Parasite Immunology, 2009, **31**, 274–282 DOI: 10.1111/j.1365-3024.2009.01105.x

Review Article

Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification

M. J. STEAR, B. BOAG, I. CATTADORI & L. MURPHY

Institute of Comparative Medicine, University of Glasgow, Glasgow, UK

SUMMARY

In cool temperate areas, such as Scotland, sheep are infected by a variety of nematodes but the dominant nematode is Teladorsagia circumcincta. *Resistant animals have one or more of the following features: fewer adult nematodes, more inhibited larvae, shorter adult nematodes and decreased production of nematode eggs. In lambs at the end of the first grazing season, the heritability of adult worm length is very strong, whereas the heritability of egg production is moderate. The heritability of worm number is low while there is no detectable genetic variation in the number of inhibited larvae. The major mechanisms underlying resistance to* T. circumcincta *appear to be the IgA mediated suppression of worm growth and the mast cell mediated regulation of worm number. Mast cell responses are slow to develop, possibly because they are responsible for protein loss and reduced growth of the host. Two genes have been repeatedly associated with resistance to* T. Circumcincta: *the MHC class II* DRB1 *locus on chromosome 20 and the interferon-*γ *locus on chromosome 3. Although the causative mutations are still unknown both genes are plausible candidates.*

Keywords: *sheep, nematode, genetics, immunity, pathology, breeding, MHC, IgA, IgE interferon*

Correspondence: M. J. Stear, Center for Infectious Disease Dynamics, Department of Biology, the Pennsylvania State University, University Park, PA 16802, USA
(e-mail: m.stear@vet.gla.ac.uk).
Present address: I. Cattadori, Center for Infectious Disease Dynamics, Department of Biology, the Pennsylvania State University, University Park, Pennsylvania PA 16802, USA
Received: 30 June 2008
Accepted for publication: 23 December 2008
Disclosure: The authors received an honorarium from the Publisher for preparation of this article.

INTRODUCTION

Nematodes present a serious threat to the health and welfare of humans, livestock and wild animals. They are among the most prevalent of all infections. About one-quarter of the human population is infected (1). The prevalence in grazing lambs can reach 100% (2) and they are among the most important infections faced by livestock (3). In wild grouse (4,5), mountain hares (6) and feral sheep (7) nematodes help to regulate the size of the host population.

Sheep are a particularly powerful host for studying nematode infections. Many of the major advances in understanding nematode infection have come from mathematical modelling of natural infections (8–12) or the detailed analysis of experimental infections (13–15). Sheep are one of the few species where both approaches can be easily combined. The results obtained from modelling or in the laboratory can be tested in natural infections. The high prevalence simplifies the design and analysis of experiments. The economic importance of nematodes in livestock ensures that the results are of general interest. Furthermore, nematodes and their ancestors have been infecting sheep and their ancestors ever since the divergence of the bovidae from the cervidae 20–40 million years ago. This long evolutionary history has produced a rich and complex series of co-adaptations by host and parasite.

NATURAL HISTORY

Most parasitic nematodes have a free-living stage and their growth and survival is constrained by the prevailing temperature and moisture. The nematodes of sheep form three communities. In hot regions with adequate rainfall, such as much of Australia and Africa, the parasitic nematode community is dominated by *Haemonchus contortus*. In warm climates, such as New Zealand, the nematode

community is dominated by two species: *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. In cool, temperate climates, such as Scotland, the predominant nematode is *T. circumcincta*. There are many differences among hosts and parasites in the different regions. Sheep belong to different breeds. They are managed differently; immunity is dependent on exposure, genetics and nutrition and all three differ among the communities. To avoid unnecessary confusion, this review will focus on cool temperate areas.

Often lambs are infected with several different species of nematode. In Scotland, postmortem analyses (2) found seven taxa of nematodes in naturally infected lambs from one field of an upland farm in Strathclyde: *T. circumcincta*, *T. axei* and *H. contortus* in the abomasum; *T. vitrinus*, several (undifferentiated) species from the genus *Cooperia*, several (undifferentiated) species of *Nematodirus* and *Bunostomum trigonocephalum* in the small intestine.

The life cycle of most gastrointestinal nematodes of livestock is simple and involves only one host (16). For *T. circumcincta*, adult male and female nematodes live and mate in the abomasum. Each female can breed with several different males (Gilleard, personal communication). Eggs are passed in the faeces and then develop through to third stage larvae. These third-stage larvae leave the faeces and a proportion of these larvae are ingested by grazing sheep. They shed their sheath, moult into fourth-stage larvae and move into the gastric glands. The time taken to mature in the gastric glands varies but in naïve lambs, larvae emerge as young adults after about a week. The adults live and breed in the abomasal mucus.

In most natural host–nematode systems, the infection cycle is, of necessity, well-adapted to the host. There is wide variation among countries and regions in the way sheep are kept. In a typical Scottish upland farm, lambs are born in late March to early April. They are weaned at 3–4 months, when they or their mothers are removed to separate pastures. At 6–7 months of age when the grass has stopped growing, the lambs are sorted. Some lambs are kept to replace old ewes but most lambs are sold for meat or to lowland farms for finishing.

The infection cycle begins when lambs start to graze. A small number of free-living parasites will survive on pasture over the winter but most infective larvae come from freshly deposited eggs. Adult ewes normally lay very few eggs but round about lambing time there is a marked increase in the number of nematode eggs produced in the faeces – a phenomenon known as the periparturient or spring rise (17,18). This increase in nematode egg production appears to be signalled by the poor nutritional status of the mother in late pregnancy and early lactation. It can be reversed by supplementary feeding of the pregnant ewe (17–21). Whether the nematode responds directly to the poor nutritional status of the ewe or the poor nutritional status weakens the immune response and allows the nematode to escape immune regulation is unknown.

The rate at which nematode eggs are produced by lambs changes during the grazing season. It is strongly influenced by the weather, especially rainfall and temperature, and the way sheep are managed, especially the frequency of anthelmintic treatment, the stocking density and the number of times that sheep are moved onto new fields during the grazing season. In a typical year, egg output starts at a low level, rises to a peak in mid-season as the number of infective larvae contaminating the pasture increases then declines as the immune response starts to control egg output (22). Often, but not always, there is a second rise at the end of the grazing season and this appears to be due the development of additional species of nematodes such as *Cooperia* and *Trichostrongylus* species (23).

THE SOURCES OF VARIATION IN RESISTANCE TO NEMATODES

Lambs vary in the number of eggs that they produce. The distribution of nematode egg counts among hosts is usually skewed and overdispersed. Most lambs produce relatively few eggs while a small proportion of lambs produce very high numbers of eggs. These 'supershedders' make a disproportionate contribution to the transmission of nematode infections.

Two methods have been used to characterize the variation among hosts in egg production. Taylor's power law models the variance of a population as a function of the mean number of individuals; specifically the variance is equal to $a\,(\text{mean})^b$ where a and b are specific for each population. For most wild populations, b lies between 1 and 2. For egg counts in Scottish sheep, $b = 1{\cdot}23 \pm 0{\cdot}08$ (24), suggesting that the variance in egg numbers is similar to the variance in other populations.

The negative binomial distribution has also been used to quantify the variation (25,26). The negative binomial is characterized by two parameters: the mean and the shape parameter k which is an inverse index of overdispersion. The mean and k are usually positively correlated (27,28). Mean egg counts in Scottish sheep ranged from 50 to 570 eggs per gram of faeces while k ranged from 0·09 to 2·59 (29).

A combination of statistical analysis and the techniques of quantitative genetics can identify and quantify the sources of variation among lambs in the intensity of nematode infection (30). Five cohorts of 200 lambs were sampled every 28 days from 1 to 5 months of age and again 6 weeks later. These lambs were all from the Scottish Blackface breed and were raised since weaning on the same field on the same Scottish upland farm.

The analyses demonstrated that the variation in faecal egg output could be assigned to just eight sources: additive genetic variation (30%), maternal common environmental effects (14%), factors unique to each individual (22%), measurement variation (22%), type of birth (single or twin 5%) early faecal egg count (4%) sex (2%) and date of birth (1%). These values refer to 5-month-old lambs. The effects are dynamic. For example, genetic variation increases from essentially zero at 1 and 2 months of age while maternal effects decline as the lambs mature.

The largest source of variation is additive genetic variation. Additive genetic variation is the sum of the average effects of genes and is estimated from the similarity among relatives (31). The ratio of additive genetic variation to the total variation, excluding fixed effects, is known as the heritability. The heritability of faecal nematode egg counts is similar to the heritability of milk production in dairy cattle and the heritability of growth rate in beef cattle. These heritabilities are high enough to justify the selective breeding of sheep for reduced nematode egg output (32).

The combination of reduced environmental contamination and enhanced genetic resistance creates a virtuous spiral (33). As selection proceeds, sheep become more resistant and produce fewer eggs which lowers the number of infective larvae on pasture. Lower numbers of infective larvae and increased resistance among hosts mean that even fewer nematodes establish and they produce fewer eggs.

Genetic variation among sheep in nematode egg production was first demonstrated and quantified in Australia (34). Both Australia and New Zealand have well-established experimental and commercial breeding schemes for reduced nematode egg production (35–39). The UK is now following their lead.

Faecal egg count is currently the method of choice to identify animals that are relatively resistant to *T. circumcincta*. However, sampling faeces can be hazardous, the heritability of faecal egg count is not very high and egg counts have a complex relationship with the worm burden (40). Interestingly, other markers of resistance such as parasite specific IgA activity, peripheral eosinophilia and pepsinogenaemia are safer and simpler to measure and can be more heritable (41–44). These traits could serve as alternative or complementary markers of nematode resistance.

THE MECHANISMS UNDERLYING RESISTANCE TO INFECTION

The production of nematode eggs is determined by the number of adult female worms in each host and by their mean fecundity. The mean number of adult *T. circumcincta* in Scottish lambs at the end of the grazing season varied over four successive years from 1548 to 6570. The number of nematodes was overdispersed. The distribution of adult *T. circumcincta* among lambs was similar to a negative binomial distribution. For each year k lay between 1 and 2. These values of k are similar to those found in naturally infected Australian sheep (45).

For Scottish Blackface lambs at the end of the grazing season, the heritability of the number of adult *T. circumcincta* in lambs was $0{\cdot}14 \pm 0{\cdot}10$ (46). This is a relatively low value and not significantly different from zero. One explanation for the low heritability of worm number is that lambs are slow to develop the ability to control the number of abomasal parasites (47,48).

The major mechanism controlling the number of adult *T. circumcincta* appears to be mast cell degranulation (48,49). Variation in the number of discharged mast cells (globule leucocytes) accounts in a statistical sense for about one-third of the total variation in worm number following deliberate infection (49). Presumably some of the remaining variation in worm number is due to variation in IgE concentration and specificity. Measurement variation will also account for some of the variation in worm number. It is difficult to obtain accurate and precise estimates of globule leukocyte concentrations and nematode numbers.

The length of adult female nematodes is an indication of their fecundity (50): In Scottish sheep, adult *T. circumcincta* range between 0·6 and 1·2 cm in length with longer worms laying more eggs per day (50). The estimated fecundity ranged from 0 to 350 eggs per worm per day. Most females fell in the lower part of this range.

The heritability of worm length in Scottish Blackface lambs at the end of the grazing season is remarkably high at $0{\cdot}62 \pm 0{\cdot}20$ (46). The major mechanism regulating the growth and development of *T. circumcincta* appears to be IgA activity against fourth-stage larvae (41,49), possibly in conjunction with eosinophils (42). Variation in IgA activity in deliberately infected Scottish sheep accounted for about 40% of the variation in worm length (49). Much of the remaining variation in worm length is due to variation in IgA specificity although this is confounded with IgA activity. Density-dependent regulation of worm length also accounts for about 20% of the variation.

There is a density-dependent relationship between the number of adult *T. circumcincta* and their mean fecundity. As the number of adult nematodes increases the fecundity declines but it is unclear whether this is due to competition among nematodes for food and other resources or to increased immune responses in more heavily infected lambs.

The density-dependent decline in nematode fecundity creates a complex relationship between egg production and the number of adult *T. circumcincta*. The relationship is similar to a gamma-distribution (40). As the number of adult *T. circumcincta* increases from zero, egg production

also increases but peaks at a relatively low intensity of infection. Beyond a total burden of 3000–4000 worms, egg production declines as worm numbers increase and at high intensities of infection very few eggs are produced at all.

Teladorsagia circumcincta can inhibit development at the early fourth larval stage. This inhibition is influenced by the season, genetic variation in the parasite, the host immune response and the number of adult nematodes (51). Inhibition is thought to be an adaptation to unfavourable conditions. Greater numbers of incoming larvae undergo inhibition at the end of the grazing season to avoid producing offspring during the winter when their chances of surviving to infect hosts is low. Different strains of parasite are known to vary in the proportion of larvae that undergo inhibition. Lambs with higher IgA activity against fourth stage larvae have greater numbers of inhibited larvae (51,52). Although IgA may be produced too slowly to be directly responsible (53). Finally deliberate infections with large numbers of larvae produces disproportionately more inhibited larvae (54). The heritability of the number of fourth-stage larvae in naturally infected Scottish sheep at the end of the grazing season is 0 (46), indicating that host factors are relatively unimportant at this time.

There are several measures of susceptibility to nematode infection: egg output, mean length of adult worms, number of adult nematodes and the number of inhibited larvae. These measures are all useful in the right context but they do not measure the same thing.

PATHOLOGY

The high heritabilities of egg production following nematode infection demonstrate that selective breeding is feasible. The desirability of selective breeding for resistance to nematode infection depends upon the severity of the infection.

Different species of nematodes produce different clinical signs. *Haemonchus contortus* produces gastropathy exacerbated by anaemia but mixed, predominantly *T. circumcincta* infection of sheep is characterized by gastroenteropathy. The abomasal mucosa is damaged during infection (55). The epithelial barrier is breached as the tight junctions between epithelial cells are destroyed. The mucosa is hyperplastic and many cells are dedifferentiated. There is an increase in the pH of the abomasal fluid and an increased production of mucus. The destruction of the abomasal architecture results in increased concentrations of gastrin and pepsinogen in the plasma and concurrent decreases in the amounts of albumin and fructosamine.

Ultimately, infection produces a relative protein deficiency with poor growth. The relative protein deficiency has four causes. Infected animals eat less. Ingested protein is digested less efficiently. There is a leakage of protein into the gastrointestinal tract and infection increases protein demand as protein is diverted into repair processes and immune and inflammatory responses (56). The clinical signs can be reduced or abolished by supplementary feeding with protein or nonprotein sources of nitrogen before and during the infection (57,58).

There are many similarities between gastric ulceration and abomasal nematode infection (59). Both pathologies are characterized by disruption of cell junctions, hyperplasia, increased mucus production, decreased acid production, gastrinaemia, pepsinogenaemia and inappetance. Our current hypotheses on the mechanisms responsible for nematode pathology in sheep are derived largely from studies in experimental models and humans.

The key event appears to be the degranulation of mast cells (60). Mucosal mast cells have surface receptors for IgE and when IgE is cross-linked by binding foreign antigens, mast cells release a variety of mediators including histamine, leukotrienes, prostaglandins, and proteases. In experimental models, one of these proteases (a granule chymase, mast cell protease II) has been shown to digest proteins in the tight junctions between epithelial cells (61,62).

The destruction of the tight junctions allows epithelial growth factor, produced in the salivary glands, to bind to its receptor on the inner surface of the epithelial cells (63). This initiates increased mucus production, decreased acid production, increased cell division and migration. Normally, these are adaptive responses to repair the epithelium but with sustained insult the responses become deleterious. Decreased acid production inhibits the autocatalytic conversion of pepsinogen to pepsin and causes pepsinogenaemia. The decreased acid production leads to hypergastrinaemia which causes inappetance (64,65). The loss of proteins into the intestinal lumen is responsible for the decreased levels of albumin and fructosamine (66,67). Normally proteins, peptides and amino acids lost in the stomach can be resorbed in the small intestine but the presence of other nematodes inhibits their uptake. This explains why mixed nematode infections are usually more pathogenic (68).

Mixed, predominantly *T. circumcincta* infection can severely reduce growth rates even in overtly healthy lambs with moderate infections (69). The genetic correlation of growth rate with faecal egg count in lambs naturally infected with mixed, predominantly *T. circumcincta* infection was –0·8 (70). A similar study in Polish sheep gave a genetic correlation of –0·6 (71). In feral sheep, there was also a favourable genetic correlation between reduced egg counts and the size of adult sheep (72). These strong genetic correlations demonstrate that resistant lambs grow more quickly and suggest that the most important genes for growth rate in the presence of an endemic high intensity infection are the genes for nematode resistance.

MOLECULAR GENETIC ANALYSIS

Identifying the genes responsible for nematode infection sheds light on the mechanisms underlying resistance to nematode infection and simplifies selective breeding.

For mixed, predominantly *T. circumcincta* infection two genes have been repeatedly associated with resistance to infection. One lies in or around the class II region of the major histocompatibility complex (mhc) on chromosome 20 (73–77). The other was first discovered in New Zealand sheep (78) and lies in or around the interferon-γ gene on chromosome three (77,79–81).

Quantitative trait loci (qtl) have been reviewed recently (82–84). In addition to the qtl on chromosomes 3 and 20, additional loci have been reported on chromosomes 1 and 6 for resistance to *T. colubriformis* (82). A more recent study identified a qtl on chromosome 8 for resistance to *T. colubriformis* (85) whereas a genome-wide scan of an island population of naturally infected feral Scottish sheep did not find any qtl for reduced faecal nematode egg counts (86). The failure to find any qtl in feral sheep was surprising given previous reports that this population did contain qtl on chromosomes 3 and 20. The failure may reflect the low power of the study and the difficulty in working with unmanaged populations. Alternatively, the previous reports of associations may have been an artefact caused by non-random breeding in the population.

The causative mutations have not been identified for any qtl for resistance to nematodes but the interferon-γ gene and mhc genes within the class II region are plausible candidates. Research in experimental models of nematode infection has clearly shown that resistant animals mount Th2 type responses (13,14,87). A similar situation exists in sheep (88). Interferon-γ is a key cytokine in the decision to produce type 1 or type 2 responses. A polymorphism that regulates interferon-γ production could readily affect resistance to nematode infection.

The role of the mhc is more subtle. There is considerable variation among hosts of the same species in the parasite molecules that they recognize. This is true for a wide variety of host–parasite systems (89–92) including *T. circumcincta* (93,94). The products of class II mhc genes play a critical role in the recognition of parasite molecules and individuals with different mhc genes recognize different sets of parasite molecules. Therefore resistant individuals not only produce strong immune responses, these responses are directed against specific sets of parasite molecules.

Both candidate genes show nonadditive genetic variation. Heterozygotes at the *DRB1* locus are more resistant than homozygotes (95) while the susceptible F allele at the *ifng* locus is dominant to the S allele (80). Interactions among alleles such as heteozygote advantage and dominance are forms of nonadditive gene action. Both loci are more strongly associated with differences in the number of adult *T. circumcincta* than with differences in parasite growth and development (unpublished observations).

EVOLUTIONARY BIOLOGY

There are several unusual features of the interaction between sheep and *T. circumcincta*. They include the slow development of immunity, the periparturient rise in faecal egg production and the relatively high levels of genetic variation in host resistance to infection.

Lambs rapidly develop the ability to control intestinal nematodes such as *Nematodirus battus* and *T. colubriformis* (47). Lambs can also mount IgA responses that slow worm development and regulate worm fecundity. The slow development of immunity on closer inspection turns out to be the slow development of immediate hypersensitivity reactions (IgE mediated mast cell degranulation) against abomasal parasites.

One possible explanation is that the slow development of the ability to mount effective immediate hypersensitivity reactions in the abomasum is a trade-off between pathology and immunity. As mast cell degranulation produces a relative protein deficiency which impairs growth and immunity, possibly the cost of this immune response is too high. Slower growing and smaller sheep are likely to have fewer offspring than their contemporaries.

The periparturient rise in egg count is obviously beneficial to the parasite but may also benefit the host. It ensures that lambs receive a small dose of nematodes when they are suckling and benefit from passive immunity. This controlled exposure generates immune responses with minimal disease.

The fundamental theorem of natural selection suggests that evolution will fix genes that improve fitness (96). This will reduce additive genetic variation. The existence of substantial genetic variance in resistance to nematodes is surprising. One explanation is that there is considerable nonadditive gene action. Balancing selection could maintain genetic variation. Different forms of balancing selection include overdominance (heterozygote advantage), frequency-dependent selection and fluctuating selection.

Conventional wisdom suggests that mhc polymorphism is maintained by heterozygote advantage; there is a trade-off between resistance to different diseases (97). Some alleles confer resistance to one disease while other alleles confer resistance to other diseases. However, this is mathematically implausible. Mathematical modelling has shown that heterozygous advantage can only maintain polymorphisms if the alleles have similar but not necessarily identical fitnesses (98). This result implies that in order for polymorphism to be maintained by a balance between different diseases, the

selective forces exerted by different diseases must remain in rough balance over millions of years. This is unlikely. An alternative explanation is that heterozygotes recognize a greater variety of parasite molecules and this makes them more resistant than homozygotes to each disease (95).

FUTURE RESEARCH

The aim of working with parasitic diseases of economically important species is to understand the processes of infection and disease and to use this understanding to control infection. Our knowledge of the interaction between sheep and gastrointestinal nematodes is as comprehensive as any other host–parasite interaction, but much remains to be understood. In particular, our knowledge of the key molecular interactions between host and parasites is inadequate. Fortunately, the new technologies of genomics (especially large-scale sequencing), functional genomics (especially microarray analysis) and proteomics (especially two-dimensional gel electrophoresis and mass spectrometry) have much to offer.

Identifying the causative mutations underlying the qtl for nematode resistance is a high priority because it will lead to a better understanding of the mechanisms underlying variation in resistance to infection. The virtual sheep genome created by matching existing linkage groups with the bovine and other sequences was an intellectual tour-de-force that has proved very useful (99). Undoubtedly, a full genome sequence will become available for one or more sheep in the near future and this will assist the identification and use of defined mutations. These analyses will be helped by the development of powerful statistical methods that combine linkage and linkage disequilibrium (100,101).

Sheep DNA chips with the capacity to simultaneously study 50 000 single nucleotide polymorphisms are under development. These chips will greatly assist in the molecular genetic analysis of resistance to infection. They also provide the possibility of whole genome selection for a variety of economically important traits, including resistance to nematode infection. This has the potential to transform animal breeding. Veterinary medicine will also be transformed if the selective breeding of relatively disease-resistant animals becomes widespread.

Microarray analysis has been used to identify differences in gene expression between resistant and susceptible sheep. The results so far are promising (102,103), although detailed analyses of the response to *T. circumcincta* have still to be published. The results include confirmation of an important role for one or more genes in the class II region of the major histocompatibility complex on chromosome 20 (104). Similarly, real time PCR was used to differentiate the response to *H. contortus* and *T. colubriformis* between resistant and susceptible sheep; although multiple pathways were expressed in the mucosa following nematode infection, there were shared features among the resistant animals (105). Although there are differences in the response to *H. contortus* and *T. colubriformis* (105), there do not appear to be important differences in the response to different strains of *H. contortus* (106). The advent of sheep DNA chips that include genes of importance in the gastrointestinal tract is likely to give considerable impetus to this area.

A major application of proteomic approaches is to identify parasite proteins, especially the targets of the host immune response. This technique has been used to identify proteins from *H. contortus* (107) and *T. colubriformis* (108) and is currently being used on *T. circumcincta* (unpublished observations). Ultimately, this procedure may help to distinguish proteins that generate protective responses from those that generate pathological responses.

In conclusion, in cool temperate areas the dominant nematode of sheep is *T. circumcincta* although other species of nematodes exacerbate the pathology of infection. Quantitative immunogenetic analyses suggest that there are two major mechanisms of resistance: an IgA-mediated suppression of worm growth and fecundity and type I immediate hypersensitivity reactions that regulate worm number. Both the quantity and specificity of local antibody are important. Suppression of worm growth develops before regulation of worm number. At the end of the first grazing season, worm length is under stronger genetic control than worm number. Genetic variation in egg production by lambs is largely determined by genetic variation in worm development and fecundity. The genetic variation is sufficiently strong to make selective breeding for reduced egg output feasible and this will produce healthier and faster growing lambs. Markers for two of the genes that determine resistance genes have been identified: one resistance gene lies in or around the major histocompatibility complex on chromosome 20; the other lies in or around the interferon-γ gene on chromosome three.

REFERENCES

1 Chan MS, Medley GF, Jamison D & Bundy DA. The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology* 1994; **109**: 373–387.
2 Stear MJ, Bairden K, Bishop SC, *et al*. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. *Parasitology* 1998; **117**: 165–171.
3 Perry BD & Randolph TF. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Vet Parasitol* 1999; **84**: 145–168.
4 Hudson PJ, Dobson AP & Newborn D. Prevention of population cycles by parasite removal. *Science* 1998; **282**: 2256–2258.

5 Cattadori IM, Haydon DT & Hudson PJ. Parasites and climate synchronize red grouse populations. *Nature* 2006; **433**: 737–741.

6 Newey S, Shaw DJ, Kirby A, Monteith P, Hudson PJ & Thirgood SJ. Prevalence, intensity and aggregation of intestinal parasites in mountain hares and their potential impact on population dynamics. *Int J for Parasitol* 2005; **35**: 367–373.

7 Gulland FM, Albon SD, Pemberton JM, Moorcroft PR & Clutton-Brock TH. Parasite-associated polymorphisms in a cyclic ungulate population. *Proc Royal Soc London B Biol Sciences* 1993; **254**: 7–13.

8 Cornell SJ, Isham VS & Grenfell BT. Stochastic and spatial dynamics of nematode parasites in farmed ruminants. *Proc Royal Soc London B Biol Sciences* 2004; **71**: 1243–1250.

9 Grenfell BT, Wilson K, Finkenstadt BF, *et al*. Noise and determinism in synchronized sheep dynamics. *Nature* 1998; **394**: 674 – 677.

10 Kao RR, Leathwick DM, Roberts MG & Sutherland IA. Nematode parasites of sheep: a survey of epidemiological parameters and their application in a simple model. *Parasitology* 2000; **121**: 85–103.

11 Gettinby G, Bairden K, Armour J & Benitez-Usher C. A prediction model for bovine ostertagiasis. *Vet Rec* 1979; **105**: 57–59.

12 Paton G, Thomas RJ & Waller PJ. A prediction model for parasitic gastro-enteritis in lambs. *Int J for Parasitol* 1984; **14**: 439–445.

13 Grencis RK. Cytokine regulation of resistance and susceptibility to intestinal nematode infection – from host to parasite. *Vet Parasitol* 2001; **100**: 45–50.

14 Grencis RK. Th2-mediated host protective immunity to intestinal nematode infections. *Philosoph Transactions Royal Soc London Series B- Biol Sci* 1997; **352**: 1377–1384.

15 Wakelin D. Immunity and immunogenetics – new approaches to controlling worm infections in sheep. *Br Vet J* 1995; **151** (2): 111–113.

16 Urquhart GM, Armour J, Duncan JL, Dunn AM & Jennings FW. *Veterinary Parasitology*. Avon, Longman Scientific and Technical, 1987.

17 Huntley JF, Jackson F, Coop RL, *et al*. The sequential analysis of local inflammatory cells during abomasal nematode infection in periparturient sheep. *Vet Immunol Immunopathol* 2004; **97**: 163–176.

18 Xie HL, Stankiewicz M, Huntley JF, *et al*. The effects of cold exposure, food allowance and litter size on immunity of periparturient sheep to *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Anim Sci* 2004; **78**: 149–158.

19 Donaldson J, van Houtert MFJ & Sykes AR. The effect of nutrition on the periparturient parasite status of mature ewes. *Anim Sci* 1998; **67**: 523–533.

20 Coop RL & Kyriazakis I. Influence of host nutrition on the development and consequences of parasitism in livestock. *Trends Parasitol* 2001; **17**: 325–330.

21 Houdijk JGM, Kyriazakis I, Jackson F & Coop RL. The relationship between protein nutrition, reproductive effort and breakdown in immunity to *Teladorsagia circumcincta* in periparturient ewes. *Anim Sci* 2006; **72**: 595–606.

22 Stear MJ, Strain SAJ & Bishop SC. How lambs control infection with *Ostertagia circumcincta*. *Vet Immunol Immunopathol* 1999; **72**: 213–218.

23 Boag B & Thomas RJ. Epidemiological studies on gastrointestinal nematode parasites of sheep: the seasonal number of generations and succession of species. *Res Vet Sci* 1977; **22**: 62–67.

24 Stear MJ, Abuargob O, Ben Othman M, *et al*. Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. *Parasitology* 2006; **132**: 275–280.

25 Bliss CI & Fisher RA. Fitting the negative binomial distribution to biological data. *Biometrics* 1953; **9**: 176–200.

26 Hunter GC & Quenouille MH. A statistical examination of the worm egg count sampling technique for sheep. *J Helminthol* 1952; **26**: 157–170.

27 Scott ME. Temporal changes in aggregation: a laboratory study. *Parasitology* 1987; **94**: 583–595.

28 Grenfell BT, Wilson K, Isham VS, Boyd HEG & Dietz K. Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode–ruminant interactions as a case study. *Parasitology* 1995; **111**: S135–S151.

29 Stear MJ, Bairden K, Duncan JL, *et al*. The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. *Parasitology* 1995; **110**: 573–581.

30 Stear MJ, Park M & Bishop SC. The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitol Today* 1996; **12**: 438–441.

31 Falconer DS & Mackay TFC. *Introduction to Quantitative Genetics*. Harlow: Longman, 1996.

32 Bishop SC & Stear MJ. Modelling responses to selection for resistance to gastro-intestinal parasites in sheep. *Anim Sci* 1997; **64**: 469–478.

33 Bishop SC & Stear MJ. Genetic and epidemiological relationships between productivity and disease resistance: gastrointestinal parasite infection in growing lambs. *Anim Sci* 1999; **69**: 515–524.

34 Albers GAA, Gray GD, Piper LR, Barker JSF, Lejambre L & Barger IA. The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. *Int J for Parasitol* 1987; **17**: 1355–1363.

35 Eady SJ, Woolaston RR & Barger IA. Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. *Livestock Prod Sci* 2002; **81**: 11–23.

36 Woolaston RR & Windon RG. Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. *Anim Sci* 2001; **73**: 41–48.

37 Morris CA, Watson TG, Bisset SA, Vlassoff A, Douch PGC. Breeding sheep in New Zealand for resistance or resilience to nematode parasites. In Gray GD, Woolaston RR, Eaton BD (eds): *Breeding for Resistance to Infectious Diseases of Small Ruminants*. Canberra, Australia: ACIAR Monograph No 34, 2000: 77–98.

38 McEwan JC, Dodds KG, Greer GJ, *et al*. Genetic estimates for parasite resistance traits in sheep and their correlations with production traits. *NZ J Zool* 1995; **22**: 177.

39 Bisset SA, Morris CA, McEwan JC & Vlassoff A. Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. *NZ Vet J* 2001; **49**: 236–246.

40 Bishop SC & Stear MJ. The use of a γ-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* 2000; **121**: 435–440.

41 Strain SAJ, Bishop SC, Henderson NG, *et al*. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology* 2002; **124**: 545–552.

42 Henderson NG & Stear MJ. Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. *Vet Immunol Immunopathol* 2006; **112**: 62–66.

43 Stear MJ, Henderson NG, Kerr A, *et al*. Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. *Parasitology* 2002; **124**: 553–560.

44 Stear MJ, Bairden K, McKellar QA, Scott I & Strain SAJ. The relationship between the number and size of nematodes in the abomasum and the concentration of pepsinogen in ovine plasma. *Res Vet Sci* 1999; **67**: 89–92.

45 Barger IA. The statistical distribution of trichostrongylid nematodes in grazing lambs. *Int J for Parasitol* 1985; **15**: 645–649.

46 Stear MJ, Bairden K, Duncan JL, *et al*. How hosts control worms. *Nature* 1997; **389**: 27.

47 McClure SJ, Emery DL, Bendixsen T & Davey RJ. Attempts to generate immunity against *Trichostrongylus colubriformis* and *Haemonchus contortus* in young lambs by vaccination with viable parasites. *Int J for Parasitol* 1998; **28**: 739–746.

48 Seaton DS, Jackson F, Smith WD & Angus KW. Development of immunity to incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta*. *Res Vet Sci* 1989; **46**: 241–246.

49 Stear MJ, Bishop SC, Doligalska M, *et al*. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunol* 1995; **17**: 643–652.

50 Stear MJ & Bishop SC. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *Int J for Parasitol* 1999; **29**: 777–780.

51 Stear MJ, Bairden K, Innocent GT, Mitchell S, Strain SAJ & Bishop SC. The relationship between IgA activity against fourth-stage larvae and density-dependent effects on the number of fourth-stage larvae of *Teladorsagia circumcincta* in naturally infected sheep. *Parasitology* 2004; **129**: 363–369.

52 Smith WD, Jackson F, Jackson E, Williams J & Miller HRP. Manifestations of resistance to ovine ostertagiasis associated with immunological responses in the gastric lymph. *J Comparative Pathol* 1984; **94**: 591–601.

53 Halliday AM, Routledge CM, Smith SK, Matthews JB & Smith WD. Parasite loss and inhibited development of *Teladorsagia circumcincta* in relation to the kinetics of the local IgA response in sheep. *Parasite Immunol* 2007; **29**: 425–434.

54 Dunsmore JD. Retarded development of *Ostertagia* species in sheep. *Nature* 1960; **186**: 986–987.

55 McKellar QA. Interactions of ostertagia species with their bovine and ovine hosts. *Int J for Parasitol* 1993; **23**: 451–462.

56 Stear MJ, Bishop SC, Henderson NG & Scott I. A Key Mechanism of Pathogenesis in Sheep Infected with the Nematode *Teladorsagia circumcincta*. *Anim Health Res Rev* 2003; **4**: 45–52.

57 Coop RL, Huntley JF & Smith WD. Effect of dietary-protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. *Res Vet Sci* 1995; **59** (1): 24–29.

58 Stear MJ, Bairden K, Duncan JL, *et al*. The influence of relative resistance and urea-supplementation on deliberate infection with *Teladorsagia circumcincta* during winter. *Vet Parasitol* 2000; **94**: 45–54.

59 Scott I. Biochemical and structural changes in response to abomasal nematode infections in ruminants. 1999; PhD thesis: University of Glasgow.

60 Schubert ML. Regulation of gastrin secretion. *Current Opinion Gastroenterol* 1997; **13**: 441–450.

61 Scudamore CL, Thornton EM, McMillan L, Newlands GFJ & Miller HRP. Release of the mucosal mast cell granule chymase, rat mast cell protease II during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995; **182**: 1871–1881.

62 McDermott JR, Bartram RE, Knight PA, Miller HRP, Garrod DR & Grencis RK. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc Natl Acad Sci USA* 2003; **100**: 7761–7766.

63 Playford RJ. Peptides and gastrointestinal mucosal integrity. *Gut* 1995; **37**: 595–597.

64 Fox MT, Gerrelli D, Shivalkar P & Jacobs DE. Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. *Res Vet Sci* 1989; **46**: 280–282.

65 Bado A, Levasseur S, Attoub S, *et al*. The stomach is a source of leptin. *Nature* 1998; **394**: 790–793.

66 Heath MF & Connan RM. Interaction of *Ostertagia* and *Nematodirus* species in sheep and the potential of serum fructosamine determination in monitoring gastrointestinal parasitism. *Res Vet Sci* 1991; **51**: 322–326.

67 Stear MJ, Eckersall PD, Graham PA, McKellar QA, Mitchell S & Bishop SC. Fructosamine concentration and resistance to natural, predominantly *Teladorsagia circumcincta* infection. *Parasitology* 2001; **123**: 211–218.

68 Parkins JJ, Taylor LM, Holmes PH, Bairden K, Salman SK & Armour J. Pathophysiological and parasitological studies on a concurrent infection of *Ostertagia ostertagi* and *Cooperia oncophora* in calves. *Res Vet Sci* 1990; **48**: 201–208.

69 Coop RL, Graham RB, Jackson F, Wright SE & Angus KW. Effect of experimental *Ostertagia circumcincta* infection on the performance of grazing lambs. *Res Vet Sci* 1985; **38**: 282–287.

70 Bishop SC, Bairden K, McKellar QA, Park M & Stear MJ. Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with liveweight in young lambs. *Anim Sci* 1996; **63**: 423–428.

71 Bouix J, Krupinski J, Rzepecki R, *et al*. Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. *Int J for Parasitol* 1998; **28**: 1797–1804.

72 Coltman DW, Pilkington J, Kruuk LEB, Wilson K & Pemberton JM. Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution* 2001; **55**: 2116–2125.

73 Schwaiger FW, Gostomski D, Stear MJ, *et al*. An ovine *major histocompatibility complex DRB1* allele is associated with low faecal egg counts following natural predominantly *Ostertagia circumcincta* infection. *Int J for Parasitol* 1995; **25**: 815–822.

74 Buitkamp J, Filmether P, Stear MJ & Epplen JT. Class I and class II major histocompatibility complex alleles are associated with faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Parasitol Res* 1996; **82**: 693–696.

75 Stear MJ, Belch A, Donskow-Schmelter K, *et al*. Detection of genes with moderate effects on disease resistance using ovine mhc and resistance to nematodes as an example. *Vet Immunol Immunopathol* 2007; **120**: 3–9.

76 Paterson S, Wilson K & Pemberton JM. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*). *Proc Natl Acad Sci USA* 1998; **95**: 3714–3719.

77 Davies G, Stear MJ, Benothman M, *et al*. Quantitative trait loci associated with parasitic infection in Scottish Blackface sheep. *Heredity* 2006; **96**: 252–258.

78 Crawford AM & McEwan JC. Identification of Animals Resistant to Nematode Parasite Infection. NZ patent GB2337587. 1999 Nov 1999.

79 Coltman DW, Wilson K, Pilkington J, Stear MJ & Pemberton JM. A microsatellite polymorphism in the γ interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology* 2001; **122**: 571–582.

80 Abuargob O. The influence of sex on nematode infection in Scottish Blackface lambs. PhD Thesis. University of Glasgow 2006, 2006.

81 Sayers G, Good B, Hanrahan JP, Ryan M & Sweeney T. Intron 1 of the interferon γ gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. *Res Vet Sci* 2005; **79**: 191–196.

82 Dominik S. Quantitative trait loci for internal nematode resistance in sheep: a review. *Genetics, Selection, Evolution* 2005; **37** (Suppl. 1): S83–S96.

83 Sayers G & Sweeney T. Gastrointestinal nematode infection in sheep – a review of the alternatives to anthelmintics in parasite control. *Anim Health Res Rev* 2005; **6**: 159–171.

84 Stear MJ, Doligalska M & Donskow-Schmelter K. Alternatives to anthelmintics for the control of nematodes in livestock. *Parasitology* 2007; **134**: 139–151.

85 Crawford AM, Paterson KA, Dodds KG, *et al*. Discovery of quantitative trait loci for resistance to parasitic nematode infection in sheep: I. Analysis of outcross pedigrees. *BMC Genomics* 2006; **7**: 178.

86 Beraldi D, McRae AF, Gratten J, *et al*. Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (*Ovis aries*). *Int J for Parasitol* 2007; **37**: 121–129.

87 Else KJ & Finkelman FD. Intestinal nematode parasites, cytokines and effector mechanisms. *Int J for Parasitol* 1998; **28**: 1145–1158.

88 Pernthaner A, Cole S-A, Morrison L & Hein WR. 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*. *Infect Immun* 2005; **73**: 2175–2183.

89 McKeand JB, Knox DP, Duncan JL & Kennedy MW. Genetic control of the antibody repertoire against excretory/secretory products and acetylcholinesterases of *Dictyocaulus viviparus*. *Parasite Immunol* 1994; **16**: 251–260.

90 Britton C, Canto GJ, Urquhart GM & Kennedy MW. The antibody repertoire in infection and vaccination with *Dictyocaulus viviparus*: heterogeneity in infected cattle and genetic control in guinea pigs. *Vet Immunol Immunopathol* 1992; **31**: 313–322.

91 Kennedy MW, Fraser EM & Christie JF. MHC class II (I-A) region control of the IgE antibody repertoire to the ABA-1 allergen of the nematode *Ascaris*. *Immunology* 1991; **72**: 577–579.

92 Hilderson H, De Graaf DC, Vercruysse J & Berghen P. Characterisation of *Ostertagia ostertagi* antigens by the different bovine immunoglobulin isotypes. *Res Vet Sci* 1993; **55**: 203–208.

93 McCririe L, Bairden K, Britton C, Buitkamp J, McKeand JB & Stear MJ. Heterogeneity in the recognition of *Ostertagia circumcincta* antigens by serum antibody from mature, infected sheep. *Parasite Immunol* 1997; **19**: 235–242.

94 Strain SAJ & Stear MJ. The recognition of molecules from fourth-stage larvae of *Ostertagia circumcincta* by IgA from infected sheep. *Parasite Immunol* 1999; **21**: 163–168.

95 Stear MJ, Innocent GT & Buitkamp J. The evolution and maintenance of polymorphism in the major histocompatibility complex. *Vet Immunol Immunopathol* 2005; **108**: 53–57.

96 Fisher RA. *The Genetical Theory of Natural Selection*. Oxford, Oxford University Press, 1930.

97 Doherty PC & Zinkernagel RM. A biological role for the major histocompatibility antigens. *Lancet* 1975; **28 June 1975**: 1406–1409.

98 De Boer RJ, Borghans JAM, van Boven M, Kesmir C & Weissing FJ. Heterozygote advantage fails to explain the high degree of polymorphism of the mhc. *Immunogenetics* 2004; **55**: 725–731.

99 Dalrymple BP, Kirkness EF, Nefedov M, *et al*. Using comparative genomics to reorder the human sequence into a virtual sheep genome. *Genome Biol* 2007; **8**: R152.

100 Meuwissen THE, Karlsen A, Lien S, Olsaker I & Goddard ME. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. *Genetics* 2002; **161**: 373–379.

101 Farnir F, Grisart B, Coppieters W, *et al*. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. *Genetics* 2002; **161**: 275–287.

102 Diez-Tascón C, Keane OM, Wilson T, *et al*. Microarray analysis of selection lines from outbred populations to identify genes involved with nematode parasite resistance in sheep. *Physiol Genomics* 2005; **21**: 59–69.

103 Keane OM, Zadissa A, Wilson T, *et al*. Gene expression profiling of naive sheep genetically resistant and susceptible to gastrointestinal nematodes. *BMC Genomics* 2006; **7** (42): 1–12.

104 Keane OM, Dodds KG, Crawford AM & McEwan JC. Transcriptional profiling of *Ovis aries* identifies *Ovar-DQA1* allele frequency differences between nematode-resistant and susceptible selection lines. *Physiol Genomics* 2007; **30**: 253–261.

105 Ingham A, Reverter A, Windon R, Hunt P & Menzies M. Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. *Int J for Parasitol* 2008; **38**: 431–442.

106 Rowe A, Gondro C, Emery D & Sangster N. Genomic analyses of *Haemonchus contortus* infection in sheep: Abomasal fistulation and two *Haemonchus* strains do not substantially confound host gene expression in microarrays. *Vet Parasitol* 2008; **154**: 71–81.

107 Yatsuda AP, Krijgsveld J, Cornelissen AWCA, Heck AJR & de Vries E. Comprehensive analysis of the secreted proteins of the parasite *Haemonchus contortus* reveals extensive sequence variation and differential immune recognition. *J Biol Chem* 2005; **278**: 16941–16951.

108 Kiel M, Josh P, Jones A, Windon R, Hunt P & Kongusuwan K. Identification of immuno-reactive proteins from a sheep gastrointestinal nematode, *Trichostrongylus colubriformis*, using two-dimensional electrophoresis and mass spectrometry. *Int J Parasitol* 2007; **37**: 1419–1429.

