). In the UK, SCOPS (Sustainable Control of Parasites in Sheep) principles are geared towards minimising the number of animals that are subjected to treatment at one time, either through targeted selective treatment or targeted treatment. The selection of animals that are going to be left untreated is aimed at recognising those exhibiting greater resistance/resilience, since these animals should be able to cope with words without the need for treatment (Stubbings *et al*., 2020). In New Zealand, there are also guidelines in regards to maintain *refugia* (Wormwise Technical Advisory Group, 2019). A strategy related to *refugia* has been implemented in New Zealand whereby ewes with lower condition-scores are left untreated and transferred to superior pastures with their drenched lambs, and thus their condition can improve without the use of anthelmintic treatment while they provide a source of *refugia* to the treated lambs (Leathwick, 2014).

1.4.3 Grazing management

The control of worm infestation through the use of grazing management schemes is also an important tool to prevent host contact with infective larvae. Pasture rotations as a form of grazing management has been used in sheep production systems for decades to minimise the threat of nematodes (Jackson *et al*., 2009). Despite its importance, grazing management requires detailed understanding of the farm and parasites affecting it in order to be successful (Hoste and Torres-Acosta, 2011; Jackson *et al*., 2009) and the complexity associated with this management is an important reason as to why this strategy is less well exploited (Jackson *et al*., 2009). Pasture rotation is very effective at reducing infection and/or reinfection rates by only allowing animals to graze a pasture for a limited period of time before moving them (Bartley, 2008). Rotational grazing can be used to exploit the biological rates of the free-living stage of parasites and introduce ruminants to a pasture after the bulk of L3 that emerge from eggs in the soil has significantly decreased due to their natural death rate (Hoste and Torres-Acosta, 2011). Additionally, mixed grazing with other species (e.g. cattle) presents a significantly low risk of cross-infection since the parasite species infecting cattle and sheep are largely different, and in this way, pasture contamination can also be reduced since it reduces stocking density of sheep. Thus, nematode eggs excreted will be deposited over a wider area and reduce the density of infective larvae present on herbage (Stubbings *et al*., 2020; Wormwise Technical Advisory Group, 2019).

1.4.4 Nutrition

The nutritional status of naïve hosts has a significant impact on the severity of parasitism and resilience to infections is enhanced through increased metabolisable nutrient supply. This increased resilience helps the animal by allowing it to develop resistance with significantly reduced pathological consequences (Van Houtert and Sykes, 1996). Nutrient availability reduction is caused by GI nematodes through reduced feed intake and/or reduced efficiency of nutrient absorption (Coop and Kyriazakis, 1999). Generally, food intake increases as animals acquire resistance to infection. The hosts' nutrition is therefore one of the crucial elements influencing the development and maintenance of host acquired immunity (Jackson *et al*., 2009). It has been hypothesised that the improved ability of the host to tolerate the negative effects incurred by parasitic infections and respond to parasites might be the result of feed complementation, particularly with nutrients constituting limiting factors of the diet (Hoste and Torres-Acosta, 2011). The approach of increasing dietary protein in order to manage parasitic infections is an attractive alternative to the use of anthelmintics since they have long-term potential and reduce the level of pharmaceutical compounds entering the food chain. Such method requires prior knowledge of the biology and epidemiology of nematodes infecting livestock, and is not easy to communicate to the wider farming industry (Wilkie, 2016). Better control of parasitism by the host and reduced impacts on their performance lessens the need for treatment (Laurenson, 2012).

1.4.5 Vaccines

While a viable alternative strategy to the use of anthelmintics there have only been a few available vaccines produced to control nematodes (Ellis, 2014). Vaccines developed against GI nematodes originally failed to protect young and susceptible animals (Vercruysse *et al.,* 2007). A vaccine to control *Haemonchus contortus* for calves in Australia (Bassetto *et al.,* 2014) has been extended for used in sheep (Bassetto *et al.,* 2020) and commercialised in Australia as '*Barbevax*' and in South Africa as '*Wirevax'*.

In the case of coccidian parasites, stimulations of development of immunity was successful and achieved using strains that were selected for short but complete life cycles (Vercruysse *et al.,* 2007). However, there are no commercial vaccines against ovine coccidiosis currently available.

1.5 Breeding for disease resistance

Resistance to nematodes is a trait of primary interest for the livestock industry due to its economic impact on sheep breeding. These costs relate to anthelmintic treatment, increased labour and pasture management, onto which the indirect costs of decreased production are added (Krawczyk and Słota, 2009). As a goal for many breeding programmes, disease resistance has become ubiquitous and is regularly nominated by breeders as a high priority trait (Bishop, 2012a). Resistance refers to the ability of the host to control the life cycle of the invading parasite, with measurements of parasite burden often considered to be indicators of resistance (Bishop, 2012a). In other words, resistance implies that hosts exert a deleterious influence on the

pathogen's fitness (Bishop and Woolliams, 2014). Resistance can be defined by the host's capacity to reduce the risk of infection through avoidance (genetically resistant animals are able to avoid parasites to a greater extent than susceptible animals), improved recovery and control infection, by limiting the rate of replication, and thus reduce parasite burden (Best *et al*., 2008; Hayward *et al*., 2014; Hutchings *et al*., 2007; Kutzer and Armitage, 2016).

It has been argued that parasite populations, such as nematodes, could evolve at a faster rate than the host (Stear *et al*., 2001, 2011), which can quickly overcome host resistance, comparable to the evolution of anthelmintic resistance when anthelmintics are indiscriminately used (Bishop, 2012a). While this has been considered a potential concern (Bishop, 2012b; Stear *et al*., 2001), the coevolution between host and parasite is not dominated by the parasite: it has been demonstrated that also animals have evolved high levels of resistance to disease (Stear *et al*., 2011). Thus, unlike what is observed with anthelmintic resistance, there is no evidence suggestive of nematodes evolving rapidly as a response to increasingly resistant hosts (Kemper *et al*., 2009; McManus *et al*., 2014; Zvinorova *et al*., 2016) probably due to the multifaceted nature of parasite resistance meaning that parasite coevolution is slower than anthelmintic resistance (Bishop, 2012b). In the case of nematodes, little doubt exists about their potential to evolve faster than their hosts. Of the three concepts determining response to selection, nematodes have shorter generation intervals, larger population sizes and their mortality rates higher, which translate to greater selection intensity. Nevertheless, despite the evolutionary potential of parasites, hosts can evolve resistance because the selection pressure on

individual nematode antigens is low (Stear *et al*., 2011). In their study, Woolaston *et al*., (1992) found no evidence that nematodes (*Haemonchus contortus*) are able to adapt to sheep selected for resistance. Additionally, there is a possibility that breeding for resistance to a specific disease might predispose host to rely on one category of immune response in detriment to others and thus, a futile endeavour (Stear *et al*., 2001).

Resistance to parasites represents a physiologically complex (Ahbara *et al*., 2021) and polygenic trait (Zvinorova *et al.*, 2018). Indicator traits may not represent all pathways involved and, as such, the mechanisms underlying genetic differences in resistance are still shrouded in mystery. Evidence for host genetic variability has been observed for resistance against GI parasites in ruminant livestock, which suggests that selective breeding for increased resistance is feasible (Assenza *et al*., 2014; Benavides *et al*., 2016a; Bishop, 2012b; Bishop *et al*., 2004; Bishop and Stear, 1999; Kemper *et al*., 2011; Sechi *et al*., 2009). These studies have suggested that host genetics greatly affect the number of GI nematodes in faeces, enabling estimates of heritability for resistance (Kim *et al*., 2015), with heritability corresponding to the proportion of total (phenotypic) variance that is attributable to genetics, i.e. it represents ratio of additive genetic variance in relation to phenotypic variance (Falconer and Mackay, 1996). This suggests that selective breeding for resistance against GI parasites is valid to help the control of parasitism (Doeschl-Wilson *et al*., 2008). In sheep resistance to GI nematodes is known to be heritable with most faecal egg count heritabilities from 0.2 and 0.4 (Bishop, 2012b). Nevertheless, studies involving complex diseases have generally been unsuccessful in explaining most of

the genetic variation, which results in the so-called '*missing heritability*' (Zvinorova *et al.,* 2018). Disease resistance traits are an attractive target for genomic studies, and this approach has the benefit of being able to select animals based on their DNA without the need to expose them to infection in a challenge test, or for them to be involved in a natural epidemic (Bishop and Woolliams, 2014).

Breeding programmes become successful when estimates of breeding values accurate, heritabilities are at least moderate and large databases containing large numbers of animal records (including genetic relationships) are used (Rauw *et al.,* 1998). In breeding programmes, the most profitable animals are targeted for future production which translates to increased profit, improved health in order to the decrease associated costs and meeting product requirements necessary to retain licence to operate (Dominik *et al.,* 2017).

Traits related to GIN disease resistance are difficult to measure and selection is only possible through the use of alternative, indirect methods like FEC and immunological indicators to identify resistant animals, without the need to sacrificing them (Sayers and Sweeney, 2005). Selectively breeding for GIN resistance using FEC as an indicator trait has already been successfully undertaken in sheep, but the classical selection method using this trait is hindered by the time-consuming and costly process of collecting data and the necessity for animals to be infected (Atlija *et al.,* 2016).

Selection of animals for increased resistance is associated with improved immunological capacity and both lower nematode burdens and concentrations of eggs in the faeces, while resilient animals still perform well even during parasitic

challenge despite at times harbouring greater parasite burden and FEC (Greer *et al.*, 2018). Even though resilient animals perform well and require lower drenching levels, they lead to more pasture contamination as a result of there being no reduction in FEC, which will cause non-resilient animals to be subjected to a higher parasitic challenge (McManus *et al*., 2014).

1.6 Commonly used resistance indicator traits

1.6.1 Faecal egg and faecal oocyst counts (FEC/FOC)

Faecal counts are the most used method to indirectly measure resistance to parasite, mainly because it is relatively easy to measure in animals, due to its reliability, and it offers direct estimates of pasture contamination (McRae, 2015; Zvinorova *et al.*, 2018). Their interpretation is dependent on the knowledge of the relative faecal dry matter content, feed intake and how the animals were fed at the time of sampling (Sargison, 2013). FEC is influenced by factors like the level of larval challenge, species, worm burden and the degree to which worm establishment and fecundity is affected by the host's immune responses (Douch *et al.,* 1996).

This trait is considered good indicator of worm burdens for various nematode species (including *T. circumcincta*), but the relationship starts to break down at high worm burdens due to density-dependent constraints on worm fecundity (Bishop, 2012a). Reported correlations between FEC and worm burdens are high, but these correlations vary depending on the host breed and species of GIN in study (Amarante *et al*., 2004; Beraldi *et al*., 2008; Bisset *et al*., 1996; Stear *et al*., 1995). Previous reports in SBF lambs have indicated that the variation observed ion FEC

after natural infection appear to be primarily a result of the variation in worm fecundity rather than worm burden (Stear *et al*., 1996, 1997, 1999a). FEC is dependent on host health, as diarrhoea can be associated to a dilution of eggs, and poor body condition can result in low egg excretion but, despite this, this trait still a useful tool to evaluate flock contamination (Guillaume, 2012).

Genetic progress in FEC continues to be made in flocks where selection is applied (Morris, 2011), since a moderate proportion of the variance in FEC has been shown to be due to host genotype (McRae, 2015). Selecting for decreased FEC both directly and indirectly benefits animal production by decreasing pasture contamination and, therefore, decreasing infectious challenge at the flock level (Bishop, 2012a). The use FEC for selection is not without drawbacks: animals are required to being exposed to relatively high challenges in order to reliably estimate their phenotype, which in turn can result in decreased productivity, samples cannot be stored for extended periods of time, and the labour-intensive nature of collecting samples makes automation unlikely (Douch *et al.*, 1996).

Oocysts in animals infected with coccidian parasites are counted much in the same manner as faecal egg counts. In general, a large number of inoculating oocysts is required in order to generate immune responses against coccidian parasites, but some exceptions have been noted (Yun *et al.*, 2000). Almost all young sheep will become infested with this parasite at some point (Andrews, 2013). There are several potential sources of infection in young lambs, with oocysts originating in excretions from periparturient ewes and/or other lambs, or surviving oocysts from previous contamination of the lambing area (Platzer *et al*., 2005). The risk of environmental

contamination with oocysts is greater with high stock rates, and with it, there is also a higher risk of infection and outbreak of clinical coccidiosis (Gauly *et al.,* 2004). The mean number of oocysts per gram of faeces has been shown to have a good correlation with the rate of infection (Gauly *et al.,* 2001).

1.6.2 Faecal soiling scores or dagginess (DAG scores)

Dagginess refers to the accumulation of faecal matter around the hind quarter of an animal, due to soiling and has a substantial economic importance in some countries since financial penalties can be applied at slaughter when animals exhibit excessive dagginess (O'Brien *et al.,* 2017). Additionally, it poses a risk of flystrike and is a management constraint (Pickering *et al.,* 2012), and has ethical and welfare implications (Pickering *et al.,* 2015a).

DAG scores and FEC are often considered as being genetically correlated traits in sheep (Pickering *et al.,* 2015b), though some studies have yielded poor genetic correlations between faecal soiling and FEC (Larsen *et al.,* 1994; Pickering *et al.,* 2012; Pollott *et al.,* 2004). Nevertheless, evidence has been found for both unfavourable (Douch *et al.,* 1995; Watson *et al.,* 1986) and favourable (Bisset *et al.,* 1992) relationships between FEC and DAG scores. Moreover, as mentioned before, evidence supporting the association between those two traits has been found in studies where anthelmintic administration effectively reduced the occurrence of faecal soiling (Allerton *et al.,* 1998; Larsen *et al.,* 1994; Watts *et al.,* 1978). If soiling is a reliable way of identifying worm burdens in sheep, it may serve as an indicator trait for selective treatment of individual animals (Broughan and Wall, 2007).

1.7 Immune responses

Parasitic infection clearance from the host depends largely on pathogen burden, and the magnitude and nature of the specific immune responses, which is itself largely regulated by the activity of helper T cells (London *et al*., 1998), as it shall be discussed later in this section. Furthermore, immune responses also depend on the nature of the pathogen infecting the host (Cox, 2001) as there are significant differences when it comes how the immune system responds to infection. This section explores the nature of immune responses and how they affect helminths and protozoan parasites affecting sheep and that are the focus of this thesis.

1.7.1 Innate immunity

Innate immunity corresponds to the first line of defence and is mediated by cells and mechanisms that are not antigen-specific do not confer any long lasting protection and may account for some of the host differences observed in primary susceptibility to infections (Bartley, 2008). The innate immune response is fast-acting, using non-specific lines of defence against invading pathogens and acts as a barrier to prevent infection (Ellis, 2014). Innate immune responses exhibit a level of antigen specificity (McNeilly *et al.,* 2008) and rely on a limited number of receptors known as pattern recognition receptors which recognises conserved molecular patterns that are associated with pathogens and their activation can result in phagocytosis by cells such neutrophils and macrophages (Sparks, 2017). At mucosal surfaces, epithelial cells form a physical barrier and their associated glands are responsible for producing innate defences, including mucins which form a mucus layer at the epithelial surface (McNeilly *et al.,* 2008). These glycoproteins can trap

microorganisms, stopping them before they reach the epithelium (McNeilly *et al.*, 2008). There are various cells involved in innate responses, including mast cells, eosinophils and natural killer (NK) cells (Marshall *et al.*, 2018). Mast cells are activated during innate immune responses, and beyond the recruitment of effector cells, they also induce physiological changes such as increased intestinal motility (Marshall and Jawdat, 2004). Eosinophils represent innate immune leukocytes which, when located at mucosal surfaces, are able to quickly identify and respond to invading pathogens (Shamri *et al.*, 2011). NK cells represent lymphocytes that act as limiting factors during microbial infections, preventing their spread and associated tissue damage that might occur (Vivier *et al.*, 2008)

1.7.2 Adaptive immunity

Adaptive immunity is a highly specific system that allows a stronger and more effective immune response to be mounted by the host after repeated exposure to a specific pathogen (Bartley, 2008). These responses take longer to develop, are highly specific and otherwise termed memory response and can be sub-divided into cellular-mediated and humoral response (Ellis, 2014). Humoral responses are involved with the production of antibodies specific to antigens from the invading pathogen (Ellis, 2014). Adaptive immune responses depend on lymphocytes, specifically B cells, linked with antigen recognition and antibody production, and T cells, which requires recognition of antigens bound to MHC proteins on antigen-presenting cells and leads to further mounting appropriate immune responses (Sparks, 2017).

1.7.2.1 T helper 1 cells (Th1)

Th1 cells are responsible for the activation of macrophages, NK cells and $CD8^+$ cells in order to combat intracellular pathogens, with several cytokines having been implicated in the differentiation towards Th1 responses (e.g. IFN-γ) (Carty *et al.,* 2018). Once differentiated, these effector cells continually produce pro-inflammatory cytokines, promoting cell production that leads to pathogen clearance (Maynard and Weaver, 2015). Th1 function and associated pro-inflammatory responses need to be balanced, as abnormally strong responses can lead to tissue damage (Carty *et al.,* 2018). Th1 cells can inhibit the effect of Th2 responses through the production of the aforementioned IFN-γ cytokine by inhibiting Th2 cell production and associated cytokines (Cohn *et al.,* 2014; Hohl, 2014).

1.7.2.2 T helper 2 cells (Th2)

Th2 cells play a vital role in the immune responses against extracellular parasites, achieved via the production of several Th2-specific cytokines (e.g. IL-4) (Maynard and Weaver, 2015). The cytokines involved in these responses act on innate immune cells and naïve $CD4^+$ cells, thus promoting Th2 differentiation and IL-4, in particular acts in a positive feedback loop that result in further Th2 cells differentiation (Carty *et al.,* 2018; Goswami and Kaplan, 2017). Th2 cytokines are crucial for the development of humoral immune responses, and Th2-mediated inflammation is characterised by the presence of eosinophils and basophils (Xu *et al.,* 2019). While important for the immunity against extracellular parasites, excessive Th2 responses are associated with several pathological conditions (Carty *et al.,* 2018).

1.7.2.3 Regulatory T cells (Treg)

Treg cells make up a subset of $CD4^+$ T cells that function as suppressors of proliferation and cytokine production of activated T cells (Carty *et al.*, 2018; Goswami and Kaplan, 2017). The main goal of the immune system is the establishment of a balance between defence and the avoidance of autoimmune responses, and this is possible partly due to Tregs (Xu *et al.*, 2019). As inflammation related processes are ongoing, an anti-inflammatory response is initiated by factors such as IL-10 in order to counterbalance inflammatory responses (Nilsson *et al.*, 2015). Treg cells are able limit immune responses while maintaining effective immune responses, but in some cases responses can be limited to a point where chronic infection can develop (Thornton, 2010). Nevertheless, regulatory responses can be beneficial as they prevent detrimental inflammatory pathology that can be a result of severe immune responses to pathogens (Thornton, 2010). The role of this particular subset of T cells in the defence against microbial infections is significant and, depending on the type of pathogen and the type of inflammatory responses elicited, Tregs can play a positive or negative role (Hohl, 2014).

1.7.2.4 Interplay between subsets of T helper cells

A differential interplay between Th1/Th2 and Treg genes has been suggested to modulate immune responses to GIN, as opposed to a straightforward Th1 or Th2 pathway (McRae *et al.*, 2015). In the 1980s, the hypothesis of the existence of a Th1/Th2 dichotomy was put forward, coming to be regarded as a cornerstone of immune responses (Van Oosterhout and Motta, 2005). This dichotomy is characterised by the contrasting effector pathways that the respective associated

cytokines induced (Maizels *et al*., 2004). Most studies of immune responses to nematodes have focused on Th1/Th2 dichotomy (Hayward, 2013), but he view that Th1 responses are linked to susceptibility to nematodes and Th2 responses are associated with resistance and that the balance between susceptibility and resistance is due to this dichotomy has been put into question (Venturina *et al*., 2013). Some studies in ruminants revealed a constant or even increased expression of Th1-associated cytokines despite predominant Th2 responses (Meeusen *et al*., 2005; Pernthaner *et al*., 2005) following nematode infection while it has also been shown that the expression of Th1 cytokine expression remains unaffected during nematode infection of immune or naïve lambs (Craig *et al*., 2007). On the other hand, Gill *et al*., (2000) results support the evidence for the existence of a Th1/Th2 dichotomy in ruminants due to strong Th2 immune responses observed following nematode infection. While this dichotomy has not been definitely demonstrated in sheep, the components involved in responses to GIN infection are typical of a Th2 pathway (McRae *et al*., 2014).

The variation in resistance could be the result of this differential interplay in the expression of genes involved in Th1, Th2 and Treg responses (Hassan *et al.*, 2011). Failure to observe consistency of gene expression profiles between resistant and susceptible animals could be to variation in response times between studies (McRae *et al.*, 2015). The simultaneous expression of both Th1 and Th2 genes is evident, and the lack of a clear Th1/Th2 phenotype in sheep might be explained by the expression of immunoregulatory responses (Hassan *et al.*, 2011).

Nematode and coccidian parasites are quite different in their morphology and in the way they interact with the host: nematode are extracellular parasites, generally considered to be controlled by Th2 immune responses (McNeilly and Nisbet, 2014), while coccidian parasites infect the host at an intracellular level and generally elicit the development of a Th1 immune response (Engwerda *et al.*, 2014). In that sense, breeding for resistance requires moderation: heavy reliance on Th2 markers in order to select resistant lambs could lead to the development of strong Th2 response to against nematodes, which would enable the clearance of helminth parasitic infections, but would be detrimental to bacterial or viral infections that require Th1 responses for resolution (Wilkie, 2016). For this reason, markers resistance from all T helper cell pathways should be considered to be included in breeding programmes so that this scenario is avoided (Wilkie, 2016).

1.8 Key cytokines and Immunoglobulin A as immunological traits

Few studies have focused on the genetic control of cytokine production in sheep. Corripio-Miyar *et al*., (2022) assessed different T cell phenotypes predicting resistance to different parasites in wild Soay sheep in Scotland, but most similar studies have been performed in humans (e.g. Brodin *et al*., 2015; Li *et al*., 2016) and have shown that serum levels of cytokines are moderately heritable. This presents the possibility of using cytokine production as novel traits to select sheep for increased disease resistance. Furthermore, immunoglobulin (Ig)A has already been used as an immunological indicator trait in sheep selective breeding (Davies *et al*., 2006; Sparks *et al*., 2019) and also shown to be heritable.

1.8.1 Interferon gamma (*IFN-γ*)

A key role for this cytokine is the activation of macrophages to increase phagocytosis and intracellular killing of pathogens, in particular bacteria and IFN-γ is also able to increase expression of MHC antigens by macrophages and this facilitates antigen presentation (Griffin, 2008; Rus and Via, 2007). This cytokine inhibits proliferation of Th2 cells and respective derived cytokines (including IL-4), which would block proliferation and cytokine release by Th1 cells (Bae *et al.*, 2016). In addition to inhibiting Th2 responses, IFN-γ also inhibits IL-10 synthesis and promotes cell mediated cytotoxicity (Burleson *et al.*, 2015).

1.8.2 Interleukin 4 (*IL-4*)

The classic Th2 cytokine IL-4 is considered to be the most important cytokine when it comes to the control of GI parasite infections (Venturina *et al.*, 2013). IL-4 establishes a circuit that maintains Th2 cell expression, also serving as a promoter of proliferation and differentiation of B cells, and induces the synthesis of IgE (Benveniste, 2014). IL-4 is known for promoting antigen presentation by increasing MHC class II expression on antigen presenting cells (Burleson *et al.*, 2015; Smiley and Grusby, 1998). This cytokine is regarded as an anti-inflammatory cytokine because as it antagonises the effects of IFN-γ on macrophages and down-regulates Th1 cell responses (Griffin, 2008). IL-4 is secreted from basophils, mast cells and eosinophils (Kubo *et al.*, 2017; Legård and Pedersen, 2019).

1.8.3 Interleukin 10 (*IL-10*)

This cytokine is produced by a whole host of cells, including Th1, Th2, Tregs, cytotoxic T cells, mast cells and activated monocytes (Burleson *et al.*, 2015). The primary function of IL-10 is to limit inflammatory responses (Griffin, 2008). IL-10 is a key anti-inflammatory cytokine and plays a critical role in maintaining the intestinal mucosa homeostasis (Shouval *et al.*, 2014). It suppresses Th1 and Th2 cell activation, and its levels are inversely correlated with incidence and severity of disease (Kubo *et al.*, 2017). As an anti-inflammatory cytokine, IL-10 maintains the balance of immune responses and allows the clearance of infection while minimising the damage inflicted on the host, and it can potentially lead to chronic infection (Howes *et al.*, 2014).

1.8.4 Immunoglobulin A (*IgA*)

IgA is a key antibody associated with the humoral mucosal immune system that can bind selectively to antigens and can prevent invading organisms from penetrating or interacting with the mucosal epithelium (Sparks *et al.*, 2018; Sutherland *et al.*, 2019). Its activity is also considered an indicator of host immune response (Riggio *et al.*, 2013). IgA is produced at mucosal surfaces and, in sheep, serum IgA is mainly derived from the intestine, being the isotype most closely associated with intestinal immune responses (McRae *et al.*, 2015). Higher levels of this immunoglobulin have been positively associated with resistance to *T. circumcincta*, and regulate worm length as well as fecundity (Karrow *et al.*, 2014; McRae *et al.*, 2015). The reduction in worm length is associated with a significantly heritable parasite-specific IgA response (Hayward, 2013). Resistance is mediated by IgA activity against 4th-larvae

and the presence of arrested L4 larvae is positively correlated with worm burden and the local IgA immune response in SBF sheep (McRae *et al.,* 2015).

1.9 The use of quantitative genetics in parasite control strategies

According to Falconer and McKay (1996), quantitative genetics refers to the study of quantitative traits likely to be influenced by large numbers of genes, with each of these genes having a small effect on the trait. Instead of considering changes in the frequencies of specific alleles, quantitative genetics quantify the changes in frequency distribution of traits, which cannot be placed within discrete phenotypic classes (Reshma and Das, 2021). Genetic variation among important traits in livestock can be explained by the infinitesimal model which is the basis of quantitative genetics. It assumes traits are determined by an infinite number of un-linked and additive loci, each with an infinitesimally small effect (Fisher, 1918).

Low heritability traits such as parasite resistance are, and will remain challenging even in the genomic era, and this is due to the need of large reference populations that require thousands of animals and thousands of single nucleotide polymorphisms (SNPs) to achieve breeding values that are predictive of genetic merit (Dominik *et al.,* 2017).

1.9.1 Advancements in the field of molecular genetics and genomic selection

Since the 1990s, advancements in the field of molecular genetics have held the promise that information at the DNA level would result in greater genetic improvement, compared to only using phenotypic and pedigree information (Meuwissen *et al*., 2016). The advancement of high-throughput genotyping and

sequencing technologies have enabled the discovery of hundreds of thousands of genetic markers, both in the human genome as well as that for plants and animals (De los Campos *et al.*, 2013) opening up the opportunity to create high density 'SNP chips', the data from which are used in several breeding programmes internationally. Such technologies present the opportunity of using genomic selection, which integrates genetic markers such as SNPs into breeding programmes (Dominik *et al*., 2017), thus presenting an alternative to traditional selection methodologies and can improve the rate of genetic with the use of these SNPs in order to predict breeding values of animals (Meuwissen *et al*., 2001). An important feature of genomic selection is the use of a reference population, for which genotyping and phenotyping of animals that are genetically related to the wider population in undertaken, in order to maximise the impact of undertaking the genotypic information (Rupp *et al.*, 2016). As a consequence of the current widespread use of such DNA information, there have been a few important breakthroughs: many thousands of SNPs markers were identified, SNP-chip genotyping technologies have evolved, making the genotyping of SNPs cost effective and allowed genomic selection methodologies to be established (Meuwissen *et al*., 2016). With dense panels of molecular markers, where their numbers can often exceed the number of records, the exploitation of multi-locus linkage disequilibrium between QTL and SNPs to predict genetic values is possible (De los Campos *et al.*, 2013). In that sense, effectively exploiting molecular information increases the chances of developing the protocols that will enable the successful selection of animals with increased resistance (Atlija *et al*., 2016). Incorporating genotype information via the use of genetic markers is frequently cited as a means of making selection decisions more quickly and

accurately, while not requiring all animals to be challenged with parasitic infections (Bishop, 2012b). The costs associated with genotyping are crucial for the success of breeding programmes, and these are set to continue to decline with new technological advances (Dominik *et al.*, 2017).

Accurate descriptions of the genome can be achieved using molecular information at a large number of loci (De los Campos *et al*., 2013), and animals can be selected at earlier ages and the rate of genetic improvement is expected to increase with enhanced accuracies of breeding value predictions (Blasco and Toro, 2014; Kaseja *et al.*, 2023). In the context of disease resistance, genomic selection brings ethical benefits as it reduces the number of animals exposed to disease, thus reducing the suffering of future generations and is therefore a credible goal for animal genetic improvement (Rupp *et al.*, 2016).

1.9.2 Genome-wide association studies

Genomic selection uses all available information from all SNPs in the prediction of genetic merit of individuals for further breeding. Genome-wide association studies (GWAS) identify loci in association with a specific trait, based on the correlation of allele frequencies at each of the thousands of markers spanning the entire genome, with trait variation in a population sample (Leung *et al.*, 2019). This process can highlight significant genomic regions which then can be targeted further for fine-mapping to determine causative alleles. When it comes to mapping genetic markers to traits of economic importance in livestock, GWAS has become a popular method, ideal for the identification of genes associated with various traits and to shed light on the mechanisms of complex traits (Sharma *et al.*, 2015). Evidence points to the

existence of 2,000 to 3,000 genes controlling host defence in mammals, providing these animals with a large repertoire of immune responses to combat invading pathogens (Mallard *et al.,* 2015).

Some genotypes can sometimes be missing following SNP-chip genotyping, an issue often solved through imputation, whereby missing genotypes can be read from the genotypes of the other animals that carry the same haplotype (Meuwissen *et al*., 2016). Genotyping of key ancestors with denser and more expensive chips allow the identification of haplotypes in the population. Thus, imputation can be used in combination with less dense, but otherwise cheaper SNP chips to genotype a large number of descendants (Meuwissen *et al*., 2016).

Genome-wide association studies for parasitic infections suggest that infections can evoke several host responses that enhance innate and acquired immune responses, gastric mucosal protection, haemostasis pathways, and delay in parasite development and reduction in the number of eggs produced by GI parasites (Ahbara *et al.,* 2021). Animals that graze under natural conditions and are exposed to a range of GI parasites, are expected to exhibit a large repertoire of defence mechanisms which may be reflected in the functions of genes located in the candidate regions (Ahbara *et al.,* 2021).

These studies are not without its limitations, often lacking in statistical power, particularly when it comes to detecting mutations that have small effects on the phenotype; for complex traits that are controlled by a large number of loci each contributing a very small effect, GWAS are less likely to be fruitful (Kent *et al.,* 2019). Two of the most widely cited challenges of next generation sequencing are

the complexity of the data and the costs associated with it, but the continual decrease in the costs and the emergence of available bioinformatics and computational methods is proof that these challenges can be resolved (Raszek *et al.,* 2016).

1.10 Aims of the thesis

This thesis aimed to disentangle complex interaction between the genetic and genomic architecture of disease and immunological traits and animal performance. The work presented in this thesis is novel in that it includes new immunological traits: here I also assess the genetic and genomic architecture of immune responses by analysing several associated key cytokines, in addition to the analysis of IgA. To my knowledge, this is one of the first studies to include genetic parameter estimates and genomic evaluation of immunological responses based on cytokine expression, alongside disease indicator traits. This thesis attempts to evaluate the feasibility of using the traits described so far in selection programmes aimed at breeding sheep with increased resistance to GI parasitism.

Therefore, the work presented here sets out to find solutions for the improvement of disease resistance in sheep by:

- Assessing the genetic background of traits related to GI parasitic co-infection of SBF sheep with nematode and coccidian parasites through genetic parameters of commonly used parasitic infection indicator traits (FEC_S, FEC_N FOC and DAG) and assessing the relationships between disease traits and productivity, as measured by lamb live weight (LWT) (**Chapter 2**).

- Examining the genetic background of traits related to animal immune responses, manifested in IFN-γ, IL-4 and IL-10 cytokines, corresponding to Th1, Th2 and Treg responses, respectively, in addition to nematode specific IgA levels in SBF lambs, and also analysing and determining the relationships between immunological traits, and the traits analysed in Chapter 2 (**Chapter 3**).
- Identifying SNPs and examining possible candidate genes associated with resistance to GI parasitism (**Chapter 4**).

Chapter 2 - Genetic parameters of animal traits associated with coccidian and nematode parasite load and growth in Scottish Blackface sheep

2.1 Chapter introduction

Gastrointestinal parasites are an important factor contributing to productivity losses in sheep around the world. While some forms of control are available, they are usually not sustainable and may incur additional costs. Selecting sheep for increased resistance represents a good alternative method of achieving the goal of increasing resistance against GI parasites. This Chapter addresses the estimation of genetic parameters of commonly used disease indicator traits for gastrointestinal parasitic burden, namely faecal counts of Strongyles, *Nematodirus* and *Coccidia*, while also determining the genetic parameters of DAG scores (another disease trait) and live weight as a measure of productivity of Scottish Blackface sheep lambs. The chapter presented here has been published in Animal, The International Journal of Animal Biosciences (https://doi.org/10.1016/j.animal.2021.100185). The results presented in this section address the first objective of this thesis. The work was completed by the PhD candidate under guidance and in cooperation from the supervisors, listed as co-authors in the manuscript.

2.2 Manuscript

Genetic parameters of animal traits associated with coccidian and nematode parasite load and growth in Scottish Blackface sheep

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Abstract

Gastrointestinal parasitism is a global problem for grazing ruminants which can be addressed in a sustainable way through breeding animals to be more resistant to disease. This study estimates the genetic parameters of common and new disease phenotypes associated with natural nematode and coccidian infection in Scottish Blackface sheep to underpin future genetic improvement strategies for parasite control. Data on faecal egg counts (FEC) from different species of strongyle parasites and faecal oocyst counts (FOC) from coccidian parasites were collected on 3-month-old lambs together with a faecal soiling score in the breech area dagginess (DAG) and live weight (LWT). Faecal count data were obtained for *Strongyles* (FEC_S), *Nematodirus* (FEC_N) and *Coccidia* (FOC). Data from 3 731 lambs sampled between 2011 and 2017 were included. Faecal egg counts and DAG records were log-transformed prior to analysis. Data were analysed using linear mixed models. Average age at sampling was 92 days with a mean LWT of 24.5 kg. Faecal soiling was not evident in 69% of lambs. *Coccidia* were the most prevalent parasite (99.5%), while *Strongyles* and *Nematodirus* had a prevalence of 95.4% and 72.7%, respectively. Heritability estimates (±SE) were 0.16 ± 0.03, 0.17 ± 0.03, 0.09 ± 0.03, 0.09 ± 0.03 and 0.33 ± 0.04 for FEC_S, FEC_N, FOC, DAG and LWT, respectively. *Strongyles* faecal egg count had a strong and positive genetic correlation with FEC_N (0.74 ± 0.09) and a moderate positive correlation with FOC (0.39 ± 0.15) while DAG was negatively genetically correlated with LWT (−0.33 ± 0.15). The significant positive genetic correlations between FEC_S, FEC_N and FOC at 3 months of age show that co- selection of sheep for resistance to these different parasites is feasible.

Selection for increased resistance to parasite infection is not expected to adversely affect live body weight, as no significant antagonistic genetic correlations were found between LWT and FEC. There were significant antagonistic phenotypic and genetic relationships between DAG and LWT being −0.19 ± 0.02 and −0.33 ± 0.15, respectively, indicating that the expression of the manifestation of disease in lambs may be a more meaningful indicator of the impact of parasite burden on productivity.

Keywords: Gastrointestinal parasites, Genetic resistance, Heritability, Selection, Sheep

Implications

Successful implementation of breeding programmes that include disease-related traits described in this paper is a promising method to control important parasites responsible for gastrointestinal parasitic infections affecting sheep. It is a long-term, sustainable alternative to the use of anthelmintics as a way of reducing infection rates, preventing production losses and improving animal efficiency as well as improving animal health and well-being.

Introduction

Animal infection from gastrointestinal (GI) parasites constitutes an important contributor to economic losses for sheep production across the world, with those in the United Kingdom estimated on average to be at around £84 million in 2005 (Nieuwhof and Bishop, 2005) with more than 2/3 being due to losses in rate of growth. However, lamb prices have increased since 2005 and financial losses are also likely to have increased. Assuming a 10% reduction in daily weight gain, losses have been estimated at approximately £4.40 per lamb (Wright, 2013). For this reason, GI parasitic infection is a serious constraint in small ruminant production that may greatly reduce the animals' productivity levels (Benavides *et al.,* 2015). In sheep, the highest susceptibility to parasite infection is observed in weaned lambs during their first grazing season (Gossner *et al.,* 2012). Lower resistance is observed in young lambs during their first grazing season compared to older sheep, with faecal strongyle egg counts typically peaking at the end of the first grazing season (Stear *et al*., 1999b). This delay in acquisition of immunity may partly reflect age-dependent effects on the anti-parasite immune response as well as parasite-induced immune suppression (McNeilly and Nisbet, 2014).

The use of anthelmintics is often the favoured choice to control GI nematodes mainly because they are widely available, cost-effective and convenient to use (Venturina, 2012), but the continuous use of these drugs has led to the emergence of resistant strains against the commonly used forms of control (Ellis, 2014; Benavides *et al.,* 2015). The pressure falls on breeders to reduce reliance on the usage of anthelmintics as a means of control (Bishop and Woolliams, 2014). In the United

Kingdom, the true extent of resistance of pathogens to drugs is difficult to determine as there are no routine surveys being conducted, and generally, resistance is only diagnosed when signs of drug failure are reported (Ellis, 2014).

One viable option to control infection by GI parasites is the development of vaccines as an alternative strategy to anthelmintics, but there has only been a few available vaccines produced to control nematodes (Ellis, 2014). In the case of coccidian parasites, stimulation of development of immunity was successful and achieved using strains that were selected for short but complete life cycles (Vercruysse *et al.,* 2007). There have also been vaccines developed against GI nematodes that originally failed to protect young and susceptible animals (Vercruysse *et al.,* 2007). A vaccine to control *Haemonchus contortus* for calves in Australia (Bassetto *et al.,* 2014) has now been extended for use in sheep Bassetto *et al*. (2020) and commercialised in Australia as 'Barbervax' and in S. Africa as 'Wirevax'.

Selective breeding programmes have traditionally focussed on the genetic improvement of production traits (Oltenacu and Broom, 2010), which has had a dramatic positive effect on livestock productivity. There is, however, a downside to this improvement, as selection for production traits alone may cause animals to be more susceptible to pathogen infections, with resultant infection-associated production losses (Flori *et al.,* 2011). Indeed, genetic selection focusing on increasing production efficiency was found to restrict the availability of the resources needed for maintenance, reproduction and growth (Rauw, 2012).

The development of breeds selected for resistance to GI parasites appears to be the most promising alternative method to control worm infections (Venturina, 2012).

There is evidence of genetic variation among individual sheep in resistance to nematodes, which has been documented in different breeds (Sechi *et al.,* 2009). The genetic control methods to select more resistant individuals relies on the existence of genetic variation (Falconer, 1965) Traditionally, breeding strategies for enhanced resistance to parasites are based on indicator traits like faecal egg counts (FEC), for which genetic variation among animals may be manifested even with moderate levels of infection (Zvinorova *et al.,* 2016). Selection of sheep for enhanced resistance to GI parasites is considered feasible under the normal commercial sheep conditions in the United Kingdom in which sheep face natural parasite challenge (Bishop *et al.,* 2004). Nieuwoudt *et al*. (2002) stated that resistance to GI parasites should be integrated into a broader control programme. The inclusion of GI parasite resistance traits in breeding goals may be of benefit for sheep production enterprises (Bishop *et al.,* 2004). Bishop *et al*. (2004) also suggested that extra benefit will be achieved if the selection is based on both *Strongyles* and *Nematodirus*. Although there has been extensive work investigating the feasibility of selecting sheep for increased resistance to nematodes based on faecal counts, and to a lesser extent, investigating the feasibility of increased resistance to coccidian parasites, there is a clear lack of studies that focus on co-infection between these two distinct classes of parasites.

Nematodes and coccidian parasites are quite different in their morphology and in the way they interact with the host: nematodes are extracellular parasites and are considered to be controlled by Th2 immune responses (McNeilly and Nisbet, 2014), while coccidian parasites infect the host at an intracellular level and generally elicit

the development of a Th1 immune response (Engwerda *et al.*, 2014). Stear *et al.* (2001) raised the possibility of unfavourable consequences for production traits, as well as for other disease traits. In the latter case, there is a concern that increasing resistance to one disease may result in increased susceptibility to another disease. This is important due to the possible antagonism between Th1 and Th2 responses. However, this relationship between these types of immunity, commonly referred to as Th1/Th2 dichotomy has not been successfully proven in sheep (McRae *et al.*, 2014), with the involvement of immunoregulatory genes in animals infected by nematodes further putting into question the existence of such dichotomy (Hassan *et al.*, 2011).

For that reason, the objective of the present study was to estimate genetic parameters of parasitic infection indicator traits. Heritability estimates of faecal counts of different parasitic genera/species were derived to assess the feasibility of genetic selection for enhanced resistance. Heritability of faecal soiling (dagginess (DAG) score), which is indicative of GI pathology, and live weight (LWT) as a measure of productivity were also estimated, and genetic correlations between parasitic infection indicator traits and DAG scores and LWT were also estimated to determine what impact selection for parasite resistance would have on pathology and production.

Materials and Methods

Animals

A total of 3,731 SBF sheep lambs were sampled from 2011 to 2017 (Table 2.1) and did not receive anthelmintic treatment prior to sampling. The animals were a part of the SRUC experimental Castlelaw hill farm flock located in the Pentland hills, Midlothian, Scotland. Lambs were managed under typical hill farm conditions and were exposed to natural infection. Animals were allocated randomly to different grazing locations associated with their heft (home range) which resulted in their sire not being confounded with grazing area. The description of the genetic line is detailed in (Lambe *et al.,* 2008). In summary, the flock was split in to three selection lines of animals as *'Selection'* (S), *'Control'* (C) and *'Industry'* (I). The S and C lines were selected using the selection indexes described by (Conington *et al.,* 2001) as being high- or average-performing, respectively. The I line was selected on visual appearance only with no regard to performance data.

Table 2.1. Number of lambs recorded per year.

	Years						
	2011	2012	2013	2014	2015	2016	2017
Animals	548	548	512	493	554	640	436

Traits and measurements

Data collection was performed post weaning when animals were, on average, approximately 3 months of age (August in 2011; July from 2012 to 2017). Separate faecal counts faecal counts measurements were obtained for Strongyles (FEC_S),

Nematodirus (FEC_N) and *Coccidia* (FOC) using the McMaster technique (Whitlock, 1948) and were scattered and analysed by eight different SRUC labs. Additionally, LWT were measured and dagginess (DAG) (faecal soiling scores), characterized by an accumulation of faecal matter around the perineum were evaluated visually at the time of FEC sampling, using a 5-point scale (0–4), where 0 means no evidence of faeces, and 4 means a significant accumulation of faeces.

Data analysis

Preliminary analyses determined the fixed effects affecting each of the studied traits (Table 2.2). Subsequently, using ASReml v.3.0 (Gilmour *et al.*, 2009), mixed models were used to obtain trait heritability (h^2) estimates in univariate analyses, and genetic (r_G) and phenotypic (r_P) correlations between traits in bivariate analyses. All analyses were based on the following model:

$$Y = X\beta + Za + e$$

Where:

- Y = the studied trait (FEC_S, FEC_N, FOC, DAG and LWT) record
- β = vector of statistically significant fixed effects
- a = vector of additive genetic effects including animal pedigree,
- e = vector of random residual effects
- X and Z = design matrices relating records to fixed or random effects.

Statistically significant fixed effects, summarised in Table 2.2 by trait, include sex of the animal, grazing location at marking age (approximately 2 months of age), birth-

rearing rank, year of birth, lab where faeces were analysed, genetic line and age of dam at parturition. Age of lambs at the time of sampling was fitted as a covariate. Data for FEC_S, FEC_N, FOC and DAG had 1 added to each value and were log-transformed prior to analysis in order to get the distributions closer to normality. Analyses were conducted with ASReml v3.0 (Gilmour *et al.*, 2009) within the statistical package R.

Table 2.2. Statistically significant fixed effects included for each trait in the model.

Traits	Fixed effects
FEC_S	sx, lab, fec_age, yr, brrnk, mk_graz, yr.brrnk, yr.mk_graz, brrnk.mk_graz, yr.lab
FEC_N	sx, lab, fec_age, yr, brrnk, mk_graz, yr.brrnk, yr.mk_graz, yr.lab
FOC	sx, lab, fec_age, yr, brrnk, mk_graz, yr.brrnk, yr.mk_graz, yr.lab
DAG	sx, yr, brrnk, mk_graz, yr.mk_graz, brrnk.mk_graz
LWT	sx, dage, line, yr, brrnk, fec_age, mk_graz, yr.brrnk, yr.mk_graz, brrnk.mk_graz

Abbreviations: FEC_S = *Strongyles* faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* faecal egg count; DAG = dag score; LWT = live weight.
Sex of lamb (2 levels, male and female); lab = laboratory (8 levels); fec_age = age at sampling; yr = year of sampling (7 levels 2011-2017); brrnk = birth-rearing rank (10 levels); mk_graz = grazing location (12 levels); dage = age of dam; line = genetic line. "." is used to indicate an interaction.

Results

Table 2.3 summarises the descriptive statistics for all studied traits. *Coccidia* were the most prevalent parasite, infecting nearly all lambs (99.5%), followed by *Strongyles* found in 95.4% of lambs and *Nematodirus* with 72.7% of animals having eggs in faeces. The fixed effects showed some interesting trends as male lambs had significantly higher levels of FEC_S and FEC_N (by 0.225 and 0.198 s.d., respectively), tended to have higher FOC and DAG, and were 926 g heavier than females lambs.

Compared to lambs born and raised as being single, twin born and raised lambs had 0.119 s.d. higher FEC_S and 0.123 s.d. higher for FEC_N and were 2.65 kg lighter. Twin born and raised lambs also tended to have higher levels of all worm species compared to twin lambs raised as a single lambs; the latter category was still higher by 376 g compared to single born and raised lambs. Compared to the base of 2011, the year of birth effect (7 levels) ranged between -0.014 and 0.19 s.d. for FEC_S, -1.09 to 0.35 s.d. for FEC_N, -0.55 to 0.58 s.d. for FOC, -0.79 to 0 s.d. for DAG, and -3.054 to 0.425 kg for LWT. Similarly, the range of s.d. difference for grazing location (12 levels) compared to the base was -0.055 to 0.77, -0.43 to 0.31 and -0.59 to 0.20 s.d. difference for FEC_S, FEC_N, and FOC, respectively. The age of lamb at the time of measurement for all parasites was important. For every unit increase (day) in age, FEC_S, FEC_N and FOC declined by 0.014, 0.015 and 0.011 s.d., respectively, with the daily LWT gain being 178g.

Table 2.3. Descriptive statistics for each trait recorded.

	No. of animals	Mean	CV (%)	Range
FEC_S (eggs/g)	3,731	714.66	136	0 – 37,500
FEC_N (eggs/g)	3,731	226.57	142	0 – 2,800
FOC (eggs/g)	3,731	30,631.82	152	0 – 1,053,000
DAG (score)	3,183	0.38	245	0 – 4
LWT (kg)	3,725	24.49	17	14 – 42.1

Abbreviations: FEC_S = *Strongyles* faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag score; LWT = live weight. CV = coefficient of variation.

Trait h^2 and genetic and phenotypic correlations between traits are summarised in Table 2.4. All h^2 estimates were significantly greater than zero ($P < 0.05$). Faecal counts estimates differed between parasite genera. Disease trait h^2 (FEC_S, FEC_N,

FOC and DAG) were low. Estimates for FEC_S and FEC_N yielded practically the same h^2 for these traits, both measuring nematode eggs in faeces (0.16±0.03 and 0.17±0.3, respectively). Additionally, the estimates for FOC, measuring oocysts in faeces, and DAG scores were exactly the same at 0.09±0.03. On the other hand, LWT h^2, as a measure of productivity, was found to be moderate in this population (0.33 ± 0.04).

Table 2.4. Genetic parameters corresponding to faecal counts (FEC_S, FEC_N and FOC), dag scores (DAG) and live weights (LWT) in lambs.

	FEC_S (SE)	**FEC_N** (SE)	**FOC** (SE)	**DAG** (SE)	**LWT** (SE)
FEC_S	0.16 (0.03)[1]	0.23 (0.02)[1]	0.13 (0.02)[1]	0.02 (0.02)	-0.06 (0.02)[1]
FEC_N	0.74 (0.09)[1]	0.17 (0.03)[1]	0.09 (0.02)[1]	0.00 (0.02)	-0.06 (0.02)[1]
FOC	0.39 (0.15)[1]	0.23 (0.16)	0.09 (0.03)[1]	-0.06 (0.02)[1]	0.02 (0.02)
DAG	0.06 (0.18)	0.02 (0.18)	0.03 (0.21)	0.09 (0.03)[1]	-0.19 (0.02)[1]
LWT	-0.01 (0.13)	-0.08 (0.12)	0.25 (0.15)	-0.33 (0.15)[1]	0.33 (0.04)[1]

Abbreviations: FEC_S = *Strongyles* faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag score; LWT = live weight. Phenotypic (r_P) and genetic (r_G) correlations are presented above and below the diagonal (heritability), respectively. [1]Estimates are significantly different from zero ($P < 0.05$)

The results indicate there is a strong positive r_G between the two nematode genera: FEC_S and FEC_N (0.74 ± 0.09). Additionally, a moderate r_G between FEC_S and FOC (0.39 ± 0.15) was also found. A further, moderate and negative r_G between LWT and DAG (-0.33 ± 0.15) was estimated. All previously mentioned genetic correlations are statistically significant ($P < 0.05$). Phenotypic correlations (r_P) were generally weaker than their genetic counterparts. All parasites were found to have weak and positive r_P between them: 0.23 ± 0.02 between FEC_S and FEC_N, and 0.13 ± 0.02 and 0.09 ± 0.02 between FOC and FEC_S, and FOC and FEC_N, respectively. Both

nematode genera were equally correlated with LWT (-0.06 ± 0.02). Lastly, LWT and FOC were negatively correlated with DAG (-0.19 ± 0.02 and -0.06 ± 0.02, respectively). All previous estimates are statistically significant ($P < 0.05$).

Discussion

The present chapter aimed at assessing the genetic background of traits related to GI parasitic infection in Scottish Blackface sheep lambs, as well as assessing the relationships between diseases related traits and productivity. To that end, genetic parameters on faecal counts on three major parasite genera, namely, *Strongyles*, *Nematodirus* and *Coccidia*, were derived along with genetic parameters on DAG scores and LWT. Results revealed significant and heritable genetic variation among animals for all these traits.

FEC_S h^2 estimates are in line with those estimated for the Appeninica Italian breed, but FOC estimate is somewhat lower to that found for this Italian breed (Filippini *et al.,* 2006). Heritability for FEC_N falls within the range of McManus *et al.,* (2009), while oocyst counts h^2 estimates were also lower than those in their multi-breed experiment in Brazil. Nematode h^2 estimates (FEC_S and FEC_N) were broadly similar to results for a New Zealand study using the information on more than 2 million pedigreed animals that include records on sheep breeder and research flock (Pickering *et al.,* 2012).

Moderate h^2 estimates of nematode faecal counts have been found in Merino lambs raised in Brazil (Benavides *et al.,* 2016a), in Texel sheep in the United Kingdom (Bishop *et al.,* 2004) and in back-cross lambs derived from purebred populations of

Martinique Black Belly and Romane (Assenza *et al.,* 2014). Nematode FEC h^2 of 0.29 was estimated for Scottish Blackface sheep by Bishop and Stear (1999) at 6 months of age, but lower estimates have been reported for the same breed (0.14 to 0.22) (Bishop *et al.,* 1996). According to (Bishop, 2012b), most egg count h^2 for egg excretion in sheep generally falls within a range of 0.20 to 0.40. Although outside of this range, our estimates for nematode FEC are not far from the lower limit of this interval. Estimates of FOC h^2 are in line with results for goats (Rout *et al.,* 2015; Sharma *et al.,* 2017). In contrast, Reeg *et al.,* (2005), studying Merinoland lambs, found remarkably high estimates beyond 2 months of age (0.54 to 0.79). While it may not be advisable to select Merino sheep for resistance at younger ages than 2 months, there is evidence to suggest that there are strong influences, at a genetic level, on oocyst excretion in older lambs.

Although significant, our DAG h^2 estimate was low compared to previous estimates in other sheep breeds (Bisset *et al.,* 1992; Pickering *et al.,* 2012). One explanation for this could be that, in our data, approximately 70% of lambs showed no signs of soiling around the breech area, while only 4% had a DAG score of 3 or higher. It is possible that the level of parasite challenge was lower in this study and/or the sheep used in this study generated a less severe inflammatory response to the parasites. LWT heritability estimates obtained here are in line with previous reports on the same and other sheep breeds (McEwan *et al.,* 1992; Bishop *et al.,* 1996; Safari *et al.,* 2005; Benavides *et al.,* 2016a).

We found strong genetic correlations between FEC_S and FEC_N, revealing that FEC_S and FEC_N are largely under the same genetic control. The results are well within the

range of previous estimates (Bisset *et al.*, 1992; McEwan *et al.*, 1992; Amarante *et al.*, 2004; Bishop *et al.*, 2004; Morris *et al.*, 2004; Wolf *et al.*, 2008; Pickering *et al.*, 2012). Correlated responses in resistance to *Nematodirus* are expected when selecting for low FEC_S. To the best of the authors' knowledge, there are few studies involving both nematode parasites and *Coccidia*. In our study, we found that at 3 months of age FEC_S and FOC are moderately and positively correlated, suggesting these two parasites are partially under the same genetic control and that there is no antagonistic relationship between these parasites. Consistently negative r_G between FEC_S and FOC were found previously in Appenninica sheep breed (Filippini *et al.*, 2006). In a Brazilian study involving different sheep breeds no meaningful r_G between these parasites was reported (McManus *et al.*, 2009). Differences with results previously reported may be attributed to different lamb age and breed populations. Using Spearman's rank correlation tests, Craig *et al.*, (2008) found positive associations between *Strongyles* and *Coccidia* in yearling and adult St. Kilda Soay sheep, although they state this may simply be a result of similar responses to the host condition or due to co-variation in parasite intake. Nevertheless, the same authors highlight the importance of genetic studies to produce h^2 estimates as well as correlations between different parasites, possibly allowing for a balanced selection. In Nellore cattle, a strong correlation between nematodes and coccidian parasites was found across a range of ages, which could indicate the possibility of genes regulating immune defence having pleiotropic effects that alter resistance to different parasites in the same direction, or alternatively, linked genes could be responsible for the defence mechanisms (Passafaro *et al.*, 2015).

No meaningful r_G between FEC traits and LWT was found. From a genetic selection standpoint, such results are favourable; this suggests that selection for increased resistance will not adversely affect animal production and growth. The impact of parasitism on lamb LWT have been a point of contention in the literature where unfavourable (McEwan *et al.,* 1992; Morris *et al.,* 2005; Pickering *et al.,* 2012) as well as favourable genetic correlations between nematode FEC and LWT have been previously reported (Eady, 1998; Gauly *et al.,* 2004). There is also evidence that the correlation may shift from strongly positive at low FEC to negative at high FEC levels (Rashidi *et al.,* 2014). With studies yielding such a wide range of genetic correlations between these traits, it is hard to generalize, as there are multiple factors that might contribute to differing results including variation in breeds, parasite genera and species, the intensity of the infection, method of statistical analysis, treatment protocols, and selection history.

In addition to the lack of antagonism between resistance to parasitic infection and productivity (LWT) found in the present study, we emphasize on the clear favourable (negative) genetic correlation between DAG and LWT. DAG is an indicator of diarrhoea, which in young lambs is assumed to be caused by GI parasites. The significance of dag scores associated with increased risk of flystrike was highlighted by Greeff *et al.,* (2014) in Australian Merino. That study estimated the heritability of DAG scores at different ages and genetic correlations with flystrike in the breech area of the sheep with a view to it being an indicator trait that could potentially be used to breed indirectly for resistance to breech strike. Although the correlations between dag and worm egg counts were not reported in that study,

strong, significant genetic correlations (0.64 to 0.81) were estimated between dag and flystrike in the breech area rendering it a very useful indicator trait to breed breech strike resistant sheep in a Mediterranean environment. In the United Kingdom, farmers have to crutch (remove wool) the breech area prior to lambs being sent for slaughter to remove any faecal soiling therefore faecal soiling is a trait of economic importance. Additionally, recent evidence showed (Zhao *et al.,* 2019) showed that animals selected for low FEC were subject to what is termed 'hypersensitivity-associated diarrhoea' resulting in higher soiling of the breech area (DAG) compared to unselected controls. While the current study found weak correlations between dag score and parasite faecal counts, they agree with those of Brown *et al.,* (2010) which were also reported to be not significantly different from zero.

These conclusions suggest that selection to improve resistance to GI parasitism in sheep would not necessarily lead to a reduction in faecal soiling therefore dag score also should be included in breeding programmes. The interactions among immunological parameters and disease should be explored further as cited by Zhao *et al.,* (2019) and indeed are currently the subject of further study with the same animal population reported in this study. In addition, in a study of New Zealand sheep, some genetic correlations between DAG and nematode egg counts have been reported to be negative at 3 months but positive at 8 months of age (Pickering *et al.*, 2012) which further complicates the messages around this issue. Genetic correlations between dag scores and faecal counts were not significant in the present study perhaps due to the low number of instances of DAG scores higher than 0 in our

population, although there was a tendency for there to be a positive genetic correlation of DAG with FEC_S and FEC_N. A significant positive correlation between FEC and DAG was also reported in Romney sheep (Bisset *et al.*, 1992). Finally, a negative r_G between DAG and LWT in this study indicates that lower DAG scores are associated with higher lamb weights, which will have a positive impact on productivity; this was also reported in the study of Brown *et al.*, (2010).

In conclusion, our study reveals that nematode and coccidian counts in Scottish Blackface lamb faeces are lowly heritable, but there is significant genetic variation among individuals to underpin a selective breeding programme aiming to enhance animal resistance to infection. Our results suggest that there is a consistently strong genetic correlation between the two species of nematodes, implying that selecting to reducing output for one will also affect the other. Furthermore, there is no evidence of antagonism between nematode FEC and FOC. These results are encouraging because the implementation of a breeding programme focusing on these traits will result in animals with greater overall resistance. The inclusion of these traits in a breeding programme will allow the development of a selection index.

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2.3 Chapter conclusion

This Chapter addressed the first aim of the present thesis. Results show that all traits studied have sufficient genetic variation among the Scottish Blackface sheep, revealing the possibility of including them in breeding programme with the aim of selecting sheep for increased resistance to GI parasitic infection. Crucially, selection will result in more resistance to all parasite species analysed here. Lastly, the incorporation of traits related to parasitic infection within breeding programmes is unlikely to result in reduced productivity. The results presented here pave the way for the development of a selection index for enhanced resistance.

Chapter 3 - Genetic profile of adaptive immune traits and relationship with animal health and productivity in Scottish Blackface sheep

3.1 Introduction

Gastrointestinal (GI) co-infections with coccidian (protozoan) and nematode parasites pose a serious constraint on sheep production. Infections with these parasites are widespread in grazing sheep and co-infection with coccidian and nematode parasites is common (Burgess *et al.*, 2012; Craig *et al.*, 2008). While both infect the GI tract, there are considerable differences in their interaction with the host, with single cell *Coccidia* infecting and replicating in epithelial cells and larger, multicellular nematodes residing within the GI lumen or closely associated with the GI mucosa (Chartier and Paraud, 2012; McRae *et al.*, 2015).

The success or failure of immune response of the host to infection depends on a range of different factors, such as pathogen burden and the scale of the immune response itself, with the latter being regulated by the activity of T helper cells (London *et al.*, 1998). These cells are recognised as key coordinators of the adaptive immune response which recognises and specifically responds to pathogens (Eagar and Miller, 2019). The expansion and differentiation of two of the most important T helper cells, T helper 1 (Th1) and 2 (Th2) from their precursors, naïve T cells occurs following effective antigen presentation (Eagar and Miller, 2019). Different functions are associated with Th1 and Th2 cells: while the former are primarily involved in inflammatory responses and controlling intracellular pathogens, the latter are mainly involved in inducing humoral responses, typically to extracellular pathogens (Butcher and Zhu, 2021; Mosmann *et al.*, 1986; Walker and McKenzie, 2018; Zhu and Paul, 2010). In the context of ovine GI parasite infections, coccidian parasites are thought to be controlled by Th1 immune responses (Engwerda *et al.*,

2014), whereas Th2 cells play a key role in controlling parasitic nematode infections (McNeilly and Nisbet, 2014). Of critical importance to the adaptive immune response is another subset of T cells, referred to as regulatory T cells (Tregs). Tregs are responsible for regulating the immune responses by preventing or inhibiting immune responses, partially through production of inhibitory cytokines such as IL-10 and Transforming Growth Factor-beta (TGF-β). Treg play a key role in preventing over-activation of the immune response and subsequent immunopathology (Thornton, 2010), but may also be actively induced by certain parasitic ovine nematodes as part of their immune evasion strategy (Grainger *et al.,* 2010).

These three T cell subtypes (Th1, Th2 and Treg) are characterised by the secretion of key prototypic cytokines following T cell activation. One of the main cytokines produced by Th1 cells is IFN-γ, which plays a vital role in establishing cellular immunity (Payne, 2017) and is recognised as a key limiting factor in coccidian infections (Ovington *et al.,* 1995; Ozmen *et al.,* 2012). A direct role for this cytokine in coccidian immunity is through classical activation of macrophages which clear the parasite within the intestinal mucosa via phagocytosis (Taubert *et al.,* 2009). This cytokine also promotes Th1 differentiation and is an inhibitor of Th2 cell proliferation (Bae *et al.,* 2016; O'Shea *et al.,* 2019).

A key cytokine involved in Th2 immune responses against nematodes is IL-4 (McNeilly and Nisbet, 2014; Venturina *et al.,* 2013). Produced by Th2 polarised cells, this cytokine is involved in promoting antibody responses and B cell class switching to IgE. It also serves as an inhibitor of Th1 immunity and classical

macrophage activation whilst promoting the Th2 responses through a positive feedback loop (O'Shea *et al.,* 2019; Zhou *et al.,* 2009).

Initially believed to be produced by Th2 cells (Fiorentino *et al.,* 1989), IL-10 has been shown to have an immuno-modulatory role, controlling and mediating inflammatory responses during infections by a wide range of pathogens including protozoa and nematodes (Ng *et al.,* 2013) but also key in controlling autoimmune diseases and allergy (Hawrylowicz, 2005). This cytokine exerts an antagonistic effect on Th1 and Th2, affecting both the innate and adaptive responses (Haritova and Stanilova, 2012). IL-10 is able to actively supress IFN-γ induced macrophage activity against both intracellular and extracellular pathogens allowing their survival (Gazzinelli *et al.,* 1992). In addition to immune-regulatory functions, IL-10 is also involved in promotion of plasma cell differentiation and antibody production (Maseda *et al.,* 2012).

Parasite specific antibodies, in particular IgA, are also key for immune responses against GI parasites (De la Chevrotière *et al.,* 2012). IgA is the most abundant antibody isotype at mucosal surfaces and nematode-specific IgA is known to be linked to resistance to nematodes in sheep, being associated with reduced worm size and fecundity in natural infections (Stear *et al.,* 1995; Strain *et al.,* 2002). IgA is a key antibody that has been associated with the humoral mucosal immune system that can bind selectively to antigens and can prevent invading organisms from penetrating or interacting with the mucosal epithelium (Sparks *et al.,* 2018). It is produced at mucosal surfaces and, in sheep, serum IgA is mainly derived from the

intestine, being the isotype most closely associated with intestinal immune responses (McRae *et al.,* 2015).

It has recently been shown that, independent of age, sex, and each other, the production of IL-4, derived from T cell mitogen-stimulated whole blood, negatively predicted GI nematode faecal egg count in a wild population of Soay sheep, whereas production of IFN-γ negatively predicted coccidian faecal oocyst (Corripio-Miyar *et al.,* 2022). This suggests that Th1 and Th2 immune traits derived from circulating lymphocytes may be useful selection marker for parasite resistance. Additionally, the previous chapter revealed a significant positive genetic correlation between FEC and FOC (Pacheco *et al*., 2021), revealing that Th1 and Th2 responses may be positively correlated at the genetic level. As cellular immune traits can be obtained from routine blood samples and have been shown to be repeatable in other species (Denholm *et al*., 2017), these may be more prove to be more useful selection markers than faecal parasitology measures, which are variable over time and can be difficult to record at scale. While parasite specific IgA is known to be heritable in sheep (Davies *et al*., 2006; Gutiérrez-Gil *et al*., 2010; Sparks *et al*., 2019; Strain *et al*., 2002), the genetics underlying variation in different types of T cell immune responses and how these relate to productivity and disease is currently unknown.

While results from the Chapter 2 confirm the feasibility of including disease traits in breeding programmes, there are still some lingering questions on the nature of immune responses against the two parasite *genera* studied. Therefore, immunological data was analysed alongside parasitic infection phenotypes. The aims of the present chapter were to (i) evaluate T cell cytokine production from whole

blood (as a measure of T cell polarisation) and nematode parasite specific IgA levels in lambs, (ii) examine the host genetic background for these traits, and (iii) assess their relationship with animal disease and production traits. In this regard, the immune status of more than 1,000 pedigree sheep was determined, the amount of genetic variance between animals and trait heritability was estimated and the genetic and phenotypic correlations with parasitic infection phenotypes and live weight of the animals (Chapter 2) were derived.

3.2 Materials and Methods

3.2.1 Animals and traits

Blood samples for cellular and serum antibody analyses were collected from a total of 1040 SBF lambs averaging approximately 53 days of age, born in 2016 and 2017. Faecal samples were collected from the same individuals roughly one month later for logistical reasons. These animals are part of the SRUC experimental hill farm flock (Pentland hills, Midlothian, Scotland). Animals were managed in typical hill farm conditions throughout the year and continually exposed to natural GI infections. The flock has been continually monitored since 1990 for aspects of performance and health from which several genetic studies have been published (Conington *et al.*, 2001, 2006; Lambe *et al.*, 2006, 2014; McLaren *et al.*, 2012). The genetic lines are detailed by (Lambe *et al.*, 2008), and in the previous chapter. The flock was split into three selection lines defined as: ‘Selection’ (S), ‘Control’ (C) and ‘Industry’ (I). S and C lines were selected based on the selection index described by Conington *et al*., (2001) as high- or average-performing, respectively. Finally, the I line was selected based on visual appearance, disregarding performance data. All experiments were

approved by the SRUC Animal Experiments Committee and were performed to Home Office Guidelines under Project Licence numbers (60/4358 and P90111799).

At the first sampling time-point, when animals averaged approximately 53 days of age (pre-weaning), whole blood stimulation assays were used to characterise the adaptive immune response traits of this flock in response to Pokeweed mitogen (PWM) and the common GI nematode *Teladorsagia circumcincta* (T-ci) by measuring release of the cytokines interferon-gamma (IFN-γ), interleukin (IL)-4, and IL-10, which relate to T-helper type 1 (Th1), Th2 and regulatory T cell (Treg), respectively. PWM is a mitogenic lectin which stimulates B and T lymphocytes irrespective of antigenic specificity (Janossy and Greaves, 1971) and thus provoking a generalised immune response: this represents innate immune responses. Conversely, *T. circumcincta* somatic antigen from fourth stage larvae (Tci-L4) was used to activate parasite specific lymphocytes and assess cellular immune responses that are specific to a parasite that is prevalent and pathogenic in UK sheep. This antigen was chosen since antibody responses to L4 are most correlated with protection against this parasite and thus the most relevant cellular immune response to quantify. Levels of *T. circumcincta* specific immunoglobulin (Ig)A in serum were also quantified by ELISA. Designations for the different cytokines and respective stimulants are shown in Table 3.1. All blood analyses were performed by Moredun Research Institute. Additionally, at the second sampling time-point, with lambs averaging 92 days of age, individual animal data were collected on faecal counts of *Strongyles* (FEC_S) and *Nematodirus* (FEC_N) eggs and *Coccidia* oocysts (FOC), along

with records on faecal soiling (DAG score) and live weight (LWT) as described in (Pacheco *et al.,* 2021).

Table 3.1. Cytokine and stimulant used and trait designations

Stimulant	Cytokine	Designation
Pokeweed mitogen (PWM)	IFN-γ	IFN-$\gamma_{(PWM)}$
	IL-4	IL-$4_{(PWM)}$
	IL-10	IL-$10_{(PWM)}$
***T. circumcincta* antigen (T-ci)**	IFN-γ	IFN-$\gamma_{(T\text{-}ci)}$
	IL-4	IL-$4_{(T\text{-}ci)}$
	IL-10	IL-$10_{(T\text{-}ci)}$

Faecal samples were collected from the same individuals. Crucially, this data was obtained roughly one month after the blood samples were collected when lambs were on average 92 days of age. Faecal data recorded includes faecal egg counts of Strongyles (FEC_S) and *Nematodirus* (FEC_N) and faecal *Coccidia* oocyst counts (FOC) determined using the McMaster technique (Whitlock, 1948), in addition to faecal soiling (DAG) scores and live weight (LWT), which were assessed at the same time faecal samples were collected. Faecal counts, DAG scores and LWT and respective genetic parameter estimates for these animals have been described and discussed in the previous chapter.

3.2.1 Data collection

3.2.1.1 Whole blood stimulation assays

Blood was collected aseptically into serum and lithium heparin vacutainers (Becton Dickinson, Oxford, UK) by jugular venepuncture. Whole blood stimulation assays were carried out by mixing 100µl of whole blood with 100µl of complete medium [RPMI-1640 (Gibco, ThermoFisher Scientific) supplemented with 2mM L-glutamine, 100 U/mL penicillin, 100µg/mL streptomycin and 50µM 2-mercaptoethanol (all from Sigma-Aldrich, UK)] containing 10µg/mL final concentration of pokeweed mitogen (PWM), 5µg/mL of *T. circumcincta* L4(T-ci) or phosphate buffered saline (PBS) as control to account for any non-specific cytokine secretion. Samples were plated in triplicate in tissue culture grade round bottom 96-well plates (Corning Costar, Sigma-Aldrich, UK). The plates were then incubated at 37˚C with 5% of CO_2 in air for 48h. After the incubation period, plates were spun at 1500rpm for 5 minutes and supernatants were stored at -20˚C for cytokine analysis.

3.2.1.2 Cytokine ELISA

Capture ELISAs were performed to examine the secretion of IFN-γ, IL-4 and IL-10, following stimulation with PWM or T-ci. All incubations were carried out at room temperature, unless stated otherwise. IFN-γ and IL-4 were quantified using commercial ELISA kits according to the manufacturer's instructions (MABTECH AB, Augustendalsvägen, Sweden). Mouse monoclonal anti-bovine IL-10 capture and detection antibodies (clones CC318 and CC320b, respectively, BioRad, UK) and standard curves produced using supernatants from COS-7 cells transfected with

bovine IL-10 (Kwong *et al.,* 2002) were used to quantify IL-10 secretion. Washing steps for all ELISAs were performed 6 times with 350µL of washing buffer (PBS + 0.05% Tween20) using a Thermo Scientific Wellwash™ Versa (ThermoFisher Scientific). High-binding capacity ELISA plates (Immunolon™ 2HB 96-well microtiter plates, ThermoFisher Scientific) were incubated with coating antibodies overnight at 4˚C. Plates were then washed and blocked for 1 hour with PBS containing 0.05% Tween20 (Sigma-Aldrich, UK) and 0.1% Bovine Serum albumin (BSA) (Sigma-Aldrich, UK) for IFN-γ, IL-4 or PBS containing 3% of BSA for IL-10. Following a further washing step, 50µL of supernatants or standards were added in duplicate for 1 hour. Subsequently, plates were washed and detection antibodies added for 1 hour. This was followed by washing and addition of Streptavidin-HRP (Dako, Agilent, Santa Clara, US) for 45 minutes. After the final washing step SureBlue TMB substrate (Insight Biotechnology, London, UK) was added and the reaction was stopped by the addition of TMB stop solution (Insight Biotechnology, London, UK). Absorbance values were read at O.D. 450nm. Standard curves were included in all plates and were constructed using seven serial dilutions of recombinant cytokines ranging from 400 to 6.25 pg/mL for IFN-γ (MABTECH AB); 2,000 to 62.5pg/mL for IL-4 (MABTECH AB) and 13.2 to 0.206 BU/mL for IL-10 (Kwong *et al.,* 2002).

3.2.1.3 Ovine *T. circumcincta* IgA ELISA

Indirect ELISAs were carried out to detect antigen specific T-ci IgA presence in sera. Briefly, high-binding capacity ELISA plates (Immunolon™ 2HB 96-well microtiter plates, ThermoFisher Scientific) were incubated with 5µg/mL of parasite antigen (*T.*

circumcincta L3 in 0.5M bicarbonate buffer, pH 9.6) at 4˚C overnight. Washing steps were carried out as detailed for cytokine ELISAs and incubations carried out at 37˚C unless stated differently. Following overnight incubation, plates were washed and blocked with 200µL of blocking buffer (PBS plus 3% fish gelatin, Sigma-Aldrich, UK) for 1 hour. Following a further washing step, sera samples diluted 1:4 in dilution buffer (PBS + 0.5% Tween-80 + 0.5M NaCl) were added in duplicate and incubated for 1 hour. Each plate also included a positive control serum sample. Following washing, 100µL of 1:15000 polyclonal rabbit anti-ovine IgA conjugated to horse radish peroxidase (AHP949P, BioRad, UK) was added to all wells and incubated for 1 hour. After a final wash, 100µL of TMB substrate (TMB substrate kit, ThermoFisher Scientific) was added and reaction stopped after 5 minutes by the addition of TMB stop solution provided within the TMB substrate kit. Absorbance values were read at O.D. 450nm. All values were then normalised using the positive control.

3.2.2 Data analysis

Preliminary analyses were carried out to determine significant fixed effects affecting the immunological traits of study. A stepwise backward elimination approach was followed, leaving only significant fixed effects in the model. Thus, fixed effects with p-values <0.05 were included in the final model for each trait. Subsequently, the following model was used to derive (co)variance components and genetic parameters:

$$y = X\beta + Za + e,$$

Where:

- y = trait record of each animal (IFN-$\gamma_{(PWM)}$, IL-$4_{(PWM)}$, IL-$10_{(PWM)}$, IFN-$\gamma_{(T\text{-}ci)}$, IL-$4_{(T\text{-}ci)}$, IL-$10_{(T\text{-}ci)}$ and IgA)
- β = vector of statistically significant fixed effects
- a = vector of additive genetic effects, including the animal pedigree
- e = vector of random residual effects
- X and Z correspond to the design linking relating records to fixed or random effects, respectively.

The pedigree in this population and respective relationship matrix included 6,458 animals in total. Statistically significant fixed effects (Table 3.2) include sex of the animal, grazing location at marking age (approximately 2 months of age), birth-rearing rank (single or twins), year of birth, genetic line, and age of dam at the time of parturition. Age of lambs at the time of sampling was fitted as a covariate. When appropriate, significant interactions were also fitted. Immunological data were log-transformed (Log + 1) prior to all analyses in order to get distributions closer to normality.

Univariate analyses were first performed for each immunological trait separately in order to derive estimates of genetic variance. Trait heritability estimates were then derived as the proportion of phenotypic variance explained by genetic variation between individuals (random effect in model above). Subsequently, bivariate analyses were undertaken in order to estimate genetic (r_G) and phenotypic (r_P) correlations among the immune traits and between immune traits and parasitic infection (FEC_S, FEC_N, FOC and DAG) and production (LWT) traits analysed

previously in Chapter 2 (Pacheco *et al.*, 2021). All analyses were conducted using the software ASReml v3.0 (Gilmour *et al.,* 2009) software.

Table 3.2. Statistically significant fixed effects included for each trait in the model.

Traits	Fixed effects
IFN-γ$_{(PWM)}$	yr, mk_graz, cyt_age, yr.mk_graz
IL-4$_{(PWM)}$	yr, brrnk, sx, mk_graz
IL-10$_{(PWM)}$	yr, sx, mk_graz, cyt_age, yr.mk_graz
IFN-γ$_{(T\text{-}ci)}$	yr, brrnk, mk_graz, cyt_age, yr.mk_graz
IL-4$_{(T\text{-}ci)}$	yr, mk_graz
IL-10$_{(T\text{-}ci)}$	yr, dage, mk_graz
IgA	yr, dage, mk_graz, cyt_age, yr.mk_graz

Abbreviations: PWM = pokeweed mitogen; T-ci = *T. circumcincta specific antigen*. IFN-γ = Interferon gamma; IL-4 = Interleukin 4; IL-10 = Interleukin 10; IgA = Immunoglobulin A. sx = sex of lamb (2 levels, male and female); cyt_age = age at sampling; yr = year of sampling (2 levels 2016-2017); brrnk = birth-rearing rank (10 levels); mk_graz = grazing location (12 levels); dage = age of dam; line = genetic line. "." is used to indicate an interaction.

3.3 Results

3.3.1 Genetic parameters

3.3.1.1 Heritability

Heritability estimates from univariate analyses for cytokine and IgA expression are summarised in Table 3.3. These estimates varied considerably between different cytokines but also differed with stimulation assays. Cytokine h^2 estimates in this study ranged from low to high. IFN-y$_{(PWM)}$ and IFN-γ$_{(T\text{-}ci)}$ estimates are somewhat close in value (0.33 ± 0.10 and 0.27 ± 0.08, respectively). IL-4$_{(PWM)}$ and IL-4$_{(T\text{-}ci)}$ registered the biggest difference in h^2 with the highest (0.77 ± 0.09) and lowest (0.14

± 0.06) estimates, respectively, of all cytokines analysed. Finally, IL-$10_{(PWM)}$ and IL-$10_{(T\text{-}ci)}$ estimates are also relatively close (0.16 ± 0.07 and 0.22 ± 0.08, respectively. Lastly, h^2 estimate was moderate (0.41±0.09) for IgA. All estimates are significant ($P < 0.05$). Furthermore, h^2 from bivariate analyses were broadly similar.

Table 3.3. Immunological trait heritability (h^2) estimates.

Traits	$\boldsymbol{h^2}$ (s.e.)
IFN-$\gamma_{(PWM)}$	0.33 (0.10)
IL-$4_{(PWM)}$	0.77 (0.09)
IL-$10_{(PWM)}$	0.16 (0.07)
IFN-$\gamma_{(T\text{-}ci)}$	0.27 (0.08)
IL-$4_{(T\text{-}ci)}$	0.14 (0.06)
IL-$10_{(T\text{-}ci)}$	0.22 (0.08)
IgA	0.41 (0.09)

3.3.1.2 Genetic and phenotypic correlations

The estimates of genetic and phenotypic correlations between traits for this chapter are summarised in Table 3.4. FOC has been found to be strongly and positively correlated (r_G) with IFN-$\gamma_{(PWM)}$ (0.67±0.30, $P < 0.05$). Additionally, the same trait was also found to have a negative, but still strong r_G with IL-$10_{(T\text{-}ci)}$. This correlation was estimated at -0.84±0.31 and is also significant ($P < 0.05$). LWT has been estimated to have an overall negative r_G with IFN-γ (-0.54±0.18 and -0.51±0.20 for IFN-$\gamma_{(PWM)}$ and IFN-$\gamma_{(T\text{-}ci)}$, respectively). The results also show that there is an overall positive r_G between IFN-γ and IL-4 expression. In other words, Th1 and Th2 responses are positively correlated (0.57±0.15 between IFN-$\gamma_{(PWM)}$ and IL-$4_{(PWM)}$;

0.74±0.21 between IFN-$\gamma_{(T\text{-}ci)}$ and IL-$4_{(T\text{-}ci)}$; and 0.50±0.15 between IFN-$\gamma_{(T\text{-}ci)}$ and IL-$4_{(PWM)}$). A significant r_G between IL-$10_{(PWM)}$ and IL-$4_{(T\text{-}ci)}$ is also reported, showing a correlation between Th2 and regulatory responses (0.53±0.23). Finally, IgA expression was found to be genetically correlated with IL-$4_{(PWM)}$ and IL-$10_{(PWM)}$ (0.32±0.17 and 0.85±0.17, respectively; $P < 0.05$).

Phenotypic correlations for this study have been summarised in Table 3.5. There were no significant correlations between immunological and disease traits in this study. There is however, evidence antagonism between IFN-$\gamma_{(PWM)}$ and LWT as denoted by the negative correlation between these traits (-0.09±0.04, $P < 0.05$). Additionally, the results show that there is a positive correlation between IL-$10_{(PWM)}$ and LWT (0.10±0.04, $P < 0.05$). Immunoglobulin A was found to have a positive r_P with all cytokines released following polyclonal T cell activation with PWM stimulation.

Table 3.4. Genetic correlations between immunological (IFN-γ, IL-4, IL-10 and IgA), parasitic infection (FEC_S, FEC_N, FOC and DAG) and production traits (LWT).

	Genetic correlations						
	IFN-$\gamma_{(PWM)}$	**IL-4$_{(PWM)}$**	**IL-10$_{(PWM)}$**	**IFN-$\gamma_{(T\text{-}ci)}$**	**IL-4$_{(T\text{-}ci)}$**	**IL-10$_{(T\text{-}ci)}$**	**IgA**
FEC_S	-0.20 (0.34)	-0.18 (0.24)	0.01 (0.33)	-0.27 (0.33)	0.01 (0.40)	-0.16 (0.34)	-0.17 (0.32)
FEC_N	-0.16 (0.43)	0.17 (0.33)	-0.16 (0.44)	0.02 (0.40)	0.01 (0.51)	0.20 (0.42)	0.29 (0.40)
FOC	0.67 (0.30)[1]	-0.17 (0.27)	0.51 (0.41)	-0.28 (0.35)	-0.09 (0.48)	-0.84 (0.31)[1]	0.59 (0.39)
DAG	0.10 (0.38)	0.39 (0.24)	-0.43 (0.34)	0.34 (0.33)	0.31 (0.46)	-0.03 (0.40)	0.27 (0.31)
LWT	-0.54 (0.18)[1]	-0.11 (0.18)	0.03 (0.26)	-0.51 (0.20)[1]	-0.26 (0.32)	0.02 (0.27)	-0.07 (0.25)
IFN-$\gamma_{(PWM)}$	–	–	–	–	–	–	–
IL-4$_{(PWM)}$	0.57 (0.15)[1]	–	–	–	–	–	–
IL-10$_{(PWM)}$	0.36 (0.28)	0.23 (0.29)	–	–	–	–	–
IFN-$\gamma_{(T\text{-}ci)}$	0.19 (0.25)	0.50 (0.15)[1]	-0.22 (0.24)	–	–	–	–
IL-4$_{(T\text{-}ci)}$	-0.06 (0.33)	0.41 (0.26)	-0.53 (0.23)[1]	0.74 (0.21)[1]	–	–	–
IL-10$_{(T\text{-}ci)}$	0.00 (0.27)	0.03 (0.20)	-0.37 (0.26)	0.25 (0.25)	0.01 (0.35)	–	–
IgA	0.39 (0.23)	0.32 (0.17)[1]	0.85 (0.17)[1]	-0.06 (0.25)	-0.15 (0.32)	0.43 (0.23)	–

Abbreviations: FEC_S = *Strongyles* faecal egg counts; FEC_N = *Nematodirus* faecal egg counts; FOC = *Coccidia* oocyst counts; DAG = dag scores; LWT = live weights; IFN-y = interferon-gamma; IL-4 = interleukin-4; IL-10 = interleukin-10; IgA = immunoglobulin A. *PWM* = stimulation assay with pokeweed mitogen; *T-ci* = stimulation assay with *T. circumcincta* antigen. [1]Estimates are significantly different from zero ($P < 0.05$). (S.E).

Table 3.5. Phenotypic correlations between immunological (IFN-γ, IL-4, IL-10 and IgA), parasitic infection (FEC_S, FEC_N, FOC and DAG) and production traits (LWT).

	Phenotypic correlations						
	IFN-$\gamma_{(PWM)}$	**IL-4$_{(PWM)}$**	**IL-10$_{(PWM)}$**	**IFN-$\gamma_{(T\text{-}ci)}$**	**IL-4$_{(T\text{-}ci)}$**	**IL-10$_{(T\text{-}ci)}$**	**IgA**
FEC_S	-0.04 (0.04)	-0.04 (0.04)	-0.03 (0.04)	-0.05 (0.03)	-0.04 (0.03)	-0.03 (0.04)	0.00 (0.04)
FEC_N	0.04 (0.04)	-0.06 (0.04)	-0.05 (0.03)	-0.06 (0.03)	-0.04 (0.03)	-0.03 (0.03)	-0.03 (0.04)
FOC	-0.01 (0.04)	-0.05 (0.04)	0.04 (0.04)	0.03 (0.04)	-0.02 (0.03)	-0.01 (0.04)	0.03 (0.04)
DAG	0.02 (0.04)	0.06 (0.04)	0.01 (0.03)	0.04 (0.03)	0.03 (0.03)	-0.02 (0.03)	0.05 (0.04)
LWT	-0.09 (0.04)[1]	0.07 (0.04)	0.10 (0.04)[1]	0.01 (0.04)	-0.02 (0.36)	0.05 (0.04)	-0.04 (0.04)
IFN-$\gamma_{(PWM)}$	–	–	–	–	–	–	–
IL-4$_{(PWM)}$	0.32 (0.04)[1]	–	–	–	–	–	–
IL-10$_{(PWM)}$	-0.03 (0.04)	0.13 (0.04)[1]	–	–	–	–	–
IFN-$\gamma_{(T\text{-}ci)}$	0.24 (0.04)[1]	0.31 (0.04)[1]	0.03 (0.04)	–	–	–	–
IL-4$_{(T\text{-}ci)}$	0.08 (0.04)[1]	0.18 (0.04)[1]	-0.04 (0.04)	0.34 (0.03)[1]	–	–	–
IL-10$_{(T\text{-}ci)}$	-0.01 (0.04)	0.06 (0.04)	0.18 (0.04)[1]	0.24 (0.03)[1]	0.06 (0.03)	–	–
IgA	0.08 (0.04)[1]	0.14 (0.04)[1]	0.11 (0.04)[1]	0.02 (0.04)	0.07 (0.04)	0.07 (0.04)	–

Abbreviations: FEC_S = *Strongyles* faecal egg counts; FEC_N = *Nematodirus* faecal egg counts; FOC = *Coccidia* oocyst counts; DAG = dag scores; LWT = live weights; IFN-y = interferon-gamma; IL-4 = interleukin-4; IL-10 = interleukin-10; IgA = immunoglobulin A. *PWM* = stimulation assay with pokeweed mitogen; *T-ci* = stimulation assay with *T. circumcincta* antigen. [1]Estimates are significantly different from zero ($P < 0.05$). (S.E).

3.4 Discussion

These results have shown that there is significant genetic variability in all immunological traits investigated in this study. This suggests that individual animals vary in their genetic capacity to mount adaptive immune responses under similar conditions of natural infection. Heritability estimates for *T. circumcincta* specific IgA were similar to those reported previously (Fairlie-Clarke *et al.,* 2019; Stear *et al.,* 1995), whereas significant heritability estimates reported for the cellular (cytokine) traits have not been previously reported in sheep. Contrary to the expectations that Th1- and Th2-immunity would negatively regulate each other, the results show that Th1 and Th2 associated cytokine measures were positively correlated at genetic and phenotypic levels. No evidence of any association between any of the immune measurements and nematode FEC was found at either the genetic or phenotypic levels, but did see some associations between IFN-γ and IL-10 release and FOC at the genetic level (correlations of 0.67±0.30 between FOC and IFN-$\gamma_{(PWM),}$ and -0.84±0.31 between FOC and IL-$10_{(T\text{-}ci)}$). Importantly, significant negative genetic correlations were found between IFN-γ production and LWT, suggesting that selection for higher IFN-γ production would come with productivity costs.

While there is limited information on the genetic control of cytokine production in sheep, a number of similar studies have been performed in humans (Kariuki and Niewold, 2010; Li *et al.,* 2016; Newport *et al.,* 2004), which have reported varying levels of genetic control dependant on the specific cytokine and the type of immune response measurement. For example, studies in humans have shown that serum

levels of Th-associated cytokines can be moderately heritable, with heritability estimates of 0.49, 0.22 and 0.46 for IL-4, IL-10 and IFN-γ, respectively (Brodin *et al.,* 2015). In other studies in humans, heritability estimates of IFN-γ release from stimulated blood leukocytes ranges from 0.0 to >0.9, depending on the bacterial, fungal stimulus used (Li *et al.,* 2016). Interestingly, in this latter study, heritability of IFN-γ release from T cell mitogen-stimulated whole blood, analogous to the PWM assay employed in this study, was estimated to be ~ 0.47, which is similar to the estimate of 0.33±0.10 for IFN-$\gamma_{(PWM)}$ in this study.

In ruminant livestock, genetic studies of cellular traits have generally focused on the total numbers of proportion of different blood leukocyte populations. These studies have identified moderate heritability estimates for T cell subsets in cattle of 0.46 and 0.41 for % CD4+ and CD8+ T cells, (Denholm *et al.,* 2017) and between 0.22 and 0.5 for total blood lymphocytes (Chinchilla-Vargas *et al.,* 2020; Leach *et al.,* 2013). While such studies point to significant genetic control of T cell numbers, they do not take into account functional diversity within the T cell populations. A more functional approach to cellular immune trait analysis in cattle has focused on measuring immune responses following immunisation with antigens known to induce either Th1 or Th2 polarised responses (Thompson-Crispi *et al.,* 2012). Genetic analysis of these responses indicated that both Th1 and Th2 responses were moderately heritable, ranging from 0.16-0.38. Furthermore, a recent report assessed immune competence on Merino sheep, through their ability to mount both antibody- and cell-mediated immune responses (Hine *et al*., 2022), in which the authors found immune competence to be heritable and favourably correlated with disease indicator

traits. Taken together, studies in humans, and cattle and sheep indicate a key role for host genetics in controlling adaptive immune responses, which is also supported by the moderate to high heritability estimates for Th-associated cytokine release reported here.

In this study, blood leukocytes were stimulated with either a polyclonal T cell mitogen (PWM) or a nematode parasite antigen (T-ci-L4), to phenotype total and parasite-specific circulating T cell populations, respectively. These results identified a significant but weak phenotypic correlation between the two stimuli within each cytokine and no genetic correlation, giving confidence that polyclonal and antigen-specific cytokine release assays were evaluating distinct T cell phenotypes and were under different genetic control. This may be due to differences in the mechanism of cellular activation between the two stimuli, with PWM activation a result of direct binding to T cell surface molecules (Yokoyama *et al.*, 1977), whereas antigen-specific activation requiring presentation of processed antigen via major histocompatibility complex (MHC) molecules (Mallone and Nepom, 2004).

Contrary to the expectations that Th1 and Th2 immune responses would negatively regulate each other (Cox, 2001), significant positive correlations between Th1 and Th2 cytokine measures (IFN-γ and L-4, respectively) were found at both the phenotypic and genetic levels. Correlations were stronger at the genetic level where moderate to strong correlations were seen (r_G = 0.74 between IFN-$\gamma_{(T\text{-}ci)}$ and IL-$4_{(T\text{-}ci)}$), indicating Th1 and Th2 traits were partially under the same genetic control. Antagonism between Th1 and Th2 immunity is well established in laboratory immunology (Elliott *et al.,* 2000; Kaiko *et al.,* 2008; Su *et al.,* 2006), however,

similar strong positive associations between Th1 and Th2 cytokine responses have been reported in the St. Kilda Soay sheep population using the same whole blood stimulation assays (Corripio-Miyar *et al.,* 2022), and work in wild rodent populations has found synergistic rather than antagonistic associations between Th1 and Th2 phenotypes (Arriero *et al.,* 2017; Young *et al.,* 2020). One explanation for this observation is that the immune response is highly compartmentalised, meaning that while local antagonism between Th1 and Th2 immunity within specific anatomical locations may exist, animals may be able to mount different types of Th response at different sites of infection (Kelly *et al.,* 1991; Sukura *et al.,* 1998). Furthermore, challenge with a variety of intra- and extracellular pathogens and the need for plasticity in the immune response could lead to selection of individuals better able to mount effective immune responses to different types of parasites. Currently, there is still some debate on the true nature of immune responses against nematodes, and the existence of a T1/Th2 dichotomy has been put into question. It has been theorised that optimal host protection is achieved through the polarisation of either Th1 or Th2 immune responses, via secretion of cytokines associated with either type of immunity (Finkelman *et al.,* 2004). No evidence was found, in this study, of a polarisation towards any specific type of immune response. Rather, there is evidence that both immune responses (Th1 and Th2) are expressed in the same direction. There is no concrete evidence that ruminants are capable of displaying such polarisation of Th1/Th2 responses (Bricarello *et al.,* 2008). However, Gill *et al.,* (2000) has demonstrated that in sheep infected with *Haemonchus contortus,* a clear polarisation toward Th2 responses occurred. This is in contrast with the results

of this study. In co-infections with different parasite taxa and with different physiology and strategies, varying responses are expected (Jackson *et al*., 2004).

A moderate negative r_G between IL-$10_{(PWM)}$ and IL-$4_{(T\text{-}ci)}$ (-0.53±0.23) was reported here, which might be indicative of the regulatory function of IL-10. Evidence has shown that nematodes are able to initiate the expansion of immune-regulatory cells that act on supressing both Th1 and Th2 immune responses in order to promote their survival in the host (McNeilly *et al*., 2013; Turner *et al*., 2008). Additionally, the results also show a strong positive genetic correlation between IL-$10_{(PWM)}$ and *T. circumcincta* specific IgA (0.85±0.17), which is consistent with data from humans and mice showing that IL-10 can promote IgA class switching and production (Cerutti, 2008; Hummelshoj *et al.*, 2006; Marconi *et al.*, 1998).

In this study, there is a significant positive correlation between IL-$10_{(T\text{-}ci)}$ and FOC at the genetic level, and while this was partly unexpected as IL-10 has been shown to interfere with immunity to coccidian parasites due to its immuno-regulatory effects (Ozmen *et al.*, 2012), studies in mice and chickens have also shown a positive association between resistance to coccidian parasites and IL-10 production (Boulton *et al.*, 2018; Wakelin *et al.*, 1993). Alternatively, it may reflect the effects of nematode-parasite induced immune regulation on the ability to control immunity to coccidian parasites. Contrary to previous results (Corripio-Miyar *et al.*, 2022) in which IFN-γ and IL-4 were negatively correlated with FOC and FEC_S, respectively, this study found no significant negative correlations between these cytokine measures and parasite egg/oocyst counts. Indeed, IFN-$\gamma_{(PWM)}$ was significantly positively correlated with FOC at the genetic level (0.67±0.30). However, in the

study previously mentioned, parasitology measures were recorded at the same time as the cytokine measures whereas in this study, parasitology measures were recorded one month after cytokine analysis. Furthermore, in the previous study, immune phenotyping of lambs occurred at around 4 months of age, whereas in this study lambs were around 2 months old, an age at which immunity to GIN, and in particular nematode parasites, is not fully developed (Greer and Hamie, 2016; McRae *et al.*, 2015). It is also known that the immune response to GI parasites following initial exposure in lambs is highly dynamic and involves a complex interplay between different types of Th response which varies over time, meaning that associations between Th responses and parasite burden may only be apparent at specific time-points post-infection (Hassan *et al.*, 2011; Liu *et al.*, 2022). Thus, the lack of and/or unexpected associations between Th1 and Th2 cytokine measurements with the parasitology data may be due to either immune phenotyping lambs at an age before anti-parasite immunity has fully developed, or due to the time-lag between the immune and parasitology measures. This would also explain the lack of association between FEC_S and IgA in this study: while IgA is a trait which has been consistently shown to be negatively associated with FEC_S, sufficient parasite exposure is required prior to the IgA measurement for this association to be detectable (Borkowski *et al.*, 2020; Shaw *et al.*, 2012). The mechanisms underlying the control of immunity against GI nematodes in sheep are largely unknown (Jacobs *et al.*, 2016), although changes in the gastro-intestinal mucosa of sheep infected by nematodes are consistent with Th2 type immune responses. IL-4 plays a key role in regulating of Th2 cell differentiation and is important in controlling the efficacy of these immune responses (Fallon *et al.*, 2006). Th2 immune effectors dominate immune response

against nematode parasitic infection (Estrada-Reyes *et al.,* 2015). This is similar to studies in rodents in which resistance to nematodes is associated with an up-regulation of IL-4 and down-regulation of IFN-γ, a cytokine responsible for Th1 responses. In mice, these responses have been linked to susceptibility to adult nematodes (J. A. Jackson *et al*., 2004). The development of Th1 immune responses post infection will result in protective cellular host responses against intracellular parasites such as *Coccidia*, unlike Th2 responses (Haritova and Stanilova, 2012). Factors responsible for enhancing Th1 cytokine expression in favour of Th2 expression could have an important on the control of coccidiosis (Haritova and Stanilova, 2012). IFN-γ is one of the main cytokines that has been linked to immune responses against coccidian parasites (Lillehoj and Trout, 1993).

Given the significant genetic variance estimated in the present study and the previous observations of Th1 and Th2 traits being associated with protection against nematode and coccidian parasites, respectively (Corripio-Miyar *et al.,* 2022), immunological traits can be included in genetic selection programmes aiming to enhance the animals' inherent resistance to parasites. In this regard, consideration should be taken of how these traits correlate with productivity traits (De la Chevrotière *et al.,* 2012). The present study indicates that IFN-γ production, both to PWM and Tci-L4, at two months of age is adversely associated with LWT at three months of age. Thus, selecting for increased IFN-γ may compromise weight and LWT, potentially through increased Th1 mediated immunopathology (Venturina *et al.,* 2013; Wilkie *et al.,* 2016). This is consistent with a recent meta-analysis of heritable traits associated with GI parasite resistance in sheep which concluded that

adaptive immune traits are generally negatively correlated with performance traits (Hayward, 2022). This Th1 immunopathology could be associated to over-reactive immune responses or hypersensitivity reactions, which in this case may be caused by the overstimulation of T cells and monocytes and/or macrophages, leading to the release of cytokines that provoke inflammation (Marshall *et al.*, 2018). The overexpression of hypersensitivity reactions have been hypothesised as the cause of negative consequences described on production and animal welfare (Hoste and Torres-Acosta, 2011). The use of a selection index methodology would be advisable here to effectively combine and improve two genetically antagonistic traits. Additionally, there is some risk that selection for Th2 immune responses may also affect live weight gain as there is a positive r_G between Th1 (IFN-γ) and Th2 (IL-4) type immunity, indicating some level of genetic control is shared between IL-4 and IFN-γ traits.

3.5 Conclusion

In conclusion, these results show that there is substantial genetic variability among individual lambs with regards to all immunological traits, although it is not clear if selecting for these traits is favourable with regards to live weight. Simultaneous genetic selection on all animal traits studied here will be the preferred solution, which may be achieved with a comprehensive selection index that includes disease resistance, immunological and productivity traits, even when some of the relationships are antagonistic. Emphasis on each trait would be influenced by genetic parameters, the direction of selection and the economic importance for the programme. The results presented here can inform the building of the selection index. The results shed light on the complex mechanism of the adaptive immune response in growing lambs. Firstly, there is evidence to suggest that both Th1 and Th2 immune responses are partially under the same genetic control, demonstrating the lack of a clear Th1/Th2 dichotomy. Furthermore, consistent with other studies in non-laboratory settings, there was no marked biased polarisation towards a specific immune response. Additionally, there is evidence to suggest that Th1 immune responses at 2 months of age could be impacting the capacity for the animal to gain weight, translating in animals with lower weights at 3 months. These results form the basis of future studies that should continue to build upon the groundwork laid here, including exploring the timing of adaptive immune and parasitology trait measurements, and their association with parasite resistance and productivity. This is particularly important as this study was performed with a relatively small sample size, which may have influenced the precision of the heritability and correlation

estimates. Importantly, these results do not support genetic selection relying solely on immune traits, which may unfavourably impact production by reducing lamb live weight. Instead, immune traits should be considered as part of a comprehensive selection index that includes other animal traits of interest.

Chapter 4 - Genome-wide association studies on disease, productivity and immunological traits

4.1 Introduction

Advancements in next-generation sequencing technology have allowed for *de novo* sequencing of livestock, opening the opportunity to create high density Single Nucleotide Polymorphism (SNP) chips. The development of the *Illumina OvineSNP50 BeadChip* microarray was made possible as part of the *International Sheep Genomics Consortium (ISGC)* (Rupp *et al.,* 2016). The practicality of genomic studies focusing on disease resistance in small ruminants has been assessed internationally, including Australia, New Zealand, USA, France, Spain, UK, Burkina Faso and Tunisia (Ahbara *et al.,* 2021; Al Kalaldeh *et al.,* 2019a, 2019b; Álvarez *et al.,* 2019; Atlija *et al.,* 2016; Estrada-Reyes *et al.,* 2019a; Pickering *et al.,* 2015b; Sallé *et al.,* 2012; Sparks *et al.,* 2019).

Widespread use of DNA information has resulted in several important breakthroughs, such as the development of genomic selection methodologies and the discovery of many thousands of SNP markers associated with multiple animal traits of interest to farmers, breeders, and consumers (Meuwissen *et al*., 2016). The use of dense panels of polymorphic molecular markers has made the accurate prediction of genomic breeding values possible (De los Campos *et al.,* 2013) with potential for early life prediction breeding values of selection candidates (Blasco and Toro, 2014). Coupled with higher accuracies than those achieved when using pedigree index, animals can be selected at earlier ages leading to shortened generation intervals and increased rate of genetic improvement (Blasco and Toro, 2014). Selective breeding may bring ethical benefits by reducing the number of animals exposed to disease and thus reducing the suffering of future generations (Rupp *et al.,* 2016). Additionally,

genetic improvement of disease resistance and welfare traits is cumulative and permanent over successive generations (Dominik *et al.,* 2017). Provided that there considerable genetic variation, genetic progress can be achieved in disease resistance traits (Ciappesoni *et al*., 2013). Breeding for appropriately targeted traits brings potential welfare benefits without undesirable economic impacts or the necessity of major changes in management (Turner *et al*., 2015). The selection of sheep for increased resistance to GI parasitism is one example of how developments made in selection strategy have helped improve the genetic progress for complex traits (Hunt *et al*., 2013). The use of genomic approaches has the advantage of reducing the requirement for intrusive, on-going trait recording (Bishop, 2012a). Traits that are difficult and/or expensive to measure, such as those linked to disease, or lowly heritable traits may be especially attractive candidates for genomic studies (Dominik *et al.,* 2017).

Genome-wide association studies (GWAS) have facilitated the identification of loci associated with a specific animal trait, based on the joint analysis of animal phenotypes and genome-wide SNP genotypes (Leung *et al.,* 2019) allowing the identification of candidate genes and shedding light on the mechanisms controlling complex traits (Ahbara *et al.,* 2021; Sharma *et al.,* 2015). The field of immunogenetics has been crucial in identifying numerous genes involved in shaping the immune repertoire (Acevedo-Whitehouse and Cunningham, 2006). Microsatellite-based QTL approaches have in the past identified a wide number of chromosomal regions with small to moderate effects associated with GIN resistance (Davies *et al*., 2006; Gutiérrez-Gil *et al*., 2009; Sallé *et al*., 2012), with the

introduction of medium-density SNP chips allowing further refinement of previous results (Al Kalaldeh *et al*., 2019a; Pickering *et al*., 2015b; Sparks *et al*., 2019), but the great variety of parasite species and sheep breeds has contributed to a general lack of agreement among studies (Atlija *et al.*, 2014). This supports the hypothesis that the traits studied may represent different aspects of host-parasite interaction during infection (Gutiérrez-Gil *et al.,* 2009), and can be attributed to the complex nature of these traits and the diversity of studies. It is therefore important to gather information from different sheep populations in order to have a better understanding of the genetic architecture underlying GIN resistance (Álvarez *et al*., 2019).

Gene ontology (GO) resources can help narrowing down the search for candidate genes (Brown *et al.*, 2013). While genes could be expected to be involved in the detection and elimination of pathogens, genes involved in tissue repair, for example, can also play a crucial role in immune function (Brown *et al.*, 2013). Even though the annotation of the sheep genome is good, some regions are yet to be annotated, which makes the identification of candidate genes in regions that contain significant SNPs challenging (Mucha *et al.*, 2015).

Genetic variation in resistance to parasites has been previously demonstrated in this thesis (Chapter 2), yet the genetic architecture underlying these traits still remains to be fully understood (Kemper *et al.*, 2011). The use of genomic information for resistance to disease traits in breeding programmes depends not only on their relative economic importance, but also on their genetic architecture (Poland and Rutkoski, 2016). Using molecular data associated with disease and immunological phenotypes can reveal important genes of interest that could be associated with the traits detailed

in Chapter 2 and Chapter 3. This chapter aims at assessing the genetic architecture underlying lamb traits associated with parasitic infection (faecal counts and DAG scores), immunological profile (cytokines and parasite-specific immunoglobulin A) and production (live body weight), and to identify and characterise candidate genes affecting these traits.

4.2 Materials and Methods

4.2.1 Phenotypic data

Data from Scottish Blackface (SBF) lambs described in Chapters 2 and 3 were used for this study. Animals were managed under typical hill conditions. The flock consisted of different genetic selection lines: *'Selection'* (S) and *'Control'* (C) lines selected for high- and average-performing lambs in terms of growth, respectively, as described by Conington *et al*., (2001), and since 2011, a '*Faecal Egg Count'* (F) line, where animals are selected for parasitic resistance based on their estimated breeding values for FEC scores. A fourth group included lambs born from a selection of ewes (~40 per year) from across the three selection lines that were mated to bought-in rams linking the flock with the SBF industry breed improvement programme (L). This was to maintain genetic linkages amongst participating flocks. Parasitic infection and production data used for the present study were collected individually on lambs born from 2016 to 2018 including faecal counts for Strongyles (FEC_S), *Nematodirus* (FEC_N), *Coccidia* (FOC), a 5-point faecal soiling score (DAG) and live body weight (LWT), as described in Chapter 2. Faecal samples and DAG and LWT records were collected twice when animals were approximately 3 and 4 months of age.

Additionally, blood samples collected to assess the animal's immunological profile, as described in Chapter 3. In summary, three distinct cytokines (Interferon-gamma (IFN-γ), Interleukin (IL)-4 and IL-10) were analysed and subjected to two distinct and separate whole blood stimulation protocols in order to characterise the adaptive immune response traits of this flock in response to Pokeweed mitogen (PWM), a

mitogenic lectin capable of stimulating B and T lymphocytes irrespective of antigenic specificity, and somatic antigen of the common GI nematode *Teladorsagia circumcincta* (T-ci), to activate parasite specific lymphocytes. Cytokine phenotypes will henceforth be designated as IFN-$\gamma_{(PWM)}$, IL-$4_{(PWM)}$ and IL-$10_{(PWM)}$ to describe PWM stimulation, and IFN-$\gamma_{(T\text{-}ci)}$, IL-$4_{(T\text{-}ci)}$ and IL-$10_{(T\text{-}ci)}$ to describe a T-ci stimulation. Additionally, levels of *T. circumcincta* specific immunoglobulin (Ig)A in serum were also measured (Chapter 3).

Each set of traits was recorded twice. Chronologically, immunological traits were first recorded at an average of 53 days, followed by parasitic infection and production traits at an average of 92 days of age: these datasets will be referred to as the 1st recording occasion. The 2nd recording occasion includes measurements of parasitic infection and production traits recorded at an average age of 126 days, followed by the measurements of immunological traits at an average age of 157 days. The order and structure of trait recording is justified by logistics, due to the complexity of collecting blood samples and faeces within shorter time intervals without putting the lambs through unnecessary stress. Table 4.1 summarises data used for the present study. Preliminary bivariate analyses between traits in the two recording occasions resulted in correlation values that were significantly lower than unity and, therefore, recordings in these two age stages were considered as different traits in the ensuing analyses, with data pertaining to different phases of the lambs' growth. In the previous chapters, only one recording per trait was analysed. However, this chapter includes traits from both recording occasions. Thus, a total of 24 animal phenotypes were eventually studied.

Table 4.1. Number of animals and their respective age at the time of data collection.

Traits		Phenotypes	Recording occasion No. 1		Recording occasion No. 2	
			No. Animals	Average age (days)	No. Animals	Average age (days)
Parasitic infection traits	Parasitology	**FEC_S**	1,561	92	1,536	126
		FEC_N	1,561	92	1,536	126
		FOC	1,561	92	1,536	126
		DAG	1,561	92	1,536	126
Production		**LWT**	1,561	92	1,536	126
Immunology traits	Cytokines	**IFN-$\gamma_{(PWM)}$**	972	53	1,068	157
		IL-$4_{(PWM)}$	972	53	1,068	157
		IL-$10_{(PWM)}$	972	53	1,068	157
		IFN-$\gamma_{(T\text{-}ci)}$	972	53	1,068	157
		IL-$4_{(T\text{-}ci)}$	972	53	1,068	157
		IL-$10_{(T\text{-}ci)}$	972	53	1,068	157
		IgA	949	53	1,045	157

FEC_S = Strongyles faecal egg count; **FEC_N** = *Nematodirus* faecal egg count; **FOC** = *Coccidia* oocyst count; **DAG** = dag scores; **LWT** = live weight; **IFN-γ** = Interferon-gamma; **IL-4** = Interleukin-4; **IL-10** = Interleukin-10; **IgA** = parasite specific Immunoglobulin A. **PWM** = Pokeweed mitogen stimulant; **T-ci** = *T. circumcincta* specific antigen stimulant.

4.2.2 Genotypic data

DNA samples for genotyping assays were collected through nasal swabs. DNA was then quantified and genotyped using the Illumina OvineSNP15k BeadChip, the Illumina OvineSNP50k BeadChip or the Illumina OvineSNP HD Beadchip (ISGC) with densities of 15, 50 and 600 thousand SNPs, respectively. Table 4.2 summarises the animals genotyped per year of study and array densities. Overall, 1,006 animals were genotyped with a low-density SNP chip (15k), 778 animals were genotyped

with a 50k SNP chip and 17 animals were genotyped using a high density SNP chip (HD), totalling 1,801 animals genotypes. The genotypes used in this study were previously quality assured and imputed for a different project on the same population of Scottish Blackface sheep.

Table 4.2. Number of animals genotyped yearly and respective SNP densities.

Year	Animals/year	SNP density	No. of animals
2016	681	**15k**	370
		50k	311
2017	481	**15k**	314
		50k	150
		HD	17
2018	639	**15k**	322
		50k	317
		Total	1,801

A SNP was removed if its call rate was less than 90% or the minor allele frequency (MAF) was less than 5%. In total, 1,716 and 4,504 SNPs were removed for failing the SNP call rate and MAF thresholds, respectively. Individual sample level call rate was also used as quality check and animal genotypes with a call rate below 90% were excluded from the analyses, thereby removing 35 samples. The software PLINK v1.9 (Purcell *et al.*, 2007) was used in all previous steps.

Following the quality control steps described, all genotypes were imputed to a subset of the most informative SNPs from the 50k array panel (Ovine SNP50 chip). Imputation was executed with the software FindHap v3 (VanRaden, *et al.*, 2011). SNP positions were based on the Oar_v3.1 version of the sheep genome assembly.

SNP data was also filtered to remove SNPs located on the sex chromosomes. After these quality control and imputation steps, 45,827 SNPs and 1,766 animals were retained for analysis. Subsequently, a genomic relationship matrix was constructed based on the first method described by VanRaden (2008).

4.2.3 Population structure

Principal component analysis (PCA) was first undertaken on the genotypic data to investigate possible population structure using the genomic relationship matrix between animals to identify principal components explaining variation among individual samples and reveal potential population stratification. Possible stratification was examined across multiple factors, including genetic line, sex, year and grazing location, in order to determine the need for possible consideration in the ensuing GWAS analyses. The principal components were analysed through the Eigen-decomposition of the genomic relationship matrix to verify if any of the recorded covariates could influence the population structure at the genetic level. Relevant analyses were conducted with GEMMA v0.84.1 (Zhou and Stephens, 2012) and the resulting plots were visualised using R software v3.5.1 (R Core Team, 2021).

4.2.4 Genome-wide association studies

The data described so far were used and analysed to determine associations between genetic variants and the traits sampled from the population of study. The following model was applied to conduct GWAS separately for each of the 24 traits listed previously, in Table 4.1:

$$\boldsymbol{y = \mu + Xb + Zu + e} \qquad \textbf{[1]}$$

Where:

- y = animal record on each of the studied traits
- μ = overall mean of the trait
- b = vector of fixed effects
- u = vector of random effects
- e = vector of residual effects
- X and Z = design matrices linking records to fixed or random effects, respectively.

Table 4.3 summarises the fixed effects pertaining to each trait. A stepwise backward elimination was implemented whereby fixed effect significance was determined in preliminary analyses with model [1] using the ASReml v3.0 (Gilmour *et al*., 2009). Fixed effects with p-values <0.05 were included in the model. Fixed effects include year of birth, sex of the lamb, genetic line, location of the lamb recorded at different stages of development (location when lambs are at the mid-point between birth and weaning and location at the time of weaning), age of the lamb at the time of recording (in days), birth-rearing rank (lambs born/reared as single or twins) and age of dam (in years) at the time of parturition. Additionally, the first three principal components were included in the model [1] as covariates to account for the population stratification revealed by PCA.

The software GEMMA v0.94.1 (Zhou and Stephens, 2012) was used to first compute the genomic relationship matrix between animals and then conduct GWAS.

Significance thresholds were obtained and applied to GWAS results after an adjusted Bonferroni correction for multiple testing was applied: $-log_{10}(0.05/N)$ and $-log_{10}(1/N)$ for genome-wide and suggestive significance, respectively, where *N* represents the total number of SNPs (*N* = 45,827).

Following the Bonferroni correction, the genome-wide significant threshold (P ≤ 0.05) was set at $P = 1.09 \times 10^{-6}$ which corresponds to $-log10(P) = 5.96$, while the suggestive significance threshold level (accounting for one false positive per genome scan) was set at $P = 2.18 \times 10^{-5}$, corresponding to $-log10(P) = 4.66$.

The quantile-quantile (QQ) plots were used to verify whether the distribution of the observed $-log10(P)$ values deviated from the expected exponential distribution under the null hypothesis of no genetic association and no LD between SNPs. Individual SNP marker associations with the studied traits were visualised by plotting the resulting $-log10(P)$ values in Manhattan plots. The lambda correction factor (λ) was calculated to determine possible estimate inflation based on the method described by Amin *et al*., (2007). Factor λ checks for any systematic deviations of observed from expected p-values that could result from remaining, unaccounted for population substructure.

Table 4.3. Statistically significant fixed effects included for each trait in the model.

	Recording occasion No. 1
Traits	**Covariates**
FEC_S	Year, sex, line, brrnk, age, 3 principal components
FEC_N	Year, sex, line, mkgraz, age, 3 principal components
FOC	Year, sex, line, mkgraz, age, 3 principal components
DAG	Year, sex, line, mkgraz, 3 principal components
LWT	Year, sex, line, mkgraz, brrnk, age, 3 principal components
IFN-$\gamma_{(PWM)}$	Year, sex, line, mkgraz, age, 3 principal components
IL-$4_{(PWM)}$	Year, sex, line, mkgraz, brrnk, 3 principal components
IL-$10_{(PWM)}$	Year, sex, line, mkgraz, 3 principal components
IFN-$\gamma_{(T\text{-}ci)}$	Year, sex, line, mkgraz, brrnk, age, 3 principal components
IL-$4_{(T\text{-}ci)}$	Year, sex, line, mkgraz, 3 principal components
IL-$10_{(T\text{-}ci)}$	Year, sex, line, mkgraz, dage, 3 principal components
IgA	Year, sex, line, mkgraz, age, dage, 3 principal components
	Recording occasion No.2
Traits	**Covariates**
FEC_S	Year, sex, line, mkgraz, wngraz, brrnk, 3 principal components
FEC_N	Year, sex, line, mkgraz, wngraz, 3 principal components
FOC	Year, sex, line, mkgraz, wngraz, age, 3 principal components
DAG	Year, sex, line, mkgraz, wngraz, dage, 3 principal components
LWT	Year, sex, line, mkgraz, wngraz, brrnk, 3 principal components
IFN-$\gamma_{(PWM)}$	Year, line, mkgraz, age, 3 principal components
IL-$4_{(PWM)}$	Year, sex, line, mkgraz, age, 3 principal components
IL-$10_{(PWM)}$	Year, line, mkgraz, brrnk, age, 3 principal components
IFN-$\gamma_{(T\text{-}ci)}$	Sex, line, brrnk, age, 3 principal components
IL-$4_{(T\text{-}ci)}$	Year, sex, line, brrnk, 3 principal components
IL-$10_{(T\text{-}ci)}$	Year, line, wngraz, age, 3 principal components
IgA	Year, sex, line, wngraz, 3 principal components

Fixed effects key: year = year of birth, sex = sex of the lamb, line = genetic line of the lamb, mkgraz = location of the lamb at the mid-point between birth and weaning, wngraz = location of the lamb at weaning, age = age of the lamb at recording, brrnk = birth-rearing rank and dage = age of the dam at the time of parturition.

The magnitude of the effects of individual markers identified from each separate GWAS was estimated using model [1] with the addition of the fixed effect of the corresponding SNP locus genotype. These analyses were conducted with the software ASReml v3.0 (Gilmour *et al.*, 2009). The genotypic effect solutions were used to estimate the additive (*a*) and dominance (*d*) effects, and the proportion of additive genetic variance (PV_A) due to each SNP locus as follows:

$$a = (AA - BB)/2$$

$$d = AB - ((AA + BB)/2)$$

$$PV_A = (2pq(a + d(q - p))^2)/V_A,$$

Where:

- *AA*, *BB* and *AB* correspond to the solutions of the respective genotypic effect levels
- *p* and *q* correspond to the allelic frequencies of *A* and *B* at the SNP locus
- V_A corresponds to the additive genetic variance of the trait.

4.2.5 Gene and gene ontology annotation in associated regions

The presence of candidate genes within 500kb upstream or downstream of the significant SNPs for each of the studied traits at either genome-wide or suggestive levels was investigated. Protein-coding genes that were found within the candidate regions considered were retrieved from the Ensemble Genes 91 database, which is based on the Oar v3.1 ovine reference genome. This was achieved using the BioMart tool within the Ensembl Genome Browser (Kinsella *et al.*, 2011; Yates *et al.*, 2019).

The classification of genes in accordance with biological function was performed using the Database for Annotation, Visualisation and Integrated Discovery (DAVID) v6.8 tool (Huang *et al.,* 2009). Gene ontology (GO) terms (Ashburner *et al.,* 2000; The Gene Ontology Consortium, 2021) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways (Ogata *et al.,* 1999; Kanehisa *et al.,* 2014) were identified using DAVID. Potential candidate genes were further reviewed with access to GeneCards (Stelzer *et al.,* 2016), the Ensembl Genome Browser (Yates *et al.*, 2019) and the NCBI database resources (Sayers *et al.*, 2020).

4.3 Results

4.3.1 Principal component analysis

The PCA revealed population stratification attributed to the different genetic lines (Figure 4.1) that form the population studied. The first, second and third principal components explain 23.9%, 18.9% and 9.6% of variance, respectively. The genetic lines appear to be distinct, illustrating the effect of directional selection. The flock was split into of animals as '*Selection*' (S), '*Control*' (C) and '*Faecal Egg Count*' (F) genetic lines. A fourth group comprising fewer animals (L), produced from mating a number of ewes from each of the three lines from other recorded SBF flocks is also represented.

4.3.2 Genome-wide association studies

Manhattan plots of significant GWAS results and the corresponding Q-Q plots of observed p-values against expected P-values for each trait are shown in Figures 4.2, 4.3 and 4.4. Deviations from the identity line in Q-Q plots are indicative that the

samples contain values that arise from a true association and validate the results in the corresponding Manhattan plots. Results show that the genomic relationship matrix and the inclusion of the three first principal components were sufficient to account for near all population structure in this study, as reflected by the lambda correction factor (λ) being near unity. The average inflation factor was 1.000±0.009, ranging from 0.9802 and 1.012 for IFN-$\gamma_{(PWM)}$ (2^{nd} recording occasion) and IgA (2^{nd} recording occasion), respectively. Inflation factors reflect that the necessary adjustments have already been made.

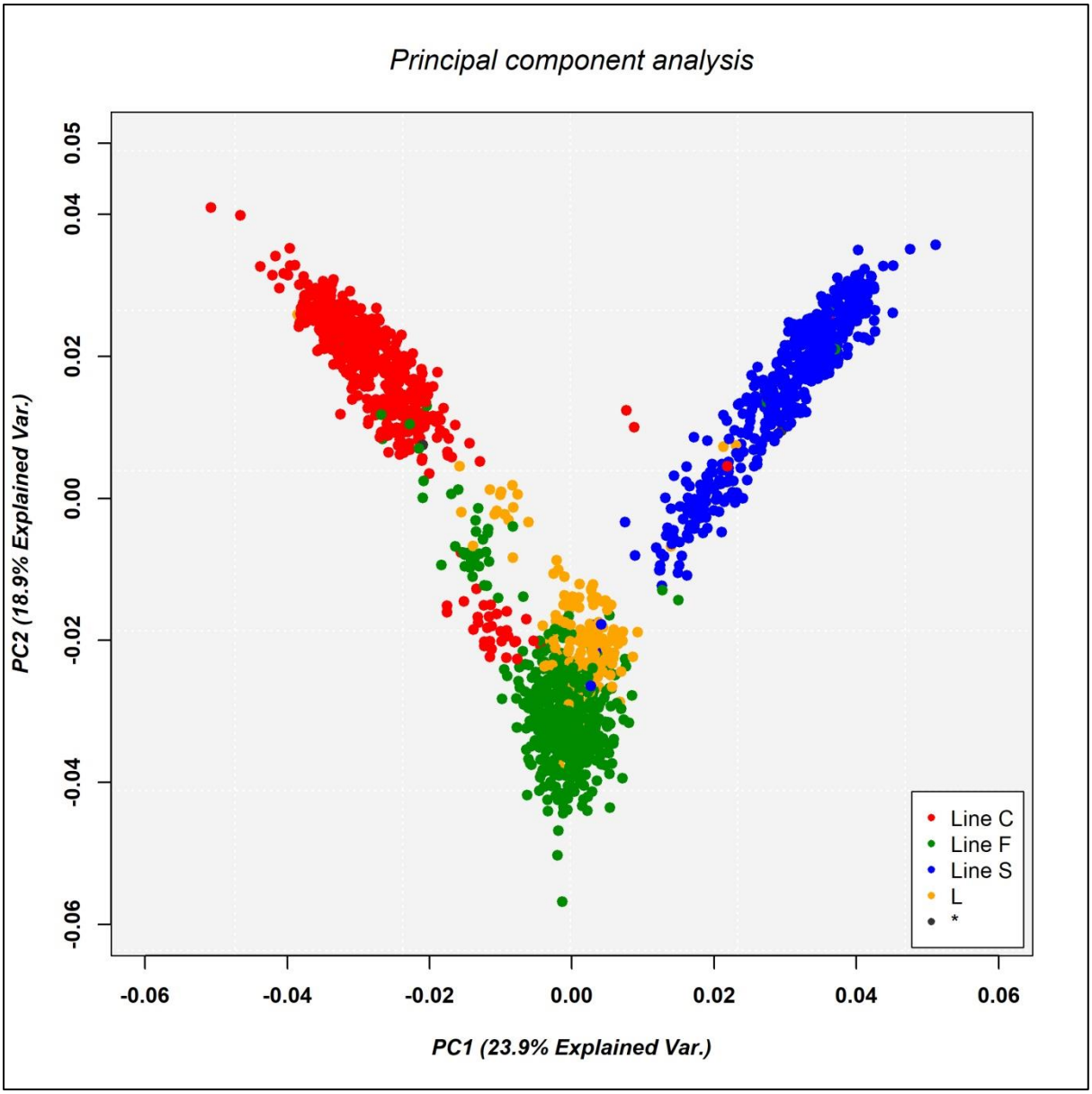

Figure 4.1 – Principal component analysis for the population of lambs genotyped in this study (n=1,766). This graph plots first vs. second principal components, revealing population stratification along genetic lines. Blue and red dots represent '*Selection*' (S) and '*Control*' lines (C) selected for high- and average-performing lambs in terms of growth, respectively. Green dots represent the '*Faecal Egg Count*' (F) line corresponding to animals selected for parasitic resistance based on their breeding values for FEC scores. Orange dots represent a group of lambs born from a selection of ewes from across the three genetic lines that were mated to bought-in rams linking the flock with the SBF industry breed improvement programme (L).

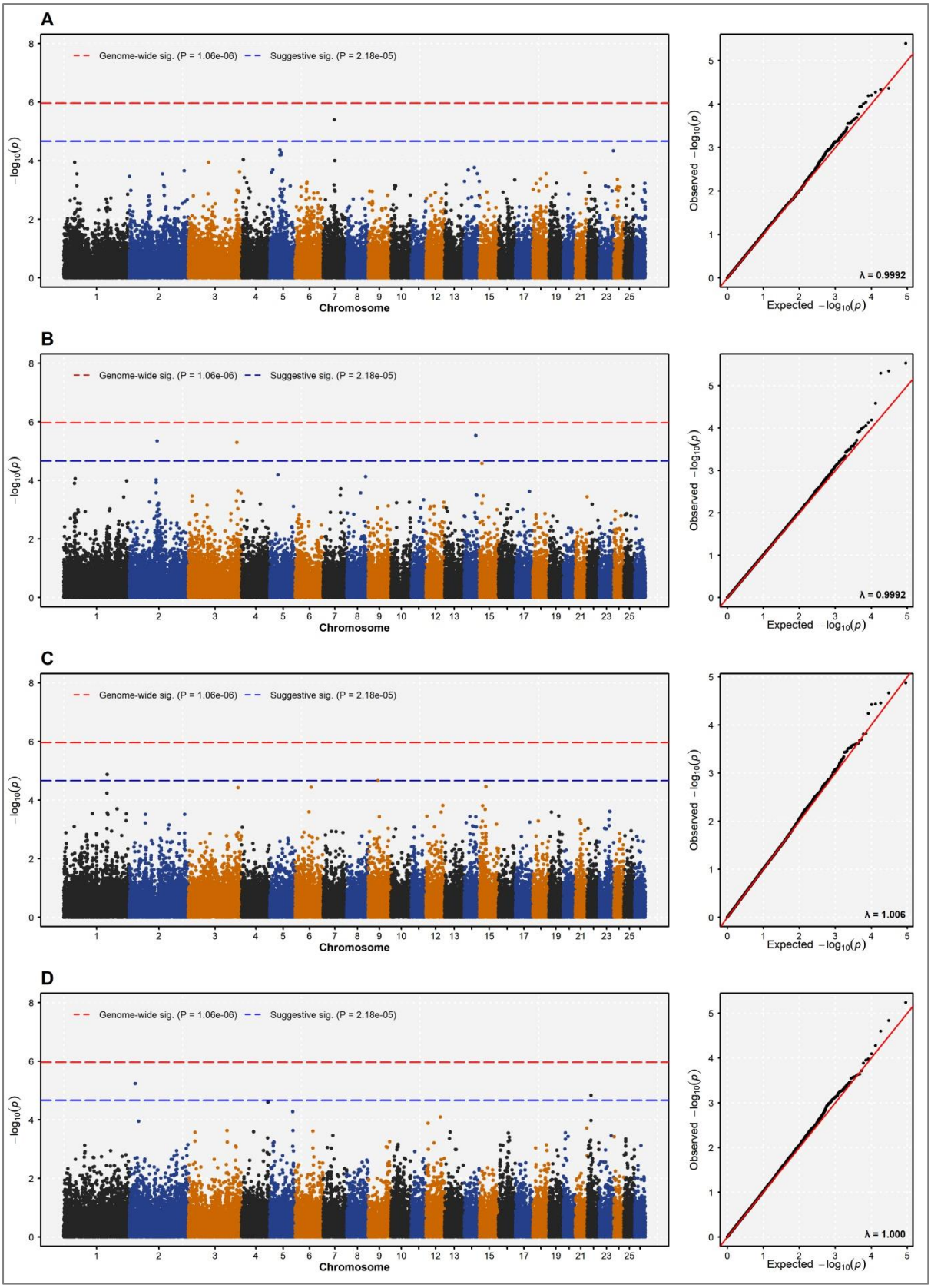

Figure 4.2 – Manhattan plots displaying GWAS results (p-values) and corresponding Q-Q plots (observed p-values against expected p-values) for **A)** FEC_S (1st recording occasion), **B)** and **C)** FEC_N (1st and 2nd recording occasion, respectively) and **D)** DAG (1st recording occasion). Red and Blue dashed lines represent the genome-wide (p-value = 1.06×10^{-6}) and suggestive (p-value = 2.18×10^{-5}) significance thresholds, respectively. FEC_S = Strongyles faecal egg counts; FEC_N = *Nematodirus* egg counts; DAG = dag scores; λ = lambda correction factor.

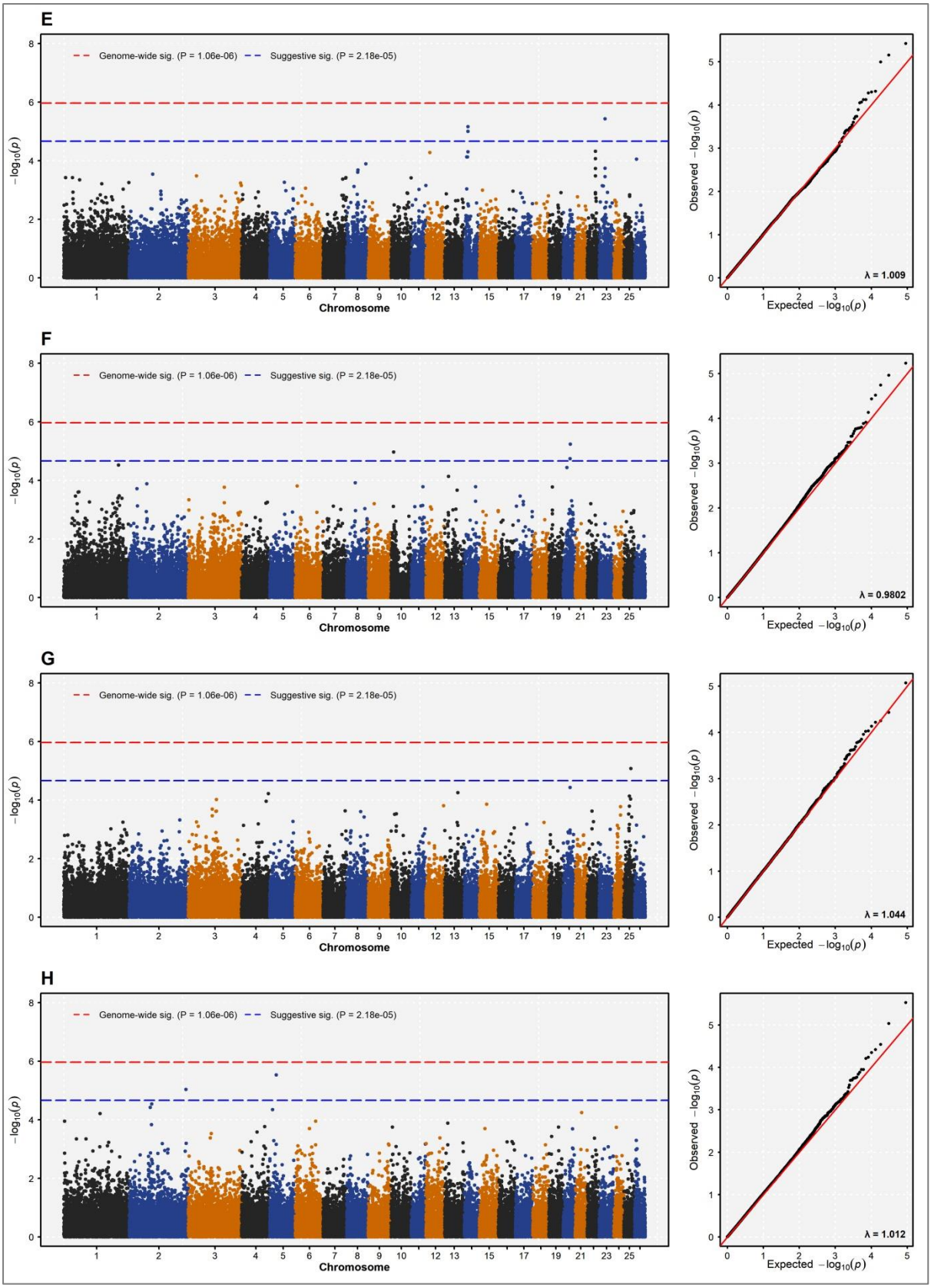

Figure 4.3 – Manhattan plots displaying GWAS results (p-values) and corresponding Q-Q plots (observed p-values against expected p-values) for **E)** IL-$4_{(PWM)}$ (1^{st} recording occasion), **F)** IFN-$\gamma_{(PWM)}$, **G)** IL-$4_{(PWM)}$ and **H)** IL-$10_{(T\text{-}ci)}$ (2^{nd} recording occasion). Red and blue dashed lines represent the genome-wide (p-value = 1.06×10^{-6}) and suggestive (p-value = 2.18×10^{-5}), respectively. IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant; λ = lambda correction factor.

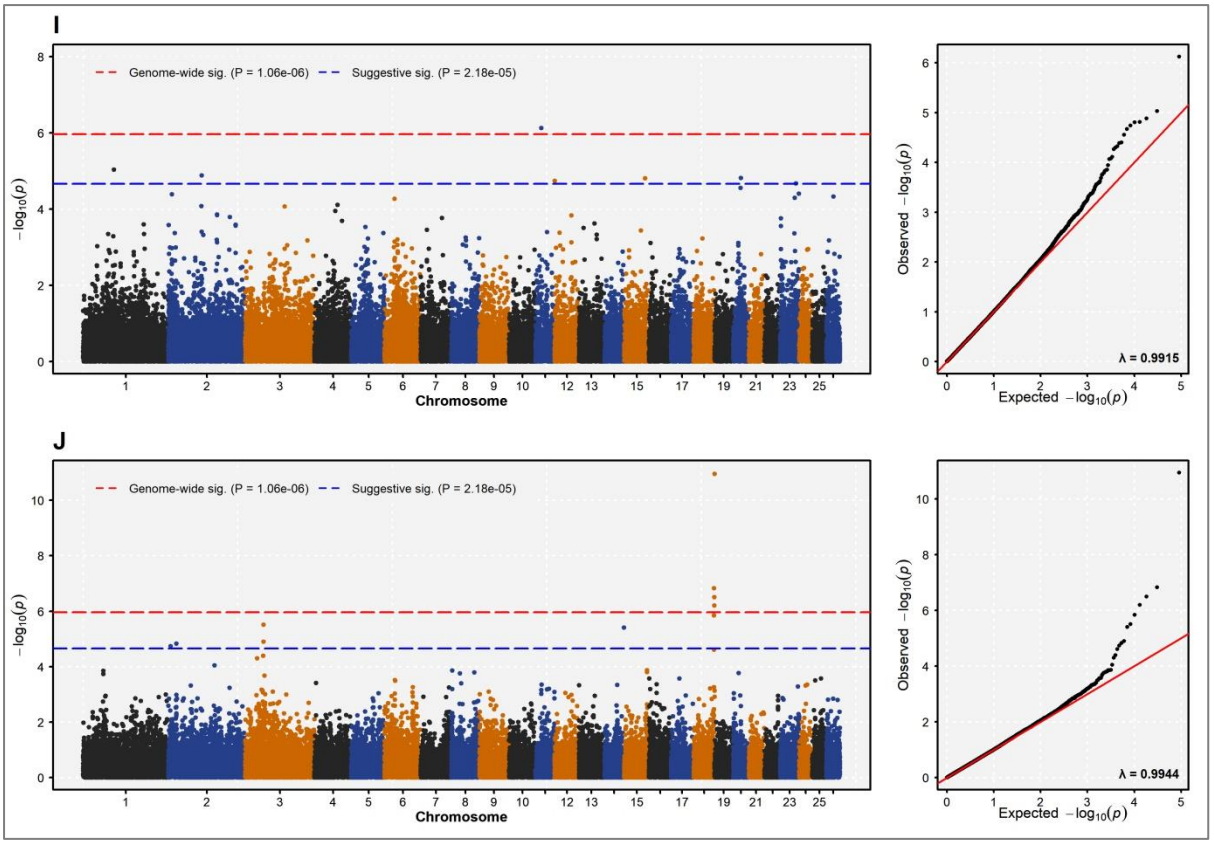

Figure 4.4 – Manhattan plots displaying GWAS results (p-values) and corresponding Q-Q plots (observed p-values against expected p-values) for **I)** and **J)** IgA (1st and second recording occasions, respectively). Red and blue dashed lines represent the genome-wide (p-value = 1.06×10^{-6}) and suggestive (p-value = 2.18×10^{-5}) significance thresholds, respectively. IgA = Immunoglobulin A; λ = lambda correction factor.

The study revealed several SNPs in genomic regions that can potentially be associated with the traits studied (Table 4.4). For most traits, SNPs were only significant at a suggestive level. A total of 15 SNPs were associated at least at a suggestive level with FEC_S, FEC_N, DAG, IFN-$\gamma_{(PWM)}$, IL-$4_{(PWM)}$, IL-$10_{(T\text{-}ci)}$ and IgA (Table 4.4). Of these 15 SNPs, only one was significant at a genome-wide level (s08970.1 on OAR 18 for IgA, 2^{nd} recording occasion), after multiple trait adjustment. Each SNP association was unique to a specific trait, and no single SNP was identified that was affecting multiple traits. No significant SNPs associated with FOC, LWT, IFN-$\gamma_{(T\text{-}ci)}$, IL-$4_{(T\text{-}ci)}$ and IL-$10_{(PWM)}$ are reported here.

The magnitude of additive and dominance genetic effects of each SNP markers was calculated, along with the proportion of total genetic variance explained by each locus (Table 4.4). Most SNPs were found to have a significant additive effect on the corresponding trait (OAR7_53177511.1, OAR3_220227474.1, s15560.1, OAR14_14894096.1, OAR1_106406464.1, OAR1_197073884.1, s67484.1, OAR20_33522555.1, OAR25_30051833.1, OAR2_252448674.1, OAR2_26992982.1 and s08970.1), and only a four had a significant dominance effect (OAR1_106406464.1, s67322.1, OAR20_33522555.1 and s08970.1).

Tables 4.4 and 4.5 summarize information on significant SNPs and individual genes neighbouring these SNPs. Individual gene information and gene function is summarised in Tables 4.6 and 4.7. The results confirmed that 52 genes neighbouring relevant SNPs are involved in immune function.

Table 4.4 - SNP information: SNPs presented show the strongest association with FEC_S, FEC_N, DAG, IFN-$\gamma_{(PWM)}$, IL-$4_{(PWM)}$, IL-$10_{(T\text{-}ci)}$ and IgA.

Traits	OAR	SNP	Position (bp)	P-value	a (se)	P-value	d (se)	P-value
FEC_S[1]	7	OAR7_53177511.1	48116542	8.91×10^{-6}	-0.1132 (0.0404)	0.0053	0.0530 (0.0653)	0.4174
FEC_N[1]	3	OAR3_220227474.1	204319907	5.31×10^{-6}	-0.3707 (0.0887)	0.0000	-0.0495 (0.1013)	0.6257
	14	s15560.1	47964362	3.11×10^{-6}	-1.0407 (0.2881)	0.0003	0.4092 (0.3016)	0.1754
DAG[1]	22	OAR22_20459522.1	16596984	1.16×10^{-5}	0.0615 (0.0785)	0.4338	0.0333 (0.0898)	0.7109
IL-$4_{(PWM)}$[1]	14	OAR14_14894096.1	14691342	8.17×10^{-6}	-0.1132 (0.404)	0.0053	0.0279 (0.0452)	0.5381
IgA[1]	1	OAR1_106406464.1	99129941	1.06×10^{-5}	-0.0170 (0.0037)	0.0000	-0.0096 (0.0040)	0.0170
	20	s67322.1	24425338	1.75×10^{-5}	0.0079 (0.0069)	0.2095	0.0213 (0.0068)	0.0018
FEC_N[2]	1	OAR1_197073884.1	182705596	1.52×10^{-5}	0.4446 (0.1060)	0.0000	-0.1301 (0.1203)	0.2797
IFN-$\gamma_{(PWM)}$[2]	20	OAR20_33522555.1	30534971	6.91×10^{-6}	-0.0924 (0.0377)	0.0144	0.0936 (0.0407)	0.0220
IL-$4_{(PWM)}$[2]	25	OAR25_30051833.1	28730885	9.09×10^{-6}	-0.2636 (0.0620)	0.0000	-0.0648 (0.0647)	0.3167
IL-$10_{(T\text{-}ci)}$[2]	2	OAR2_252448674.1	239031909	1.06×10^{-5}	-0.0890 (0.0219)	0.0001	-0.0214 (0.0253)	0.3997
IgA[2]	2	OAR2_26992982.1	26058151	1.67×10^{-5}	0.1353 (0.0573)	0.0186	-0.0184 (0.0952)	0.8472
	3	s67484.1	59634347	3.48×10^{-6}	-0.0783 (0.0304)	0.0102	0.0165 (0.1140)	0.6059
	14	s52366.1	62552503	4.51×10^{-6}	-0.1946 (0.1132)	0.0861	-0.0817 (0.1140)	0.4740
	18	s08970.1	68450168	4.49×10^{-11}	-0.0899 (0.0119)	0.0000	0.0271 (0.0135)	0.0451

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant. OAR = sheep chromosome; bp = base-pair position; a = additive effect; d = dominance effect; se = standard error.

Table 4.5 - SNP information and associated genes found in proximity to significant SNPs.

Traits	OAR	SNP	MAF	% V_A Exp.	Genes
FEC_S[1]	7	OAR7_53177511.1	0.35	16	*RNF111*; *CCNB2*
FEC_N[1]	3	OAR3_220227474.1	0.37	23	*CLEC1A*; *CLEC1B*; *CLEC1B*; *LOC101120482*; *LOC101103714*; *CLEC7A*; *LOC101123029*; *CLEC9A*; *CLEC12B*; LOC101116896; *LOC1011104216*; *LOC101123288*; *KLRD1*; *LOC101102227*; *LOC101116641*; *LOC105614844*; *LOC105613001*; *OLR1*
	14	s15560.1	0.09	31	*LGALS4*; *NFKBIB*; *ZFP36*
DAG[1]	22	OAR22_20459522.1	0.42	3	*BLNK*
IL-$4_{(PWM)}$[1]	14	OAR14_14894096.1	0.46	2	*MYLK3*
IgA[1]	1	OAR1_106406464.1	0.39	5	*FCGR1A*; *CTSS*; *ECM1*
	20	s67322.1	0.15	6	*IL17A*; *IL17F*
FEC_N[2]	1	OAR1_197073884.1	0.29	5	*IGSF11*; *CD80*; *PLA1A*
IFN-$\gamma_{(PWM)}$[2]	20	OAR20_33522555.1	0.26	8	*BTN1A1*; *BTN2A2*; *HFE*; *TRIM38*
IL-$4_{(PWM)}$[2]	25	OAR25_30051833.1	0.13	6	*PLA2G12B*
IL-$10_{(T\text{-}ci)}$[2]	2	OAR2_252448674.1	0.43	15	*IFI6*
IgA[2]	2	OAR2_26992982.1	0.04	4	*SYK*; *NFIL3*
	3	s67484.1	0.11	6	*IL1A*; *IL1B*; *IL1F10*; *IL1RN*; *IL36A*; *IL36B*; *IL36RN*; *IL37*
	14	s52366.1	0.04	4	*TRIM28*
	18	s08970.1	0.36	12	*PLD4*; *BTBD6*

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant. OAR = sheep chromosome; MAF = minor allele frequency; V_A Exp. = Genetic variance explained by individual SNP.

4.3.2.1 Disease traits – parasitic infection

The Cyclin B2 (*CCNB2*) and the Ring finger protein 111 (*RNF111*) genes were respectively found 77 kbp and 117 kbp upstream of SNP OAR7_53177511.1, which was the most significant SNP on OAR7 for FEC_S. This explains approximately 16% of genetic variance of the trait in lambs at around three months of age.

Ten genes belonging to the C-type lectin superfamily of genes were found close to SNPs associated with FEC_N. Of these, nine genes were 127-488 kbp upstream of the most significant SNP OAR3_220227474.1 (*CLEC1A*, *CLEC1B*, *CLEC2B*, *LOC101120482*, *LOC101103714*, *CLEC7A*, *CLEC9A*, *CLEC12B* and *OLR1*) and one gene (*LOC101123029*) was 363kbp downstream the same SNP. Several additional genes belonging to the killer cell lectin-like receptor family were found near OAR3_220227474.1, including *LOC101116896*, *LOC101104216*, *LOC101123288*, *KLRD1*, *LOC101116641*, *LOC105614844* and *LOC105613001* located 71-295 kbp downstream of this SNP on OAR 3. Another killer cell lectin-like encoding gene (*LOC101102227*) was found encompassing the same SNP in chromosome 3. Furthermore, three genes were found close to SNP s15560.1 on OAR14, also associated with FEC_N. The galectin 4 (*LGALS4*) and NFKB inhibitor Beta (*NFKBIB*) genes were found 238 kbp and 156 kbp downstream the highest SNP on OAR14, respectively, while the ZFP36 Ring Finger Protein (*ZFP36*) gene was 301 kbp upstream the same SNP. The SNPs OAR3_220227474.1 and s15560.1account for 23% and 31% of the genetic variance of FEC_N, respectively. Finally, the genes Immunoglobulin Superfamily Member 11 (*IGSF11*), CD80 Molecule (*CD80*) and Phospholipase A1 Member A (*PLA1A*) are located 15-463 kbp

downstream of SNP OAR1_197073884.1, which is associated FEC_N and explains 5% of the genetic variance of FEC_N in 4-month old lambs.

The gene B cell linker (*BLNK*) was found 157 kbp upstream of OAR22_20459522.1 which corresponds to the most significant SNP on chromosome 22 associated with DAG and accounted for 3% of the genetic variance.

4.3.2.2 Immunological traits - cytokines

Four genes were found around SNP OAR20_33522555.1, associated with IFN-$\gamma_{(PWM)}$. Two of these genes belong to the butyrophilin family of genes (Butyrophilin subfamily 1 member A1 - *BTN1A1* and Butyrophilin subfamily 2 member A2 - *BTN2A2*) and are located, respectively, 5kbp downstream and 8kbp upstream the SNP on OAR20. Additionally, the Homeostatic Iron Regulator (*HFE*) and Tripartite Motif Containing 38 (*TRIM38*) genes were located, respectively, 255kbp and 324kbp downstream of OAR20_33522555.1. This SNP located on OAR20 accounts for 8% of explained genetic variance of IFN- γ for lambs averaging 5 months of age.

The Myosin Light Chain Kinase 3 (*MYLK3*) gene, here associated with IL-$4_{(PMW)}$ is located 96 kbp downstream of the OAR14_14894096.1 on chromosome 14, which accounts to 2% of genetic variance for this trait. The Phospholipase A2 Group XIIB (*PLA2G12B*) gene is located 98kbp upstream the OAR_253001833.1 SNP associated with IL-$4_{(PWM)}$. This SNP explains around **6%** of the genetic variance of this trait.

Finally, a significant SNP (OAR2_252448674.1) located on OAR2, accounts for approximately 15% of the genetic variance of IL-$10_{(T\text{-}ci)}$ (2^{nd} recording occasion).

The Interferon Alpha Inducible Protein 6 (*IFI6*) is located 491 kbp downstream this SNP.

4.3.2.3 Immunological traits - IgA

Eighteen immune-related genes were found in proximity to six SNPs associated with IgA, namely OAR1_106406464.1, s67322.1, OAR2_26992982.1, s67484.1, s52366.1 and s08970.1. Three of these genes were located on OAR1 around the OAR1_106406464.1 SNP: the Fc Gamma receptor Ia (*FCGR1A*) gene is located 302kbp downstream of this SNP, while the Extracellular Matrix protein 1 (*ECM1*) and Cathepsin S (*CTSS*) genes are located 311 kbp and 493 kbp upstream of the same SNP, respectively. Two genes belonging to the IL-17 receptor family found on OAR20 are noteworthy due to their location in relation to SNP s67322.1: *IL17A* located 25 kbp downstream of the SNP and *IL17F* which encompasses s67322.1. The OAR1_106406464.1 and s67322.1 SNPs together explain 11% of genetic variance of IgA in lambs averaging 2 months of age (5% and 6%, respectively).

The Spleen Associated Tyrosine Kinase (*SYK*), and the nuclear factor Interleukin 3 (*NFIL3*) genes are respectively located 243kbp upstream and 298kbp downstream of SNP OAR2_26992982.1 on OAR2, which account for 4% of genetic variance explained for this trait. Eight potential candidate genes identified on chromosome 3 are located 74-443 kbp upstream of SNP s67484.1, accounting for 6% of explained variance of IgA at an average of 5 months of age. These genes belong to the interleukin (IL)-1 family of cytokines: Interleukin 1 Alpha and Beta (*IL1A* and *IL1B*, respectively), the Interleukin 1 Receptor Antagonist (IL1RN), the Interleukin 1 Family Member 10 (*IL1F10*), the Interleukin 36 Alpha and Beta (*IL36A* and *IL36B*,

respectively), the Interleukin 36 Receptor Antagonist (IL36RN), and the Interleukin 37 gene (*IL37*).

The Tripartite motif 28 (*TRIM28*) gene is located 119kbp upstream the s52366.1 SNP on OAR14, which explains 4% of the genetic variance of IgA (2^{nd} recording occasion). Lastly, two further genes were found in proximity to the s08970.1 SNP on OAR18, representing the only significant SNP at genome-wide level and accounting for 12% of the trait genetic variance. The Phospholipase D Family Member 4 (*PLD4*) and the BTB Domain Containing 6 (*BTBD6*) genes are located 372kbp and 182kbp downstream of the SNP, respectively.

Table 4.6. Gene information, including symbols, name and descriptions, IDs and position.

Trait	OAR	Gene symbol	Gene name and description	Ensembl ID	Start	End
FEC_S[1]	7	*CCNB2*	Cyclin B2	ENSOARG00000020836	48194193	48217973
		RNF111	Ring finger protein 111	ENSOARG00000020839	48233777	48284148
FEC_N[1]	3	*CLEC1A*	C-type lectin domain family 1 member A	ENSOARG00000021016	204498733	204517912
		CLEC1B	C-type lectin domain family 1 member B	ENSOARG00000021020	204545775	204556579
		CLEC2B	C-type lectin domain family 2 member B	ENSOARG00000021044	204722589	204738493
		LOC101120482	C-type lectin domain family 2 member F-like	ENSOARG00000021031	204655632	204665472
		LOC101103714	C-type lectin domain family 2 member H-like	ENSOARG00000021050	204808148	204813367
		CLEC7A	C-type lectin domain containing 7A	ENSOARG00000021015	204477420	204492854
		LOC101123029	C-type lectin domain family 7 member A-like	ENSOARG00000020949	203900545	203956472
		CLEC9A	C-type lectin domain containing 9A	ENSOARG00000021019	204529427	204540034
		CLEC12B	C-type lectin domain containing 12 member B	ENSOARG00000021025	204569906	204576351
		LOC101116896	NKG2-D type II integral membrane protein	ENSOARG00000021000	204242047	204248863
		LOC1011104216	NKG2-A/NKG2-B type II integral membrane protein-like	ENSOARG00000020964	203994894	204024663
		LOC101123288	NKG2-A/NKG2-B type II integral membrane protein-like	ENSOARG00000020990	204179719	204186124
		KLRD1	Natural killer cells antigen CD94-like	ENSOARG00000021002	204295733	204301675
		LOC101102227	Natural killer cells antigen CD94-like	ENSOARG00000021003	204311908	204320372
		LOC101116641	Killer cell lectin-like receptor 6	ENSOARG00000020980	204089162	204104854
		LOC105614844	Natural killer cells antigen CD94-like	ENSOARG00000020983	204121000	204129216
		LOC105613001	Natural killer cells antigen CD94-like	ENSOARG00000020985	204148504	204158538
		OLR1	Oxidised low density lipoprotein receptor 1	ENSOARG00000021012	204447104	204461390

Trait	OAR	Gene symbol	Gene name and description	Ensembl ID	Start	End
FEC_N[1]	14	*LGALS4*	Galectin 4	ENSOARG00000005839	47715560	47725503
		NFKBIB	NFKB inhibitor beta	ENSOARG00000005899	47796710	47807736
		ZFP36	ZFP36 ring finger protein	ENSOARG00000006068	48266102	48269670
DAG[1]	22	*BLNK*	B cell linker	ENSOARG00000007320	16754368	16832091
IL-$4_{(PWM)}$[1]	14	*MYLK3*	Myosin light chain kinase 3	ENSOARG00000015706	14550957	14594827
IgA[1]	1	*FCGR1A*	Fc fragment IgG receptor Ia	ENSOARG00000020667	98816248	98827299
		ECM1	Extracellular matrix problem 1	ENSOARG00000020820	99441346	99446281
		CTSS	Cathepsin S	ENSOARG00000020861	99623159	99650475
	20	*IL17A*	Interleukin 17A	ENSOARG0000002030	24394173	24399987
		IL17F	Interleukin 17F	ENSOARG00000014066	24424618	24433376
FEC_N[2]	1	*ISGF11*	Immunoglobulin superfamily member 11	ENSOARG00000019655	182069331	182242308
		CD80	CD80 molecule	ENSOARG00000019762	182614039	182637278
		PLA1A	Phospholipase A1 member A	ENSOARG00000019774	182662813	182689805
IFN-$\gamma_{(PWM)}$[2]	20	*BTN1A1*	Butyrophilin subfamily 1 member A1	ENSOARG00000000198	30524486	30529827
		BTN2A2	Butyrophilin subfamily 2 member A2	ENSOARG00000000423	30543817	30551949
IFN-$\gamma_{(PWM)}$[2]	20	*HFE*	Homeostatic iron regulator	ENSOARG00000001148	30790428	30794050
		TRIM38	Tripartite motif containing A	ENSOARG00000001519	30859856	30873824
IL-$4_{(PWM)}$[2]	25	*PLA2G12B*	Phospholipase A2 group XIIB	ENSOARG00000007339	28829649	28850317
IL-$10_{(T\text{-}ci)}$[2]	2	*IFI6*	Interferon alpha inducible protein 6	ENSOARG00000003341	238537980	238539929
IgA[2]	2	*SYK*	Spleen associated tyrosine kinase	ENSOARG00000007814	25701083	25759453
		NFIL3	Nuclear factor, interleukin 3 regulated	ENSOARG00000003352	26301791	26303179

Trait	OAR	Gene symbol	Gene name and description	Ensembl ID	Start	End
IgA[2]	3	*IL1A*	Interleukin 1 alpha	ENSOARG00000020877	60078287	60088697
		IL1B	Interleukin 1 beta	ENSOARG00000020866	60023514	60034494
		IL1F10	Interleukin 1 family member 10	ENSOARG00000020835	59778179	59795239
		IL1RN	Interleukin 1 receptor antagonist	ENSOARG00000020828	59709089	59714722
		IL36A	Interleukin 36 alpha	ENSOARG00000020848	59855920	59859301
		IL36B	Interleukin 36 beta	ENSOARG00000020843	59841799	59848492
		IL36RN	Interleukin 36 receptor antagonist	ENSOARG00000020837	59796672	59801932
		IL37	Interleukin 37	ENSOARG00000020859	59952933	59960245
	14	*TRIM28*	Tripartite motif containing 28	ENSOARG00000003949	62671612	62679807
	18	*PLD4*	Phospholipase D family member 4	ENSOARG00000007988	68072172	68077408
		BTBD6	BTB domain containing 6	ENSOARG00000008607	68260502	68268003

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; LWT = live weight; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant. OAR = sheep chromosome; Ensembl ID = gene identification; Start – End = position of the gene within the chromosome.

Table 4.7. Gene function summary description.

Trait	Gene	Gene function
FEC_S[1]	*CCNB2*	T cell homeostasis; transforming growth factor (TGF)-β receptor signalling pathway
	RNF111	positive regulation of TGF-β receptor signalling pathway
FEC_N[1]	*CLEC1A*	
	CLEC1B	
	CLEC2B	
	LOC101120482	
	LOC101103714	innate immune receptor involved in recognition of pathogen glycans and induction of inflammation
	CLEC7A	
	LOC101123029	
	CLEC9A	
	CLEC12B	
	LOC101116896	natural killer receptors (dimerise with CD94); NKG2D transmits activating signals; part of the C-type lectin *r* receptor family
	LOC101104216	natural killer receptors (dimerise with CD94); NKG2A and NKG2B transmit inhibitory signals; part of C-type lectin *r* receptor family
	LOC101123288	
	KLRD1	
	LOC101102227	natural killer cell receptor complex; dimerises with NKG2 receptors to transmit activating or inhibitory signals
	LOC101116641	
	LOC105614844	natural killer cell receptor complex; dimerises with NKG2 receptors to transmit activating or inhibitory signals
	LOC105613001	
	OLR1	encodes a low density lipoproteins receptor belonging to the C-type lectin superfamily; results in pro-inflammatory responses

Trait	Gene	Gene function
FEC_N[1]	*LGALS4*	promoter of inflammation
	NFKBIB	inhibitor of inflammation
	ZFP36	role in regulating immune response and inflammatory diseases; inhibits production of various inflammatory responses
DAG[1]	*BLNK*	regulation of B cell receptor signalling
IL-$4_{(PWM)}$[1]	*MYLK3*	regulation of vascular permeability involved in acute inflammatory response; cellular responses to interleukin (IL)-1
IgA[1]	*FCGRA1A*	Fc receptor on surface of macrophages, neutrophils, eosinophils, dendritic cells; binding of IgG in cell activation, phagocytosis and respiratory burst
	ECM1	negative regulation of cytokine mediated signalling pathway; regulation of type-2 immune responses
	CTSS	involved in processing of antigens for presentation via MHC II to T cells
	IL17A	produced by Th17 cells and involved in inflammatory responses; promotes intestinal IgA responses
	IL17F	produced by Th17 cells and involved in inflammatory responses
FEC_N[2]	*IGSF11*	up-regulated in colorectal cancers and hepatocellular carcinomas as well as intestinal-type gastric cancers
	CD80	co-stimulatory for T cell activation by antigen presenting cells
	PLA1A	involved in allergic immune responses; up-regulated in gut inflammatory responses (Crohn's disease)
IFN-$\gamma_{(PWM)}$[2]	*HFE*	negative regulation of T cell antigen processing and presentation and T cell cytokine production; negative regulation of antigen processing and presentation of endogenous peptide antigen via MHC class I
	BTN1A1 *BTN2A2*	inhibits the proliferation of CD4 and CD8 T cells activated by anti-CD3, T cell metabolism and IL-2 and IFN-γ secretion
	TRIM38	regulation of IFN-β production (type I interferon) signalling; negative regulation of Toll-like receptor (TLR) induced I-kappa B kinase/NF-kappa B signalling
IL-$4_{(PWM)}$[2]	*PLA2G12B*	role in inflammation
IL-$10_{(T\text{-}ci)}$[2]	*IFI6*	involved in type I interferon response; antiviral response

Trait	Gene	Gene function
IgA[2]	*SYK*	B cell receptors (BCR) and T cell receptor (TCR) signalling
	NFIL3	negative regulator of regulatory T cells
	IL1A	pro-inflammatory; SNPs associated with childhood IgA nephropathy; induces IgA production from gut peyers patches
	IL1B	pro-inflammatory; SNPs associated with childhood IgA nephropathy
	IL1F10	IL-1 mediated inflammatory response
	IL1RN	anti-inflammatory; SNPs associated with childhood IgA nephropathy
	IL36A *IL36B*	inflammatory response; member of the IL-1 family of cytokines
	IL36RN	antagonist of IL-36
	IL37	anti-inflammatory; member of IL-1 family of cytokines
	TRIM28	innate immune responses
	PLD4	SNP associated with autoimmunity (Systemic Lupus Erythematous – SLE)
	BTBD6	class I MHC mediated antigen processing and presentation and innate immune system (PathCards)

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; LWT = live weight; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant.

4.3.3 Gene ontology

Gene ontology (GO) terms were extracted for each of the genes discussed above that were located within 500kbp of the corresponding SNPs. This analysis is used as a gene functional classification system that allows us to describe the properties of genes. GO terms are functionally separated into three domains: biological processes, cellular components and molecular function. Most of the GO terms found in the present study were associated with biological processes. GO terms and corresponding genes relating to the various terms are summarised on Supplementary Table S4.1 found in the Appendix section of this thesis. All terms described relate to immune function.

4.3.3.1 Parasitic infection traits

There were several GO terms associated disease traits. The gene *CLEC7A*, corresponding to a C-type lectin, is associated with the signalling of pattern recognition receptor activity (GO:0008329). *LOC101116896*, a member of the killer cell lectin-like receptor family has important roles in the natural killer cell activation, positive regulation of killer cell mediated cytotoxicity and the MHC class Ib receptor activity (GO:0030101, GO:0045954 and GO:0032394). The gene *ZFP36* is an important negative regulator of inflammatory responses (GO:0050728), while *CD80* is involved in T cell co-stimulation and positive alpha-beta T cell proliferation (GO:0031295 and GO:0046641, respectively).

4.3.3.2 Immunological traits - cytokines

MYLK3, associated with IL-4, is important in the regulation of vascular permeability involved in acute inflammatory responses (GO:0002528). The gene *BTN2A2* is positive regulator of T cell differentiation (GO:0045591), but also has roles as a negative regulator of cytokine secretion and negative regulator of the T cell signalling pathway (GO:0050710 and GO:0050860), respectively. *HFE* is involved in the negative regulation of T cell antigen processing and presentation, T cell cytokine production and antigen processing and presentation of endogenous peptides antigens via MHC class I (GO:0002626, GO:0002725 and GO:1904283, respectively).

4.3.3.3 Immunological traits - IgA

Immunoglobulin A has several important associated genes related to important GO terms. *FCGR1A* is implicated in the antigen processing and presentation of exogenous peptide antigen via MHC class I and defence responses to bacterium (GO:0042590 and GO:0042742, respectively). *ECM1* is involved in the regulation of Type 2 immune responses (GO:0002828), while *CTSS* has roles in adaptive immune responses and antigen processing and presentation of peptide antigen (GO:0002250 and GO:0030574, respectively). Two genes coding for IL-17 cytokines, *IL17A* and *IL17F*, are both involved in inflammatory responses (GO:0006954) and in the positive regulation of cytokine production involved in inflammatory response (GO:1900017). *SYK* is involved in the macrophage activation involve in immune responses, as well as in innate immune responses, and is a positive regulator of B cell differentiation (GO:0002281, GO:0045087 and GO:0045579, respectively).

Several genes coding members of the IL-1 family of cytokines were found in association with IgA. *IL1A* has a role in connective tissue replacement involved in inflammatory response to wound healing and immune responses (GO:0002248 and GO:0006955). *IL1B*, *IL36A*, *IL36B* and *IL37* genes are all associated with GO terms related to inflammatory responses and immune responses (GO:0006954 and GO:0006955, respectively). *IL1RN* serves as a negative regulator of cytokine-mediated signalling pathway and a negative regulator of the IL-1-mediated signalling pathway (GO:0001960 and GO:2000660), while *IL36RN* serves as a negative regulator of the cytokine-mediated signalling pathway and a negative regulator of IL-17 production and IFN-γ secretion (GO:0001960, GO:0032700 and GO:1902714, respectively).

4.3.4 KEGG Pathways

KEGG pathway analysis results are presented in Supplementary Table S4.2 found in the Appendix section. Here, the most relevant results are summarised.

4.3.4.1 Disease traits

Four members of the killer cell lectin-like receptor family, which are *LOC101104216*, *LOC101123288*, *LOC101116896* and *LOC101102227*, are involved in the antigen process and presentation pathway (oas04612), with the latter two genes of the four also being involved in the natural killer cell mediated cytotoxicity pathway (oas04650). *NFKBIB* gene is involved in both T and B cell receptor signalling pathway (oas04660 and oas04662, respectively). The *CD80* gene is involved in the intestinal immune network for IgA production pathway (oas04672).

4.3.4.2 Immunological traits - IgA

The *CTSS* gene, here in close proximity to a SNP associated with IgA is also involved in the antigen processing and presentation pathway (oas04612), while *SYK* is involved in the B cell receptor pathway (oas04662). *IL17A*, *IL1A* and *IL1B* are all involved in the cytokine-cytokine receptor interaction pathway (oas4060). Additionally, *IL1A* and *IL1B* are involved in the mitogen-activated protein kinase (MAPK) pathway (oas4010) and the hematopoietic cell lineage pathway (oas04640). *IL1B* is also involved in the Toll-like receptor signalling pathway NF-kappa B signalling pathway (oas04620 and oas04064, respectively).

4.4 Discussion

The present study set out to investigate the genomic architecture of lamb traits related with parasitic disease resistance, immune profile, and production. Multiple suggestive significant SNP associations were identified for disease resistance and immunological traits, but not for production (LWT).

4.4.1 Parasitic infection traits

Five SNPs associated with parasitic infection traits were identified. Four SNPs were found to be associated with FEC: OAR7_53177511.1 accounting for 16% of explained genetic variance of FEC_S on recording occasion 1 (at around 3 months of age), OAR3_220227474.1 and s15560.1 explaining 23% and 31%, of genetic variance respectively for FEC_N measured on recording occasion 1, and OAR1_197073884.1 which accounts for 5% of the genetic variance of FEC_N on recording occasion 2, when animals average 4 months of age. The only SNP found in association with DAG explained 3% of genetic variance. It is important to note that, taken together, SNPs OAR3_220227474.1 and s15560.1 accounted for over 50% of the genetic variance of FEC_N during the 1st recording occasion. The results presented for FEC_N are consistent with Davies *et al.,* (2006), who reported several QTLs associated with FEC at varying time points with respective estimates of proportions of genetic variance varying from 26% to 79% for FEC traits. In contrast, the proportion of genetic variance explained by significant SNPs associated with Strongyle and *Nematodirus* FEC varied between 1.6% and 12.7% in a study by Keane *et al.,* (2018). Two other related studies also found much lower proportions of variance explained by individual SNPs. Estrada-Reyes *et al.,* (2019b) found that

individual SNPs explained a proportion of genetic variance of FEC ranging from 10% to 15% in a Florida native sheep breed. In a study by Kemper *et al.,* (2011) in a mixed breed population, the SNPs accounted collectively for 11% of the genetic variance, with each SNP explaining one hundredth of the total proportion explained. While it is possible that the size of SNP effects in genomic studies be overestimated (Mucha *et al.,* 2015), the results reported here reveal the presence of notable signals in the respective genomic regions and may harbour genes that play a substantial role in animal resistance to parasitic infection.

Indeed, multiple genes were identified following post-GWAS analyses that are located in proximity to the above-mentioned SNPs. Among them are several genes located on OAR3 and in proximity to SNP OAR3_220227474.1 (associated with FEC_N, 1st recording occasion), encoding C-type lectins (CTLs), namely *CLEC1A*, *CLEC1B*, *LOC101120482*, *LOC101103714*, *CLEC7A*, *LOC101123029*, *CLEC9A* and *CLEC12B* which are involved in the recognition of pathogen glycans as innate immune receptors and are involved in the induction of inflammation, and *OLR1* which is responsible for pro-inflammatory responses. C-type lectins (CTLs) are involved in immune responses, from initial recognition and uptake to the modulation of adaptive immunity (Drummond and Brown, 2013), also modulating inflammatory processes (Mayer *et al.,* 2017). Currently, there is a knowledge gap regarding the function of CTLs in veterinary species (Lindenwald and Lepenies, 2020). The influence of other CTL-associated alleles on immune responses against parasitic infection has been described in wild Soay sheep, with the *CLEC16A* gene being strongly correlated with specific IgA levels against *T. circumcincta* in lambs and

mature sheep (Sparks *et al.,* 2019). CTLs were shown to be up-regulated in abomasal mucosa of immune cattle following infection with *Ostertagia ostertagi* and *Cooperia oncophora*, including *CLEC12A* (Li *et al.,* 2011), supporting their role in invoking host immune responses and the development of resistance against pathogens. *CLEC12L* was significantly up-regulated C-type lectin by sheep peripheral blood mononuclear cells (PBMC) stimulated with *H. contortus* soluble extract (Wang *et al.,* 2019). Similarly, St. Croix PBMCs up-regulated several CTLs including *CLEC4D*, *CLEC1A*, *CLEC5A* and *CLEC12A* when exposed to *H. contortus* (Jacobs *et al.,* 2020). During parasitic infections, CTLs activate dendritic cells which will go on to promote Th2 differentiation (Kaisar *et al.*, 2018).

Also, in proximity SNP OAR3_220227474.1, several receptors for natural killer cell genes are reported, represented by members of the killer cell lectin-like receptor family (*LOC101116896*, *LOC1011104216*, *LOC101123288*, *KLRD1*, *LOC101102227*, *LOC101116641*, *LOC105614844* and *LOC105613001*), which are capable of transmitting activating or inhibitory signals. When bonded with CD94, these form CD94/NKG2 heterodimers of the C-type lectin receptor family (Zaghi *et al.,* 2019). These receptors enable NK cells to discriminate between healthy and infected cells by monitoring the levels of MHC class I molecules. Associations between polymorphisms within the CD94/NKG2 cluster and various diseases have been reported (Iwaszko and Bogunia-Kubik, 2011). Much like Th2 cells (Craig, 2009), innately activated NK cells produce Interleukin (IL)-13 (De Veer *et al.,* 2007), a cytokine involved in Th2 immune responses which are crucial in clearing the host of nematode parasites (Allen and Maizels, 2011). Indeed, NK cells were

proven to be an important source of IL-13 in the intestinal tissue of mice infected with *Trichinella spiralis* (McDermott *et al.,* 2005). The role of NK cells on IL-13 production during nematode infection was further shown by Hepworth and Grencis (2009).

The genes *LGALS4*, *NFKBIB* and *ZFP36* are in proximity to SNP s15560.1, associated with FEC_N, on OAR 14. The *LGALS4* gene, an inhibitor of inflammation, codes Galectin 4, a protein exclusively expressed within the GI tract. Treatment with this protein was shown to ameliorate signs of inflammation (Kim *et al.,* 2013; Paclik *et al.,* 2008). LGALS4 has been identified as one of three genes belonging to the galectin family to be GIN-activated and up-regulated in resistant sheep infected with *T. circumcincta* (Chitneedi *et al.,* 2018). Similarly, Galectin-4 was shown to be significantly up-regulated in naïve yearling lambs after being infected with *T. circumcincta* L3 (Knight *et al.,* 2011) Along with CTLs, galectins are prime candidates for the innate recognition of carbohydrates present on the surface of nematodes leading to immune activation and modulation (De Veer *et al.,* 2007).

The *NFKBIB* gene serves as a promoter of inflammation and represents a major regulator of nuclear factor kappa B (NF-κB) in mammals (Scheibel *et al.,* 2010), playing a crucial role in pro-inflammatory responses in vivo (Scheibel *et al.,* 2010). It has been shown to be highly expressed in a group of resistant goats infected with *H. contortus* (Wang *et al.,* 2022). Enhanced NF-κB activity in mice infected with *T. muris* resulted in higher levels of IL-4 and IL-13 in favour of IFN-γ (Bąska and Norbury, 2022).

The *ZFP36* gene plays a role in regulating immune responses by inhibiting the production of several inflammatory cytokines (Guo *et al.,* 2017; Makita *et al.,* 2021). IL-10 (Makita *et al.,* 2021) and IFN-γ (Jin *et al.,* 2012) cytokines have been identified as potential targets of *ZFP36*. Its regulatory function appears to control both initiation and resolution of inflammatory responses, regulating immune responses in a multitude of immune cells and through various mechanisms (Makita *et al.,* 2021). Mice lacking this gene suffer chronic inflammation (Jin *et al.,* 2012). This gene is differentially expressed in sheep infected with *H. contortus* (Rowe *et al.,* 2008).

The gene *CD80,* located on OAR 1, and close to SNP OAR1_197073884.1, is responsible for encoding CD80 molecules, involved in T cell activation by antigen presenting cells (Tatari-Calderone *et al.,* 2002). Down-regulation of *CD80* on the surface of antigen presenting cells is capable of interfering with the generation and maintenance of T cell responses (McNeilly *et al.,* 2013). Ovine dendritic cells express co-stimulatory molecules such as CD80 and are capable of presenting antigen to T cells (McNeilly *et al.,* 2009). Dendritic cells can be inhibited by the down-regulation of CD80 (McNeilly *et al.,* 2009). Galectin 4 is responsible for inhibiting *CD80* expression on PBMCs (Paclik *et al.,* 2008).

The *BLNK* gene, a regulator of B cell receptor signalling, was linked to SNP OAR22_20459522.1 on OAR22 and associated with DAG in the present study. This gene encodes a central linker protein, with a function in regulating the biological outcome of B cell function and development (Fu *et al.,* 1998). A lack of *BLNK* in mice was shown to significantly lower IL-10 expression in B cells, a cytokine crucial

for maintaining a balance between Treg cells and Th1/Th17 cells, and also suppressing antigen-presentation capacity in dendritic cells (Jin *et al.,* 2013). *BLNK* was identified as being one of the genes associated with super-shedding of E. coli in cattle (Wang et al., 2017).

4.4.2 Immunological traits - cytokines

Four SNPs were identified in association with cytokines, significant at a suggestive level. The results thus presented, show that these four SNPs account for 2% (OAR14_14894096.1), 8% (OAR20_33522555.1), 6% (OAR25_30051833.1) and 15% (OAR2_252448674.1) of explained genetic variance of IL-$4_{(PWM)}$ when lambs average approximately 2 months of age, and IFN-$\gamma_{(PWM)}$, IL-$4_{(PWM)}$ and IL-$10_{(T\text{-}ci)}$ when lambs average 5 months of age, respectively. Ahola-Olli *et al.,* (2017) reported proportions of genetic variance explained by SNPs varying from 1% to 34% in a study involving multiple cytokines. This study's results are within this range of the results reported by Ahola-Olli *et al.,* (2017).

Three noteworthy genes were identified on OAR20 close to SNP OAR20_33522555.1, which is associated with IFN-$\gamma_{(PWM)}$ in the present study. *BTN1A1* and *BTN2A2*, known inhibitors of CD4 and CD8 T cell proliferation, belong to the Butyrophilin group of MHC-associated protein encoding genes (Smith *et al.*, 2010), important due to their stimulatory and inhibitory effects on cells belonging to the immune system (Malinowska *et al.,* 2017). Proteins encoded by these genes are capable of mediating complex interactions between antigen-presenting cells and T cells (Rhodes *et al.,* 2016). Evidence suggests that butyrophilin-like proteins may play a role as regulators of intestinal inflammation

(Yamazaki *et al.,* 2010). *BTN1A1* and *BTN2A2* have been shown to inhibit the expression of cytokines involved in T cell metabolism and activation, in particular the secretion of IFN-γ (Arnett and Viney, 2014; Malinowska *et al.,* 2017; Smith *et al.,* 2010).

Additionally, the *HFE* gene, with a role in the negative regulation of T cell antigen processing and presentation and T cell cytokine production, and also a negative regulator of antigen processing and presentation of endogenous peptide antigen, represents an MHC class I-like gene (Porto *et al.,* 2019). Primarily described for its role in iron metabolism, defining an immunological role for *HFE* has been of great interest due to its remarkable structural homology with MHC molecules (Reuben *et al.,* 2017). Restriction of iron absorption by pathogens is one of the host's defences against parasitic and bacterial growth, with evidence suggesting microbes are capable of regulating their host's iron metabolism to escape immune surveillance (Liu *et al.,* 2021). Its expression appears to be down-regulated by the production of IFN-γ secreted by T cells (Reuben *et al.,* 2015). In mice infected with *Salmonella*, IFN-γ expression limits iron availability to the microbes and strengthens macrophage immune function (Nairz *et al.,* 2008). Nairz *et al.,* (2017) highlighted the crucial role of macrophage iron homeostasis in the outcome of infections. IFN-γ is one of the main cytokines deployed in immune responses to pathogens, being responsible for the up-regulation of MHC I expression to enhance cytotoxic T lymphocytes and may also induce components of the antigen processing pathway (Reuben *et al.,* 2017). Selection strategies aimed at maintaining diversity and increase heterozigosity in

MHC genes may be important to breed robust animals that respond adequately to invading pathogens (Thompson-Crispi *et al.,* 2014).

4.4.3 Immunological traits - IgA

Furthermore, these results show that the proportion of IgA genetic variance explained by 6 SNPs varies from 4% and 12%. Two SNPs (OAR1_106406464.1 on OAR1 and s67322.1 on OAR20) account, respectively, for 5% and 6% of the explained genetic variance of IgA on recording occasion 1, while SNPs OAR2_26992982.1 (OAR2), s67484.1 (OAR3), s52366.1 (OAR14) and s08970.1 (OAR18) respectively account for 4%, 5%, 4% and 12% of the explained genetic variance of IgA on recording occasion 2. The proportion of genetic variance explained by SNPs associated with IgA levels in Soay sheep lambs were found to vary between 10% and 20% for three SNPs located in OAR18, OAR20 and OAR24 in a population of Soay sheep (Sparks *et al.,* 2019). In their earlier study, Davies *et al.,* (2006) determined the proportion of genetic variance explained by two significant QTLs for IgA to vary between 41% and 51%. The results of this study are more comparable to the results of Sparks *et al.,* (2019).

Multiple genes were found to be associated with IgA in this study. Th17 differentiation results in the up-regulation of Th17-related cytokines, such as IL-17A and IL-17F, respectively encoded by *IL17A* and *IL17F* genes (Evans *et al.,* 2009), found neighbouring SNP s67322.1 on OAR20, associated with IgA. These IL-17 cytokine coding genes have been described previously in a genome-wide association study investigating immune response traits in Canadian Holstein cattle (Thompson-Crispi *et al*., 2014), and more recently they have also been described in Sarda dairy

sheep (Casu *et al*., 2022). In the GI tract, *IL17A* is necessary for protective immunity against several pathogens, being an important regulator of mucosal immune defence associated with inflammation (Dann *et al.,* 2015). It is involved in the protective immunity against protozoan parasites, triggering the production of microbial peptides and complement factors, and regulates the secretion of parasite-specific intestinal IgA (Paerewijck *et al.,* 2019). *IL17F* shares the highest sequence homology with *IL17A* and is a weaker inducer of pro-inflammatory cytokine expression (Iwakura *et al.,* 2008; Samiei *et al.,* 2018). *IL17F* is capable of promoting the synthesis of inflammatory cells, causing tissue damage (Chen *et al.,* 2019). Th17 cells are crucial for the production of secretory IgA at mucosal surfaces in the intestine, with IgA class switching being dependent on these cells (Hirota *et al.,* 2013) and intestinal IgA being impaired in mice deficient in IL-17 (Cao *et al.,* 2015). Secretory IgA work in tandem with Th17 cells in order to protect mucosal surfaces against invading microbes (Saha *et al.,* 2017). IL-17 has been shown to be a contributing cytokine involved in increased secretion of IgA in the intestine of chickens (Karaffová *et al.,* 2015). The activation of inflammatory cells belonging to the Th17 subset has been linked to the inability to control L3 larval colonisation, infection by adult *T. circumcincta* worms and egg production in Blackface sheep (Gossner *et al.,* 2012). Liu *et al.,* (2022) results support the notion that the effects of IL-17A is time dependent, with early secretion during infection being good for parasitic control, while late IL-17 secretion resulting in poor worm control, GI barrier disruption and inflammation.

The *SYK* protein coding gene, involved in both B cell receptor and T cell receptor signalling and located on OAR2 in proximity to SNP OAR2_26992982.1 (IgA) is known for its role in adaptive immune receptor signalling and has been implicated in the mediation of innate immune recognition (Mócsai *et al.,* 2010). The gene is involved in the induction of maturation of dendritic cells into effector antigen-presenting cells, capable of eliciting the differentiation of Th17 and Th1 cells (LeibundGut-Landmann *et al.,* 2007). A signalling pathway involving *SYK* is crucial for the development of Th17 responses in infections with *C. albicans*, leading to potent Th17 responses (Mócsai *et al.,* 2010). The *SYK* protein may be involved in driving inflammation in patients suffering from IgA nephropathy (McAdoo and Tam, 2018).

Finally, several genes encoding members for the Interleukin-1 family of cytokines, located on OAR3 near the SNP s67484.1, also found to be associated with IgA. IL-1 is a primary cytokine implicated in acute and chronic inflammatory diseases, playing a significant role in immune regulation and inflammatory responses (Hahn *et al.,* 2009). *IL1A* and *IL1B*, involved in pro-inflammatory responses, respectively encode IL-1α, which represents a known agonist of the IL-1 receptor and is has a pro-inflammatory action (Hahn *et al.,* 2009), and IL-1β, one of the typical cytokines mediating Th1 immune responses, alongside with cytokines such as IFN-γ (Rad *et al.,* 2004). The *IL1RN* gene encodes a receptor antagonist which inhibits IL-1α and IL-1β function, neutralising the activity of these cytokines in immune responses (Cruz-Robles *et al.,* 2009). The *IL1F10* gene regulates adaptive and innate immune responses by inhibiting the production of T cell cytokines (Fonseca-Camarillo *et al.,*

2018). The gene may also play a role in the pathogenesis of inflammatory diseases (Van De Veerdonk *et al.,* 2012) and drives the proliferation of Tregs, preventing them from transforming into Th17 (Xie *et al.,* 2019). Moreover, the *IL36A*, *IL36B* and *IL36RN* genes encode two IL-36 cytokines with agonistic activity (IL-36α and IL-36β) and IL-36Ra, a receptor antagonist, which are responsible for the regulation of IL-36 signalling (Han *et al.,* 2020; Saito *et al.,* 2020). The *IL37* gene, also a member of the IL-1 cytokine family**,** encodes IL-37 cytokine with a role in limiting innate inflammation and in suppressing acquired immunity (Dinarello *et al.,* 2016). IL-37 is unique since it limits inflammation by dampening the production of pro-inflammatory cytokines (Cavalli and Dinarello, 2018). The IL-1 family of cytokines is considered to play crucial roles in the progression of IgA nephropathy (Hahn *et al.,* 2009). Crucially, the cytokine IL-1β has been shown to be an important eosinophil-derived modulator of IgA class switching, with a decrease of this cytokine in the intestine resulting in a reduced production of IgA in mice (Jung *et al*., 2015).

4.5 Conclusions

The present study identified multiple potential candidate genes with known biological functions related to the studied animal traits. The results reported here highlight the importance of several C-type lectins associated with animal resistance to *Nematodirus* infection and several genes encoding a number of cytokines belonging to the IL-1 family and IL-17 were associated with IgA levels. The present study revealed a largely complex and polygenic genetic control on resistance to parasitic infection and immunological traits in the studied population. Nevertheless, certain genomic regions identified may hold larger genomic loads than others, thus requiring further attention. Insights into these regions may increase the accuracy of genomic evaluation and selection towards enhancing disease resistance. Future work should examine the significance of these results on independent data and further investigate the reported genomic regions associated with each trait.

Chapter 5 - General Discussion

5.1 Thesis overview

5.1.1 Genetic parameters for traits associated with GI parasitic co-infection and growth in Scottish Blackface sheep

The economic impact stemming from GI parasitism due to losses in production and the pitfalls of continuous anthelmintic use, make the inclusion of disease traits in breeding programmes an attractive and promising complementary measure to achieve improved disease resistance. In the past, breeding programmes have focussed on improving productive efficiency, with the consequence of animals becoming more susceptible to infection. The development of animal populations that are selected for increased resistance to GI parasitism has thus become a more common approach to control such economic losses.

Evidence for the necessary genetic variation in disease resistance is required for selective breeding strategies to be successful. These selective breeding strategies for enhanced resistance to GI parasitism are traditionally based on indicator traits such as FEC, for which there is enough genetic variation among individual animals, even with moderate levels of infection. Enhancing resistance to parasites in sheep with selective breeding is therefore feasible. Nematode and coccidian parasites vary both in their morphology and on how they interact with their host, generally eliciting distinct immune responses. This has raised the concern that an increase of resistance against one disease might result in susceptibility to another, if genetic antagonism between different immune responses were at play. However, this antagonism is yet to be definitely proven. The involvement of immunoregulatory genes further puts this potential antagonism into question.

The first aims of this thesis were therefore:

- To assess the genetic background of traits related of GI parasitic infection (FEC, FOC and DAG) and productivity (LWT) in SBF sheep and assess the relationships between disease traits and productivity.

To address these objectives, genetic parameters were estimated for three traits related to major parasite genera, Strongyles, *Nematodirus* and *Coccidia*, and for faecal soiling scores (DAG), which corresponds to another measure of parasitic infection. Genetic parameters for live body weight (LWT), a trait related to sheep productivity, were also calculated. Analyses were performed for records collected at an average lamb age of 3 months.

The results revealed significant and heritable genetic variation among animals for all traits considered. Heritability (h^2) estimates derived are generally in line with previous estimates of FEC and FOC, albeit a bit lower than most reported cases, particularly in the case of FOC. FEC h^2 estimates fall outside the most agreed range for this trait (0.20 and 0.40). Furthermore, while low, DAG h^2 might be explained by there being a lower proportion of animals with signs of faecal soiling. Despite this, according to these h^2 estimates, the studied traits were significantly heritable and therefore suitable for inclusion into breeding programmes. The LWT h^2 was found to be moderate and significantly greater than zero.

The strong genetic correlation between faecal counts of Strongyles and *Nematodirus* reveals that these traits are largely under the same genetic control, which is in line with previous results (e.g.: Bishop *et al.*, 2004; Morris *et al.*, 2004; Pickering *et al.*,

2012; Wolf *et al.,* 2008). It is expected that correlated responses in resistance to Strongyles will also result in selection for low levels of *Nematodirus*. Few studies have focused on co-infections with nematode and coccidian parasites, and the genetic and phenotypic relationships between them. The results presented her show a moderate and positive correlation between Strongyles and *Coccidia* faecal counts which is in contrast with previous reports of either negative or no correlation. At an average of three months of age, these results point towards Strongyles and *Coccidia* being partially under the same genetic control. A positive phenotypic association was reported between Strongyles and *Coccidia* in yearling and adult Soay sheep and the importance of genetic studies to assess the h^2 and the correlation between these two distinct genera of parasite were highlighted (Craig *et al.,* 2008). A strong correlation between nematode and coccidian parasites has also been found in cattle, indicating the possibility of genes regulating immunity having pleiotropic effects, favourably altering resistance to different parasites in the same direction.

No meaningful genetic correlations between faecal egg count traits and live weight were found. Such results are desirable from a genetic standpoint because selection for increased resistance at this age will not adversely affect animal production and growth, and vice versa. Both unfavourable and favourable correlations have been described before (Gauly *et al.,* 2004; Pickering *et al.,* 2012) and it is possible that this correlation may change depending on the intensity of infection with animals only losing live weight when severely infected (Rashidi *et al.,* 2014). There are a multitude of factors that might contribute to differing results, such as different breeds studied, parasite genera and species affecting the animals, intensity of infection,

method of analysis, treatment protocols followed, and selection history (Coltman *et al.,* 2001).

In addition to the lack of genetic antagonism between FEC and LWT observed here, the clear and favourable negative correlation between DAG and LWT should be emphasised. The negative correlation between these two traits observed in the present study indicates that low faecal soiling scores are associated with higher lamb body weight at 3 months of age, which translates to a positive impact on productivity. DAG is an indicator of diarrhoea, which in young lambs are assumed to be the result of GI infection. Therefore, DAG could be considered as an indicator of infection. The lack of meaningful correlations between egg counts and DAG scores could be explained by the increased moisture on diarrhoeic faeces, which may dilute the number of eggs observed. If this is the case, faecal counts will produce a low number of eggs per gram of faeces, underestimating the true number of eggs (Le Jambre *et al.,* 2007). While correlations between DAG and FEC are considered important due to the generally held view that these traits are strongly associated (Pickering *et al*., 2012), this study failed to show such correlation, as did the study by Pickering *et al*., (2012) despite the fact that their study included more than 90,000 DAG records and more than 100,000 FEC records. According to these authors, it is possible that the wide range of previous estimates and large SE may have led to a misinterpretation the correlations. Nevertheless, although no significant correlation between FEC and DAG has been found, the latter is still important as it is associated with elevated risk of flystrike as well as having an economic penalty. This study suggests that selection to improve resistance to FEC might not necessarily lead to a

reduction in faecal soiling. For its correlation with flystrike, this trait should be considered for inclusion in breeding programmes due to its economic importance.

The present study has revealed that, despite the lowly heritable nature of faecal counts of both nematodes and coccidian parasites in SBF sheep, there is a significant amount of genetic variance among individuals to underpin a selective breeding programme aimed at enhancing resistance to GI parasitic infection. A strong genetic correlation between both nematode species was observed, meaning that selection aimed at reducing the egg output of Strongyles is expected to reduce the egg output of *Nematodirus*. Importantly, there is no evidence of antagonism between faecal counts of nematode eggs and faecal counts of coccidian of oocysts. Such results are encouraging for the development and implementation of a breeding programme including both faecal egg and oocyst counts and resulting in animals with greater overall resistance to parasitic infection, without negatively influencing productivity.

Chapter 2 was based on faecal traits related to infection with Strongyles, *Nematodirus*, and *Coccidia*, but the immune mechanisms involved in each case were not addressed. Indeed, immune responses to nematode infestation reportedly differ from immune responses that occur after *Coccidia* infection (Engwerda *et al.,* 2014; McNeilly and Nisbet, 2014). For this reason, the next part of the study focussed on key immunological parameters associated with parasitism in sheep.

5.1.2 Genetic profile of adaptive immune traits and relationship with animal health and productivity in Scottish Blackface sheep

Co-infections involving parasitic nematodes and *Coccidia* are widespread. While both parasites can infect the GI tract, there are important differences on how these two distinct genera affect and interact with the host. *Coccidia* (FOC) infect and replicate inside epithelial cells while nematodes reside within the GI lumen or mucosa. The success or failure of immune responses is dependent on factors such as pathogen burden and the scale of the response, which is regulated by T cell activity.

Two of the most important subsets of T cells, Th1 and Th2, play different roles. Th1 immune responses are mainly inflammatory in nature and control intracellular pathogens. Th2 immune responses induce humoral responses and typically act against extracellular pathogens. Interferon gamma (IFN-γ) is associated with Th1 responses while Interleukin 4 (IL-4) cytokine is expressed during Th2 responses. Regulatory T cells (Treg) are also critically important for their regulation and inhibition of immune responses such as interleukin 10 (IL-10). These responses act to prevent over-activation of immune responses and immunopathology.

Parasite specific antibodies, such as Immunoglobulin A (IgA) also play an important role during infection. IgA has previously been linked to parasite resistance and specifically, has been shown to reduce worm size and negatively affect worm fecundity. This trait is known to be heritable in sheep as well as being involved in immunity against GI parasites. However, the genetics underlying variation in different types of immune responses and how these relate to parasitic burdens and productivity are still largely unknown.

Further to estimating genetic parameters of parasitic infection and productivity phenotypes in Chapter 2, this thesis also aimed at:

- Examining the host genetic background of traits related to immune response with the inclusion of novel traits in the form of expression of IFN-γ, IL-4 and IL-10 cytokines corresponding to Th1, Th2 and Treg responses, respectively, and nematode specific IgA levels in lambs, and analysing and determining the relationship between these traits with lamb disease and production traits.

This was achieved by deriving the genetic parameters for all three cytokines and IgA. The study required whole blood stimulation assays to be carried out in order to investigate cytokine responses to different stimuli. Two distinct stimulants were used: pokeweed mitogen (PWM) which results in non-specific immune responses, and *T. circumcincta* (T-ci) antigen to activate parasite-specific lymphocytes. Cytokine ELISAs were performed to quantify cytokine expression following stimulation with PWM and T-ci. Additionally, indirect ELISAs were carried out to detect and quantify antigen specific T-ci IgA presence in sera.

The results revealed significant and heritable genetic variation for all seven immunological traits in animals averaging of two months of age considered in this study, indicating that individual animals vary genetically in their capacity to mount adaptive immune responses when reared under similar conditions of natural infection. Corresponding heritability estimates varied from low to high across the traits of study. Information on genetic control of cytokine production in sheep is limited, but similar studies have been performed in humans reporting varying levels of genetic control depending on the specific cytokine and immune response

measurement. Heritability estimates for cytokines in humans are generally in line with the results presented here (Brodin *et al.,* 2015; Li *et al.,* 2016). Genetic studies in ruminant livestock have mainly focussed on the proportions of leukocyte populations, with moderate h^2 estimates regarding different T cell subsets (Denholm *et al.*, 2017), although there has been a more functional approach focusing on immune responses that are capable of inducing polarised responses. These findings point out an important role for host genetics in the control of adaptive immune responses.

The positive correlations between Th1 and Th2 cytokine measures at phenotypic and genetic levels subverted the notion that Th1 and Th2 responses cross-regulate each other. The antagonism between Th1 and Th2 immunity has been well established in laboratory trials (Kaiko *et al.*, 2008) and the notion of Th1/Th2 dichotomy has been around since the late 1980s. That has been critical for the understanding of immune responses (Van Oosterhout and Motta, 2005), with Th1 cells driving cell-mediated responses through the secretion of cytokines such as IFN-γ, and Th2 cells driving humoral immune responses via the expression of specific cytokines such as IL-4 (Van Oosterhout and Motta, 2005). Current knowledge exists of the role of Th2 immune responses on protective immunity against GI nematodes and the mediation of such immune responses is orchestrated by the secretion of several cytokines, including IL-4, one of the main drivers of Th2 immunity (Ahbara *et al.*, 2021).The theory is, that optimal host protection should be mediated through the polarisation of effector cells into Th1 or Th2 immune responses, with secretion of specific cytokines (Finkelman *et al.,* 2004). Thus, factors that drive Th1 responses in favour of Th2

could be crucial in controlling coccidiosis (Haritova and Stanilova, 2012). The results of this study report positive correlations between Th1 and Th2 responses, which indicate that these immune responses are partially under the same genetic control. Such results are in line with previous reports in wild sheep and rodent populations (Corripio-Miyar *et al.,* 2022; Young *et al.*, 2020). The results of present study could be explained by the compartmentalised nature of immune responses, with animals being capable of mounting differing immune response, even if local antagonism between Th1 and Th2 responses exists. Co-infections with different parasite taxa, with distinct physiology and strategies are expected to produce varied responses (Jackson *et al*., 2004).

The moderate, negative correlation between IL-$10_{(PWM)}$ and IL-$4_{(T\text{-}ci)}$ might be indicative of the regulatory function of IL-10 as nematodes have been shown to be capable of initiating the expansion of regulatory responses that suppress Th1 and Th2 responses (McNeilly *et al.,* 2013). Additionally, IL-$10_{(PWM)}$ is also positively correlated with parasite specific IgA, which is in line with data from human and mice studies. On the other hand, the positive correlation between IL-$10_{(T\text{-}ci)}$ and FOC was somewhat unexpected, since this cytokine has been shown to interfere with immune responses against *Coccidia*, which are in line with previous reports in chicken and mice (Boulton *et al.,* 2018; Wakelin *et al.,* 1993). Additionally, they could be an indication of regulation of immunity on the ability to control immunity against *Coccidia*.

The results are in contrast with previous reports (Corripio-Miyar *et al.,* 2022), since no negative correlations between IFN-γ and FOC have been found, or with IL-4 and

FEC_S/FEC_N as no correlation was found between IL-4 and nematode egg counts, while a positive correlation was found between IFN-γ and oocyst counts. In this study, immunological data was recorded approximately one month before any parasitological measurement was made. Additionally, phenotyping of immune traits was carried out at an age when immunity is still not fully developed. Immune responses following exposure are highly dynamic, varying greatly over time. For these reasons, any unexpected association (or lack thereof) between cytokine measurements and parasitology data could be due to the fact lambs were phenotyped before immunity against GI parasites has fully developed, or due to the time-lag between immunological and parasitological data recording. Similarly, this could also explain the lack of association nematode egg counts and IgA.

The existence of significant genetic variance for the immunological traits analysed here and the earlier observations that Th1 and Th2 are associated with protection against coccidian and nematode parasites mean that these immunological traits can be included in selection programmes aimed at increasing resistance to parasitic infection. However, careful consideration should also be given at how immunological traits might affect production and the timing at which parasitological and immunological traits are conducted.

The results from this study indicate that IFN-γ production at the early stages of development is unfavourably associated with LWT at indicating that Th1 mediated immunity negatively affects production. These results support the notion that mounting immune responses may also be the cause of losses in productivity. Furthermore, there is some risk that productivity might also be affected when

selecting for improved Th2 immunity as well, due to the positive correlations between IL-4 and IFN-γ. In this sense, both Th1 and Th2 might affect LWT in practice. For this reason, steps should be taken to combine genetically antagonistic traits into breeding programme to avoid their deterioration due to selection. This can be achieved with a comprehensive genetic index that includes all economically important traits, even when some relationships are antagonistic.

The results reported here shed some light on the complexity of mechanisms involved in immune responses. The evidence points towards the fact that both Th1 and Th2 immune responses are at positively correlated. The results presented in this thesis point to the inexistence of a clear Th1/Th2 dichotomy in this SBF population. This means there is no markedly biased polarisation of specific immune response in favour of another. Despite the existence of enough genetic variability for all immunological traits among lambs in this study, there are some reservations regarding the effect of immune responses on the animals' growth and therefore on productivity. From the above results, it becomes obvious that the practicality of undertaking detailed immunological assays and using them as indicators of disease status in breeding programmes would have limited appeal in practice. This stems not only from the complexity of the findings reported here, with no clear picture emerging from the data set used for these analyses, but also because there are significant costs associated with collecting and analysing immunological data that would have to be routinely obtained from candidate animals. Consequently, the use of molecular data associated with the immunological and disease phenotypes could reveal important genes of interest that are implicated in the control and expression of

disease. In the next study, the findings from the interpretation of genomic information generated for the same animals could shed additional light into some of the mechanisms involved in the identification of genetically resistant animals to disease.

5.1.3 Genome-wide associations studies on disease, productivity and immunological traits in Scottish Blackface sheep

The *de novo* sequencing technology and its advancements were made possible by the creation of genome-wide SNP chips like the Illumina Ovine SNP50 BeadChip microarray. The practicality of genomic studies has been assessed and demonstrated worldwide, leading to important breakthroughs. The use and incorporation of genomic data into structured breeding programmes to enhance the prediction accuracy is particularly ideal for lowly heritable and complex traits. The higher accuracy from genomic studies allows for potential early life prediction of breeding values. Selective breeding using genomic information can lead to a reduction in the number of animals exposed to disease and the suffering of successive generations, as genetic improvement is cumulative and permanent. Furthermore, genomic approaches are advantageous in that they reduce the need for intrusive trait recording, while proving to be a viable option for diseases that have historically been difficult to incorporate into breeding programmes.

Genome-wide association studies have enabled the identification of loci associated with specific traits, thus becoming a useful tool in identifying candidate genes and elucidating on the mechanisms underlying complex traits. A number of chromosomal regions with effects associated with infection have been already

identified. However, there is a general lack of consensus due to the great variety of parasite species and sheep breeds, as well as the diversity of studies and the complexity of these traits. The majority of additive variance of complex diseases is often left unexplained, and while genetic variation in resistance exists, the genetic architecture of such traits is still unclear.

Molecular data on disease and immunological phenotypes can prove useful in revealing important genes associated with these traits. Therefore, the final aims of this thesis were as follows:

- Identifying SNPs and examining possible candidate genes associated with resistance to GI parasitism.

A wide range of chromosome regions holding small to moderate effects associated with resistance has been identified in other studies internationally. Previous GWAS studies (Álvarez *et al.,* 2019; Benavides *et al.,* 2015; Benavides *et al.,* 2016b; Estrada-Reyes *et al.,* 2019b; Keane *et al.,* 2018; Kemper *et al.,* 2011; Kim *et al.,* 2015; Sparks *et al.,* 2019) identified a considerable number of genes with significant effects on disease and parasitism traits, including genes in the MHC and cytokine genes with different genes being important at different ages and different stages of infection and disease, and with evidence pointing to the existence of a repertoire of thousands of genes controlling immune responses to combat invading pathogens in mammals (Mallard *et al.,* 2015).

As was the case with Beraldi *et al.,* (2007), for the most part, the regions identified did not reach high enough significance beyond genome-wide level to allow in depth

speculations about the genetic architecture of the traits analysed. Nevertheless, the candidate genes, and their related pathways and networks involved in underlying molecular mechanisms of resistance to parasite have been identified. Heritable genetic variation and genomic regions affecting some of the traits were identified.

A number of regions uncovered are correlated, at a suggestive significance level, with disease traits, namely faecal counts of nematode eggs, with several genes associated with immune function identified. There is a possibility that some SNP effect sizes associated with faecal counts in this study might be overestimated (Mucha *et al.*, 2015). However, the results confirmed the presence of noteworthy signals in these genomic regions which include genes with important roles in animal resistance.

The GWAS analyses in this study revealed multiple genes linked to the significant regions found to be related to the traits in this study, confirming their polygenic nature. Several genes coding C-type lectin expression were found to been correlated to FEC_N, and while not much is known about their role in veterinary species, CTL coding genes have been already described in sheep. These proteins are notable for their roles in the defence against pathogens and maintain immune homeostasis, while also modulating inflammatory responses. Similarly, genes belonging to the killer cell lectin-like receptor family are also reported here in correlation *Nematodirus* FEC. Such receptors allow NK cells to differentiate between healthy and infected cells. NK cells are also involved in the production IL-13, crucial in driving Th2 immune responses and eliminating nematode parasites. Furthermore, the gene *LGALS4* represents an inhibitor of inflammation exclusively expressed in the GI tract and has

been shown to be up-regulated in sheep that have been infected with *T. circumcincta*. On the other hand, *NFKBIB* promotes inflammation and regulates nuclear factor kappa B (NF-κB) in mammals. Higher levels of IL-4 and IL-13 (Th2 immune responses) in favour of IFN-γ (Th1 immune responses) have been observed as a result of enhanced NF-κB activity. *ZFP36* regulates immune response while inhibiting several inflammatory cytokines with IFN-γ and IL-10 being two cytokines potentially targeted by this gene. Finally, *CD80* gene codes CD80 molecules involved in the activation of T cells, and its down-regulation can interfere with generating and maintain T cell responses.

When it comes to immunological traits, the results also identified some noteworthy genes. Butyrophilin belong to a group of MHC-associated genes, of which *BTN1A1* and *BTN2A2* are known inhibitors of T cell proliferation. These proteins may play a role in the regulation of intestinal inflammation and inhibit the expression of cytokines, including IFN-γ. HFE represents a gene remarkable in its homology with MHC 1 molecules and belongs to the MHC on OAR20. The protein coded by this gene is a negative regulator of iron absorption, which is a vital element for the survival of pathogens. Reuben *et al.,* (2015) suggests the expression of this gene may be down-regulated by IFN-γ secreted by T cells.

Furthermore, the present study found a number of genes that were in close proximity to SNPs associated with IgA and that have been linked to this trait. Two of these genes, IL17A and IL17F, encode Th17 pro-inflammatory IL-17A and IL-17F cytokines, respectively. IL-17A is capable of regulating IgA. IL-17F is a weaker inducer of inflammatory responses but shares a high sequence homology with IL-

17A. Th17 cells play a vital role in the production of secretory IgA at mucosal surfaces in the intestine. These genes have previously been implicated in immune responses against parasitic infections in mice and cattle, but not in sheep (Benavides *et al.,* 2016b).

Lastly, IL-1 represents an important family of cytokines involved in acute and inflammatory diseases, having major roles in immune regulation and inflammation. IL1A and IL1B are two examples of genes coding proteins (IL-1α and IL-1β) belonging to this family, involved in pro-inflammatory responses. The IL-1 family of cytokines is thought to play an important role in the progression of IgA nephropathy. Importantly, cytokine IL-1β appears to be an important modulator of IgA class switching, and therefore crucial for the production of this immunoglobulin.

There is a consensus on chromosomal regions where genetic markers associated with GI parasite resistance have been found, in particular the regions surrounding the IFNG locus on OAR3 and the regions found within or adjacent to the MHC on OAR20 (Benavides *et al.,* 2016b). MHC is probably the single most important genetic system concerning resistance and accounts for the largest amount of genetic variation. The results presented here include a number of key genes that can be found within the MHC or at least in close proximity to this region. Maintaining the diversity and increasing the heterozygosity in genes that belong to the MHC may prove useful in the quest to breed animals capable of adequately respond to several challenges.

Gene ontology is useful in narrowing down the search for candidate genes. Genes with important roles in resistance may be involved as a part of a cascade or

signalling pathway. Beyond genomic regions directly implicated in resistance to parasites, there is mounting evidence pointing towards genes not strictly related to immunity, but rather linked to GI mucus production, parasite expulsion and the regulation of homeostasis. Some regions of the sheep genome remain to be annotated, making the identification of some candidate genes a challenge (Mucha *et al.,* 2015). The evidence of these results confirms the polygenic nature of FEC and IgA, i.e., these traits are likely not influenced by genes with major effect.

Associations with candidate genes can only be considered as an indication. The results presented here require further research to be validated, which would probably require more animals to be genotyped. While genetic associations exist, it is unlikely that a single resistance marker could serve all sheep breeds. In dairy cattle, where powerful QTL mapping studies have been undertaken, less than 10% of genetic variance has been explained for most traits, while the remaining genetic variance is handled through traditional methods (Meuwissen *et al*., 2016). In general, the proportion of captured genetic variance related to several diseases has remained low, even with more powerful studies. Many economically important traits, such as the resistance to infection, are influenced by many genes. Tracking only a fraction of these genes will only serve to explain a small percentage of the variation.

Providing that there is substantial variation underpinning genomic regions containing relevant genes associated with immunity, the phenotypes in study can potentially respond to selection. The results laid out here allowed the identification of certain genomic regions that require further attention, which may serve to increase the accuracy of genetic evaluation and the selection of Scottish Blackface sheep with

enhanced parasite resistance. Several potential candidate genes with known biological functions to immunity were identified. This study provides an insight towards genomic regions affecting lambs and their immune responses at different stages of development, allowing a broader picture on the development of resistant animals in the future.

5.2 Concluding remarks

The results of the present research confirmed the existence of sufficient genetic variation for traits related to parasitic infection and productivity, highlighting the correlations between distinct types of parasites. Additionally, the inclusion of novel traits related to immune responses shed light on the complex nature of adaptive immune responses of Scottish Blackface sheep lambs, and show that there is enough genetic variation underlying the immunological traits analysed allowing their inclusion within breeding programmes aiming at enhancing resistance to parasitic infection in Scottish Blackface sheep. To my knowledge, this is one of the first studies including novel traits of cytokine expression and that focuses on the genetic control of cytokine production in sheep. The results hereby presented, suggest the lack of a clear polarisation of immune responses. Careful consideration should, however, be given to the correlations between immune responses and productivity as there is a risk of these associations impacting the lamb growth. Nevertheless, there is enough genetic variation to underpin a selective breeding programme. In this case, the desired solution would be the simultaneous genetic selection through a comprehensive selection index that includes disease resistance, immunological and productivity traits, even when some of the relationships are antagonistic.

Furthermore, genomic association studies on disease and immunological phenotypes revealed several genes with known functions within the immune system and involved in immune responses. The results have confirmed the polygenic nature of FEC, with several genes with known immune functions associated with this trait. The results also show that IgA is a polygenic trait, further highlighting the complexity of host immune responses. The results of genomic association studies paint a clearer picture of the immune mechanisms at a genomic level and could potentially be used within a breeding programme aiming at developing more resistant sheep to GIN.

5.3 Implications of this study

Gastrointestinal parasites pose a severe risk of both morbidity and mortality in grazing ruminants. Such infections remain a major constraint on the production and welfare of domestic sheep around the world, including the UK. The control of infections has been achieved, for the most part, through the use of chemical compounds. In the case of nematode parasites, anthelmintic drenching has been the method of choice since for over 50 years. Implementing successful strategies for selection requires the knowledge of the underlying biological processes of the traits that are used.

Selective breeding for increased resistance is generally accepted as a sustainable, long-term solution to this problem. Breeding for resistance to parasitic infections has been a working concept in livestock production for several decades. Overall, the literature so far has been clear: selection of animals for increased resistance to infections can be achieved, and has been proven to be successful in a number of

countries around the world. Progress to achieve this is slow, taking several generations of animals, which could be explained by the highly complex nature of traits related to resistance. Consensus exists on there being a large number of genes underlying these traits.

Breeding for increased resistance to parasites is expected to lead to sustained improvements in both the animals' health and performance as demonstrated in the first chapter. As demonstrated in Chapter 2, an improvement in the resistance to nematodes is expected to improve resistance to coccidian infections. Additionally, these results confidently show that this will translate to improved productivity by decreasing the risk of infection. Chapter 3 results offer some insight at the possibility of including new immunological traits in breeding programmes by assessing the genetic background of different immune responses by analysing three key cytokines. In theory, these traits can be included in such programmes with the best approach involving the development of a comprehensive selection index that includes all traits studied here. Further evidence was also found for the non-existent dichotomy between Th1 and Th2 immune response, due to the overall positive correlations between IFN-γ and IL-4 cytokines, varying from 0.50 to 0.74 in this study. This is encouraging in that it confirms that selection for improved immune responses against nematodes is likely to lead to improved responses against *Coccidia*, and also means that only one type of response needs to be phenotyped. The overall health of the population is expected to improve since selection will decrease the rates of infection and decrease pasture contamination, which is an important aspect of parasitic infestation affecting livestock production. The flock's exposure to infection is

therefore expected to decrease. Breeding for resistance is the ideal alternative strategy to traditional methods of control, such as anthelmintic drenching. Successful and efficient implementation should come without the need for additional work for breeders.

Finally, even though GWAS failed to reach Bonferroni significance for almost all traits, this study was able to identify genes that have role in the genetic control of immunity. The confirmation that these genes underlie the outcome of immune responses could prove useful. Gene ontology also helped to uncover some noteworthy roles these genes have in immunity. Additionally, these results report that the genetic variance explained by individual SNPs was generally low, but significant. Genomic approaches allow for new opportunities, as it becomes possible to decouple phenotype recording from selection.

5.4 Limitations

Phenotypic markers require the animals to be infected in order to be expressed. There are some limitations in using these phenotypes in lieu of molecular markers. Recording parasitic infection and immunological phenotypes requires the animals' exposure to the infectious agent, and parasite exposure will lead to potential losses of production, particularly if anthelmintics are not administered. In the case of FEC/FOC traits, other limitations include the labour-intensive nature of collecting samples, the inability of storing these samples for long periods of time and difficulty to measure FEC/FOC automatically and accurately. DAG scores are also a trait that has been linked with parasitic infection, even though it is generally not correlated with faecal counts, which is explained by the fact that eggs and oocysts in diarrhoea

are often diluted due to increased fluid in the faeces, underlining the need to include faecal consistency scores (FCS) in the analyses.

This study is novel, in that it included IgA analyses and introduced the measurements of three important cytokines, in addition to widely-used disease phenotypes such as faecal counts and DAG scores. The goal was not only to determine if these immunological traits were viable for inclusion in a breeding programme with a goal of increasing resistance, but also find correlations with the aforementioned disease phenotypes. In that sense, one of the major limitations of this study relates to timing and logistics. The project set out to explore disease and immunological traits and determine potential correlations amongst them. Due to the labour intensity and time-consuming nature of collecting both faecal samples, evaluate dag scores and collecting blood samples on hundreds of sheep, data were collected for the different traits at different time points, roughly one month apart. Therefore, each collection occurred one month apart. For this reason, caution should be exercised when interpreting these results since the correlation between disease and immune responses cannot be accurately determined when there is such a gap between collection dates. While it is possible to include immunological traits within breeding programmes alongside other traits analysed here, a different strategy must be adopted when it comes to data recording. Ideally, disease and immunological traits should be collected within a much smaller timeframe. The costs of undertaking of immunological analyses could also become a limiting factor. Lastly, the risk of immunopathology affecting the animals' growth cannot be discarded.

With the exception of a few examples, most of the candidate genes identified in this study have not yet been discussed in the literature in the context of GI parasites, neither in sheep nor other livestock. The genome analyses results presented in this thesis require further research in order to be validated, which would probably require more animals to be genotyped. It is important to note that associations with significant SNPs can only be considered as an indication. Estimates cannot be taken at face value when they are overestimated, but they provide a good basis for future studies.

5.5 Future work recommendations

One important recommendation for future studies focusing on the same traits analysed here should be recording disease and immunological traits within a closer timeframe. While the logistics of undertaking this step will still remain an important factor preventing data collection from being completed at the same time, an interval of one week between collecting faecal records and immunological traits would be a good compromise. This is especially important if the aim is to determine the correlations between disease and immunological traits.

Further research is necessary in order to determine and confirm the underlying genetic mechanisms of resistance to GI parasitism in this population. While higher SNP density or full sequencing are possibilities, the costs of so-doing are currently a rate-limiting factor, making this option not worthwhile. Further analyses should be conducted in the genes identified to either confirm or validate the role they might have in mechanisms that result in immune responses. Regional Heritability Mapping should be considered for future research (Nagamine *et al.*, 2012). This approach

provides heritability estimates for genomic segments that contain both common and rare allelic effects and that, individually, contribute too little variance to be detected by GWAS. Regional Heritability Mapping has been considered as a superior approach to capture more of the underlying genetic effects (Al Kalaldeh *et al.*, 2019b). Lastly, low-pass sequencing (Li *et al.*, 2021) is an alternative becoming available in cattle and chicken. The costs associated with imputations are lower than those of DNA array genotyping and this methodology can offer the opportunity to perform genomic analyses with a wider number of SNPs.

Bibliography

Acevedo-Whitehouse, K., and Cunningham, A. A. (2006). Is MHC enough for understanding wildlife immunogenetics? *Trends in Ecology and Evolution*, *21*(8), 433–438.

Ahbara, A. M., Rouatbi, M., Gharbi, M., Rekik, M., Haile, A., Rischkowsky, B., and Mwacharo, J. M. (2021). Genome-wide insights on gastrointestinal nematode resistance in autochthonous Tunisian sheep. *Scientific Reports*, *11*(1), 9250.

Ahola-Olli, A. V., Würtz, P., Havulinna, A. S., Aalto, K., Pitkänen, N., Lehtimäki, T., Kähönen, M., Lyytikäinen, L.-P., Raitoharju, E., Seppälä, I., Sarin, A.-P., Ripatti, S., Palotie, A., Perola, M., Viikari, J. S., Jalkanen, S., Maksimow, M., Salomaa, V., Salmi, M., Kettunen, J., and Raitakari, O. T. (2017). Genome-wide Association Study Identifies 27 Loci Influencing Concentrations of Circulating Cytokines and Growth Factors. *The American Journal of Human Genetics*, *100*(1), 40–50.

Al Kalaldeh, M., Gibson, J., Duijvesteijn, N., Daetwyler, H. D., MacLeod, I., Moghaddar, N., Lee, S. H., and van der Werf, J. H. J. (2019a). Using imputed whole-genome sequence data to improve the accuracy of genomic prediction for parasite resistance in Australian sheep. *Genetics Selection Evolution*, *51*(1), 32.

Al Kalaldeh, M., Gibson, J., Lee, S. H., Gondro, C., and van der Werf, J. H. J. (2019b). Detection of genomic regions underlying resistance to gastrointestinal parasites in Australian sheep. *Genetics Selection Evolution*, *51*(1), 37.

Allen, J. E., and Maizels, R. M. (2011). Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, *11*(6), 375–388.

Allerton, G., Gogolewski, R., Rugg, D., Plue, R., Barrick, R., and Eagleson, J. (1998). Field trials evaluating ivermectin controlled-release capsules for weaner sheep and for breeding ewes. *Australian Veterinary Journal*, *76*(1), 39–43.

Álvarez, I., Fernández, I., Soudré, A., Traoré, A., Pérez-Pardal, L., Sanou, M., Tapsoba, S. A. R., Menéndez-Arias, N. A., and Goyache, F. (2019). Identification of genomic regions and candidate genes of functional importance for gastrointestinal parasite resistance traits in Djallonké sheep of Burkina Faso. *Archives Animal Breeding*, *62*(1), 313–323.

Amarante, A. F. T., Bricarello, P. A., Rocha, R. A., and Gennari, S. M. (2004). Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. *Veterinary Parasitology*, *120*(1–2), 91–

106.

Amin, N., van Duijn, C. M., and Aulchenko, Y. S. (2007). A Genomic Background Based Method for Association Analysis in Related Individuals. *PLoS ONE*, *2*(12), e1274.

Andrews, A. H. (2013). Some aspects of coccidiosis in sheep and goats. *Small Ruminant Research*, *110*(2–3), 93–95.

Arnett, H. A., and Viney, J. L. (2014). Immune modulation by butyrophilins. *Nature Reviews Immunology*, *14*(8), 559–569.

Arriero, E., Wanelik, K. M., Birtles, R. J., Bradley, J. E., Jackson, J. A., Paterson, S., and Begon, M. (2017). From the animal house to the field: Are there consistent individual differences in immunological profile in wild populations of field voles (Microtus agrestis)? *PLoS ONE*, *12*(8), 1–10.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., and Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nature Genetics*, *25*(1), 25–29.

Assenza, F., Elsen, J.-M., Legarra, A., Carré, C., Sallé, G., Robert-Granié, C., and Moreno, C. R. (2014). Genetic parameters for growth and faecal worm egg count following *Haemonchus contortus* experimental infestations using pedigree and molecular information. *Genetics Selection Evolution*, *46*(1), 13.

Atlija, M., Arranz, J.-J., Martinez-Valladares, M., and Gutiérrez-Gil, B. (2016). Detection and replication of QTL underlying resistance to gastrointestinal nematodes in adult sheep using the ovine 50K SNP array. *Genetics Selection Evolution*, *48*(1), 4.

Atlija, M., Arranz, J. J., Martinez-Valladares, M., and Gutierrez-Gil, B. (2014). Search of genomic regions influencing faecal egg count, as an indicator of resistance to gastrointestinal nematode infections, based on the analysis of the OvineSNP50 BeadChip. *Proceedings of 10th World Congress of Genetics Applied to Livestock Production, 2011*.

Bae, H., Barlow, A. T., Young, H., and Valencia, J. C. (2016). Interferon γ: An Overview of Its Functions in Health and Disease. In *Encyclopedia of Immunobiology* (Vol. 2, pp. 494–500). Elsevier.

Bartley, D. J. (2008). *Prevalence, characterisation and management of anthelmintic resistance in gastro-intestinal nematodes of Scottish sheep*. The University of

Edinburgh.

Bąska, P., and Norbury, L. J. (2022). The Role of Nuclear Factor Kappa B (NF-κB) in the Immune Response against Parasites. *Pathogens*, *11*(3), 1–24.

Bassetto, C. C., Almeida, F. A., Newlands, G. F. J., Smith, W. D., and Amarante, A. F. T. (2020). Repeated vaccination against *Haemonchus contortus* provides continuous protection to young grazing sheep. *Veterinary Parasitology*, *287*(July), 109273.

Bassetto, C. C., Silva, M. R. L., Newlands, G. F. J., Smith, W. D., Ratti Júnior, J., Martins, C. L., and Amarante, A. F. T. (2014). Vaccination of grazing calves with antigens from the intestinal membranes of *Haemonchus contortus*: effects against natural challenge with Haemonchus placei and *Haemonchus similis*. *International Journal for Parasitology*, *44*(10), 697–702.

Benavides, M. V., de Souza, C. J. H., Moraes, J. C. F., and Berne, M. E. A. (2016a). Is it feasible to select humid sub-tropical Merino sheep for faecal egg counts? *Small Ruminant Research*, *137*, 73–80.

Benavides, M. V., Sonstegard, T. S., and Van Tassell, C. (2016b). Genomic Regions Associated with Sheep Resistance to Gastrointestinal Nematodes. *Trends in Parasitology*, *32*(6), 470–480.

Benavides, M. V., Sonstegard, T. S., Kemp, S., Mugambi, J. M., Gibson, J. P., Baker, R. L., Hanotte, O., Marshall, K., and Van Tassell, C. (2015). Identification of Novel Loci Associated with Gastrointestinal Parasite Resistance in a Red Maasai x Dorper Backcross Population. *PLOS ONE*, *10*(4), e0122797.

Benveniste, E. N. (2014). Cytokines. In *Encyclopedia of the Neurological Sciences* (Vol. 1, pp. 921–925). Elsevier.

Beraldi, D., Craig, B. H., Bishop, S. C., Hopkins, J., and Pemberton, J. M. (2008). Phenotypic analysis of host-parasite interactions in lambs infected with *Teladorsagia circumcincta*. *International Journal for Parasitology*, *38*(13), 1567–1577.

Beraldi, D., McRae, A. F., Gratten, J., Pilkington, J. G., Slate, J., Visscher, P. M., and Pemberton, J. M. (2007). Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (Ovis aries). *International Journal for Parasitology*, *37*(1), 121–129.

Best, A., White, A., and Boots, M. (2008). Maintenance of host variation in tolerance to pathogens and parasites. *Proceedings of the National Academy of*

Sciences, *105*(52), 20786–20791.

Bishop, S. C. (2012a). A consideration of resistance and tolerance for ruminant nematode infections. *Frontiers in Genetics*, *3*(DEC).

Bishop, S. C. (2012b). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal*, *6*(5), 741–747.

Bishop, S. C., Bairden, K., McKellar, Q. A., Park, M., and Stear, M. J. (1996). Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. *Animal Science*, *63*, 423–428.

Bishop, S. C., Jackson, F., Coop, R. L., and Stear, M. J. (2004). Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. *Animal Science*, *78*(2), 185–194.

Bishop, S. C., and Stear, M. J. (1999). Genetic and epidemiological relationships between productivity and disease resistance: Gastro-intestinal parasite infection in growing lambs. *Animal Science*, *69*(3), 515–524.

Bishop, S. C., and Woolliams, J. A. (2014). Genomics and disease resistance studies in livestock. *Livestock Science*, *166*(1), 190–198.

Bisset, S. A., Vlassoff, A., Douch, P. G. C., Jonas, W. E., West, C. J., and Green, R. S. (1996). Nematode burdens and immunological responses following natural challenge in Romney lambs selectively bred for low or high faecal worm egg count. *Veterinary Parasitology*, *61*(3–4), 249–263.

Bisset, S. A., Vlassoff, A., Morris, C. A., Southey, B. R., Baker, R. L., and Parker, A. G. H. (1992). Heritability of and genetic correlations among faecal egg counts and productivity traits in Romney sheep. *New Zealand Journal of Agricultural Research*, *35*(1), 51–58.

Blasco, A., and Toro, M. A. (2014). A short critical history of the application of genomics to animal breeding. *Livestock Science*, *166*(1), 4–9.

Borkowski, E. A., Avula, J., Karrow, N. A., Menzies, P. I., Sears, W., Redman, E. M., Lillie, B. N., Gilleard, J. S., and Peregrine, A. S. (2020). Correlation of salivary antibody to carbohydrate larval antigen (CarLA) with health and gastrointestinal nematode parasitism in sheep under Ontario grazing conditions. *Veterinary Parasitology*, *283*(May), 109183.

Boulton, K., Nolan, M. J., Wu, Z., Psifidi, A., Riggio, V., Harman, K., Bishop, S. C.,

Kaiser, P., Abrahamsen, M. S., Hawken, R., Watson, K. A., Tomley, F. M., Blake, D. P., and Hume, D. A. (2018). Phenotypic and genetic variation in the response of chickens to *Eimeria tenella* induced coccidiosis. *Genetics Selection Evolution*, *50*(1), 63.

Bricarello, P. A., Zaros, L. G., Coutinho, L. L., Rocha, R. A., Silva, M. B., Kooyman, F. N. J., De Vries, E., Yatsuda, A. P., and Amarante, A. F. T. (2008). Immunological responses and cytokine gene expression analysis to Cooperia punctata infections in resistant and susceptible Nelore cattle. *Veterinary Parasitology*, *155*(1–2), 95–103.

Brodin, P., Jojic, V., Gao, T., Bhattacharya, S., Angel, C. J. L., Furman, D., Shen-Orr, S., Dekker, C. L., Swan, G. E., Butte, A. J., Maecker, H. T., and Davis, M. M. (2015). Variation in the human immune system is largely driven by non-heritable influences. *Cell*, *160*(1–2), 37–47.

Broom, L. J., and Kogut, M. H. (2019). Deciphering desirable immune responses from disease models with resistant and susceptible chickens. *Poultry Science*, *98*(4), 1634–1642.

Broughan, J. M., and Wall, R. (2007). Faecal soiling and gastrointestinal helminth infection in lambs. *International Journal for Parasitology*, *37*(11), 1255–1268.

Brown, D. J., Swan, A. A., and Gill, J. S. (2010). Within- and across-flock genetic relationships for breech flystrike resistance indicator traits. *Animal Production Science*, *50*(12), 1060.

Brown, E. A., Pilkington, J. G., Nussey, D. H., Watt, K. A., Hayward, A. D., Tucker, R., Graham, A. L., Paterson, S., Beraldi, D., Pemberton, J. M., and Slate, J. (2013). Detecting genes for variation in parasite burden and immunological traits in a wild population: Testing the candidate gene approach. *Molecular Ecology*, *22*(3), 757–773.

Burgess, C. G. S., Bartley, Y., Redman, E., Skuce, P. J., Nath, M., Whitelaw, F., Tait, A., Gilleard, J. S., and Jackson, F. (2012). A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. *Veterinary Parasitology*, *189*(2–4), 299–307.

Burleson, S. C. M., Fick, R. B., Mannie, M. D., Olmstead, S. G., and Van Scott, M. R. (2015). The Immune Basis of Allergic Lung Disease. In *Comparative Biology of the Normal Lung* (Second Edi, Issue 1, pp. 683–719). Elsevier.

Butcher, M. J., and Zhu, J. (2021). Recent advances in understanding the Th1/Th2 effector choice. *Faculty Reviews*, *10*(30).

Cao, A. T., Yao, S., Gong, B., Nurieva, R. I., Elson, C. O., and Cong, Y. (2015). Interleukin (IL)-21 promotes intestinal IgA response to microbiota. *Mucosal Immunology*, *8*(5), 1072–1082.

Carty, S. A., Riese, M. J., and Koretzky, G. A. (2018). T-Cell Immunity. In *Hematology* (Seventh Ed, pp. 221–239). Elsevier.

Casu, S., Usai, M. G., Sechi, T., Salaris, S. L., Miari, S., Mulas, G., Tamponi, C., Varcasia, A., Scala, A., and Carta, A. (2022). Association analysis and functional annotation of imputed sequence data within genomic regions influencing resistance to gastro-intestinal parasites detected by an LDLA approach in a nucleus flock of Sarda dairy sheep. *Genetics Selection Evolution*, *54*(1), 1–16.

Cavalli, G., and Dinarello, C. A. (2018). Suppression of inflammation and acquired immunity by IL-37. *Immunological Reviews*, *281*(1), 179–190.

Cerutti, A. (2008). The regulation of IgA class switching. *Nature Reviews Immunology*, *8*(6), 421–434.

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H. W., Chartier, C., Vineer, H. R., Hinney, B., von Samson-Himmelstjerna, G., Băcescu, B., Mickiewicz, M., Mateus, T. L., Martinez-Valladares, M., Quealy, S., Azaizeh, H., Sekovska, B., Akkari, H., Petkevicius, S., Hektoen, L., Höglund, J., Morgan, E. R., Bartley, D. J., and Claerebout, E. (2020). Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. *Preventive Veterinary Medicine*, *182*(June), 105103.

Charlier, J., van der Voort, M., Kenyon, F., Skuce, P., and Vercruysse, J. (2014). Chasing helminths and their economic impact on farmed ruminants. *Trends in Parasitology*, *30*(7), 361–367.

Chartier, C., and Paraud, C. (2012). Coccidiosis due to *Eimeria* in sheep and goats, a review. *Small Ruminant Research*, *103*(1), 84–92.

Chen, Y., Yang, Z., Wu, D., Min, Z., and Quan, Y. (2019). Upregulation of interleukin-17F in colorectal cancer promotes tumor invasion by inducing epithelial-mesenchymal transition. *Oncology Reports*, *42*(3), 1141–1148.

Chinchilla-Vargas, J., Kramer, L. M., Tucker, J. D., Hubbell, D. S., Powell, J. G., Lester, T. D., Backes, E. A., Anschutz, K., Decker, J. E., Stalder, K. J., Rothschild, M. F., and Koltes, J. E. (2020). Genetic Basis of Blood-Based Traits and Their Relationship With Performance and Environment in Beef Cattle at Weaning. *Frontiers in Genetics*, *11*(July), 1–13.

Chitneedi, P. K., Suárez-Vega, A., Martínez-Valladares, M., Arranz, J. J., and Gutiérrez-Gil, B. (2018). Exploring the mechanisms of resistance to *Teladorsagia circumcincta* infection in sheep through transcriptome analysis of abomasal mucosa and abomasal lymph nodes. *Veterinary Research*, *49*(1), 39.

Ciappesoni, G., Goldberg, V., and Gimeno, D. (2013). Estimates of genetic parameters for worm resistance, wool and growth traits in Merino sheep of Uruguay. *Livestock Science*, *157*(1), 65–74.

Cohn, L., Hawrylowicz, C., and Ray, A. (2014). Biology of Lymphocytes. In *Middleton's Allergy* (Eighth Edi, Vols. 1–2, pp. 203–214). Elsevier.

Coltman, D. W., Pilkington, J., Kruuk, L. E. B., Wilson, K., and Pemberton, J. M. (2001). Positive Genetic Correlation between Parasite Resistance and Body Size in a Free-Living Ungulate Population. *Evolution*, *55*(10), 2116–2125.

Conington, J., Bishop, S. C., Grundy, B., Waterhouse, A., and Simm, G. (2001). Multi-trait selection indexes for sustainable UK hill sheep production. *Animal Science*, *73*(3), 413–423.

Conington, J., Bishop, S. C., Lambe, N. R., Bünger, L., and Simm, G. (2006). Testing selection indices for sustainable hill sheep production – lamb growth and carcass traits. *Animal Science*, *82*(4), 445–453.

Coop, R. L., and Kyriazakis, I. (1999). Nutrition – parasite interaction. *Veterinary Parasitology*, *84*, 187–204.

Corripio-Miyar, Y., Hayward, A., Lemon, H., Sweeny, A. R., Bal, X., Kenyon, F., Pilkington, J. G., Pemberton, J. M., Nussey, D. H., and McNeilly, T. N. (2022). Functionally distinct T-helper cell phenotypes predict resistance to different types of parasites in a wild mammal. *Scientific Reports*, *12*(1), 3197.

Cox, F. E. G. (2001). Concomitant infections, parasites and immune responses. *Parasitology*, *122*(SUPPL.).

Craig, B. H., Tempest, L. J., Pilkington, J. G., and Pemberton, J. M. (2008). Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep. *Parasitology*, *135*(4), 433–441.

Craig, N. M. (2009). *Cytokine gene expression in naïve and previously infected sheep and lambs after challenge with the abomasal nematode Teladorsagia circumcincta*. http://hdl.handle.net/1842/4433

Craig, N. M., Miller, H. R. P., Smith, W. D., and Knight, P. A. (2007). Cytokine expression in naïve and previously infected lambs after challenge with

Teladorsagia circumcincta. *Veterinary Immunology and Immunopathology*, *120*(1–2), 47–54.

Cruz-Robles, D., Chvez-Gonzlez, J. P., Cavazos-Quero, M. M., Prez-Mndez, O., Reyes, P. A., and Vargas-Alarcn, G. (2009). Association between IL-1B and IL-1RN gene polymorphisms and chagas' disease development susceptibility. *Immunological Investigations*, *38*(3–4), 231–239.

Dann, S. M., Manthey, C. F., Le, C., Miyamoto, Y., Gima, L., Abrahim, A., Cao, A. T., Hanson, E. M., Kolls, J. K., Raz, E., Cong, Y., and Eckmann, L. (2015). IL-17A promotes protective IgA responses and expression of other potential effectors against the lumen-dwelling enteric parasite Giardia. *Experimental Parasitology*, *156*(858), 68–78.

Davies, G. (2006). *Investigating genetic aspects of the variation in the host response to gastrointestinal parasites in sheep*. University of Glasgow.

Davies, G., Stear, M. J., Benothman, M., Abuagob, O., Kerr, A., Mitchell, S., and Bishop, S. C. (2006). Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. *Heredity*, *96*(3), 252–258.

De la Chevrotière, C., Bambou, J. C., Arquet, R., Jacquiet, P., and Mandonnet, N. (2012). Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats. *Veterinary Parasitology*, *186*(3–4), 337–343.

De los Campos, G., Hickey, J. M., Pong-Wong, R., Daetwyler, H. D., and Calus, M. P. L. (2013). Whole-genome regression and prediction methods applied to plant and animal breeding. *Genetics*, *193*(2), 327–345.

De Veer, M. J., Kemp, J. M., and Meeusen, E. N. T. (2007). The innate host defence against nematode parasites. *Parasite Immunology*, *29*(1), 1–9.

Denholm, S. J., McNeilly, T. N., Banos, G., Coffey, M. P., Russell, G. C., Bagnall, A., Mitchell, M. C., and Wall, E. (2017). Estimating genetic and phenotypic parameters of cellular immune-associated traits in dairy cows. *Journal of Dairy Science*, *100*(4), 2850–2862.

Dinarello, C. A., Nold-Petry, C., Nold, M., Fujita, M., Li, S., Kim, S., and Bufler, P. (2016). Suppression of innate inflammation and immunity by interleukin-37. *European Journal of Immunology*, *46*(5), 1067–1081.

Doeschl-Wilson, A. B., Vagenas, D., Kyriazakis, I., and Bishop, S. C. (2008). Exploring the assumptions underlying genetic variation in host. *Genetics Selection Evolution*, *40*(June 2002), 241–264.

Dominik, S., Smith, J. L., Conington, J., Daetwyler, H. D., Olesen, I., and Bunter, K. L. (2017). Genetic solutions. In *Advances in Sheep Welfare* (pp. 107–130). Elsevier Ltd.

Douch, P. G. C., Green, R. S., Morris, C. A., Bisset, S. A., Vlassoff, A., Baker, R. L., Watson, T. G., Hurford, A. P., and Wheeler, M. (1995). Genetic and phenotypic relationships among anti-*Trichostrongylus colubriformis* antibody level, faecal egg count and body weight traits in grazing Romney sheep. *Livestock Production Science*, *41*(2), 121–132.

Douch, P. G. C., Green, R. S., Morris, C. A., McEwan, J. C., and Windon, R. G. (1996). Phenotypic markers for selection of nematode-resistant sheep. *International Journal for Parasitology*, *26*(8–9), 899–911.

Drummond, R. A., and Brown, G. D. (2013). Signalling C-Type Lectins in Antimicrobial Immunity. *PLoS Pathogens*, *9*(7), e1003417.

Duncanson, G. R. (2012). Veterinary treatment of sheep and goats. In G. R. Duncanson (Ed.), *Veterinary Treatment of Sheep and Goats*. CAB International.

Eady, S. J. (1998). Worm resistance in a Merino breeding objective - Influence of genetic correlations between resistance and production traits. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production*, 141–144.

Eagar, T. N., and Miller, S. D. (2019). Helper T-Cell Subsets and Control of the Inflammatory Response. In *Clinical Immunology* (Fifth Edit, Issue Chapter 4, pp. 235-245.e1). Elsevier.

Elliott, D. E., Urban, J. F., Argo, C. K., and Weinstock, J. V. (2000). Does the failure to acquire helminthic parasites predispose to Crohn's disease? *The FASEB Journal*, *14*(12), 1848–1855.

Ellis, S. E. E. (2014). *Interactions of Teladorsagia circumcincta with the ovine immune system – mimicry and vaccine development*. University of Edinburgh.

Engwerda, C. R., Ng, S. S., and Bunn, P. T. (2014). The Regulation of CD4+ T Cell Responses during Protozoan Infections. *Frontiers in Immunology*, *5*(OCT), 1–9.

Estrada-Reyes, Z. M., López-Reyes, A. G., Lagunas-Martínez, A., Ramírez- Vargas, G., Olazarán-Jenkins, S., Hernández-Romano, J., Mendoza-de-Gives, P., and López-Arellano, M. E. (2015). Relative expression analysis of IL-5 and IL-6 genes in tropical sheep breed Pelibuey infected with *Haemonchus contortus*. *Parasite Immunology*, *37*(9), 446–452.

Estrada-Reyes, Z. M., Rae, O., Postley, C., Jiménez Medrano, M. B., Leal Gutiérrez,

J. D., and Mateescu, R. G. (2019a). Association study reveals Th17, Treg, and Th2 loci related to resistance to *Haemonchus contortus* in Florida Native sheep. *Journal of Animal Science*, *97*(11), 4428–4444.

Estrada-Reyes, Z. M., Tsukahara, Y., Goetsch, A. L., Gipson, T. A., Sahlu, T., Puchala, R., and Mateescu, R. G. (2019b). Association analysis of immune response loci related to *Haemonchus contortus* exposure in sheep and goats using a targeted approach. *Livestock Science*, *228*(August), 109–119.

Evans, H. G., Gullick, N. J., Kelly, S., Pitzalis, C., Lord, G. M., Kirkham, B. W., and Taams, L. S. (2009). In vivo activated monocytes from the site of inflammation in humans specifically promote Th17 responses. *Proceedings of the National Academy of Sciences*, *106*(15), 6232–6237.

Fairlie-Clarke, K., Kaseja, K., Sotomaior, C., Brady, N., Moore, K., and Stear, M. (2019). Salivary IgA: A biomarker for resistance to *Teladorsagia circumcincta* and a new estimated breeding value. *Veterinary Parasitology*, *269*(April), 16–20.

Falconer, D. S. (1965). The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Annals of Human Genetics*, *29*(1), 51–76.

Falconer, D. S., and Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics* (4th Editio). Longman Grup Limited.

Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McIlgorm, A., Jolin, H. E., and McKenzie, A. N. J. (2006). Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *The Journal of Experimental Medicine*, *203*(4), 1105–1116.

Filippini, G., Aloisio, F., Cecchi, F., Biagetti, M., Macchioni, F., Ciampolini, R., Venditti, G., Ciani, E., Mazzanti, E., Sebastiani, C., and Cianci, D. (2006). An investigation on the genetic resistance to parasitical fauna in the Appenninica sheep breed. *8th World Congress on Genetics Applied to Livestock Production*, 8–11.

Finkelman, F. D., Morris, S. C., Gildea, L., Strait, R., Madden, K. B., and Schopf, L. (2004). Interleukin-4- and interleukin- 13-mediated host protection against intestinal nematode parasites. *Immunological Reviews*, *201*, 139–155.

Fiorentino, D. F., Bond, M. W., and Mosmann, T. R. (1989). Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *Journal of Experimental Medicine*, *170*(6), 2081–2095.

Fisher, B. A. (1918). The correlations between Relatives on the Supposition of Mendelian Inheritance. *Transactions of the Royal Society of Edinburgh*, *52*, 399–433.

Flori, L., Gao, Y., Laloë, D., Lemonnier, G., Leplat, J. J., Teillaud, A., Cossalter, A. M., Laffitte, J., Pinton, P., de Vaureix, C., Bouffaud, M., Mercat, M. J., Lefèvre, F., Oswald, I. P., Bidanel, J. P., and Rogel-Gaillard, C. (2011). Immunity traits in pigs: Substantial genetic variation and limited covariation. *PLoS ONE*, *6*(7).

Fonseca-Camarillo, G., Furuzawa-Carballeda, J., Iturriaga-Goyon, E., and Yamamoto-Furusho, J. K. (2018). Differential Expression of IL-36 Family Members and IL-38 by Immune and Nonimmune Cells in Patients with Active Inflammatory Bowel Disease. *BioMed Research International*, *2018*(Cd), 1–12.

Fu, C., Turck, C. W., Kurosaki, T., and Chan, A. C. (1998). BLNK: a Central Linker Protein in B Cell Activation. *Immunity*, *9*(1), 93–103.

Gauly, M., Krauthahn, C., Bauer, C., and Erhardt, G. (2001). Pattern of *Eimeria* Oocyst Output and Repeatability in Naturally Infected Suckling Rhon Lambs. *Journal of Veterinary Medicine Series B*, *48*(9), 665–673.

Gauly, M., Reeg, J., Bauer, C., and Erhardt, G. (2004). Influence of production systems in lambs on the *Eimeria* oocyst output and weight gain. *Small Ruminant Research*, *55*(1–3), 159–167.

Gazzinelli, R. T., Oswald, I. P., James, S. L., and Sher, A. (1992). IL-10 inhibits parasite killing and nitrogen oxide production by IFN-gamma-activated macrophages. *Journal of Immunology*, *148*(6), 1792–1796.

Gill, H. S., Altmann, K., Cross, M. L., and Husband, A. J. (2000). Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology*, *99*(3), 458–463.

Gilmour, A. R., Gogel, B. J., Cullis, B. R., and Thompson, R. (2009). ASReml user guide release 3.0. In *VSN International Ltd, Hemel Hempstead, HP1 1ES, United Kingdom*. www.vsni.co.uk

Gossner, A. G., Venturina, V. M., Shaw, D. J., Pemberton, J. M., and Hopkins, J. (2012). Relationship between susceptibility of Blackface sheep to *Teladorsagia circumcincta* infection and an inflammatory mucosal T cell response. *Veterinary Research*, *43*(1), 26.

Goswami, R., and Kaplan, M. H. (2017). STAT Transcription Factors in T Cell Control of Health and Disease. In *International Review of Cell and Molecular*

Biology (1st ed., Vol. 331, pp. 123–180). Elsevier Inc.

Grainger, J. R., Smith, K. A., Hewitson, J. P., McSorley, H. J., Harcus, Y., Filbey, K. J., Finney, C. A. M., Greenwood, E. J. D., Knox, D. P., Wilson, M. S., Belkaid, Y., Rudensky, A. Y., and Maizels, R. M. (2010). Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. *Journal of Experimental Medicine*, *207*(11), 2331–2341.

Greeff, J. C., Karlsson, L. J. E., and Schlink, A. C. (2014). Identifying indicator traits for breech strike in Merino sheep in a Mediterranean environment. *Animal Production Science*, *54*(2), 125.

Greer, A. W. (2008). Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. *Parasite Immunology*, *30*(2), 123–132.

Greer, A. W., and Hamie, J. C. (2016). Relative maturity and the development of immunity to gastrointestinal nematodes in sheep: An overlooked paradigm? *Parasite Immunology*, *38*(5), 263–272.

Greer, A. W., McKenzie, J. L., McAnulty, R. W., Huntley, J. F., and McNeilly, T. N. (2018). Immune development and performance characteristics of Romney sheep selected for either resistance or resilience to gastrointestinal nematodes. *Veterinary Parasitology*, *250*(December 2017), 60–67.

Griffin, D. E. (2008). Cytokines and Chemokines. In *Encyclopedia of Virology* (pp. 620–624). Elsevier.

Guillaume, S. (2012). *Functional validation and detection regions of the genome that affect the resistance to gastrointestinal strongyles in sheep*. Institut National Polytechnique de Toulouse.

Guo, J., Qu, H., Chen, Y., and Xia, J. (2017). The role of RNA-binding protein tristetraprolin in cancer and immunity. *Medical Oncology*, *34*(12), 196.

Gutiérrez-Gil, B., Pérez, J., Álvarez, L., Martínez-Valladares, M., de la Fuente, L.-F., Bayón, Y., Meana, A., Primitivo, F. S., Rojo-Vázquez, F.-A., and Arranz, J.-J. (2009). Quantitative trait loci for resistance to trichostrongylid infection in Spanish Churra sheep. *Genetics Selection Evolution*, *41*(1), 46.

Gutiérrez-Gil, B., Pérez, J., De La Fuente, L. F., Meana, A., Martínez-Valladares, M., San Primitivo, F., Rojo-Vázquez, F. A., and Arranz, J. J. (2010). Genetic parameters for resistance to trichostrongylid infection in dairy sheep. *Animal*, *4*(4), 505–512.

Hahn, W. H., Cho, B. S., Kim, S. Do, Kim, S. K., and Kang, S. (2009). Interleukin-1 cluster gene polymorphisms in childhood IgA nephropathy. *Pediatric Nephrology*, *24*(7), 1329–1336.

Han, Y., Huard, A., Mora, J., da Silva, P., Brüne, B., and Weigert, A. (2020). IL-36 family cytokines in protective versus destructive inflammation. In *Cellular Signalling* (Vol. 75, Issue July). Elsevier.

Haritova, A. M., and Stanilova, S. A. (2012). Enhanced expression of IL-10 in contrast to IL-12B mRNA in poultry with experimental coccidiosis. *Experimental Parasitology*, *132*(3), 378–382.

Hassan, M., Hanrahan, J. P., Good, B., Mulcahy, G., and Sweeney, T. (2011). A differential interplay between the expression of Th1/Th2/Treg related cytokine genes in *Teladorsagia circumcincta* infected *DRB1*1101* carrier lambs. *Veterinary Research*, *42*(1), 45.

Hawrylowicz, C. M. (2005). Regulatory T cells and IL-10 in allergic inflammation. *Journal of Experimental Medicine*, *202*(11), 1459–1463.

Hayward, A. D. (2013). Causes and consequences of intra- and inter-host heterogeneity in defence against nematodes. *Parasite Immunology*, *35*(11), 362–373.

Hayward, A. D. (2022). Genetic parameters for resistance to gastrointestinal nematodes in sheep: a meta-analysis. *International Journal for Parasitology*, *52*(13–14), 843–853.

Hayward, A. D., Nussey, D. H., Wilson, A. J., Berenos, C., Pilkington, J. G., Watt, K. A., Pemberton, J. M., and Graham, A. L. (2014). Natural Selection on Individual Variation in Tolerance of Gastrointestinal Nematode Infection. *PLoS Biology*, *12*(7), 1–13.

Hepworth, M. R., and Grencis, R. K. (2009). Disruption of Th2 Immunity Results in a Gender-Specific Expansion of IL-13 Producing Accessory NK Cells during Helminth Infection. *The Journal of Immunology*, *183*(6), 3906–3914.

Hine, B. C., Acton, G. A., Elks, D. J., Niemeyer, D. D. O., Bell, A. M., Colditz, I. G., Ingham, A. B., and Smith, J. L. (2022). Targeting improved resilience in Merino sheep – Correlations between immune competence and health and fitness traits. *Animal*, *16*(7), 100544.

Hirota, K., Turner, J., Villa, M., Duarte, J. H., Demengeot, J., Steinmetz, O. M., and Stockinger, B. (2013). Plasticity of TH17 cells in Peyer's patches is responsible for the induction of T cell–dependent IgA responses. *Nature Immunology*,

14(4), 372–379.

Hohl, T. M. (2014). Cell-Mediated Defense against Infection. In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (Eighth Edi, Vol. 1, pp. 50–69). Elsevier Inc.

Hoste, H., and Torres-Acosta, J. F. J. (2011). Non chemical control of helminths in ruminants: Adapting solutions for changing worms in a changing world. *Veterinary Parasitology*, *180*(1–2), 144–154.

Howes, A., Gabryšová, L., and O'Garra, A. (2014). Role of IL-10 and the IL-10 Receptor in Immune Responses. In *Reference Module in Biomedical Sciences* (Vol. 1, pp. 1–11).

Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, *4*(1), 44–57.

Hummelshoj, L., Ryder, L. P., and Poulsen, L. K. (2006). The role of the interleukin-10 subfamily members in immunoglobulin production by human B cells. *Scandinavian Journal of Immunology*, *64*(1), 40–47.

Hunt, P. W., Kijas, J., and Ingham, A. (2013). Understanding parasitic infection in sheep to design more efficient animal selection strategies. *Veterinary Journal*, *197*(2), 143–152.

Hutchings, M., Knowler, K., McAnulty, R., and McEwan, J. (2007). Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1620), 1839–1844.

Idris, A., Moors, E., Sohnrey, B., and Gauly, M. (2012). Gastrointestinal nematode infections in German sheep. *Parasitology Research*, *110*(4), 1453–1459.

ISGC. (n.d.). *International Sheep Genomics Consortium*. International Sheep Genomics Consortium. Retrieved September 27, 2022, from https://www.sheephapmap.org/

Iwakura, Y., Nakae, S., Saijo, S., and Ishigame, H. (2008). The roles of IL-17A in inflammatory immune responses and host defense against pathogens. In *Immunological Reviews* (Vol. 226, Issue 1, pp. 57–79).

Iwaszko, M., and Bogunia-Kubik, K. (2011). Clinical Significance of the HLA-E and CD94/NKG2 Interaction. *Archivum Immunologiae et Therapiae Experimentalis*, *59*(5), 353–367.

Jack, C., Hotchkiss, E., Sargison, N. D., Toma, L., Milne, C., and Bartley, D. J. (2017). A quantitative analysis of attitudes and behaviours concerning sustainable parasite control practices from Scottish sheep farmers. *Preventive Veterinary Medicine*, *139*, 134–145.

Jackson, F., Bartley, D., Bartley, Y., and Kenyon, F. (2009). Worm control in sheep in the future. *Small Ruminant Research*, *86*(1–3), 40–45.

Jackson, J. A., Turner, J. D., Rentoul, L., Faulkner, H., Behnke, J. M., Hoyle, M., Grencis, R. K., Else, K. J., Kamgno, J., Bradley, J. E., and Boussinesq, M. (2004). Cytokine response profiles predict species-specific infection patterns in human GI nematodes. *International Journal for Parasitology*, *34*(11), 1237–1244.

Jacobs, J. R., Middleton, D., Greiner, S. P., and Bowdridge, S. A. (2020). RNA-Sequencing of ovine PBMC after exposure to *Haemonchus contortus* larval antigen. *Parasite Immunology*, *42*(5), 1–8.

Jacobs, J. R., Sommers, K. N., Zajac, A. M., Notter, D. R., and Bowdridge, S. A. (2016). Early IL-4 gene expression in abomasum is associated with resistance to *Haemonchus contortus* in hair and wool sheep breeds. *Parasite Immunology*, *38*(6), 333–339.

Jacobson, C., Larsen, J. W., Besier, R. B., Lloyd, J. B., and Kahn, L. P. (2020). Diarrhoea associated with gastrointestinal parasites in grazing sheep. *Veterinary Parasitology*, *282*(May), 109139.

Janossy, G., and Greaves, M. F. (1971). Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. *Clinical and Experimental Immunology*, *9*(4), 483–498.

Jawasreh, K. I. Z., Mukbel, R. M., Qader, A. A., and Mayyas, M. A. (2013). Coccidiosis in Awassi, Romanov, Charollais and Suffolk sheep breeds during the winter and summer seasons in Jordan. *International Journal of Applied Science and Technology*, *3*(6), 10–15.

Jin, W.-J., Chen, C.-F., Liao, H.-Y., Gong, L.-L., Yuan, X.-H., Zhao, B.-B., Zhang, D., Feng, X., Liu, J.-J., Wang, Y., Chen, G.-F., Yan, H.-P., and He, Y.-W. (2012). Downregulation of the AU-Rich RNA-Binding Protein ZFP36 in Chronic HBV Patients: Implications for Anti-Inflammatory Therapy. *PLoS ONE*, *7*(3), e33356.

Jung, Y., Wen, T., Mingler, M. K., Caldwell, J. M., Wang, Y. H., Chaplin, D. D., Lee, E. H., Jang, M. H., Woo, S. Y., Seoh, J. Y., Miyasaka, M., and

Rothenberg, M. E. (2015). IL-1β in eosinophil-mediated small intestinal homeostasis and IgA production. *Mucosal Immunology*, *8*(4), 930–942.

Kaiko, G. E., Horvat, J. C., Beagley, K. W., and Hansbro, P. M. (2008). Immunological decision-making: How does the immune system decide to mount a helper T-cell response? *Immunology*, *123*(3), 326–338.

Kaisar, M. M. M., Ritter, M., del Fresno, C., Jónasdóttir, H. S., van der Ham, A. J., Pelgrom, L. R., Schramm, G., Layland, L. E., Sancho, D., Prazeres da Costa, C., Giera, M., Yazdanbakhsh, M., and Everts, B. (2018). Dectin-1/2–induced autocrine PGE2 signaling licenses dendritic cells to prime Th2 responses. *PLOS Biology*, *16*(4), e2005504.

Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2014). Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Research*, *42*(D1), D199–D205.

Karaffová, V., Bobíková, K., Husáková, E., Levkut, M., Herich, R., Revajová, V., Levkutová, M., and Levkut, M. (2015). Interaction of TGF-β4 and IL-17 with IgA secretion in the intestine of chickens fed with E. faecium AL41 and challenged with S. Enteritidis. *Research in Veterinary Science*, *100*, 75–79.

Kariuki, S. N., and Niewold, T. B. (2010). Genetic regulation of serum cytokines in systemic lupus erythematosus. *Translational Research*, *155*(3), 109–117.

Karrow, N. A., Goliboski, K., Stonos, N., Schenkel, F., and Peregrine, A. (2014). Review: Genetics of helminth resistance in sheep. *Canadian Journal of Animal Science*, *94*(1), 1–9.

Kaseja, K., Mucha, S., Smith, E., Yates, J., Banos, G., and Conington, J. (2023). Including genotypic information in genetic evaluations increases the accuracy of sheep breeding values. *Journal of Animal Breeding and Genetics*, *140*(4), 462–471.

Keane, O. M., Hanrahan, J. P., McRae, K. M., and Good, B. (2018). An independent validation study of loci associated with nematode resistance in sheep. *Animal Genetics*, *49*(3), 265–268.

Kelly, E. A., Cruz, E. S., Hauda, K. M., and Wassom, D. L. (1991). IFN-gamma- and IL-5-producing cells compartmentalize to different lymphoid organs in Trichinella spiralis-infected mice. *Journal of Immunology (Baltimore, Md. : 1950)*, *147*(1), 306–311.

Kemper, K. E., Elwin, R. L., Bishop, S. C., Goddard, M. E., and Woolaston, R. R. (2009). *Haemonchus contortus* and *Trichostrongylus colubriformis* did not

adapt to long-term exposure to sheep that were genetically resistant or susceptible to nematode infections. *International Journal for Parasitology*, *39*(5), 607–614.

Kemper, K. E., Emery, D. L., Bishop, S. C., Oddy, H., Hayes, B. J., Dominik, S., Henshall, J. M., and Goddard, M. E. (2011). The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. *Genetics Research*, *93*(03), 203–219.

Kent, C. F., Tiwari, T., Rose, S., Patel, H., Conflitti, I. M., and Zayed, A. (2019). Studying the Genetics of Behavior in the Genomics Era. In *Encyclopedia of Animal Behavior* (Second Edi, Vol. 2, pp. 223–233). Elsevier.

Kim, E.-S., Sonstegard, T. S., da Silva, M. V. G. B., Gasbarre, L. C., and Van Tassell, C. P. (2015). Genome-Wide Scan of Gastrointestinal Nematode Resistance in Closed Angus Population Selected for Minimized Influence of MHC. *PLOS ONE*, *10*(3), e0119380.

Kim, S. W., Park, K. C., Jeon, S. M., Ohn, T. B., Kim, T. Il, Kim, W. H., and Cheon, J. H. (2013). Abrogation of galectin-4 expression promotes tumorigenesis in colorectal cancer. *Cellular Oncology*, *36*(2), 169–178.

Kinsella, R. J., Kahari, A., Haider, S., Zamora, J., Proctor, G., Spudich, G., Almeida-King, J., Staines, D., Derwent, P., Kerhornou, A., Kersey, P., and Flicek, P. (2011). Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database*, *2011*.

Knight, P. A., Griffith, S. E., Pemberton, A. D., Pate, J. M., Guarneri, L., Anderson, K., Talbot, R. T., Smith, S., Waddington, D., Fell, M., Archibald, A. L., Burgess, S. T., Smith, D. W., Miller, H. R., and Morrison, I. W. (2011). Novel gene expression responses in the ovine abomasal mucosa to infection with the gastric nematode *Teladorsagia circumcincta*. *Veterinary Research*, *42*(1), 78.

Krawczyk, A., and Słota, E. (2009). Genetic markers to gastrointestinal nematode resistance in sheep: a review. *Helminthologia*, *46*(1), 3–8.

Kubo, T., Morita, H., Sugita, K., and Akdis, C. A. (2017). Introduction to Mechanisms of Allergic Diseases. In *Middleton's Allergy Essentials* (pp. 1–27). Elsevier.

Kutzer, M. A. M., and Armitage, S. A. O. (2016). Maximising fitness in the face of parasites: a review of host tolerance. *Zoology*, *119*(4), 281–289.

Kwong, L. S., Hope, J. C., Thom, M. L., Sopp, P., Duggan, S., Bembridge, G. P.,

and Howard, C. J. (2002). Development of an ELISA for bovine IL-10. *Veterinary Immunology and Immunopathology*, *85*(3–4), 213–223.

Lambe, N. R., Bünger, L., Bishop, S. C., Simm, G., and Conington, J. (2008). The effects of selection indices for sustainable hill sheep production on carcass composition and muscularity of lambs, measured using X-ray computed tomography. *Animal*, *2*(1), 27–35.

Lambe, N. R., Conington, J., McLean, K. A., Navajas, E. A., Fisher, A. V., and Bunger, L. (2006). In vivo prediction of internal fat weight in Scottish Blackface lambs, using computer tomography. *Journal of Animal Breeding and Genetics*, *123*(2), 105–113.

Lambe, N. R., Wall, E., Ludemann, C. I., Bünger, L., and Conington, J. (2014). Genetic improvement of hill sheep – Impacts on profitability and greenhouse gas emissions. *Small Ruminant Research*, *120*(1), 27–34.

Larsen, J., Anderson, N., Vizard, A., Anderson, G., and Hoste, H. (1994). Diarrhoea in Merino ewes during winter: association with trichostrongylid larvae. *Australian Veterinary Journal*, *71*(11), 365–372.

Larsen, J. W., Anderson, N., Vizard, A. L., Anderson, G. A., and Hoste, H. (1994). Diarrhoea in merino ewes during winter: association with trichostrongylid larvae. *Australian Veterinary Journal*, *71*(11), 365–372.

Laurenson, Y. C. S. M. (2012). *Modelling Nematode Infections in Sheep and Parasite Control Strategies*. University of Edinburgh.

Le Jambre, L. F., Dominik, S., Eady, S. J., Henshall, J. M., and Colditz, I. G. (2007). Adjusting worm egg counts for faecal moisture in sheep. *Veterinary Parasitology*, *145*, 108–115.

Leach, R. J., Chitko-McKown, C. G., Bennett, G. L., Jones, S. A., Kachman, S. D., Keele, J. W., Leymaster, K. A., Thallman, R. M., and Kuehn, L. A. (2013). The change in differing leukocyte populations during vaccination to bovine respiratory disease and their correlations with lung scores, health records, and average daily gain. *Journal of Animal Science*, *91*(8), 3564–3573.

Leathwick, D. M. (2014). Sustainable control of nematode parasites - A New Zealand perspective. *Small Ruminant Research*, *118*(1–3), 31–34.

Legård, G. E., and Pedersen, B. K. (2019). Muscle as an Endocrine Organ. In *Muscle and Exercise Physiology* (pp. 285–307). Elsevier.

LeibundGut-Landmann, S., Groß, O., Robinson, M. J., Osorio, F., Slack, E. C.,

Tsoni, S. V., Schweighoffer, E., Tybulewicz, V., Brown, G. D., Ruland, J., and Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nature Immunology*, *8*(6), 630–638.

Leung, C.-K., Zhao, H., Lv, Y., Lu, G., and Chen, Z.-J. (2019). Genome-Wide Association Studies of Ovarian Function Disorders. In *The Ovary* (3rd ed., pp. 311–325). Elsevier.

Li, J. H., Mazur, C. A., Berisa, T., and Pickrell, J. K. (2021). Low-pass sequencing increases the power of GWAS and decreases measurement error of polygenic risk scores compared to genotyping arrays. *Genome Research*, *31*(4), 529–537.

Li, R. W., Rinaldi, M., and Capuco, A. V. (2011). Characterization of the abomasal transcriptome for mechanisms of resistance to gastrointestinal nematodes in cattle. *Veterinary Research*, *42*(1), 114.

Li, Y., Oosting, M., Smeekens, S. P., Jaeger, M., Aguirre-Gamboa, R., Le, K. T. T., Deelen, P., Ricaño-Ponce, I., Schoffelen, T., Jansen, A. F. M., Swertz, M. A., Withoff, S., van de Vosse, E., van Deuren, M., van de Veerdonk, F., Zhernakova, A., van der Meer, J. W. M., Xavier, R. J., Franke, L., Joosten, L. A. B., Wijmenga, C., Kumar, V., and Netea, M. G. (2016). A Functional Genomics Approach to Understand Variation in Cytokine Production in Humans. *Cell*, *167*(4), 1099–1110.

Lillehoj, H. S., Min, W., and Dalloul, R. A. (2004). Recent progress on the cytokine regulation of intestinal immune responses to *Eimeria*. *Poultry Science*, *83*(4), 611–623.

Lillehoj, H. S., and Trout, J. M. (1993). Coccidia: A review of recent advances on immunity and vaccine development. *Avian Pathology*, *22*(1), 3–31.

Lindenwald, D. L., and Lepenies, B. (2020). C-type lectins in veterinary species: Recent advancements and applications. In *International Journal of Molecular Sciences* (Vol. 21, Issue 14, pp. 1–16).

Liu, J., Wu, X., Wang, H., Wei, J., Wu, Q., Wang, X., Yan, Y., Cui, J., Min, J., Wang, F., and Zhou, J. (2021). HFE inhibits type I IFNs signaling by targeting the SQSTM1-mediated MAVS autophagic degradation. *Autophagy*, *17*(8), 1962–1977.

Liu, W., McNeilly, T. N., Mitchell, M., Burgess, S. T. G., Nisbet, A. J., Matthews, J. B., and Babayan, S. A. (2022). Vaccine-induced time- and age-dependent mucosal immunity to gastrointestinal parasite infection. *Npj Vaccines*, *7*(1), 1–

9.

London, C. A., Abbas, A. K., and Kelso, A. (1998). Helper T cell subsets: Heterogeneity, functions and development. *Veterinary Immunology and Immunopathology*, *63*(1–2), 37–44.

MacKinnon, K. M. (2007). *Differential gene expression and immune regulatory mechanisms in parasite-resistant hair and susceptible wool sheep infected with the parasitic nematode, Haemonchus contortus*. Virginia Polytechnic Institute and State University.

Maizels, R. M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M. D., and Allen, J. E. (2004). Helminth parasites - masters of regulation. *Immunological Reviews*, *201*(1), 89–116.

Makita, S., Takatori, H., and Nakajima, H. (2021). Post-Transcriptional Regulation of Immune Responses and Inflammatory Diseases by RNA-Binding ZFP36 Family Proteins. *Frontiers in Immunology*, *12*(July), 1–8.

Malinowska, M., Tokarz-Deptula, B., and Deptula, W. (2017). Butyrophilins: An important new element of resistance. *Central European Journal of Immunology*, *42*(4), 399–403.

Mallard, B. A., Emam, M., Paibomesai, M., Thompson-Crispi, K., and Wagter-Lesperance, L. (2015). Genetic selection of cattle for improved immunity and health. *Japanese Journal of Veterinary Research*, *63*, S37–S44.

Mallone, R., and Nepom, G. T. (2004). MHC Class II tetramers and the pursuit of antigen-specific T cells: Define, deviate, delete. *Clinical Immunology*, *110*(3), 232–242.

Marconi, M., Plebani, A., Avanzini, M. A., Maccario, R., Pistorio, A., Duse, M., Stringa, M., and Monafo, V. (1998). IL-10 and IL-4 co-operate to normalize in vitro IgA production in IgA- deficient (IgAD) patients. *Clinical and Experimental Immunology*, *112*(3), 528–532.

Marshall, J. S., and Jawdat, D. M. (2004). Mast cells in innate immunity. *Journal of Allergy and Clinical Immunology*, *114*(1), 21–27.

Marshall, J. S., Warrington, R., Watson, W., and Kim, H. L. (2018). An introduction to immunology and immunopathology. *Allergy, Asthma and Clinical Immunology*, *14*(s2), 1–10.

Maseda, D., Smith, S. H., DiLillo, D. J., Bryant, J. M., Candando, K. M., Weaver, C. T., and Tedder, T. F. (2012). Regulatory B10 Cells Differentiate into Antibody-

Secreting Cells After Transient IL-10 Production In Vivo. *The Journal of Immunology*, *188*(3), 1036–1048.

Mavrot, F., Hertzberg, H., and Torgerson, P. (2015). Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. *Parasites & Vectors*, *8*(1), 557.

Mayer, S., Raulf, M. K., and Lepenies, B. (2017). C-type lectins: their network and roles in pathogen recognition and immunity. In *Histochemistry and Cell Biology* (Vol. 147, Issue 2, pp. 223–237). Springer Berlin Heidelberg.

Maynard, C. L., and Weaver, C. T. (2015). Effector CD4 + T Cells in the Intestines. In *Mucosal Immunology* (Vols. 1–2, pp. 721–732). Elsevier.

McAdoo, S., and Tam, F. W. K. (2018). Role of the Spleen Tyrosine Kinase Pathway in Driving Inflammation in IgA Nephropathy. *Seminars in Nephrology*, *38*(5), 496–503.

McDermott, J. R., Humphreys, N. E., Forman, S. P., Donaldson, D. D., and Grencis, R. K. (2005). Intraepithelial NK Cell-Derived IL-13 Induces Intestinal Pathology Associated with Nematode Infection. *The Journal of Immunology*, *175*(5), 3207–3213.

McEwan, J. C., Mason, P., Baker, R. L., Clarke, J. N., Hickey, S. M., and Turner, K. (1992). Effect of selection for productive traits on internal parasite resistance in sheep. *Proceedings of the New Zealand Society of Animal Production*, *52*, 53–56.

McLaren, A., Lambe, N. R., Brotherstone, S., Conington, J., Mrode, R., and Bünger, L. (2012). Investigation into the presence of genotype by environment interactions (G×E) in Scottish Blackface lamb traits. *Small Ruminant Research*, *105*(1–3), 46–52.

McManus, C., Louvandini, H., Paiva, S. R., de Oliveira, A. A., Azevedo, H. C., and de Melo, C. B. (2009). Genetic factors of sheep affecting gastrointestinal parasite infections in the Distrito Federal, Brazil. *Veterinary Parasitology*, *166*(3–4), 308–313.

McManus, C., Paim, T. do P., de Melo, C. B., Brasil, B. S. A. F., and Paiva, S. R. (2014). Selection methods for resistance to and tolerance of helminths in livestock. *Parasite*, *21*, 56.

McNeilly, T. N., Devaney, E., and Matthews, J. B. (2009). Teladorsagia circumcincta in the sheep abomasum: defining the role of dendritic cells in T cell regulation and protective immunity. *Parasite Immunology*, *31*(7), 347–356.

McNeilly, T. N., McClure, S. J., and Huntley, J. F. (2008). Mucosal immunity in sheep and implications for mucosal vaccine development. *Small Ruminant Research*, *76*(1–2), 83–91.

McNeilly, T. N., and Nisbet, A. J. (2014). Immune modulation by helminth parasites of ruminants: implications for vaccine development and host immune competence. *Parasite*, *21*(51), 51.

McNeilly, T. N., Rocchi, M., Bartley, Y., Brown, J. K., Frew, D., Longhi, C., McLean, L., McIntyre, J., Nisbet, A. J., Wattegedera, S., Huntley, J. F., and Matthews, J. B. (2013). Suppression of ovine lymphocyte activation by *Teladorsagia circumcincta* larval excretory-secretory products. *Veterinary Research*, *44*(1), 1–18.

McRae, K. M. (2015). *Identification of Variation in the Sheep Genome Controlling Gastrointestinal Nematode Resistance*. Dublin City University.

McRae, K. M., McEwan, J. C., Dodds, K. G., and Gemmell, N. J. (2014). Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genomics*, *15*(1), 637.

McRae, K. M., Stear, M. J., Good, B., and Keane, O. M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology*, *37*(12), 605–613.

Meeusen, E. N. T., Balic, A., and Bowles, V. (2005). Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Veterinary Immunology and Immunopathology*, *108*(1-2 SPEC. ISS.), 121–125.

Meuwissen, T. H. E., Hayes, B. J., and Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, *157*(4), 1819–1829.

Meuwissen, T., Hayes, B., and Goddard, M. (2016). Genomic selection: A paradigm shift in animal breeding. *Animal Frontiers*, *6*(1), 6.

Mócsai, A., Ruland, J., and Tybulewicz, V. L. J. (2010). The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nature Reviews Immunology*, *10*(6), 387–402.

Morris, C. A. (2011). Review of genetic parameters for disease resistance in sheep in New Zeland and Australia. *Proceedings Association for the Advancement of Animal Breeding and Genetics*, *18*, 263–271.

Morris, C. A., Bisset, S. A., Vlassoff, A., West, C. J., and Wheeler, M. (2004).

Genetic parameters for Nematodirus spp egg counts in Romney lambs in New Zealand. *Animal Science*, *79*, 33–39.

Morris, C. A., Wheeler, M., Watson, T. G., Hosking, B. C., and Leathwick, D. M. (2005). Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. *New Zealand Journal of Agricultural Research*, *48*(1), 1–10.

Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., and Coffman, R. L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology*, *136*(7), 2348–2357.

Mucha, S., Bunger, L., and Conington, J. (2015). Genome-wide association study of footrot in Texel sheep. *Genetics Selection Evolution*, *47*(1).

Nagamine, Y., Pong-Wong, R., Navarro, P., Vitart, V., Hayward, C., Rudan, I., Campbell, H., Wilson, J., Wild, S., Hicks, A. A., Pramstaller, P. P., Hastie, N., Wright, A. F., and Haley, C. S. (2012). Localising Loci underlying Complex Trait Variation Using Regional Genomic Relationship Mapping. *PLoS ONE*, *7*(10).

Nairz, M., Fritsche, G., Brunner, P., Talasz, H., Hantke, K., and Weiss, G. (2008). Interferon-γ limits the availability of iron for intramacrophage Salmonella typhimurium. *European Journal of Immunology*, *38*(7), 1923–1936.

Nairz, M., Schroll, A., Haschka, D., Dichtl, S., Tymoszuk, P., Demetz, E., Moser, P., Haas, H., Fang, F. C., Theurl, I., and Weiss, G. (2017). Genetic and Dietary Iron Overload Differentially Affect the Course of Salmonella Typhimurium Infection. *Frontiers in Cellular and Infection Microbiology*, *7*(APR).

National Sheep Association, and National Farmers Union. (2014). *A Vision for British lamb production*. http://www.nationalsheep.org.uk/workspace/pdfs/nsanfuvisiondocument28072014195406_1.pdf

Newport, M. J., Goetghebuer, T., Weiss, H. A., Allen, A., Banya, W., Sillah, D. J., McAdam, K. P. W. J., Mendy, M., Ota, M., Vekemans, J., Jobe, K., Bennett, S., Aaby, P., Stockton, J. C., Cadau, G., Lambert, P. H., Schlegel-Hauter, S., Valenti, P., Whittle, H., Siegrist, C. A., and Marchant, A. (2004). Genetic regulation of immune responses to vaccines in early life. *Genes and Immunity*, *5*(2), 122–129.

Ng, T. H. S., Britton, G. J., Hill, E. V., Verhagen, J., Burton, B. R., and Wraith, D.

C. (2013). Regulation of adaptive immunity; the role of interleukin-10. *Frontiers in Immunology*, *4*(May), 1–13.

Nieuwhof, G. J., and Bishop, S. C. (2005). Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science*, *81*(1), 23–29.

Nieuwoudt, S. W., Theron, H. E., and Kruger, L. P. (2002). Genetic parameters for resistance to *Haemonchus contortus* in Merino sheep in South Africa. *Journal of the South African Veterinary Association*, *73*(1), 4–7.

Nilsson, J., Gonçalves, I., and Edsfeldt, A. (2015). Chronic Inflammation and Atherosclerosis. In *Early Vascular Aging (EVA)* (pp. 157–167). Elsevier.

O'Brien, A. C., McHugh, N., Wall, E., Pabiou, T., McDermott, K., Randles, S., Fair, S., and Berry, D. P. (2017). Genetic parameters for lameness, mastitis and dagginess in a multi-breed sheep population. *Animal*, *11*(6), 911–919.

O'Shea, J. J., Gadina, M., and Siegel, R. M. (2019). Cytokines and Cytokine Receptors. In *Clinical Immunology: Principles and Practice* (Fifth Edit, Vol. 1, pp. 127-155.e1). Elsevier Ltd.

Odden, A., Enemark, H. L., Robertson, L. J., Ruiz, A., Hektoen, L., and Stuen, S. (2017). Treatment against coccidiosis in Norwegian lambs and potential risk factors for development of anticoccidial resistance—a questionnaire-based study. *Parasitology Research*, *116*(4), 1237–1245.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., and Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, *27*(1), 29–34.

Oltenacu, P., and Broom, D. (2010). The impact of genetic selection for increased milk yield on the welfare of dairy cows. *Animal Welfare*, *19*(S1), 39–49.

Ovington, K. S., Alleva, L. M., and Kerr, E. A. (1995). Cytokines and immunological control of *Eimeria spp. International Journal for Parasitology*, *25*(11), 1331–1351.

Ozmen, O., Adanir, R., and Haligur, M. (2012). Immunohistochemical detection of the cytokine and chemokine expression in the gut of lambs and kids with coccidiosis. *Small Ruminant Research*, *105*(1–3), 345–350.

Pacheco, A., McNeilly, T. N., Banos, G., and Conington, J. (2021). Genetic parameters of animal traits associated with coccidian and nematode parasite load and growth in Scottish Blackface sheep. *Animal*, *15*(4), 100185.

Paclik, D., Danese, S., Berndt, U., Wiedenmann, B., Dignass, A., and Sturm, A. (2008). Galectin-4 controls intestinal inflammation by selective regulation of peripheral and mucosal T cell apoptosis and cell cycle. *PLoS ONE*, *3*(7), e2629.

Paerewijck, O., Maertens, B., Gagnaire, A., De Bosscher, K., and Geldhof, P. (2019). Delayed development of the protective IL-17A response following a Giardia muris infection in neonatal mice. *Scientific Reports*, *9*(1), 8959.

Papadopoulos, E. (2008). Anthelmintic resistance in sheep nematodes. *Small Ruminant Research*, *76*(1–2), 99–103.

Papadopoulos, E., Gallidis, E., and Ptochos, S. (2012). Anthelmintic resistance in sheep in Europe: A selected review. *Veterinary Parasitology*, *189*(1), 85–88.

Passafaro, T. L., Carrera, J. P. B., Santos, L. L. dos, Raidan, F. S. S., Santos, D. C. C. dos, Cardoso, E. P., Leite, R. C., and Toral, F. L. B. (2015). Genetic analysis of resistance to ticks, gastrointestinal nematodes and *Eimeria spp.* in Nellore cattle. *Veterinary Parasitology*, *210*(3–4), 224–234.

Payne, S. (2017). Immunity and Resistance to Viruses. In *Viruses* (pp. 61–71). Elsevier.

Pernthaner, A., Cole, S. A., Morrison, L., and Hein, W. R. (2005). Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with *Trichostrongylus colubriformis*. *Infection and Immunity*, *73*(4), 2175–2183.

Pickering, N., Blair, H., Hickson, R., Johnson, P., Dodds, K., and McEwan, J. (2015a). Estimates of genetic parameters for breech strike and potential indirect indicators in sheep. *New Zealand Veterinary Journal*, *63*(2), 98–103.

Pickering, N. K., Auvray, B., Dodds, K. G., and McEwan, J. C. (2015b). Genomic prediction and genome-wide association study for dagginess and host internal parasite resistance in New Zealand sheep. *BMC Genomics*, *16*(1), 958.

Pickering, N. K., Dodds, K. G., Blair, H. T., Hickson, R. E., Johnson, P. L., and McEwan, J. C. (2012). Genetic parameters for production traits in New Zealand dual-purpose sheep, with an emphasis on dagginess. *Journal of Animal Science*, *90*(5), 1411–1420.

Platzer, B., Prosl, H., Cieslicki, M., and Joachim, A. (2005). Epidemiology of *Eimeria* infections in an Austrian milking sheep flock and control with diclazuril. *Veterinary Parasitology*, *129*(1–2), 1–9.

Poland, J., and Rutkoski, J. (2016). Advances and Challenges in Genomic Selection for Disease Resistance. *Annual Review of Phytopathology*, *54*(1), 79–98.

Pollott, G. E., Karlsson, L. J. E., Eady, S., and Greeff, J. C. (2004). Genetic parameters for indicators of host resistance to parasites from weaning to hogget age in Merino sheep. *American Society of Animal Science*, *82*, 2852–2864.

Porto, G., Cruz, E., Teles, M. J., and de Sousa, M. (2019). HFE related hemochromatosis: Uncovering the inextricable link between iron homeostasis and the immunological system. *Pharmaceuticals*, *12*(3), 1–12.

Purcell, S., Neale, B., Todd-brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., Bakker, P. I. W. De, Daly, M. J., and Sham, P. C. (2007). PLINK : A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics*, *81*(September), 559–575.

R Core Team. (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Viena, Austria*. https://www.r-project.org/

Rad, R., Dossumbekova, A., Neu, B., Lang, R., Bauer, S., Saur, D., Gerhard, M., and Prinz, C. (2004). Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during Helicobacter pylori infection. *Gut*, *53*(8), 1082–1089.

Rashidi, H., Mulder, H. A., Arendonk, J. A. M. van, Jong, M. C. M. de, and Stear, M. J. (2014). Genetics of Tolerance and Resistance to Nematode Infection in Sheep. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production*, 7–9.

Raszek, M. M., Guan, L. L., and Plastow, G. S. (2016). Use of Genomic Tools to Improve Cattle Health in the Context of Infectious Diseases. *Frontiers in Genetics*, *7*(MAR), 1–15.

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, *3*, 1–14.

Rauw, W. M., Kanis, E., Noordhuizen-Stassen, E. N., and Grommers, F. J. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science*, *56*(1), 15–33.

Reeg, K. J., Gauly, M., Bauer, C., Mertens, C., Erhardt, G., and Zahner, H. (2005). Coccidial infections in housed lambs: Oocyst excretion, antibody levels and genetic influences on the infection. *Veterinary Parasitology*, *127*(3–4), 209–

219.

Reshma, R. S., and Das, D. N. (2021). Molecular markers and its application in animal breeding. In *Advances in Animal Genomics* (pp. 123–140). Elsevier.

Reuben, A., Chung, J. W., Lapointe, R., and Santos, M. M. (2017). The hemochromatosis protein HFE 20 years later: An emerging role in antigen presentation and in the immune system. *Immunity, Inflammation and Disease*, *5*(3), 218–232.

Reuben, A., Godin-Ethier, J., Santos, M. M., and Lapointe, R. (2015). T lymphocyte-derived TNF and IFN-γ repress HFE expression in cancer cells. *Molecular Immunology*, *65*(2), 259–266.

Rhodes, D. A., Reith, W., and Trowsdale, J. (2016). Regulation of Immunity by Butyrophilins. *Annual Review of Immunology*, *34*(1), 151–172.

Riggio, V., Matika, O., Pong-Wong, R., Stear, M. J., and Bishop, S. C. (2013). Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. *Heredity*, *110*(5), 420–429.

Rout, P. K., Bishop, S. C., Chauhan, K. K., and Singh, S. K. (2015). Genetic resistance to natural coccidiosis infection in goats in a semi-arid region of India. *Cogent Food & Agriculture*, *51*(1), 1–7.

Rowe, A., Gondro, C., Emery, D., and Sangster, N. (2008). Genomic analyses of *Haemonchus contortus* infection in sheep: Abomasal fistulation and two *Haemonchus* strains do not substantially confound host gene expression in microarrays. *Veterinary Parasitology*, *154*(1–2), 71–81.

Rupp, R., Mucha, S., Larroque, H., McEwan, J., and Conington, J. (2016). Genomic application in sheep and goat breeding. *Animal Frontiers*, *6*(1), 39.

Rus, V., and Via, C. S. (2007). Cytokines in Systemic Lupus Erythematosus. In *Systemic Lupus Erythematosus* (First Edit, pp. 109–120). Elsevier.

Safari, E., Fogarty, N. M., and Gilmour, A. R. (2005). A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livestock Production Science*, *92*(3), 271–289.

Saha, C., Das, M., Patil, V., Stephen-Victor, E., Sharma, M., Wymann, S., Jordi, M., Vonarburg, C., Kaveri, S. V, and Bayry, J. (2017). Monomeric immunoglobulin A from plasma inhibits human Th17 responses in vitro independent of FcαRI and DC-SIGN. *Frontiers in Immunology*, *8*(MAR).

Saito, K., Iwata, Y., Fukushima, H., Watanabe, S., Tanaka, Y., Hasegawa, Y., Akiyama, M., and Sugiura, K. (2020). IL-36 receptor antagonist deficiency resulted in delayed wound healing due to excessive recruitment of immune cells. *Scientific Reports*, *10*(1), 14772.

Sallé, G., Jacquiet, P., Gruner, L., Cortet, J., Sauvé, C., Prévot, F., Grisez, C., Bergeaud, J. P., Schibler, L., Tircazes, A., François, D., Pery, C., Bouvier, F., Thouly, J. C., Brunel, J. C., Legarra, A., Elsen, J. M., Bouix, J., Rupp, R., and Moreno, C. R. (2012). A genome scan for QTL affecting resistance to *Haemonchus contortus* in sheep. *Journal of Animal Science*, *90*(13), 4690–4705.

Samiei, G., Yip, W. K., Leong, P. P., Jabar, M. F., Dusa, N. M., Mohtarrudin, N., and Seow, H. F. (2018). Association between polymorphisms of interleukin-17A G197A and interleukin-17F A7488G and risk of colorectal cancer. *Journal of Cancer Research and Therapeutics*, *14*(9), S299–S305.

Sargison, N. (2004). Differential diagnosis of diarrhoea in lambs. *In Practice*, *26*(1), 20–27.

Sargison, N. D. (2008). Appendix B: Overview of the Structure and Economics of the UK Sheep Industry, Highlighting the Increasing Need for Efficient Flock Health Management. In *Sheep Flock Health* (pp. 451–456). Blackwell Publishing, Ltd.

Sargison, N. D. (2013). Understanding the epidemiology of gastrointestinal parasitic infections in sheep: What does a faecal helminth egg count tell us? *Small Ruminant Research*, *110*(2–3), 78–81.

Sargison, N. D. (2014). Sustainable helminth control practices in the United Kingdom. *Small Ruminant Research*, *118*(1–3), 35–40.

Sargison, N. D., Jackson, F., Bartley, D. J., Wilson, D. J., Stenhouse, L. J., and Penny, C. D. (2007). Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. *Veterinary Parasitology*, *145*(1–2), 65–76.

Sayers, E. W., Beck, J., Brister, J. R., Bolton, E. E., Canese, K., Comeau, D. C., Funk, K., Ketter, A., Kim, S., Kimchi, A., Kitts, P. A., Kuznetsov, A., Lathrop, S., Lu, Z., McGarvey, K., Madden, T. L., Murphy, T. D., O'Leary, N., Phan, L., Schneider, V. A., Thibaud-Nissen, F., Trawick, B. W., Pruitt, K. D., and Ostell, J. (2020). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *48*(D1), D9–D16.

Sayers, G., and Sweeney, T. (2005). Gastrointestinal nematode infection in sheep – a review of the alternatives to anthelmintics in parasite control. *Animal Health Research Reviews*, *6*(2), 159–171.

Scheibel, M., Klein, B., Merkle, H., Schulz, M., Fritsch, R., Greten, F. R., Arkan, M. C., Schneider, G., and Schmid, R. M. (2010). IκBβ is an essential co-activator for LPS-induced IL-1β transcription in vivo. *Journal of Experimental Medicine*, *207*(12), 2621–2630.

Sechi, S., Salaris, S., Scala, A., Rupp, R., Moreno, C., Bishop, S., and Casu, S. (2009). Estimation of (co)variance components of nematode parasites resistance and somatic cell count in dairy sheep. *Italian Journal of Animal Science*, *8*(Suppl. 2), 156–158.

Shamri, R., Xenakis, J. J., and Spencer, L. A. (2011). Eosinophils in innate immunity: An evolving story. *Cell and Tissue Research*, *343*(1), 57–83.

Sharma, A., Lee, J. S., Dang, C. G., Sudrajad, P., Kim, H. C., Yeon, S. H., Kang, H. S., and Lee, S.-H. (2015). Stories and Challenges of Genome Wide Association Studies in Livestock — A Review. *Asian-Australasian Journal of Animal Sciences*, *28*(10), 1371–1379.

Sharma, D., Paul, S., Rout, P., Mandal, A., Bhusan, S., Sharma, N., and Kushwah, Y. (2017). Caprine coccidiosis in semi-arid India: Dynamics and factors affecting fecal oocysts count. *Journal of Advanced Veterinary and Animal Research*, *4*(1), 1.

Shaw, R. J., Morris, C. A., Wheeler, M., Tate, M., and Sutherland, I. A. (2012). Salivary IgA: A suitable measure of immunity to gastrointestinal nematodes in sheep. *Veterinary Parasitology*, *186*(1–2), 109–117.

Shouval, D. S., Ouahed, J., Biswas, A., Goettel, J. A., Horwitz, B. H., Klein, C., Muise, A. M., and Snapper, S. B. (2014). Interleukin 10 Receptor Signaling. In *Advances in Immunology* (1st ed., Vol. 122, pp. 177–210). Elsevier Inc.

Smiley, S. T., and Grusby, M. J. (1998). Interleukin 4. In *Encyclopedia of Immunology* (Vol. 1998, Issue May, pp. 1451–1453). Elsevier.

Smith, I. A., Knezevic, B. R., Ammann, J. U., Rhodes, D. A., Aw, D., Palmer, D. B., Mather, I. H., and Trowsdale, J. (2010). BTN1A1, the Mammary Gland Butyrophilin, and BTN2A2 Are Both Inhibitors of T Cell Activation. *The Journal of Immunology*, *184*(7), 3514–3525.

Sparks, A. M. (2017). *Evolutionary genetics of immunity to helminths in wild Soay sheep*. University of Edinburgh.

Sparks, A. M., Watt, K., Sinclair, R., Pilkington, J. G., Pemberton, J. M., Johnston, S. E., McNeilly, T. N., and Nussey, D. H. (2018). Natural selection on antihelminth antibodies in a wild mammal population. *American Naturalist*, *192*(6), 745–760.

Sparks, A. M., Watt, K., Sinclair, R., Pilkington, J. G., Pemberton, J. M., McNeilly, T. N., Nussey, D. H., and Johnston, S. E. (2019). The genetic architecture of helminth-specific immune responses in a wild population of Soay sheep (Ovis aries). *PLOS Genetics*, *15*(11), e1008461.

Stear, M., Bishop, S., Mallard, B., and Raadsma, H. (2001). The sustainability, feasibility and desirability of breeding livestock for disease resistance. *Research in Veterinary Science*, *71*(1), 1–7.

Stear, M. J., Bairden, K., Duncan, J. L., Holmes, P. H., McKellar, Q. A., Park, M., Strain, S., Murray, M., Bishop, S. C., and Gettinby, G. (1997). How hosts control worms. *Nature*, *389*(6646), 27–27.

Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E., and Murray, M. (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology*, *17*(12), 643–652.

Stear, M. J., Boag, B., Cattadori, I., and Murphy, L. (2009). Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: From heritabilities to gene identification. In *Parasite Immunology* (Vol. 31, Issue 5, pp. 274–282).

Stear, M. J., Park, M., and Bishop, S. C. (1996). The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitology Today*, *12*(11), 438–441.

Stear, M. J., Singleton, D., and Matthews, L. (2011). An evolutionary perspective on gastrointestinal nematodes of sheep. *Journal of Helminthology*, *85*(2), 113–120.

Stear, M. J., Strain, S., and Bishop, S. C. (1999a). Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology*, *29*(1), 51–56.

Stear, M. J., Strain, S., and Bishop, S. C. (1999b). How lambs control infection with *Ostertagia circumcincta*. *Veterinary Immunology and Immunopathology*, *72*(1–2), 213–218.

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., and

Lancet, D. (2016). The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Current Protocols in Bioinformatics*, *54*(1), 1.30.1-1.30.33.

Strain, S. A. J., Bishop, S. C., Henderson, N. G., Kerr, A., McKellar, Q. A., Mitchell, S., and Stear, M. J. (2002). The genetic control of IgA activity against Teladorsagia circumcincta and its association with parasite resistance in naturally infected sheep. *Parasitology*, *124*(5), 545–552.

Stubbings, L., Bartley, D., Busin, V., Lovatt, F., Page, P., Rose Vineer, H., and Skuce, P. (2020). *SCOPS Internal Parasite Technical Manual*.

Su, Z., Segura, M., and Stevenson, M. M. (2006). Reduced protective efficacy of a blood-stage malaria vaccine by concurrent nematode infection. *Infection and Immunity*, *74*(4), 2138–2144.

Sukura, A., Higgins, J., and Pedersen, N. C. (1998). Compartmentalization of Th1/Th2 cytokine responses to experimental Yersinia pseudotuberculosis infection in cats. *Veterinary Immunology and Immunopathology*, *65*(2–4), 139–150.

Sutherland, M., Dowling, S., Shaw, R., Hickey, J., Fraser, D., Cameron, C., and Sutherland, I. (2019). Stress-induced immunomodulation in low and high reactive sheep. *Animals*, *9*(3), 1–13.

Sweeney, T., Hanrahan, J. P., Ryan, M. T., and Good, B. (2016). Immunogenomics of gastrointestinal nematode infection in ruminants – breeding for resistance to produce food sustainably and safely. *Parasite Immunology*, *38*(9), 569–586.

Tatari-Calderone, Z., Semnani, R. T., Nutman, T. B., Schlom, J., and Sabzevari, H. (2002). Acquisition of CD80 by Human T Cells at Early Stages of Activation: Functional Involvement of CD80 Acquisition in T Cell to T Cell Interaction. *The Journal of Immunology*, *169*(11), 6162–6169.

Taubert, A., Behrendt, J. H., Sühwold, A., Zahner, H., and Hermosilla, C. (2009). Monocyte- and macrophage-mediated immune reactions against *Eimeria bovis*. *Veterinary Parasitology*, *164*(2–4), 141–153.

Taylor, M. (1995). Diagnosis and control of coccidiosis in sheep. *In Practice*, *17*, 172–177.

The Gene Ontology Consortium. (2021). The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Research*, *49*(D1), D325–D334.

Thompson-Crispi, K. A., Sargolzaei, M., Ventura, R., Abo-Ismail, M., Miglior, F.,

Schenkel, F., and Mallard, B. A. (2014). A genome-wide association study of immune response traits in Canadian Holstein cattle. *BMC Genomics*, *15*(1), 559.

Thompson-Crispi, K. A., Sewalem, A., Miglior, F., and Mallard, B. A. (2012). Genetic parameters of adaptive immune response traits in Canadian Holsteins. *Journal of Dairy Science*, *95*(1), 401–409.

Thornton, A. M. (2010). Regulatory T Cells. In *Comprehensive Toxicology* (pp. 87–107). Elsevier.

Traversa, D., and von Samson-Himmelstjerna, G. (2016). Anthelmintic resistance in sheep gastro-intestinal strongyles in Europe. *Small Ruminant Research*, *135*, 75–80.

Turner, J. D., Jackson, J. A., Faulkner, H., Behnke, J., Else, K. J., Kamgno, J., Boussinesq, M., and Bradley, J. E. (2008). Intensity of Intestinal Infection with Multiple Worm Species Is Related to Regulatory Cytokine Output and Immune Hyporesponsiveness. *The Journal of Infectious Diseases*, *197*(8), 1204–1212.

Turner, S. P., Conington, J., and Dwyer, C. M. (2015). Opinion paper: Is there a role for breeding for welfare improvement? *Animal*, *9*(8), 1265–1267.

Van De Veerdonk, F. L., Stoeckman, A. K., Wu, G., Boeckermann, A. N., Azam, T., Netea, M. G., Joosten, L. A. B., Van Der Meer, J. W. M., Hao, R., Kalabokis, V., and Dinarello, C. A. (2012). IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(8), 3001–3005.

Van Dijk, J., Sargison, N. D., Kenyon, F., and Skuce, P. J. (2010). Climate change and infectious disease: Helminthological challenges to farmed ruminants in temperate regions. *Animal*, *4*(3), 377–392.

Van Houtert, M. F. J., and Sykes, A. R. (1996). Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology*, *26*(11), 1151–1167.

Van Oosterhout, A. J. M., and Motta, A. C. (2005). Th1/Th2 paradigm: not seeing the forest for the trees? *The European Respiratory Journal*, *25*(4), 591–593.

VanRaden, P. M., O'Connell, J. R., Wiggans, G. R., and Weigel, K. A. (2011). Genomic evaluations with many more genotypes. *Genetics Selection Evolution*, *43*(1), 10.

VanRaden, P. M. (2008). Efficient Methods to Compute Genomic Predictions. *Journal of Dairy Science*, *91*(11), 4414–4423.

Venturina, V. M. (2012). *Relationship between an inflammatory mucosal T cell response and susceptibility of sheep to Teladorsagia circumcincta infection*. University of Edinburgh.

Venturina, V. M., Gossner, A. G., and Hopkins, J. (2013). The immunology and genetics of resistance of sheep to *Teladorsagia circumcincta*. *Veterinary Research Communications*, *37*(2), 171–181.

Vercruysse, J., Schetters, T. P. M., Knox, D. P., Willadsen, P., and Claerebout, E. (2007). Control of parasitic disease using vaccines: an answer to drug resistance? *Revue Scientifique et Technique (International Office of Epizootics)*, *26*(1), 105–115. http://www.ncbi.nlm.nih.gov/pubmed/17633296

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., and Ugolini, S. (2008). Functions of natural killer cells. *Nature Immunology*, *9*(5), 503–510.

Wakelin, D., Rose, M. E., Hesketh, P., Else, K. J., and Grencis, R. K. (1993). Immunity to coccidiosis: genetic influences on lymphocyte and cytokine responses to infection with *Eimeria vermiformis* in inbred mice. *Parasite Immunology*, *15*(1), 11–19.

Walker, J. A., and McKenzie, A. N. J. (2018). TH2 cell development and function. *Nature Reviews Immunology*, *18*(2), 121–133.

Wang, O., McAllister, T. A., Plastow, G., Stanford, K., Selinger, B., and Guan, L. L. (2017). Host mechanisms involved in cattle Escherichia coli O157 shedding: A fundamental understanding for reducing foodborne pathogen in food animal production. *Scientific Reports*, *7*(1), 1–15.

Wang, S., Hu, D., Wang, C., Tang, X., Du, M., Gu, X., Suo, J., Hu, M., Fang, R., Zhu, X., Zhang, X., Du, A., Suo, X., and Liu, X. (2019). Transcriptional profiling of innate immune responses in sheep PBMCs induced by *Haemonchus contortus* soluble extracts. *Parasites & Vectors*, *12*(1), 182.

Wang, W., Xie, T., Jiang, F., Jiang, Y., Jin, Z., Yang, Z., Kong, M., Cheng, L., Fu, L., and Du, X. (2022). Protein interactions network of goat innate immune signalling pathway proteins and *Haemonchus contortus* excretory-secretory proteins. *Parasite Immunology*, *44*(12), 1–11.

Watson, T. G., Baker, R. L., and Harvey, T. G. (1986). Genetic variation in resistance or tolerance to internal nematode parasites in strains of sheep at Rotomahana. *Proceedings of the New Zealand Society of Animal Production*,

23–26.

Watts, J. E., Dash, K. M., and Lisle, K. A. (1978). The Effect of Anthelmintic Treatment and Other Management Factors on the Incidence of BReech Strike In Merino Sheep. *Australian Veterinary Journal*, *54*(7), 352–355.

Whitlock, H. V. (1948). Some modifications of the McMaster helminth egg-counting technique and apparatus. *Journal of the Council for Scientific and Industrial Research*, *21*(3), 177–180.

Wilkie, H. (2016). *Identifying and Exploiting the Molecular Basis of Resistance to Gastro-intestinal Nematodes in Sheep*. University of Edinburgh.

Wilkie, H., Nicol, L., Gossner, A., and Hopkins, J. (2016). Mucosal Expression of T Cell Gene Variants Is Associated with Differential Resistance to *Teladorsagia circumcincta*. *PLOS ONE*, *11*(12), e0168194.

Williams, A. R., and Palmer, D. G. (2012). Interactions between gastrointestinal nematode parasites and diarrhoea in sheep: Pathogenesis and control. *The Veterinary Journal*, *192*(3), 279–285.

Wolf, B. T., Howells, K., Nakielny, C., Haresign, W., Lewis, R. M., Davies, O., and Davies, M. H. (2008). Genetic parameters for strongyle and Nematodirus faecal egg counts in lambs and their relationships with performance traits. *Livestock Science*, *113*(2–3), 209–217.

Woolaston, R. R., Elwin, R. L., and Barger, I. A. (1992). No adaptation of *Haemonchus contortus* to genetically resistant sheep. *International Journal for Parasitology*, *22*(3), 377–380.

Wormwise Technical Advisory Group. (2019). *Wormwise - National Worm Management Strategy* (Issue July). https://beeflambnz.com/knowledge-hub/PDF/wormwise-resource-book

Wright, N. (2013). *Economic Impact of Health and Welfare Issues in Beef Cattle and Sheep in England*. http://beefandlamb.ahdb.org.uk/wp/wp-content/uploads/2013/04/Economic-Impact-of-Health-Welfare-Final-Rpt-170413.pdf

Xie, L., Huang, Z., Li, H., Liu, X., Zheng, S., and Su, W. (2019). IL-38: A New Player in Inflammatory Autoimmune Disorders. *Biomolecules*, *9*(8), 345.

Xu, H., Timares, L., and Elmets, C. A. (2019). Host Defenses in Skin. In *Clinical Immunology* (Fifth Edit, Vol. 2, Issue Chapter 5, pp. 273-283.e1). Elsevier.

Yamazaki, T., Goya, I., Graf, D., Craig, S., Martin-Orozco, N., and Dong, C. (2010). A Butyrophilin Family Member Critically Inhibits T Cell Activation. *The Journal of Immunology*, *185*(10), 5907–5914.

Yates, A. D., Achuthan, P., Akanni, W., Allen, J., Allen, J., Alvarez-Jarreta, J., Amode, M. R., Armean, I. M., Azov, A. G., Bennett, R., Bhai, J., Billis, K., Boddu, S., Marugán, J. C., Cummins, C., Davidson, C., Dodiya, K., Fatima, R., Gall, A., Giron, C. G., Gil, L., Grego, T., Haggerty, L., Haskell, E., Hourlier, T., Izuogu, O. G., Janacek, S. H., Juettemann, T., Kay, M., Lavidas, I., Le, T., Lemos, D., Martinez, J. G., Maurel, T., McDowall, M., McMahon, A., Mohanan, S., Moore, B., Nuhn, M., Oheh, D. N., Parker, A., Parton, A., Patricio, M., Sakthivel, M. P., Abdul Salam, A. I., Schmitt, B. M., Schuilenburg, H., Sheppard, D., Sycheva, M., Szuba, M., Taylor, K., Thormann, A., Threadgold, G., Vullo, A., Walts, B., Winterbottom, A., Zadissa, A., Chakiachvili, M., Flint, B., Frankish, A., Hunt, S. E., Iisley, G., Kostadima, M., Langridge, N., Loveland, J. E., Martin, F. J., Morales, J., Mudge, J. M., Muffato, M., Perry, E., Ruffier, M., Trevanion, S. J., Cunningham, F., Howe, K. L., Zerbino, D. R., and Flicek, P. (2019). Ensembl 2020. *Nucleic Acids Research*, *48*(D1), D682–D688.

Yokoyama, K., Terao, T., and Osawa, T. (1977). Isolation and characterization of membrane receptors for pokeweed mitogens from mouse lymphocytes. *Biochemical Journal*, *165*(3), 431–437.

Young, S., Fenn, J., Arriero, E., Lowe, A., Poulin, B., MacColl, A. D. C., and Bradley, J. E. (2020). Relationships between immune gene expression and circulating cytokine levels in wild house mice. *Ecology and Evolution*, *10*(24), 13860–13871.

Yun, C. H., Lillehoj, H. S., and Lillehoj, E. P. (2000). Intestinal immune responses to coccidiosis. *Developmental & Comparative Immunology*, *24*(2–3), 303–324.

Zaghi, E., Calvi, M., Marcenaro, E., Mavilio, D., and Di Vito, C. (2019). Targeting NKG2A to elucidate natural killer cell ontogenesis and to develop novel immune-therapeutic strategies in cancer therapy. *Journal of Leukocyte Biology*, *105*(6), 1243–1251.

Zhao, Z., Wang, M., Liu, S., Palmer, D., Shaw, R., Karlsson, J., Vercoe, P. E., Martin, G. B., and Greeff, J. (2019). Heritabilities of IgA and IgE activities against *Teladorsagia* and *Trichostrongylus* L3 larval antigens correlated with traits for faecal worm egg count, health and productivity in Merino sheep. *Animal Production Science*, *59*(10), 1792.

Zhou, L., Chong, M. M. W., and Littman, D. R. (2009). Plasticity of CD4+ T Cell

Lineage Differentiation. *Immunity*, *30*(5), 646–655.

Zhou, X., and Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics*, *44*(7), 821–824.

Zhu, J., and Paul, W. E. (2010). Heterogeneity and plasticity of T helper cells. *Cell Research*, *20*(1), 4–12.

Zvinorova, P. I., Halimani, T. E., Muchadeyi, F. C., Matika, O., Riggio, V., and Dzama, K. (2016). Breeding for resistance to gastrointestinal nematodes – the potential in low-input/output small ruminant production systems. *Veterinary Parasitology*, *225*, 19–28.

Zvinorova, P. I., Jonsson, N., and Dzama, K. (2018). The genetics of parasite resistance in tropical animal production: Some insights. *Proceedings of the World Congress on Genetics Applied to Livestock Production*.

Appendix – Supplementary tables of results

Supplementary Table S4.1 – Gene ontology results summary.

Trait	Gene	GO term	GO term name
FEC_S[1]	*CCNB2*	GO:0043029	T cell homeostasis
	RNF111	GO:0030511	Positive regulation of TGF-β receptor signalling pathway
FEC_N[1]	*CLEC7A*	GO:0008329	Signalling pattern recognition receptor activity
	LOC101116896	GO:0002223	Stimulatory C-type lectin signalling pathway
		GO:0030101	Natural killer cell activation
		GO:0032729	Positive regulation of IFN-γ production
		GO:0045954	Positive regulation of killer cell mediated cytotoxicity
		GO:0032394	MHC class Ib receptor activity
	ZFP36	GO:0045085	Negative regulation of IL-2 biosynthetic process
		GO:0050728	Negative regulation of inflammatory response
		GO:0032680	Regulation of tumour necrosis factor production
$IL\text{-}4_{(PWM)}$[1]	*MYLK3*	GO:0002528	Regulation of vascular permeability involved in acute inflammatory response
		GO:0071347	Cellular response to IL-1
IgA[1]	*FCGR1A*	GO:0001788	Antibody-dependent cellular cytotoxicity
		GO:0042590	Antigen processing and presentation of exogenous peptide antigen via MHC class I
		GO:0042742	Defence response to bacterium
		GO:0050766	Positive regulation phagocytosis
		GO:0019770	IgG receptor activity
	CTSS	GO:0002250	Adaptive immune response
		GO:0030574	Antigen processing and presentation of peptide antigen

Trait	Gene	GO term	GO term name
IgA[1]	*ECM1*	GO:0001960	Negative regulation of cytokine mediated signalling pathway
		GO:0002828	Regulation of Type 2 immune response
		GO:0006954	Inflammatory response
		GO:0043123	Positive regulation of I-kappa B kinase/NK-kappa B signalling
		GO:2000404	Regulation of T cell migration
	IL17A	GO:0006954	Inflammatory response
		GO:0032747	Positive regulation of IL-23 production
		GO:0071347	Cellular response to IL-1
		GO:1900017	Positive regulation of cytokine production involved in inflammatory response
		GO:2000778	Positive regulation of IL-6 secretion
	IL17F	GO:0006984	Inflammatory response
		GO:0017015	Regulation of TGF-β receptor signalling pathway
		GO:0045076	Regulation of IL-2 biosynthetic process
		GO:0045408	Regulation of IL-6 biosynthetic process
		GO:0045414	Regulation of IL-8 biosynthetic process
		GO:1900017	Positive regulation of cytokine production involved in inflammatory response
		GO:2000778	Positive regulation of IL-6 secretion
FEC_N[2]	*CD80*	GO:0031295	T cell co-stimulation
		GO:0046641	Positive alpha-beta T cell proliferation
IFN-$\gamma_{(PWM)}$[2]	*BTN2A2*	GO:0045591	Positive regulatory T cell differentiation
		GO:0046007	Negative regulation of activated T cell proliferation

Trait	Gene	GO term	GO term name
IFN-$\gamma_{(PWM)}$[2]	*BTN2A2*	GO:0050710	Negative regulation of cytokine secretion
		GO:0050860	Negative regulation of T cell receptor signalling pathway
	HFE	GO:0002626	Negative regulation of T cell antigen processing and presentation
		GO:0002725	Negative regulation of T cell cytokine production
		GO:1904283	Negative regulation of antigen processing and presentation of endogenous peptides antigens via MHC class I
		GO:2001186	Negative regulation of CD8-positive, alpha-beta T cell activation
		GO:1990712	HFE-transferrin receptor complex
	TRIM38	GO:0032648	Regulation of IFN-β production
		GO:0043123	Positive regulation I-kappa B/NF-kappa B signalling
		GO:0050687	Negative regulation of defence response to virus
		GO:0051092	Positive regulation of NF-kappa B transcription factor activity
IL-$4_{(PWM)}$[2]	*PLA2G12B*	GO:0004623	Phospholipase A2 activity
IgA[2]	*SYK*	GO:0002281	Macrophage activation involved in immune response
		GO:0002283	Neutrophil activation involved in immune response
		GO:0032481	Positive regulation of type I interferon production
		GO:0042742	Defence response to bacterium
		GO:0045087	Innate immune response
		GO:0045401	Positive regulation of IL-3 biosynthetic process
		GO:0045579	Positive regulation of B cell differentiation
		GO:0045588	Positive regulation of gamma-delta T cell differentiation

Trait	Gene	GO term	GO term name
IgA[2]	*SYK*	GO:0046638	Positive regulation of alpha-delta T cell differentiation
		GO:0046641	Positive regulation of alpha-delta T cell proliferation
		GO:0050715	Positive regulation of cytokine secretion
		GO:0050853	B cell receptor signalling pathway
		GO:0019815	B cell receptor complex
		GO:0032009	Early phagosome
		GO:0042101	T cell receptor complex
	NFIL3	GO:0006955	Immune response
		GO:0071353	Cellular response to IL-4
	IL1A	GO:0002248	Connective tissue replacement involved in inflammatory response to wound healing
		GO:0006955	Immune response
		GO:0032755	Positive regulation of IL-6 production
		GO:0045086	Positive regulation of IL-2 biosynthetic process
		GO:0050715	Positive regulation of cytokine secretion
		GO:0070498	IL-1-mediated signalling pathway
	IL1B	GO:0006954	Inflammatory response
		GO:0006955	Immune response
		GO:0042742	Defence response to bacterium
	IL1RN	GO:0001960	Negative regulation of cytokine-mediated signalling pathway
		GO:2000660	Negative regulation of IL-1-mediated signalling pathway
	IL36A	GO:0006954	Inflammatory response

Trait	Gene	GO term	GO term name
IgA[2]	*IL36A*	GO:0006955	Immune response
		GO:0032755	Positive regulation of IL-6 production
	IL36B	GO:0006954	Inflammatory response
		GO:0006955	Immune response
		GO:0032755	Positive regulation of IL-6 production
		GO:0045582	Positive regulation of T cell differentiation
	IL36RN	GO:0001960	Negative regulation of cytokine-mediated signalling pathway
		GO:0019732	Antifungal humoral response
		GO:0032700	Negative regulation of IL-17 production
		GO:0032715	Negative regulation of IL-6 production
		GO:1902714	Negative regulation of IFN-γ secretion
	IL37	GO:0006954	Inflammatory response
		GO:0006955	Immune response
	TRIM28	GO:0045087	Innate immune response
	PLD4	GO:0006909	Phagocytosis

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; LWT = live weight; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant; GO term = Gene Ontology term.

Supplementary Table S4.2 – KEGG pathway results summary.

Trait	Gene	KEGG	KEGG name
FEC_N[1]	*CLEC7A*	oas04145	Phagosome
	LOC101116896	oas04650	Natural killer cell mediated cytotoxicity
	LOC101104216	oas04612	Antigen processing and presentation
	LOC101123288	oas04612	Antigen processing and presentation
	KLRD1	oas04612	Antigen processing and presentation
		oas04650	Natural killer cell mediated cytotoxicity
	LOC101102227	oas04612	Antigen processing and presentation
		oas04650	Natural killer cell mediated cytotoxicity
	OLR1	oas04145	Phagosome
	NFKBIB	oas04062	Chemokine signalling pathway
		oas04621	NOD-like receptor signalling pathway
		oas04660	T cell receptor signalling pathway
		oas04662	B cell receptor signalling pathway
DAG[1]	*BLNK*	oas04064	NF-kappa B signalling pathway
		oas04380	Osteoclast
		oas04662	B cell receptor signalling pathway
		oas05340	Primary immunodeficiency
IgA[1]	*FCGR1A*	oas04145	Phagosome
		oas04380	Osteoclast differentiation
		oas04640	Hematopoietic cell lineage
		oas04666	Fc gamma R-mediated phagocytosis
	CTSS	oas04145	Phagosome
		oas04612	Antigen processing and presentation
	IL17A	oas04060	Cytokine-cytokine reception interaction
		oas05321	Inflammatory bowel disease (IBD)
	IL17F	oas05321	Inflammatory bowel disease (IBD)
FEC_N[2]	*CD80*	oas04514	Cell adhesion molecules (CAMs)
		oas04620	Toll-receptor signalling pathway
		oas04672	Intestinal immune network for IgA production
IgA[2]	*SYK*	oas04064	NF-kappa B signalling pathway
		oas04380	Osteoclast differentiation
		oas04650	Natural killer cell mediated cytotoxic

Trait	Gene	KEGG	KEGG name
IgA[2]	*SYK*	oas04662	B cell receptor signalling pathway
		oas04666	Fc gamma R-mediated phagocytosis
	IL1A	oas04010	MAPK signalling pathway
		oas04060	Cytokine-cytokine receptor interaction
		oas04380	Osteoclast differentiation
		oas04640	Hematopoietic cell lineage
		oas05321	Inflammatory bowel disease (IBD)
	IL1B	oas04010	MAPK signalling pathway
		oas04060	Cytokine-cytokine receptor interaction
		oas04064	NF-kappa B signalling pathway
		oas04380	Osteoclast differentiation
		oas04620	Toll-like receptor signalling pathway
		oas04621	NOD-like receptor signalling pathway
		oas04640	Hematopoietic cell lineage
		oas04668	TNF signalling pathway
		oas04750	Inflammatory mediator regulation of TRP channels
		oas05321	Inflammatory bowel disease (IBD)

[1]Recording occasion no.1; [2]Recording occasion no.2. FEC_S = Strongyles faecal egg count; FEC_N = *Nematodirus* faecal egg count; FOC = *Coccidia* oocyst count; DAG = dag scores; LWT = live weight; IFN-γ = Interferon-gamma; IL-4 = Interleukin-4; IL-10 = Interleukin-10; IgA = parasite specific Immunoglobulin A; PWM = Pokeweed mitogen stimulant; T-ci = Larval (L4) *T. circumcincta* specific antigen stimulant. KEGG = pathway identification.