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Breeding Sheep for Resistance to Nematode Infections

[1]A.F.T. Amarante and [2]M.R.V. Amarante
[1]Departamento de Parasitologia - Instituto de Biociências; [2]Departamento de Nutrição e Melhoramento Animal - Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Campus de Botucatu - SP, CEP 18618-000, Brazil

Abstract: Control of gastrointestinal nematodes in sheep relies heavily on anthelmintic treatments of the flocks. This strategy has been frequently useless as a consequence of the appearance of nematode populations with resistance to drugs. An alternative to alleviate the problems associated with chemotherapeutics is the breeding of sheep for resistance to nematodes. As the resistance to parasites is heritable, the efficiency of worm control can be increased through objective and accurate identification of genetically resistant individuals and an understanding of mechanisms underlying such genetic resistance. Mechanisms responsible for resistance are not fully understood. However, the development of resistance against nematodes has been associated with a response mediated by lymphocytes Th2 $CD4^+$, increase in mast cell numbers in mucosa, eosinophilia, production of specific antibodies, increased mucus production and the presence of inhibitory substances in the mucus. Since resistant breeds are generally poorly productive, simple substitution of a susceptible, productive breed by a resistant breed is not always a viable option. Mapping genes for resistance will possibly allow marker-assisted selection based on individual quantitative trait loci. It may be possible to utilize resistance genes from a resistant breed by introgression through backcrossing into a more productive breed using marker assisted selection.

Key words: sheep, genetic resistance, helminths and *Haemonchus*

Introduction

Control of gastrointestinal nematodes in sheep relies heavily on anthelmintic treatments of the flocks. This strategy has been frequently useless as a consequence of the appearance of nematode populations with resistance to drugs (Amarante, 2001). Since the first reports of resistance to the broad spectrum anthelmintics were made some three decades ago, this phenomenon has changed from being considered merely as a parasitological curiosity to a state of industry crisis in certain livestock sectors. This extreme situation exists with the small ruminant industry of the tropical/sub-tropical region of southern Latin America where resistance to the entire broad spectrum anthelmintic arsenal now occurs (Amarante *et al.*, 1992; Waller, 1997).

It appears that because of the very high costs and risks associated with taking a new active drug down the development track to marketing, that the pharmaceutical industry has, in general, turned away from this activity. By implication, the international small ruminant industry is too small for these companies to make the necessary investment to develop new drugs (Waller, 1997). Therefore, if sheep breeding keep requiring the exclusive use of anthelmintics to control parasites, it will walk into failure. An alternative for that problem is the selection and raising of sheep genetically resistant to gastrointestinal nematode infections (Woolaston, 1992; Woolaston and Baker, 1996; Zajac, 1995; Bisset and Morris, 1996; Miller *et al.*, 1998; Amarante *et al.*, 1999a).

The ability of sheep to acquire immunity and express resistance varies substantially among and within breeds and is under genetic control (Stear and Murray, 1994). A distinction between "resistance to infection" and "resistance to the effects of infection" should be made: "resistance" would be the ability to suppress establishment and/or subsequent development of infection, and "resilience" - the ability to maintain a relatively undepressed production level when infected (Alberts *et al.*, 1987).

In Tropical environment, where the most important parasite is *Haemonchus contortus*, a nematode that can cause severe loss of blood, selection of the host appears to be better aimed at increasing the ability of the animal to limit an infection (selection for resistance) than to try to select a resilient flock. At some level of *H. contortus* infection even the most resilient host must reach a point where it can no longer continue to produce enough blood to compensate for the loss due to parasites. It would be like selecting an animal that can live with open-ended arteries (Le Jambre, 1995).

Therefore, the efficiency of worm control can be

increased through objective and accurate identification of genetically resistant individuals and an understanding of mechanisms underlying such genetic resistance.

Parasite-resistant sheep breeds: Several sheep breeds exhibit genetic resistance to gastrointestinal nematode infection (Table 1). In South Brazil, lambs of Crioula breed showed high resistance to *H. contortus* infection in comparison with Corriedale lambs (Bricarello, 1999). Similarly, hair sheep of the Santa Inês breed raised in several regions of Brazil were more resistant to *H. contortus* and *Oesophagostomum columbianum* than Suffolk and Ile de France lambs (Amarante, 2002).

This is also the case of Florida Native, St. Croix, Barbados Blackbelly and Gulf Coast Native breeds, called exotic breeds in United States. Sheep of exotic breeds develop resistance in an early age to *Haemonchus* infection (Courtney *et al.*, 1985; Gamble and Zajac, 1992; Bahirathan *et al.*, 1996). Suckling Gulf Coast Native lambs, for instance, developed resistance to *H. contortus* infection during their first exposure to infection, before weaning (Bahirathan *et al.*, 1996). Courtney *et al.* (1985) observed that breed differences between lambs of exotic breeds, as St. Croix, Florida Native and Barbados Blackbelly, and lambs of domestic breeds (Rambouillet e Rambouillet x Finn-Dorset) were most pronounced prior to puberty, with domestic lambs highly susceptible to secondary infections. After puberty, domestic lambs were better able to resist infections. Age apparently had little or no effect on the parasite resistance of exotic breeds.

Amarante *et al.* (1999a) observed that F1 lambs (½ Florida Native x ½ Rambouillet) and Rambouillet were more susceptible to *H. contortus* challenge than Florida Native lambs. In contrast, adult F1 ewes were resistant to gastrointestinal nematode infections just as Florida Native ewes (Amarante *et al.*, 1999b). In this last experiment, Rambouillet ewes exhibited high susceptibility to infections. The authors suggested that there are apparently two mechanisms playing a role in worm resistance, namely, innate resistance and acquired resistance. Innate resistance is possibly the case of Florida Native sheep and acquired resistance would be the case of F1 sheep. F1 animals would be susceptible while young, but with a good ability for developing acquired resistance.

Genetic resistance of hosts and its influence on epidemiology: Parasite burdens have been generally found to be overdispersed, with the negative binomial distribution providing a good empirical description of observed distributions of parasite burdens within host populations. In overdispersed distributions, most hosts carry few parasites, while a few heavily infected hosts harbor a large proportion of the total parasite population (Barger, 1985; Amarante *et al.*, 1998; Stear *et al.*, 1998). This pattern of distribution occurs because the immune response is not uniform in the animals of one particular flock.

This resistance to parasites is heritable. Despite the large number of breeds and species examined, estimates of heritability of resistance in sheep are remarkably consistent, and range from 0.3 to 0.5, values similar in magnitude to heritabilities of production characters such as fleece weight or body weight, for which selection has been demonstrably successful (Barger, 1989).

Merino sheep selected for resistance to *H. contortus* showed also resistance to *Trichostrongylus colubriformis* infections. These results suggest that by selecting sheep for high responsiveness to a certain species of nematodes, one can also achieve a substantial improvement of resistance against a wide range of other nematode species (Sréter *et al.*, 1994). Besides, there is no evidence of adaptation of parasites in sheep selected for resistance. *Haemonchus contortus* showed no adaptation to genetically resistant sheep after 14 generations in lines of Merino sheep bred to have either increased or decreased resistance (Woolaston *et al.*, 1992).

The use of genetically resistant hosts has a great influence on parasite infections epidemiology. Seasonal peaks in parasite burdens were greatly reduced in genetically resistant sheep, as a consequence, larval numbers on pasture were reduced even further (Barger, 1989; Bishop and Stear, 1997; Bisset *et al.*, 1997, Bishop and Stear, 1999). The use of genetically resistant hosts should permit a reduced frequency of anthelmintic treatment, and hence reduce the rate of development of anthelmintic resistance in the parasite. In addition, there is a favorable correlation between weight gain in sheep and resistance to gastrointestinal nematode infections, estimated by FEC (Table 2).

Bishop and Stear (1999) developed a model of the gastrointestinal parasitism in lambs to include between-animal variation for live-weight gain, food intake, larval establishment rate in the host, worm fecundity and worm mortality

Table 1: Sheep breeds that exhibited resistance to nematode infections

Sheep breed	Origin	Compared with	Infection with	References
Florida Native	USA	Rambouillet; Hampshire	Hoemonchus contortus	Loggins *et al.* (1965); Radhakrishnan *et al.* (1972); Bradley *et al.* (1973); Amarante *et al.* (1999a)
Florida Native	USA	Rambouillet; Finn-Dorset x Rambouillet; Dorset x Rambouillet	mix infections	Courtney *et al.* (1984); Zajac *et al.* (1988); Amarante et al. (1999a); amarante *et al.* (1999b)
St. Croix	Caribe	Rambouillet; Finn-Dorset x Rambouillet; Dorest x Rambouillet	mix infections	Courtney et al. (1984); Mansfield & Gamble (1995)
Barbados Blackbelly	Caribe	Rambouillet; Finn-Dorset x Rambouillet; Dorset	mix infections	Yazwinski *et al.* (1979); Courtney *et al.* (1984)
Gul Coast Native	USA	Suffolk	Mix infections	Bahirathan et al. (1996); Miller *et al.* (!998); Li *et al.* (2001)
Red Maasai	Africa	Blackheaded Somali; Dorper; Romney Marsh; Marino; Corriedale; Hampshire	*H. contortus*	Preston & Allonby (1979); Mugambi *et al.* (1996); Mugambi *et al.* (1997); Wanyangu et al. (1997)
Red Maasai	Africa	Dorper	mix infections	Baker *et al.* (1999)
Scottish Blackface	Scotland	Finn Dorset	*H. contortus*	Altaif & Dargie (1978a); Altaif & Dargie (1978b)
Crioula	Brazil	Corriedale	*H. contortus*	Bricarello (1999)
Santa Ines	Brazil	Suffolk; Ile de France; Poll Dorset	mix infections	Moraes *et al.* (2000); Bueno *et al.* (2002); Amarante (2002)

Table 2: Phenotypic* and genetic** correlations between fecal egg count and weight gain[1] or body weidht[2] in sheep

Country	Sheep breed	Infection with	Correlation Coefficients	References
New Zealand	Romey[1]	Several species, *Trichostrongylus* spp. was Predominant	-0.07* 0.48**	Bisset *et al.* (1992)
Pland	Polish Long-Wool[1]	*O. circumcincta* and *H. contortus*	-0.15* -0.61**	Boiux *et al.* (1998)
Australia	Merino[2]	*H. contortus*, *T. colubriformis* or natural mixed infections	-0.20**	Eady *et al.* (1998)
Scotland	Scottish Blackface[1]	O. circumcincta	-0.10 -0.27**	Bishop & Stear (1999)

rate. The model predicted a weak phenotypic correlation (mean = -0.10) between observed live weight and FEC, the indicator of resistance, but a stronger favorable (negative) genetic correlation between these traits (mean = -0.27). The severity, or epidemiology, of the disease greatly influenced the results – the genetic correlation between observed live weight and FEC strengthened from -0.02 to -0.46 as the disease severity changed from mild to severe. Selection for reduced FEC resulted in large correlated increases in live-weight gain, more than twice that predicted by quantitative genetic theory, due to the reductions in growth rate losses as the disease challenge to the animals decreased. Conversely, selection for increased live-weight gain resulted in reductions in FEC close to expectations. This asymmetry of selection response emphasizes the epidemiological benefits obtainable from selection for resistance to infectious chronic diseases – such selection will result in improvements in both animal health and productivity not seen when selection is for improved productivity, alone. Breeding goal should be designed to take account of such effects.

Different from weigh gain, it has been noticed an unfavorable correlation between resistance and wool production. This was the case of the results obtained in Merino sheep in Australia. In that country, the genetic correlation between FEC and grease fleece weight was 0.21 in 10-month old sheep (Eady *et al.*, 1998).

This unfavorable correlation between resistance to gastrointestinal nematodes and wool production was also recorded in New Zealand. One Romney line has been selected for high greasy fleece weight for 37 years, and the other has been randomly selected during the same period (control). Selected sheep are more productive than control sheep when clinical parasitism is controlled by anthelmintic treatment (Williamson *et al.*, 1995a). However,

there were clear differences between sheep selection lines in the level of establishment and/or development of *H. contortus* and *Ostertagia circumcincta*, with numbers of adult parasites at slaughter being higher in the selected line than in the control line (Williamson *et al.*, 1995b). These studies demonstrate that long-term selection for high wool production has resulted in increased FEC, suggesting that wool production and FEC may be unfavorably genetically correlated.

In conclusion, sheep selection for resistance may result in improvement of the weight gain in breeds specialized in meat production. However, this approach has to be employed with caution in sheep breeds specialized in wool production.

Immune response to gastrointestinal nematode infections: The major gastrointestinal nematode parasites of ruminants all belong to the Order Strongylida and the family Trichostrongyloidea. Despite this close evolutionary relationship, distinct differences exist in the microenvironmental niches occupied by the developmental stages of the various parasites, which may account for the variable susceptibility of the different parasite species to the immune effector mechanisms generated by the host. In addition, different manifestations of resistance have been observed against the adult and larval stages of the same parasite species, and even against the same parasite stage (Balic *et al.*, 2000).

Immunity against adult stages of gastrointestinal nematodes in ruminants is manifested as: expulsion of the adult nematode population; changes in the morphology of adult nematodes; and reduction in the fecundity of female worms. Manifestations of resistance to larval nematode parasites include developmental arrest (hypobiosis) and failure of infective larvae to establish infection (Balic *et al.*, 2000). How nematodes are damaged is unclear. Worms might be damaged directly by effector cells and molecules of the immune system. Alternatively, they might be damaged by the physiological stress of their efforts to resist attack. Thus, the interaction between worms and the host immune response can be thought of as the interaction of opposing forces in that nematodes actively attempt to persist in the face of attack by the host immune response. At extremes, the outcome is either that the infection persists (apparently unaffected) or that the worms are killed or expelled. An intermediate outcome is that infection persists, but aspects of nematode survival and fecundity are reduced below some maximum. Therefore, an alternative view of nematode infections is that the reduction in the fecundity and survival of infections in immune hosts is, at least in part, a result of the energy expended by a parasite to protect itself against immune attack (Viney, 2002).

The mechanisms responsible for resistance are not fully understood. However, the development of resistance against nematodes has been associated with a response mediated by lymphocytes Th2 $CD4^+$, increase in mast cell numbers in mucosa, eosinophilia, production of specific antibodies, increased mucus production and the presence of inhibitory substances in the mucus.

The immune response in resistant and susceptible animals: Lymphocytes T, which express $CD4^+$ markers on the surface, produce a large number of cytokines that activate other immune cells, for this reason they are called T helper cells (Franco, 1999). There are two subsets of T helper cells defined by the set of cytokines they secrete: T helper 1 (Th1) and T helper 2 (Th2). Thus Th1 type cells produce inferferon-gamma (IFN-gamma), lymphotoxin, and interleukin-2 (IL-2) whilst Th2 type cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. A naive T cell can differentiate into either a Th1 or Th2 type cell. The differentiation pathway towards either Th1 or Th2 is influenced by a number of factors, the most potent of which seems to be the immediate cytokine environment a T cell experiences at the time of antigen presentation. Thus, IL-12 promotes the development of Th1 type cells whilst Th2 type cells develop in the presence of IL-4 (Else and Finkelman, 1998). The citotoxic T cells and macrophages are stimulated respectively by IL-2 and IFN-gamma, what confers to lymphocytes Th1 a pivotal role in the immunity regulation against intracelular patogens (Franco, 1999). On the other hand, IL-4 and IL-5 promote differentiation of lymphocyte B and an elevation in a number of parameters including eosinophilia, intestinal mastocytosis and IgE what associates the Th2 lymphocytes to resistance against gastrointestinal nematode infections (Franco, 1999; Else and Grencis, 1991; Else and Finkelman, 1998; Grencis, 2001).

There is a clear evidence for the importance of Th2 cytokines, and specifically the Th2 cytokine IL-4, in resistance to infection to *Trichuris muris* in genetically resistant mice. In contrast, susceptible strains of mouse respond to a primary *T. muris* infection by mounting an immune response characterized by high levels of

IFN-gamma and parasite-specific IgG2a, both hallmarks of a Th1 type response. These mice go on to harbor long term chronic infections and are unable to expel the parasite (Else and Grencis, 1991; Else and Finkelman, 1998; Grencis, 2001). The resistance against *H. contortus* infections in sheep also involves the lymphocyte Th2 $CD4^+$ response with expressive IL-5 production. Infection with *H. contortus* initiated strong production of IL-5 by isolated lymphoid cells. Both genetically and random-bred lambs exhibited this pattern of cytokine production pos-infection, although it was noteworthy that overall levels of IL-5 production were highest in the regional lymph nodes of the gastrointestinal tract of resistant animals (Gill *et al.*, 2000). The resistant lambs, when infected, also produce higher levels of IgE and IgA anti-*Haemonchus* and present higher densities of mast cells and eosinophils in the abomasum mucosa than random-bred lambs (Gill *et al.*, 1993; Karanu *et al.*, 1997; Gill *et al.*, 2000). These results strongly argue that the protective immune response in sheep genetically resistant to *H. contortus* involves the selective expansion of Th2-type subset cells with strong IL-5-secreting activity, with minimal activation (and possibly active down-regulation) of Th1-type IFN-gamma-secreting activity (Gill *et al.*, 2000).

Local and/or systemic IgA and IgG1 anti-*Haemonchus* are associated with resistance to haemonchosis in sheep. Significantly higher IgA and IgG1 antibody levels in resistant compared with random-bred sheep and the negative correlation found between FEC and the levels of these antibodies suggest that they might play a protective role in genetically determined resistance of Merino sheep to *H. contortus* (Gill *et al.*, 1993).

Infection of sheep with *H. contortus* resulted in elevated IgE levels in serum 2-4 weeks after infection. A negative correlation between worm counts and total serum IgE levels was found in repeatedly infected sheep (Kooyman *et al.*, 1997). Infection of sheep with *Trichostrongylus colubriformis* leads to elevated levels of total and parasite-specific IgE in serum and resulted in significantly higher numbers of IgE-positive cells in gut tissue sections (Shaw *et al.*, 1998; Harrison *et al.*, 1999). A similar response is observed in the abomasum of sheep infected with *Trichostrongylus axei* (Pfeffer *et al.*, 1996).

Huntley *et al.* (2001) identified an IgE reactive antigen on the *Teladorsagia circumcincta* (= *O. circumcincta*) larval surface. IgE production was higher in animals with low FEC than in sheep with high parasite burdens. There was evidence of an IgE role in the protection of lambs to *T. circumcincta* infection. Antigens with similar molecular weight were demonstrated in *T. colubriformis* and *Cooperia curticei,* but not in *H. contortus.*

The immunological response to parasites is a complex process that can involve several mechanisms. In the case of *T. circumcincta* infections, two mechanisms are involved in the resistance: the response mediated by IgA that affects the growth and fecundity of the worms and the hipersensibility reaction mediated by IgE that affects the incoming larvae. The relative importance of the two responses can be influenced by several aspects, including the age, the genotype and the previous exposure level of the sheep to the parasites (Stear *et al.*, 1995a; Stear *et al.*, 1997; Huntley *et al.*, 2001). Following infection with *O. circumcincta,* sheep with more mast cells also had more globule leucocytes, more eosinophils, more IgA plasma cells and greater amounts of parasite-specific IgA in the abomasal mucosa (Stear *et al.*, 1995; Stear *et al.*, 1997). In lambs infected with *H. contortus*, Strain and Stear (2001) also found significant association between reduced female adult worm length and increased IgA against third-stage larvae. According to these authors, the magnitude of the IgA response is influenced by the quality of the diet and may be the major mechanism controlling fecundity of *H. contortus.*

The role of mast cells in resistance: Both mast cells and basophils originate form the same haematopoietic stem cell and have similar products and activation mechanisms, including cross-linking of high affinity receptors for IgE expressed on the cell surface. Major differences lie in the different cytokines required for their growth and differentiation and their localization. Mast cells reside and mature in the tissues while basophils circulate as mature cells in the blood stream from where they can be quickly mobilized to inflamed tissue sites (Balic *et al.*, 2000).

Granules of intraepithelial mast cells are generally larger than tissue mast cells, particularly in ruminants where they are referred to as globule leucocytes (Huntley *et al.*, 1984). Intraepithelial mast cells are generally considered to be end stage effect cells, ideally located to discharge their products into the lumen and epithelial compartment where they can affect the micro-environment of the parasite with minimal effect on the underlying tissue architecture (Balic *et al.*, 2000).

The numbers of mucosal mast cells and globule leucocytes increase during the development of

resistance to nematode parasites (Douch *et al.*, 1986; Gill, 1991; Huntley *et al.*, 1992; Douch and Morum, 1993; Pfeffer *et al.*, 1996; Winter *et al.*, 1997) and are higher in the sheep selected for low fecal egg counts (Bisset *et al.*, 1996).

Sensitization of mucosal mast cells to parasite antigen during primary infection is closely associated with the development of acquired immunity to *T. colubriformis* and *H. contortus* (Huntley *et al.*, 1992; Bendixsen *et al.*, 1995). *In vitro,* mucosal mast cell from sheep immune to *T. colubriformis* released maximal amounts of sheep mast cell protease and histamine within half hour of incubation with larval antigen whereas maximum secretion of leukotrienes occurred three hours after addition of antigen (Bendixsen *et al.*, 1995).

This fast response allows the elimination of most of the *T. colubriformis* larvae within two hours after challenge of resistant sheep (Wagland *et al.*, 1996; Harrison *et al.*, 1999). A rapid rejection was also reported in immune sheep after infection with *H. contortus* (Miller *et al.*, 1983).

Parasitic infection in one particular anatomical site induces "at distance" inflammatory reactions of the whole mucosal system (Yacob *et al.*, 2002). In sheep with immunity to either *T. colubriformis* and *H. contortus* the mast cell response is distributed from the site of parasitosis throughout the small intestine, however, mast cells release higher levels of protease in the areas of preferential parasite establishment reflecting, probably, the greater sensitization of mast cells in those regions (Bendixsen *et al.*, 1995).

Recent studies have also shown the relationship between the response against arthropods and helminths. The presence of the nasal bot fly, *Oestrus ovis,* in sheep cause significant reductions in *T. colubriformis* egg excretion, worm fecundity and worm burden. These changes were associated with significant modifications in tissular populations of mast cells, globule leucocytes and eosinophils in the respiratory and digestive tracts. In contrast, infection of the digestive tract with nematodes did not modify the biology of *Oestrus* population (Yacob *et al.*, 2002). Sheep with greater resistance to gastrointestinal parasites also tend to be less susceptible to lice (*Bovicola ovis*) (James *et al.*, 2002).

Eosinophils: Mature eosinophils are continuously recruited at low level from the bone marrow into the blood and from there into the tissues where they may survive for several days to weeks (Balic *et al.*, 2000). While eosinophils are not normally present in high numbers in peripheral blood, larger numbers are found in the tissues, in particular the gastrointestinal tract. After helminth infections, eosinophil numbers can increase dramatically (=eosinophilia) in both blood and tissues (Douch *et al.*, 1986; Dawkins *et al.*, 1989; Pfeffer *et al.*, 1996; Winter *et al.*, 1997; Amarante *et al.*, 1999a). Interleukin-5 (IL-5) is the key cytokine responsible for the dramatic, T- cell dependent increase in eosinophils in blood and tissues after helminth infections (Balic *et al.*, 2000; Meeusen and Balic, 2000).

While eosinophils play some role in tissue remodeling and immune regulation, their major function during nematode infections may lie in their direct cytotoxic potential, in particular through the release of granule proteins and production of superoxide anions. In addition, leukotrienes synthesized by eosinophils after activation may act in a similar fashion to those produced by mast cells, mediating vasodilatation, smooth-muscle contraction and mucus secretion (Balic *et al.*, 2000).

Rainbird *et al.* (1998) demonstrated the eosinophil-mediated killing of *H. contortus* larvae. The level of larval immobilization in the presence of antibody was significantly increased when complement was added to cultures containing activated eosinophils. The addition of IL-5 to larval cultures containing antibody and complement resulted in a significant increase in larval immobilization suggesting that eosinophil effector function is enhanced following priming with this cytokine. Ultrastructural analysis of the eosinophil/larvae interaction at six hours of incubation revealed degranulation of adhering eosinophils onto the surface of larvae. By 24 hours of incubation, many larvae showed signs of damage and most eosinophils had degenerated.

Eosinophils associated with antibody and complement act killing especially infective larval stages (Brigandi *et al.*, 1996; Meeusen and Balic, 2000), but not adults of most helminth parasites (Meeusen and Balic, 2000). Preexisting eosinophilia, due either to allergy or to infection with tissue-invasive helminth species, may confer some degree of immediate a nonspecific resistance in primary infections with parasitic worms (Daly *et al.*, 1999).

Some researches have shown that the resistance level in sheep presents correlation with the degree of the eosinophilia. Numbers of eosinophils were higher in high responder lambs when compared to low responders after vaccination and challenge infections with *T.*

colubriformis (Dawkins *et al.*, 1989). Comparison of the correlations in different periods indicated that the coefficients were negative and increased commensurately with the induction of immune mechanisms that cause worm rejection (Dawkins *et al.*, 1989). Buddle *et al.* (1992) also observed that lambs with the most rapid increase in eosinophil counts were the first to exhibit a decline in *T. colubriformis* FECs. Eosinophilia is associated with the expression of resistance to nematodes in Romney lambs rather than an indication of the presence of a nematode infection (Buddle *et al.*, 1992). Similarly, eosinophil responses in both skin and blood are more vigorous in high responder than in low responder Merino lambs infected with *T. colubriformis* (Rothwell *et al.*, 1993).

Breed difference in eosinophil counts in blood has been reported in susceptible and resistant sheep breeds in Africa. Red Maasai sheep are more resistant to *H. contortus* infection than Dorper sheep. After artificial infection, Red Maasai lambs shown higher eosinophil numbers compared to Dorper (Mugambi *et al.*, 1996). Similar results occurred in Dorper and Red Maasai ewes naturally infected with *H. contortus* (Wanyangu *et al.*, 1997). In contrast, Amarante *et al.* (1999a) did not find significant difference between eosinophil numbers of Florida Native (resistant) and Rambouillet (susceptible) lambs, after challenge with 6000 *H. contortus* larvae.

Woolaston *et al.* (1996), based on results from experimental and field infections, found that eosinophils can be genetically related to resistance under some, but not all, circumstances. The association appeared stronger during experimental infections, perhaps due to synchronization of the immune response with stage of infection. During field infections a number of environmental and genetic factors may interact to weaken the association.

The role of mucins in the host-parasite interactions: Mucins are the main non-aqueous components of the protective mucus barriers that cover epithelial surfaces. It is important to differentiate the term 'mucus' (which refers to an aggregated secretion) from the term 'mucin' (which identifies specific glycoprotein molecules within this secretion) (Theodoropoulos *et al.*, 2001). Mucin molecules are involved in several interactions between helminth parasites and their hosts. Some parasites locate their hosts by detecting components of host mucus, while others express mucin-like molecules, which might be involved in protecting the parasites from their hosts. By contrast, hosts themselves might alter the structure of their mucins as part of an active biosynthetic response to parasite infections (Theodoropoulos *et al.*, 2001).

Quantity of released mucus regulated by CD4+ lymphocytes has an important role in protection against *Nippostrongylus brasiliensis*-infection in mice. Significantly less intestinal mucus was released in anti-CD4 antibody treated mice than in control mice. The reduction in mucus production delayed the expulsion of intestinal worms (Khan *et al.*, 1995).

Maruyama *et al.* (2000) examinated effects of mast cell glycosaminoglycans on the establishment of the intestinal nematode, *Strongyloides venezuelensis*, in the mouse small intestine and observed that these substances could inhibit invasion of adult worms into intestinal mucosa. Because adult worms of *S. venezuelensis* were actively moving in the intestinal mucosa, probably exiting and reentering during infection, the possible expulsion mechanism for *S. venezuelensis* is inhibition by mast cell glycosaminoglycans of attachment and subsequent invasion of adult worms into intestinal epithelium.

Gastrointestinal mucus from nematode-resistant sheep possesses greater inhibitory activity to paralyze *T. colubriformis* infective larvae than mucus from nematode-susceptible sheep (Rabel *et al.*, 1994). Similar results were found by Harrison *et al.* (1999) in naive and in immune Romney sheep to *T. colubriformis*-infection. These authors observed that mucus samples from immune sheep contained increased parasite-specific antibody, histamine and anti-parasite activity than the samples from naive sheep. Similar activity of mucus was described in the abomasum of sheep infected with *T. axei* (Pfeffer *et al.*, 1996). In sheep naturally infected with nematodes, antiparasite activity of the intestinal mucus was significantly higher in animals with low egg counts than those with high counts and was associated with significantly lower burdens of fourth stage larvae (Douch *et al.*, 1984). Globule leucocyte and eosinophil numbers in mucosa were also correlated with antiparasite activity in mucus (Douch *et al.*, 1986).

The immune response as a cause of production losses: While immunity to helminth infection has evolved to mediate rapid elimination of the parasite, the strategies evolved by the parasites themselves aim to delay this rejection process and ensure the survival and distribution of their progeny. Ineffective or incomplete immunity results in persistence of parasites or their products within the host tissues, inappropriate

or chronic stimulation by parasite antigens, hyper-reactivity and tissue damage or immunopathology (Meeusen, 1999).

The inflamatory reaction, triggered in the mucosa by helminth infection, can lead to malabsortion and diarrhoea as a consequence of the combined effects of smooth muscle contraction, vasodilation, excessive mucus secretion accompanied by epithelial and vascular damage (Meeusen, 1999). Shaw *et al.* (1998) observed that marked softening of feces coincided with peaks of specific and total IgE responses during *T. colubriformis* challenge infections.

Persistent diarrhea commonly affects adult Merino sheep grazing improved pastures in the high winter rainfall areas of Australia during winter and spring. This syndrome, often called 'winter scours', is major management problem for sheep farmers because affected sheep accumulate feces around the breech area, know as 'dag'. The dags have to be removed before shearing and lambing by crutching, which is costly, and a time consuming and unpleasant task for farmers (Larsen *et al.*, 1999). It was commonly believed that diarrhea and dag formation in ewes was nutritionally induced. However, studies in Australia provided strong evidence that diarrhea was associated with a host reaction to worm infection, most probably as a consequence of challenge with *Ostertagia* spp. and *Trichostrongylus* spp. larvae (Larsen *et al.*, 1994; Larsen *et al.*, 1995). Larsen *et al.* (1994) did not find significant correlation between worm egg counts and the occurrence of severe dag in ewes. However, there were significantly more eosinophils in the small intestine of ewes affected with severe dag compared with unaffected ewes (Larsen *et al.*, 1994). Affected sheep had a hypersensitive inflammatory reaction in the pylorus and upper jejunum, characterized by the infiltration of significantly more eosinophils and changed lymphocyte populations. The changes in the lymphocyte populations include a reduced number of $CD8^+$ cells, increased $CD4^+$:$CD8^+$ T-cell ratio, and significantly reduced numbers of cells reacting to IFN-gamma (Larsen *et al.*, 1999).

Therefore, selecting 'resistant' lines of sheep (usually on the basis of low FEC) may also inadvertently select sheep that suffer significantly more diarrhea because they have an increased 'hypersensitivity' response to worm larvae. This undesirable consequence should be considered when selecting sheep for increased resistance to internal parasites on the basis of low FEC (Larsen *et al.*, 1995). As the selection of sheep with increased resistance to gut nematodes, will not prevent winter scours, phenotypic culling and genetic selection, to remove sheep susceptible to the hypersensitivity inflammatory response, is proposed by Larsen *et al.* (1999) as the most suitable long-term strategy.

Another example of immune response associated with lesion in gut occurs in *Oesophagostomum* spp. infections in sheep. The caseous nodules in small and large intestine wall appear to be a characteristic reaction to the histotropic fourth-stage larva of *O. columbianum* in a host which has been sensitized either by a previous infection or during the first histotropic phase of the same infection (Dash, 1973).

The underlying immune mechanism causing either protection or pathology is likely to be very similar, differing only in intensity, duration and specificity. The development of strategies that aim to promote parasite-specific immune responses also have to take into account the potential of enhancing the immunopathological consequences of infection (Meeusen, 1999).

An example of reduction in production traits of animals selected for resistance was recorded in New Zealand (Morris *et al.*, 2000). Divergent breeding lines of Romney sheep, selected as lambs for consistently high or low FEC following natural multi-species challenge by nematodes parasites, were established in New Zealand in 1985. In most years, *T. colubriformis, Trichostrongylus vitrinus* and *O. circumcincta* were numerically by far the most important parasites. Relative to the control line, the low FEC line experienced significant reductions in gains and weights of lambs after weaning, as well as increases in dag scores of lambs and reductions in the fleece weights of 8-month animals and yearlings. There were corresponding changes in the opposite direction in the high FEC line, suggesting that all of these changes were of biological significance, rather than chance (Morris *et al.*, 2000).

It is interesting to note that the severity of scouring in adult sheep decreases after two years of age, suggesting that the immune response can achieve a balance in the different immune effector components which drives the reaction towards protection rather than pathology (Meeusen, 1999). Selecting sheep for parasite resistance at an early age after few infections, may bias towards the selection of an extreme response in only one arm of this complex immunological network (Meeusen, 1999). New selection strategies of sheep are now in progress which apply index selection of a range of production traits as well as decreased

FEC and decreased dags under conditions of nematode challenge (Bisset and Morris, 1996; Morris *et al.*, 2000).

Mapping genes for resistance: The majority of economically important traits in livestock production are typically quantitative multifactorial, influenced by environmental factors as well as undefined number of polygenes or quantitative trait loci (QTL) (Georges *et al.*, 1993; Georges *et al.*, 1996). They are continuous traits and present a continuous phenotypic class of distribution and are often given a quantitative value. Which means individual phenotype reflects the action of several genes that are controlled by many loci and each of them segregating according to Mendel's laws.

It seems likely that parasite resistance is no exception. Analysis of individual gene effects in polygenic traits is a complex task, since a particular locus contributes only partly to the phenotype. However, it seems that the size of the effects of individual genes vary so that some genes have large effects while others have small effects (Beh and Maddox, 1996).

The improvement of these livestock traits has been an important task for many breeding programs. With a classical pedigree-breeding program it's possible to practice selection until advanced-generation lines with the proper phenotype for the quantitative trait under selection are identified. Then, selected animals would enter into a series of crossings to test them. It is expected that those animals have the best performance because they have a selected combination of alleles most favorable to the expression of the trait. But this type of program requires an intensive amount of labor and money (Shook *et al.*, 1991).

Another way to get the improvement of these animal traits is to identify those lines that contain QTLs that contribute to a high value of the trait under selection. The first step is to choose and analyze molecular markers associated with the QTL trait. Then, identify members of a random segregating population for this quantitative trait and determine the molecular genotype of each member of the population. (Shook *et al.*, 1991). Mapping markers linked to QTLs identifies regions of the genome that contain genes involved in the expression of the quantitative trait.

Several experiments are currently in progress to identify regions of the genome that confer resistance or susceptibility to parasites. These experiments involve a systematic scan of the entire genome and involve genotyping sheep families using sufficient number of hyper variable DNA markers to tag all chromosomes. With several markers on each chromosome, special linkage analysis computer programs can be used to obtain precise estimates of the position and size of the effect of the putative gene. Thus the two requirements for a linkage study are a set of evenly spaced markers selected from a genetic map and a set of families phenotypically assessed for parasite resistance (Beh and Maddox, 1996).

Microsatelite markers are the markers of choice for gene mapping because they are highly polymorphic (O'Brien *et al.*, 1991). The development of a medium-density microsatellite linkage map for the ovine genome (Maddox, *et al.*, 2001) makes marker mapping of QTL feasible.

It's necessary to determine if there is an association between any of the markers and the quantitative trait. That is called linkage disequilibrium and is based on the presence of linkage disequilibria between alleles at the marker locus and alleles at the QTL. This disequilibrium is supposed to create marker-associated quantitative effects that can be detected by appropriate statistical analysis (Strachan and Read, 1999).

Resistance to infectious disease is under polygenic control by additive QTL. Candidate genes for resistance have been sought especially within the major histocompatibility gene complex (Wilkie and Mallard, 1999).

Candidate gene approach is used when a QTL is already mapped. It is necessary to examine a specific region involved in the animal's phenotype expression searching for a particular gene that is supposed to be directly associated with the process. A candidate locus for resistance to nematodes was found by analyzing the association between the candidate gene marker allele and the animal's phenotype (Axford *et al.*, 1999). Clarke *et al.* (2001) detected association between one of three alleles of IgE and resistance to *T. colubriformis* in a sheep flock. However, this association was not confirmed in two other herds infected with *T. colubriformis* and *H. contortus* (Clarke *et al.*, 2001). Coltman *et al.* (2001) verified that reduced fecal egg counts is in association with an allele at the microsatellite locus located in the first intron of the IFN-gamma gene in Soay sheep aged four and sixteen months, mostly infected by *T. circumcincta*. The same allele was also associated with increased *T. circumcincta*-specific antibody Ig-A in lambs, but not associated

significantly with sixteen months old sheep. These results corroborate data from previous studies realized in New Zealand showing gene-mapping loci associated to QTL for resistance on the same region of domestic sheep chromosome 3 (3q23-24) (Coltman *et al.*, 2001).

Mapping QTLs should allow marker-assisted selection, which is expected to increase the rate of genetic progress (Georges *et al.*, 1993). The main goal of marker-assisted selection in animal breeding programs is to utilize genetic markers to increase the accuracy of selection and estimate the expected gains in the livestock. Marker-assisted selection is useful when traditional methods are unsuitable. Effects of genes within a locus (dominance) and across loci (epistasis) are ignored in traditional selection that is effective only on additive genetic variation (Shook *et al.*, 1991).

Two steps are necessary to implementation of marker-assisted selection: location of loci affecting the trait in question and manipulation of the QTL with the aid of genetic markers. Marker-assisted selection based on individual quantitative trait loci can contribute to increase selection intensity, decrease generation intervals, and also by increasing accuracy of evaluation utilizing epistatic and dominance genetic variation (Shook *et al.*, 1991; Axford *et al.*, 1999).

Since resistant breeds are generally poorly productive, simple substitution of a susceptible, productive breed by a resistant breed is not always a viable option. Another approach would be to utilize resistance genes from a resistant breed by introgression through backcrossing into a more productive breed using marker assisted selection.

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