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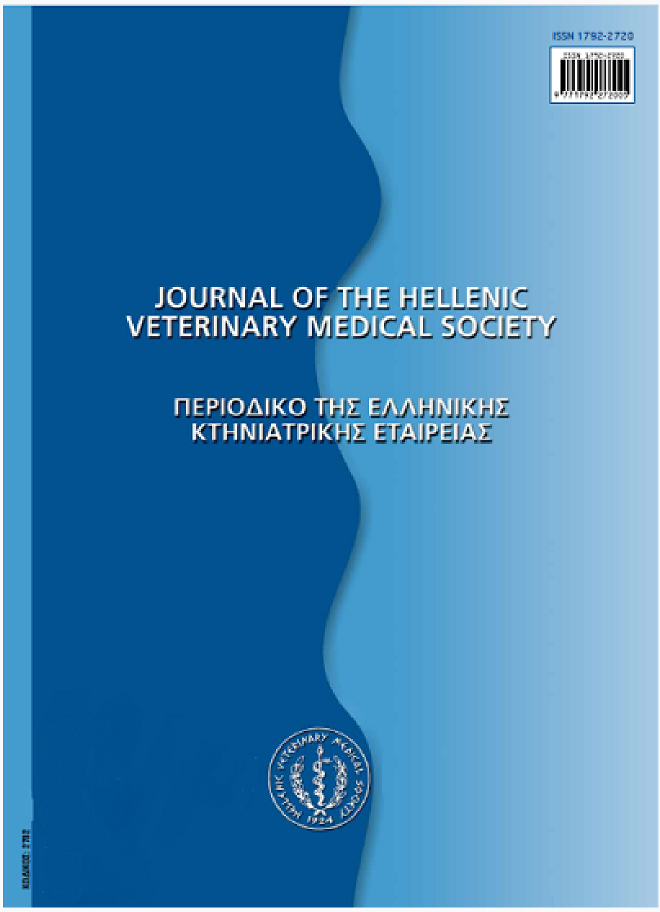

Immune and other factors modulating host resistance against gastrointestinal nematode parasites in sheep

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Review Article
Ανασκόπηση

Immune and other factors modulating host resistance against gastrointestinal nematode parasites in sheep

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Ανοσιακοί και άλλοι παράγοντες που επηρεάζουν την ανθεκτικότητα του προβάτου στα γαστρεντερικά νηματώδη παράσιτα

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ABSTRACT. Infection of small ruminants with gastrointestinal nematode (GIN) parasites is a significant problem with crucial impact on meat and milk production. The strategy of administrating anthelminthic drugs has been implemented for many years and has resulted in the development of resistant strains of parasites. Meanwhile, consumers demand for free of drugs products have led to the adoption of alternative control methods, which involve the selective breeding of animals, which are resistant to parasitism. The development of immunity and therefore, resistance against gastrointestinal parasites is based on the activation of specific host genes. Gene analysis has revealed areas (QTLs), which affect resistance or susceptibility of sheep to gastrointestinal infestations between animals of different breeds and between individuals of the same breed. The role of cytokines and T helper cells has been enhanced as research, strongly, supports the connection of Th2 cells with resistance and Th1 cells with susceptibility against GIN. Latest data implicates T regulatory cells and a specific cell type, Th17, in immune response mechanisms. Specific adhesion molecules (integrins, lectins, cadherins) are produced in the gut lumen in sufficient amounts and appear to boost immunity and reduce clinical signs in sheep. Additionally, the immunoglobulins IgA and IgE have been positively correlated with increased resistance against GIN. In several cases of GIN, where an increased number of eosinophils and mast cells in the intestinal epithelium have been recorded, the animals had a reduced number of parasite eggs in their feces. The genes of the Major Histocompatibility

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Complex have been referred to as potential resistance or susceptibility markers. Other enzymes, like chitinases, enhance the resilience of animals and protect them effectively. Animal's nutritional status is another determinant factor of immune capability against GIN in sheep, both systemic, as well as locally. Regarding the effect of reactive oxygen and nitrogen species, some researchers support their direct effect against GIN, resulting in a natural reduction of their number, while others claim the indirect action in the intestinal epithelium by reducing local immunity. Consequently, the detection of genes associated with resistance or susceptibility to gastrointestinal infestations is promising and in line with modern requirements.

Keywords: Sheep, gastrointestinal nematodes, resistance, immunity, genes

ΠΕΡΙΛΗΨΗ. Η μόλυνση των μικρών μηρυκαστικών με γαστρεντερικά νηματώδη παράσιτα αποτελεί ένα σημαντικό πρόβλημα με επίπτωση στην παραγωγή κρέατος και γάλακτος. Η αθρόα χορήγηση αντιπαρασιτικών φαρμάκων, στα πλαίσια των προγραμμάτων αποπαρασιτισμού, συνετέλεσε στην ανάπτυξη ανθεκτικών στελεχών παρασίτων. Παράλληλα, η απαίτηση των καταναλωτών για προϊόντα απαλλαγμένα από φάρμακα οδήγησε στην υιοθέτηση εναλλακτικών μεθόδων αποπαρασιτισμού, τα οποία περιλαμβάνουν την επιλεκτική αναπαραγωγή ζώων ανθεκτικών στις παρασιτώσεις. Η ενεργοποίηση του ανοσιακού συστήματος και κατά συνέπεια η ενίσχυση της ανθεκτικότητας έναντι των γαστρεντερικών νηματωδών παρασίτων βασίζεται στην ενεργοποίηση συγκεκριμένων γονιδίων του ξενιστή. Η γονιδιακή ανάλυση έχει αποκαλύψει περιοχές (QTLs), οι οποίες επηρεάζουν την ανθεκτικότητα ή την ευαισθησία του προβάτου στις παρασιτικές μολύνσεις τόσο μεταξύ των διαφορετικών φυλών όσο και μεταξύ διαφορετικών ατόμων της ίδιας φυλής. Ο ρόλος των κυτοκινών και των T βοηθητικών κυττάρων (Th) ενισχύεται καθώς έρευνες έχουν αποδείξει τη συσχέτιση των Th2 κυττάρων με ανθεκτικότητα και των Th1 με ευαισθησία έναντι των γαστρεντερικών νηματωδών παρασίτων. Πρόσφατα δεδομένα αναδεικνύουν ακόμη περισσότερο τη σημασία των T ρυθμιστικών κυττάρων (Treg) και των Th17 βοηθητικών κυττάρων στην απάντηση του ανοσιακού συστήματος έναντι των παρασιτώσεων. Ειδικά μόρια προσκόλλησης, τα οποία παράγονται στον αυλό του εντέρου σε επαρκείς ποσότητες, υποβοηθούν την ανοσία και μειώνουν τη βαρύτητα των κλινικών συμπτωμάτων στα πρόβατα. Επιπλέον, οι ανοσοσφαιρίνες IgA και IgE έχουν συσχετιστεί θετικά με αυξημένη ανθεκτικότητα των μηρυκαστικών έναντι των γαστρεντερικών νηματωδών. Σε αρκετές περιπτώσεις παρασιτώσεων όπου καταγράφηκε υψηλός αριθμός εωσινόφιλων και μαστοκυττάρων στο εντερικό επιθήλιο, τα ζώα εμφάνισαν μειωμένο αριθμό παρασιτικών στοιχείων στα κόπρανα τους. Τα γονίδια του Μείζονος Συμπλέγματος Ιστοσυμβατότητας αναφέρονται ως πιθανοί δείκτες ανθεκτικότητας ή ευαισθησίας. Ένζυμα όπως οι χιτινάσες ενισχύουν την ικανότητα αντίστασης των ξενιστών. Η διατροφή των ζώων είναι ακόμη ένας σημαντικός παράγοντας που επηρεάζει την ανοσιακή απόκριση έναντι των παρασιτώσεων στο πρόβατο, τόσο συστηματικά όσο και τοπικά. Σχετικά με την επίδραση των ελεύθερων ριζών οξυγόνου, ορισμένοι ερευνητές υποστηρίζουν την άμεση επίδρασή τους έναντι των παρασίτων, οδηγώντας σε φυσική μείωση της μόλυνσης, ενώ άλλοι την έμμεση μείωση της τοπικής ανοσίας του εντέρου. Συνεπώς, η ανίχνευση γονιδίων που σχετίζονται με ανθεκτικότητα ή ευαισθησία στα γαστρεντερικά νηματώδη παράσιτα αποτελεί σημαντική προοπτική και συμβαδίζει με τις σύγχρονες απαιτήσεις.

Λέξεις Ευρετηρίασης: πρόβατο, γαστρεντερικά νηματώδη παράσιτα, ανθεκτικότητα, ανοσία, γονίδια

INTRODUCTION

Gastrointestinal nematodes (GIN) are one of the major causes of disease in ruminants, worldwide (Familton and McAnulty, 1997; Perry and Randolph, 1999). Parasites such as *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Nematodirus* spp. impose severe constraints on sheep and goat production including heavy losses in terms of milk and meat production, direct cost of antiparasitic drugs and loss due to mortality (Miller et al., 2012). For example, annual treatment cost for *H. contortus* alone, had been estimated to be 26 million USD in Kenya, 46 million USD in South Africa and 103 million USD in India (McRae et al., 2014). Anthelminthics, such as benzimidazoles and macrocyclic lactones have been widely used to control parasitism. However, indiscriminate use of drugs has led to the emergence of GIN resistant strains to anthelminthic drugs, which further complicates the management of parasitic diseases in small ruminants (Gasbarre et al., 2009; Kim et al., 2013). Taken together the increasing incidence of anthelminthic resistance, the cost of developing new drugs and the consumers' concern to minimize drench residues in animal products, necessitate further efforts into the discovery of alternative ways to control helminthes (Sargison, 2012).

Three general approaches are available for controlling infection by disrupting the life cycle of the parasite and reducing the severity of infection (Hoste and Torres-Acosta, 2011; Li et al., 2012). These include, grazing management to reduce the number of drug resistant nematodes and host exposure, elimination of nematodes in the host by conventional anthelminthic drugs or alternative non pharmaceutical measures and enhancing host resistance (Li et al., 2012).

Nowadays, there is an increasing interest in control strategies based on the host immunogenetics, because a variable genetic host resistance exists for the major ovine GIN (Miller et al., 2006). Among sheep breeds a considerable variation on their ability to resist GIN has been demonstrated (Periasamy et al., 2014). Similarly, a genetic variation even within the animals of the same breed has been reported for sheep populations of Merino, Romney, Scottish Blackface and Feral Soay sheep (Periasamy et al., 2014). Host resistance is a heritable feature with wide variability among individuals. Host resistance is also characterized by rapid genetic progress in sheep flocks both under research and commercial conditions (Morris et al., 2000; 2005; McRae et al., 2014). Breeding for host resistance is considered a crucial method of nematode control. It is possible to manipulate breeding lines of sheep as to produce strong phenotypic differences, in well-defined pedigrees, in a relatively short space of time (Kim et al., 2013). Moreover, computer simulation models have shown that selection for host resistance, using the phenotypic characteristic low fecal egg count (FEC), is reliable because it remains constant for long time, i.e. 20 years (McRae et al., 2014).

This review summarizes the mechanisms of host immune response to GIN which are associated with resistance or susceptibility focusing on Th0 cell activation, cytokines and cell adhesion molecules that interact, antibodies produced, eosinophils and mast cells recruited and MHC products involved. Furthermore, it analyses the impact of other factors, such as activated enzymes like chitinases, oxidative status and nutrition. Finally, it highlights the molecular immunology of infection and the identification of candidate genes for selection.

The role of cytokines and T helper (Th) cells

Parasites have the ability to alter host immune response and create chronic infection by modulating and escaping host immune protective mechanisms. The hallmark of nematode infections in sheep is the Th0 cell activation, which develops in two distinct pathways. These pathways are characterized by differentiation of Th0 cells and therefore special Th1 or Th2 cell response. This model of activation is controlled by specific cytokines (Finkelman and Urban, 2001; Li et al., 2012; Venturina et al., 2013).

Studies on murine models demonstrated that GIN induce Th2 cell responses. Th2 cells produce IL-4, IL-5, IL-9, IL-13, IL-25 and IL-33, which cause differentiation and maturation of intraepithelial mast cells, eosinophilia and goblet cell development (Artis and Grencis, 2008; Li et al., 2012). These events lead to alteration of enterocyte permeability and increased enterocyte turnover. IL-13 activates goblet cells and, consequently, increases the secretion of mucus and prevents contact of parasites with the epithelial surface. Other goblet cell products (Muc5A, Relm-beta) are associated with anti-parasitic responses (Artis et al., 2004; Hasnain et al., 2011) and contribute to local inflammation in the mucosa (Li et al., 2007; Nair et al., 2008; Li et al., 2012). Additionally, IL-13 in collaboration with IL-4 activate macrophages that attack and stress, with their metabolic products, larval stage of nematodes within the intestinal mucosa (Artis and Grencis, 2008). Activated macrophages alter intestinal contractility and epithelial cell function in mouse models (Zhao et al., 2008). Nematode infection promotes a turnover of the enterocytes as a host mechanism of parasitic expulsion that is regulated by local cytokines (Li et al., 2012).

The above process has, also, been proved in ruminants. In sheep, a Th2 response induces an increase of immunoglobulin A (IgA) and immunoglobulin G (IgG), tissue eosinophilia, mucosal mast cell activation and goblet cell hyperplasia against nematode infection (Li and Gasbarre, 2009; Li et al., 2010). Eosinophils generally comprise less than 5% of total leukocytes but increase quickly at the site of infection as the infection progresses (Li and Gasbarre, 2009; Li et al., 2012). Immunological studies have shown that lambs were unable to mount immune responses against *H. contortus* because of weak Th2 responses (Schallig, 2000; Benavides et al., 2002). Th2 cytokines expression levels, especially those of IL-4, in the gastrointestinal lymphatic tissue were also crucial for the immune response in sheep genetically resistant to *T. colubriformis* after natural challenge (Pernthaner et al., 1997; Benavides et al., 2002).

The expression of Th1 response during nematode infection can determine the relative resistance or susceptibility of a genetically distinct mouse strain (Li et al., 2012). Research using murine models has underlined the role of Th1 cells in the susceptibility of the host immune system against GIN infection (Venturina et al., 2013). More precisely, C57BL/6 and AKR mice developed persistent infection against *Heligmosomoides polygyrus* and *Trichuris muris,* when a Th1 immune response was present, while Balb/c mice managed to control the infection against the same nematodes with a strong Th2 response (Reynolds et al., 2012; Venturina et al., 2013). At the same frame, an infection of mice with *H. polygyrus, T. muris* and *Nippostrongylus brasiliensis* were characterized by Th2 responses and high levels of cytokines IL4 and IL13 was linked to parasite resistance (Maizels et al., 2003; Anthony et al., 2007; Gossner et al., 2013). On the other hand, an infection characterized by Th1 responses and thus high levels of IFN-γ has been correlated with susceptibility, persistence of infection and clinical outcome of the disease (Maizels and Yazdanbakhsh, 2003; Anthony et al., 2007; Dawson et al., 2009; Venturina et al., 2013). In cattle, IFN-γ inhibited host protective antibody responses to *Strongyloides papillosus* resulting in an improvement of the larvae survival and increased egg production (Nakamura et al., 2002). This link between IFN-γ and susceptibility is attributed to IFN-γ properties of inhibiting Th2 responses and down-regulating IL-4 production (Pulendran, 2004; Venturina et al., 2013). The other major Th1 cytokine associated with nematode infection is IL-12 (Venturina et al., 2013). IL-12 induces the differentiation of Th0 to Th1 cells. Binding of IL-12 to its receptors activates the Th1 pathway and triggers the production of its transcription factors (TBX21, HLX). These in turn, promote the production of IFN-γ (Venturina et al., 2013). On the contrary, Sayers et al. (2005b) supported a relationship between IFN-γ and resistance to nematode infection in Texel breed.

The view that a Th1 response is associated with susceptibility and a Th2 response with resistance, as well as their balance, was for many years an issue of conflict. Some studies on ruminants have shown constant or increased expression of IFN-γ and IL-12 despite a predominant Th2 response in *H. contortus* infection (Meeusen et al., 2005; Pernthaner et al., 2005). In another study, IFN-γ expression was unaffected by *T. circumcincta* infection of either immune or naive lambs (Craig et al., 2007; Venturina et al., 2013). Other researchers concluded that nematodes can also induce various levels of a Th1 response (Finkelman and Urban, 2001; Li et al., 2012). However strongly polarized Th2 responses for the control of protozoan and microbial infections can down-modulate Th1 based immunity (Finkelman and Urban, 2001; Li et al., 2012).

T regulatory cells (Tregs) and Th17 responses

The profile of secreted Th1/Th2 cytokines modulates mechanisms that affect the susceptibility or resistance against GIN. One of the ways of manipulating the immune response is the development of T regulatory cells (Tregs) (Venturina et al., 2013). These cells have a double role. Firstly, they suppress prolonged immune activation with IL-10 and TGF-β cytokines, which are responsible for the flexibility of the Th1/Th2 cell activation (Belkaid and Rouse, 2005; Ouyang et al., 2011). Secondly, Tregs are crucial for the clinical outcome of the parasitism (Belkaid and Tarbell, 2009; Venturina et al., 2013).

Murine experiments showed correlation between parasite resistance and a balanced Th1/Th2/Tregs response. On the contrary, an unbalanced (high Th1/Th2/Tregs) response resulted in persistent infection. CD4+ Tregs cells usually express the IL-2 receptor α chain (IL2RACD25) and the transcription factor FOXP3 (Hori et al., 2003; Fontenot et al., 2005). In *H. polygyrus* infection in mice, an early Th2 dominated cytokine profile altered to a Tregs response by day

28. This was marked by expansion of FOXP3 cells, elevated IL-10 and higher numbers of TGF - β T cells (Finney et al., 2007; Venturina et al., 2013). Tregs activity has also been demonstrated in human infections with the filarial nematodes *Onchocerca volvulus* (Korten et al., 2008) and *Litosomoides sigmondontis* (Taylor et al., 2005) and in sheep infected with *T. circumcincta* (Craig et al., 2007), *H. contortus* and *T. colubriformis* (Ingham et al., 2008).

Human experiments have emerged Th17 cells as a distinct T cell category that produces the inflammatory cytokines IL-17A and IL-21, but not IFN-γ or IL-4. These cells are important in the induction of inflammation that controls bacterial infections, especially at mucosal sites (Korn et al., 2009). However, inappropriate activation of Th17 cells leads to autoimmune inflammatory diseases like inflammatory bowel disease (IBD), asthma and rheumatoid arthritis (Weaver et al., 2007; Peck and Mellins, 2009). The cytokine IL-6 is crucial for Th17 development (Kimura and Kishimoto, 2010), as it drives the TGF-β induced T cells to differentiate to Th17 instead of other T cell strains (Veldhoen et al., 2006). Moreover, IL-6 upregulates IL-23R and works in collaboration with IL-23, another cytokine that promotes Th17 inflammation (Ahern et al., 2010; Venturina et al., 2013).

In experiments conducted on the immunopathology of chronic *T. circumcincta* infection, Blackface lambs were infected with L3 larvae for over 12 weeks. These lambs were identified with a range of susceptibilities, as assessed by adult worm count at post mortem examination, FEC and IgA antibody levels. Noteworthy, histopathology showed no adult worms, low FEC and high amounts of IgA in the abomasal mucosa of resistant animals with a low level of lymphocyte infiltration (Venturina et al., 2013). On the contrary, in susceptible lambs there were high numbers of adult worms and FEC, low IgA production and extensive inflammatory infiltration, resulting in major pathological changes (Venturina et al., 2013). Reverse transcription quantitative PCR assays on the abomasal lymph nodes showed high levels of IL-6, IL-21 and IL-23A expression in susceptible sheep and also, a positive correlation to adult worm count and FEC. This is consistent with the hypothesis that the inability to control L3 larval colonization, adult worm infection and egg production is due to the activation of the inflammatory Th17 cell subset (Gossner et al., 2012a; b; Venturina et al., 2013).

Cell adhesion molecules

Cell adhesion molecules are mediators that are necessary for promoting cell to cell and cell to extracellular matrix interactions. These molecules, which contain cadherins, integrins, lectins and neural cell adhesion proteins, are involved in numerous biological processes in bovine gastrointestinal track, including cell proliferation and differentiation, pathogen recognition and host defense (Li and Gasbarre, 2010; Li et al., 2012). Murine experiments have shown that these substances enhance accumulation of inflammatory mediators to the site of infection and provoke immune responses, especially by mounting Th1 response to nematode infection (Bell and Else, 2008). Increased expression of cadherin-26 is positively correlated with eosinophilia and FEC in cattle infected with *Cooperia* spp. (Li et al., 2012). Integrins, particularly integrin β-7, play a key role in the accumulation of mast cells in the gastrointestinal mucosa (Pennock and Grencis, 2006). Lectins are thought to be mediators of inflammation that enhance immune response (Lasky, 1991). The role of galectins, intelectins and lectins is to recognize the special surface molecules of parasites. Galectin-11 is, strongly, induced when sheep are infected with *H. contortus* and *Trichostrongylus vitrinus* (Dunphy et al., 2000). Intelectin-2 expression is regulated by IL-4 (French et al., 2007; Li et al., 2012). Its elevated expression is observed in the sheep abomasum in response to *T. circumcincta*, *Dictyocaulus filaria* (French et al., 2009) and *H. contortus* infection (Rowe et al., 2009). Intelectin-2 is significantly higher in the abomasal mucosa of immunized sheep in response to challenge with *T. circumcincta* (Athanasiadou et al., 2008).

The role of Immunoglobulin A (IgA) and Immunoglobulin E (IgE)

Several experimental results have identified IgA as a major antibody against parasite infection and fecundity (Beraldi et al., 2008; Shaw et al., 2012). Many researchers support a negative correlation of serum IgA levels with infection parameters. This has been proved for *T. circumcincta* (Li et al., 2012; Shaw et al., 2012). While the association between serum IgA and infection exists, it is presumed that the biologically active antibody is secretory IgA, which is actively secreted across the mucosal epithelium of the abomasum (Macpherson et al. 2008; Venturina et al., 2013). The mechanisms of IgA action are not clear, even though IgA can bind to both larvae and adults or to nematode secretions, thus controlling larval development and egg production (Stear et al., 2004; Halliday et al., 2007). One report suggests that secretory IgA can inhibit larval establishment by provoking eosinophil degranulation (Li et al., 2012), despite the fact that eosinophils and antibodies are on different sides of the epithelial barrier (Venturina et al., 2013). Finally, it has been demonstrated that IgA inactivates metabolic enzymes and suppresses feeding of the parasite, which results in reduced adult worm length and fecundity (Craig et al., 2007).

IgE antibodies play a significant role in parasite expulsion as well. High levels of IgE are negatively correlated with FEC (Murphy et al., 2010). IgE demonstrate their way of action through a classical Type-1 hypersensitive reaction, mediated by mast cell proliferation and degranulation of mast cells (Greer et al., 2008; Li et al., 2012). As a result of the above, vasoactive mediators and cytokines are released (Pochanke et al., 2007), leading to contraction of blood vessels, increased mucus production by the gut mucosa (Stear et al., 2003) and upregulation of interlectins, that block larval colonization and development (French et al., 2008). This is supported by similar observations in infected, cured and re infected immunized sheep (Gossner et al., 2013; Venturina et al., 2013).

Consequently, the control of larvae establishment, worm development and egg production is assisted through the production of parasite specific IgA and IgE antibodies in sheep (Gasbarre et al., 2001; Murphy et al., 2010; Gossner et al., 2013). The levels of these two antibody classes are highly negatively correlated with worm length, fecundity and FEC, as both IgA and IgE are significantly increased in resistant sheep (Beraldi et al., 2008; Gossner et al., 2013).

The role of eosinophils (EOS) and mast cells

Eosinophils have been shown to have a crucial role in the protection against GIN infections of many animal species. Galioto et al. (2006) has proved this protective role against *Strongyloides stercoralis* in mice and Robinson et al. (2010) against *H. contortus* in sheep. Researchers have observed a rapid eosinophil recruitment around L4 larvae resulting to a considerable damage to *H. contortus* larval surface, especially in gastric pits of immunized sheep (Balic et al., 2006). In another experiment, Balic et al. (2002) have not observed any differences in eosinophils or mast cells numbers after 12 weeks of *H. contortus* infection in sheep. Terefe et al. (2005) reported eosinophilia to be correlated with protection against *H. contortus*. However, their protective role against *T. circumcincta* is unclear. Henderson and Stear (2006) found no relationship between numbers of adult *T. circumcincta* and tissue eosinophilia. At the same aspect, Beraldi et al. (2008) did not observe relationship between FEC and circulating eosinophil counts. These controversy results are due to the fact that *T. circumcincta* causes little damage to the mucosal epithelium and eosinophils cannot interact with the parasites on the lumenal side of the intestinal epithelium (Venturina et al., 2013).

Increased eosinophilia has been reported as an indicator of a greater responsiveness of hosts to *T. colubriformis* infection (Dawkins et al., 1989). Shakya et al. (2011) have found an induction of peripheral eosinophilia in Native lambs regardless of infection regimen. Higher numbers of abomasal mucosal eosinophils, mast cells and neutrophils have been observed in infected animals compared to controls. Hunter and MacKenzie (1982) reported a few eosinophils present on the seventh day of *H. contortus* infection in lambs but no mast cells or globule leukocytes. Eosinophils have been observed in the abomasal mucosa of first time infected lambs with larval stages of parasites and their numbers remained stable later as larvae developed into adults (Shakya et al., 2011). On the contrary, in a similar experiment, the numbers of mucosal mast cells increased in such lambs (Balic et al., 2000).

H.contortus sensitized sheep were seen to have greater numbers of eosinophils (Rainbird et al., 1998). In such cases, eosinophils immobilized *H. contortus* larvae in the presence of anti-*Haemonchus* antibodies in an *in vitro* study (Rainbird et al., 1998; Shakya et al., 2011). According to Pennock and Grencis (2006), post infection mastocytosis had an important role in elimination of the specific parasite in lambs. A greater number of eosinophils and mucosal mast cells in Native lambs was associated with an increased immature *H. contortus* population, thus indicating a possible contribution to inhibition of growth and development of the parasites (Shakya et al., 2011). Finally, Pesce et al. (2008) reported that high numbers of neutrophils are involved in the reduction of bacterial burden, especially in early stages of *H. contortus* infection, that co-infect with *H. contortus* nematode.

Major Histocompatibility Complex (MHC I and II)

The association of the Major Histocompatibility Complex (MHC) with differential response to parasite infection is attributed to MHC polymorphism and involvement of MHC gene products with the triggering and modulation of the immune response (Cresswell, 1994). In this sense, the main research focus has been on MHC genes, which are involved in immunological induction and regulation processes.

Several Ovar-D (MHC class II) gene alleles have shown significant associations with low FEC, under both natural and experimental infection protocols, including the allele Ovar-DRB 257 (Paterson et al., 1998) and Ovar-DY (Buitkamp et al., 1996). The latter authors found that some alleles from MHC Class I and DY (Class II) loci were associated with 8- and 218-fold decrease in FEC, respectively, in that same flock. One of the best-studied genetic markers is Ovar DRB1-1101 (Hassan et al., 2011). More precisely, Schwaiger et al. (1995) found an association between a DRB1 allele and FEC reductions of 58-fold in Scottish Blackface lambs naturally infected with *T. circumcincta*. Carrier lambs with DRB1-1101 had lower adult worm burdens and higher mast cell and plasma lymphocyte counts (Hassan et al., 2011, Venturina et al., 2013).

Susceptibility has been associated with the MHC Class II locus, where high FEC was observed in New Zealand sheep carrying the Ovar DQA2-1201 allele (Hickford et al., 2011). MHC-DQA2 allele 1201 was significantly associated with increased total FEC at both 4 and 9 months old lambs. It was especially associated with increased Strongyle and *Nematodirus* spp. counts. At weaning, allele 1001 was also found to be strongly associated with increased Strongyle, *Nematodirus* spp. and total FEC. The majority of the associations between MHC-DQA2 and various parameters of parasite resistance were age and parasite-count specific, s uggesting that immune response is both age and challenge (combination of parasites) dependent (Hickford et al., 2011).

Outteridge et al. (1985) found associations between resistance to *T. colubriformis* and MHC Class I in different lines of sheep. However, the relationship between MHC Class I and sheep selected for low FEC was not observed in a later study (Outteridge et al., 1986). Similarly, Cooper et al. (1989) and Crawford et al. (1997) found no effect of MHC on the susceptibility of sheep to GIN. Paterson et al. (1998) reported associations between variation in microsatellites associated with MHC-DRB1 and measures of FEC, with particular alleles being associated with both higher and lower FEC in Soya sheep. These results were not found to be consistent between lambs and yearlings and led to the conclusion that different MHC alleles exhibit different associations at different stages during a sheep's life. The authors, also, speculated that these results possibly reflect a "complex interplay" between GIN and the vertebrate immune system and suggested that other MHC types, which the authors had not considered in their study, may have conferred protection against parasites.

MHC allele frequencies may also be affected by other non-parasitic diseases, such as the footrot, with two reports that the MHC-DQA2 and MHC-DQA2-like genes are associated with variation in susceptibility to this bacterial disease (Ennen et al., 2009; Hickford et al., 2011).

The role of chitinase and chitinase-like proteins

Chitinases are a group of digestive enzymes that catalyse glycosidic bonds in chitin, which are present in nematodes and arthropods. Mammalian chitinases and chitinase-like proteins are upregulated and secreted in Th2 response (McRae et al., 2014). CHIA, which is a chitinase candidate gene, has been associated with the immune response against helminthic infection in mammals (Lee et al., 2011). In Th2 response, IL-13 activates CHIA, which produces chitinase and has been implicated in Th2 dominated disorders such as asthma (Zhu et al., 2004). In mice, chitin is a recognition element for tissue infiltration by innate immune cells, such as eosinophils and basophils, and this process can be negatively regulated by chitinase (McRae et al., 2014).

Chitinase-like proteins can bind chitin, but they do not have chitinolytic enzyme activity due to mutations in their active domains (Lee et al., 2011). The chitinase-like molecule CHI3L1 is upregulated in the abomasum of sheep in response to *T. circumcincta* challenge of previously infected animals (Knight et al., 2007; McRae et al., 2014). CHIA expression was examined in the same study but while expression was observed, the upregulation of transcripts was minor. Expression of CHI3L2 has been observed in the abomasum of 18 and 21 week old steers exposed to *Ostertagia ostertagi* (Sonstegard et al., 2004). Expression has also been demonstrated in the abomasal lymph node of resistant and susceptible Blackface lambs infected with *T. circumcincta* in comparison to non infected control animals (Gossner et al., 2013; McRae et al., 2014).

The role of nutrition

The nutritional status of the animal plays a determinant role in both disease reduction and enhanced host resistance to GIN. Infection with GIN is accompanied by mild to severe anorexia, impaired digestion and absorption and increased partitioning of nutrients (Sykes, 2008; Hoste and Torres-Acosta, 2011; Li et al., 2012).

Cholecystokinin is a mediator, affecting appetite status during GIN infection of lambs. In murine models, appetite depression was immunologically linked to secretion of cholecystokinin by enteroendocrine cells of the gut mucosa, mediated by Th2 cytokines IL4 and IL13 (McDermott et al., 2006; Li et al., 2012). Nutrients are required for the repair of the damaged gut mucosa and the enhancement of immune response against these infections. The boosting of immunity is due to extensive replication of lymphocytes and other immune cells as well as the synthesis of acute phase proteins (Colditz, 2008). Nutrient management can enhance host resistance against GIN infection and improve clinical signs (Wagland et al., 1984). The impact of infection on protein metabolism usually exceeds its impact on energy balance. This explains why the resilience and productivity of infected animals can be improved by protein supplementation (Coop and Kyriazakis, 1999). In addition, studies in rats and sheep concluded that the supplemental dietary protein induced transcriptome changes, which in turn increased cell turnover, growth, differentiation and finally the expulsion of nematodes from the intestine (Athanasiadou et al., 2011; Li et al., 2012). Elucidation of the extra metabolic needs of parasitized animals and adequate nutritional supplementation to improve the health and performance of infected and non-infected animals remains an important need. Coupling dietary changes to transcriptomic expression of appropriate resistance mechanisms could improve the efficiency of integrated strategies for parasite control (Li et al., 2012).

There are many references that confirm a clear relationship between dietary program and expression of specific genes. "Nutrigenomics" is a new term that supports the effects of food constituents on the expression of genes with immunomodulatory properties (Singh-Dang et al., 2014).

Oxidative status

The generation of host oxidants, especially Reactive Oxygen and Nitrogen Species (RONS) is considered significant in parasite control (Ingham et al., 2008; Patel et al., 2009). RONS are possible to exert an anti-parasitic effect through direct damage of parasitic tissues (Colasanti et al., 2002; Lees et al., 2011). Host generated RONS display high reactivity and low specificity. This is the reason why they can damage host tissues, leading to dysfunction of the immune response (Wang et al., 2007). There has been evidence that GIN specifically produce and secrete a number of protective antioxidant enzymes in response to host generated RONS (Kotze, 2003; Dzik, 2006; Lees et al., 2011). Some studies have demonstrated the requirement for effective host antioxidant defenses for the development of immunity against GIN infection (Smith et al., 2005).

Research conducted by Lees et al. (2011) provided evidence that host generated oxidants and antioxidants are major components of the response against *H. contortus* infection in sheep. They demonstrated that the natural host (sheep) triggered a specific inflammatory response to the parasite, characterized by an increase in the dual oxidase group of oxidant production (DUOX2/DUOXA2) during the first 7 days of the infection (larvae expulsion period) (Lees et al., 2011). On the contrary, they demonstrated an inverse correlation between DUOX2 and DUOXA2 expression levels, at the time when resistance in sheep was established (day 28). The above provides evidence that DUOX2 is associated with a successful host response to infection (Lees et al., 2011). Similarly, they concluded that the host antioxidant response to infection is specific to the time of challenge, involving a switch in expression between members of the glutathione peroxidase family of genes (Lees et al., 2011).

DUOX2 gene expression in the lungs has been responsive for the production of cytokine IFN-γ (Th1 pathway), while DUOX1 has been linked to the production of cytokine IL4 (Th2 pathway) (Harper et al., 2005). One interesting point is that a positive association between IFN-γ and IL4 expression was noted, despite the fact that these cytokines have been shown to have antagonistic functions (Paludan, 1998; Lees et al., 2011). Lees et al. (2011) have linked the expression of host oxidants and antioxidants in response to the parasite infection, showing that IL4 appears to be strongly linked to a proliferation of immune cells, as well as to the induction or suppression of the two arms of the host oxidant and antioxidant response, respectively.

Genomic loci associated with host resistance

Many sheep producing countries have undertaken studies to clarify the genetic variation of resistance among breeds and among animals within a breed (Bishop and Morris, 2007). Several quantitative trait loci (QTLs) mapping studies on parasite resistance characteristics have been reported in sheep. The objective of QTL studies is to identify underlying causative molecular markers, such as single nucleotide polymorphisms (SNPs) which correlate with an observed trait (Periasamy et al., 2014). The QTL analysis is a powerful method to understand the genotype - phenotype relationship and has up to date identified a number of traits indicative of resistance (Silva et al., 2012; Marshall et al., 2012). Most of the QTLs related to parasite resistance were found to be located in chromosome 3 (16 QTLs), followed by chromosome 14 (7 QTLs). The different QTLs reported in chromosome 3 for host resistance against parasites were distributed all over the chromosome, with varying overlapping regions (Marshall et al., 2009; Sayre et al., 2012).

Advances in genomic technologies and sequencing provide new opportunities to identify polymorphisms conferring host resistance. Exploration of genetic variation either within specific regions of the genome or in specific candidate genes, involved in innate and adaptive immune pathways, may help to identify a set of DNA markers strongly associated with host resistance to parasites. Results from studies support the hypothesis that host resistance to GIN is likely to be controlled by a number of loci of moderate to small effects (McRae et al., 2014). McRae et al. (2014) selectively bred divergent lines of Romney and Perendale sheep, for high and low FEC and genotyped them using the Illumina® Ovine SNP50 BeadChip. They identified fourteen novel regions associated with resistance or susceptibility to GIN, which included candidate genes, involved in chitinase activity and cytokine response.

In one of the first SNP-based QTLs detection studies for *H. contortus* host resistance in sheep, four QTLs regions in sheep chromosomes, OAR-5, -12, -13 and -21, were identified as key players among many other QTLs with small to moderate effects. A QTL on OAR-21, the first reported QTL affecting pepsinogen concentration, exactly matched the pepsinogen PGA-5 locus. The OAR-5 and OAR-13 showed QTLs with large or pleiotropic effects or both that could not be matched to any known functional candidate genes. The OAR-12 remains an interesting candidate, because of a 10-Mbp region affecting FEC both after the first and the second infection (Salle et al., 2012).

Periasamy et al. (2014) reported strong phylogeographic structure and balancing selection operating SNPs located within immune pathway genes. This study identified a total of 41 SNPs within 38 candidate genes, located in sheep chromosome 3, as well as in other genes involved in major immune pathways, in a panel of 713 unrelated sheep, belonging to 22 breeds across Asia, Europe and South America.

Conclusion

Nowadays, there is an increasing interest in control strategies based on the host immunogenetics and their underlying mechanisms, as genetic variation in host resistance exists for the major nematode species affecting sheep. Nematode infections in sheep induce significant changes in patterns of gene expression. One approach of increasing resistance may be the exploitation of genetically resistant breeds or the use of molecular markers to select resistant individuals. Further work is required to clarify the complex underlying genetic mechanisms.

It is plausible that an adaptive immune response plays a crucial role in parasite control. This has been indicated by the fact that exposure to infectious larvae leads to reduction of larval colonization, fecundity and FEC. Antibodies (IgA, IgE) and other immune mediated cells (EOS, mast cells, Tregs) are important for the protection against nematodes, thus being affected by Th1/Th2/Th17 cell immune responses, as well as their cytokines.

In addition, SNPs identified up to date were found to have potential for future large scale association studies in naturally exposed sheep populations. The complexity of this analysis is evident from the fact that multiple, significant QTLs regions have been reported across the entire genome, but the identification of candidate causative genes has remained elusive. The lack of consensus overlap among reported QTLs has hindered the identification of candidate genes and genetic markers for selection in sheep.

Future studies will aim at exploring genetic variations within genomic regions and different candidate genes involved in immunoregulatory mechanisms, identification of SNPs and genetic diversity analysis in sheep populations under different environmental conditions.

Conflict of interest statement

None of the authors have any conflicts of interest to declare.

REFERENCES

Ahern PP, Schiering C, Buonocore S, McGeachy MJ, Cua DJ, Maloy KJ, Powrie F (2010) Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity 33:279-288.

Anthony RM, Rutitzky LI, Urban JF, Stadecker MJ, Gause WC (2007) Protective immune mechanisms in helminth infection. Nat Rev Immunol 7:975–987.

Artis D, Grencis RK (2008) The intestinal epithelium: sensors to effectors in nematode infection. Muc Immunol 1:252–264.

Artis D, Wang ML, Keilbaugh SA, He W, Brenes M, Swain GP, Knight PA Donaldson DD, Lazar MA, Miller HR, Schad GA, Scott P, Wu GD (2004) RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. Proc Nat Acad Sci USA 101: 13596–13600.

Athanasiadou S, Jones LA, Burgess ST, Kyriazakis I, Pemberton AD, Houdijk JG, Huntley JF (2011) Genome-wide transcriptomic analysis of intestinal tissue to assess the impact of nutrition and a secondary nematode challenge in lactating rats. PLoS One 6 e20771.

Athanasiadou S, Pemberton A, Jackson F, Inglis N, Miller HR, Thıvenod F, Mackellar A, Huntley JF (2008) Proteomic approach to identify candidate effector molecules during the in vitro immune exclusion of infective *Teladorsagia circumcincta* in the abomasum of sheep. Vet Res 39:58.

Balic A, Bowles VM, Meeusen EN (2000) Cellular profiles in the abomasal mucosa and lymph node during primary infection with *Haemonchus contortus* in sheep. Vet Immunol Immunopathol 75:109–120.

Balic A, Bowles VM, Meeusen EN (2002) Mechanisms of immunity to *Haemonchus contortus* infection in sheep. Parasite Immunol 24:39–46.

Balic A, Cunningham CP, Meeusen EN (2006) Eosinophil interactions with *Haemonchus contortus* larvae in the ovine gastrointestinal tract. Parasite Immunol 28:107–115.

Belkaid Y, Rouse BT (2005) Natural regulatory T cells in infectious disease. Nat Immunol 6:353–360.

Belkaid Y, Tarbell K (2009) Regulatory T cells in the control of host microorganism interactions. Annu Rev Immunol 27:551–589.

Bell LV, Else KJ (2008) Mechanisms of leucocyte recruitment to the inflamed large intestine: redundancy in integrin and addressin usage. Parasite Immunol 30:163–170.

Benavides MV, Weimer TA, Borba MFS, Berne MEA, Sacco AMS (2002) Association between microsatellite markers of sheep chromosome 5 and faecal egg counts. Small Ruminant Research 46:97–105.

Beraldi D, Craig BH, Bishop SC, Hopkins J, Pemberton JM (2008) Phenotypic analysis of host-parasite interactions in lambs infected with *Teladorsagia circumcincta.* Int J Parasitol 38:1567–1577.

Bishop SC, Morris CA (2007) Genetics of disease resistance in sheep and goats. Small Rum Res 70:48–59.

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

Colasanti M, Gradoni L, Mattu M, Persichini T, Salvati L, Venturini G, Ascenzi P (2002) Molecular bases for the anti-parasitic effect of NO. Int J Mol Med 9:131–134.

Colditz IG (2008) Six costs of immunity to gastrointestinal nematode infections. Parasite Immunol 30:63–70.

Coop RL, Kyriazakis I (1999) Nutrition–parasite interaction. Vet Parasitol 84:187–204.

Cooper DW, Van Oorschot RAH, Piper LR, Le Jambre LF (1989) No association between the ovine leukocyte antigen (OLA) system in the Australian Merino and susceptibility to *Haemonchus contortus* infestation. Int J Parasitol 15:101–109.

Craig NM, Miller HR, Smith WD, Knight PA (2007) Cytokine expression in naive and previously infected lambs after challenge with *Teladorsagia circumcincta*. Vet Immunol Immunopathol 120:47–54.

Crawford AM, McEwan JC, Dodds KG, Wright CS, Bisset SA, Macdonald PA, Knowler KJ, Greer GJ, Green RS, Shaw RJ, Paterson KA, Cuthbertson RP, Vlassoff A, Squire DR, West CJ, Phua SH (1997) In: Proceedings of the 12th Conference on Resistance to Nematode Parasites in Sheep: How Important are the MHC Genes? Part 1 Dubbo, NSW, Australia, pp.58–62.

Cresswell P (1994) Assembly, transport, and function of MHC class-II molecules. Annu Rev Immunol 12:259–293.

Dawkins HJ, Windon RG, Eagleson GK (1989) Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus colubriformis*. Int J Parasitol 19:199–205.

Dawson HD, Solano-Aguilar G, Beal M, Beshah E, Vangimalla V, Jones E, Botero S, Urban JF (2009) Localized Th1, Th2, T regulatory cell and inflammation associated hepatic and pulmonary immune responses in *Ascaris suum*-infected swine are increased by retinoic acid. Infect Immun 77:2576–2587.

Dunphy JL, Balic A, Barcham GJ, Horvath AJ, Nash AD, Meeusen EN (2000) Isolation and characterization of a novel inducible mammalian galectin*.* J Biol Chem 275:32106–32113.

Dzik JM, Zielinski Z, Golos B, Walajtys-Rode E (2006) *Trichinella spiralis* infection affects p47(phox) protein expression in guinea-pig alveolar macrophages. Exp Parasitol 112:158–163.

Dzik JM (2006) Molecules released by helminth parasites in host colonization. Acta Biochim Pol 53:33–64.

Ennen S, Hamann H, Distl O, Hickford J, Zhou H, Ganter M (2009) A field trial to control ovine footrot via vaccination and genetic markers. Small Rumin Res 86:22–25.

Familton AS, McAnulty RW (1997) Life cycles and development of nematode parasites of ruminants in sustainable control of internal parasites in ruminants. Animal Industries Workshop 67–80.

Finkelman FD, Urban JF (2001) The other side of the coin: the protective role of the TH2 cytokines. J Allergy Clin Immunol 107:772–780.

Finney CA, Taylor MD, Wilson MS, Maizels RM (2007) Expansion and activation of CD4(+)CD25(+) regulatory T cells in *Heligmosomoides polygyrus* infection. Eur J Immunol 37:1874–1886.

Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY (2005) Regulatory T cell lineage specification by the forkhead transcription factor FoxP3. Immunity 22:329–341.

French AT, Bethune JA, Knight PA, McNeilly TN, Wattegedera S, Rhind S, Miller HR, Pemberton AD (2007) The expression of intelectin in sheep goblet cells and upregulation by interleukin 4. Vet Immunol Immunopathol 120:41–46.

French AT, Knight PA, Smith WD, Brown JK, Craig NM, Pate JA, Miller HR, Pemberton AD (2008) Up-regulation of intelectin in sheep after infection with *Teladorsagia circumcincta.* Int J Parasitol 38:467–475.

French AT, Knight PA, Smith WD, Pate JA, Miller HR, Pemberton AD (2009) Expression of three intelectins in sheep and response to a Th2 environment. Vet Res 40:53.

Galioto AM, Hess JA, Nolan TJ, Schad GA, Lee JJ, Abraham D (2006) Role of eosinophils and neutrophils in innate and adaptive protective immunity to larval *Strongyloides stercoralis* in mice. Infect Immun 74:5730–5738.

Gasbarre LC, Smith LL, Lichtenfels JR, Pilitt PA (2009) The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. Vet Parasitol 166: 281–5.

Gossner A, Wilkie H, Joshi A, Hopkins J (2013) Exploring the abomasal lymph node transcriptome for genes associated with resistance to the sheep nematode *Teladorsagia circumcincta.* Vet Res 44(1):68.

Gossner AG, Venturina VM, Peers A, Watkins CA, Hopkins J (2012b) Expression of sheep interleukin 23 (IL23A, alpha subunit p19) in two distinct gastrointestinal diseases*.* Vet Immunol Immunopathol 150:118–122.

Gossner AG, Venturina VM, Shaw DJ, Pemberton JM, Hopkins J (2012a) Relationship between susceptibility of Blackface sheep to *Teladorsagia circumcincta* infection and an inflammatory mucosal T cell response. Vet Res 43:26.

Greer AW, Huntley JF, MacKellar A, McAnulty RW, Jay NP, Green RS, Stankiewicz M, Sykes AR (2008) The effect of corticosteroid treatment on local immune responses, intake and performance in lambs infected with *Teladorsagia circumcincta*. Int J Parasitol 38:1717–1728.

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

Harper RW, Xu C, Eiserich JP, Chen Y, Kao CY, Thai P, Setiadi H, Wu R (2005) Differential regulation of dual NADPH oxidases/ peroxidases Duox2 and Duox2, by Th1 and Th2 cytokines in respiratory tract epithelium. FEBS Lett 579:11–17.

Hasnain SZ, Evans CM, Roy M, Gallagher AL, Kindrachuk KN, Barron L, Dickey BF, Wilson MS, Wynn TA, Grencis RK, Thornton DJ (2011) Muc5ac: a critical component mediating the rejection of enteric nematodes. J Exp Med 208:893–900.

Hassan M, Good B, Hanrahan J, Campion D, Sayers G, Mulcahy G, Sweeney T (2011) The dynamic influence of the DRB1*1101 allele on the resistance of sheep to experimental *Teladorsagia circumcincta* infection. Vet Res 42:46.

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

Hickford J, Forrest R, Zhou H, Fang Q, Frampton C (2011) Association between variation in faecal egg count for a mixed field-challenge of nematode parasites and ovine MHC-DQA2 polymorphism. Vet Immunol Immunopathol 144:312–320.

Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061.

Hoste H, Torres-Acosta JF (2011) Non chemical control of helminthes in ruminants: adapting solutions for changing worms in a changing world. Vet Parasitol 180:144–154.

Hunter AR, MacKenzie G (1982) The pathogenesis of a single challenge dose of *Haemonchus contortus* in lambs under six months of age. J Helminthol 56:135–144.

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

Kim ES, Sonstegard TS, Vinicius M, Silva GB, Gasbarre LC, Van Tassell CP (2013) Identification of quantitative trait loci affecting gastrointestinal parasite resistance in an experimental Angus population. Animal Genetics 45:117–121.

Kimura A, Kishimoto T (2010) IL-6: regulator of Treg/Th17 balance. Eur J Immunol 40:1830–1835.

Knight PA, Pate J, Smith WD, Miller HR (2007) An ovine chitinase-like molecule, chitinase-3 like-1 (YKL-40), is upregulated in the abomasum in response to challenge with the gastrointestinal nematode, *Teladorsagia circumcincta*. Vet Immunol Immunopathol 120(1–2):55–60.

Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. Annu Rev Immunol 27:485–517.

Korten S, Badusche M, Buettner DW, Hoerauf A, Brattig N, Fleischer B (2008) Natural death of adult *Onchocerca volvulus* and filaricidal effects of doxycycline induce local FOXP3+/CD4 + regulatory T cells and granzyme expression. Microbes Infect 10:313–324.

Kotze AC (2003) Catalase induction protects *Haemonchus contortus* against hydrogen peroxide in vitro. Int J Parasitol 33:393–400.

Lasky LA (1991) Lectin cell adhesion molecules (LEC-CAMs): a new family of cell adhesion proteins involved with inflammation. J Cell Biochem 45:139–146.

Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang M-J, He C-H, Takyar S, Elias JA (2011) Role of chitin and chitinase/ chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol 73(1):479–501.

Lees MS, Robinson MA, Inghama AB, Kotzea AC, Piedrafit DM (2011) Dual oxidase 2 and glutathione peroxidase gene expression are elevated in hyperimmunised sheep challenged with *Haemonchus contortus*. Vet Parasitol 179:113–122.

Li RW, Choudhary RK, Capuco AV, Urban Jr JF (2012) Exploring the host transcriptome for mechanisms underlying protective immunity and resistance to nematode infections in ruminants Vet Parasitol 190:1-11.

Li RW, Gasbarre LC (2009) A temporal shift in regulatory networks and pathways in the bovine small intestine during *Cooperia oncophora* infection. Int. J. Parasitol 39:813–824.

Li RW, Gasbarre LC (2010) Gene expression in the bovine gastrointestinal tract during nematode infection. Vet Parasitol 157–178.

Li RW, Hou Y, Li C, Gasbarre LC (2010) Localized complement activation in the development of protective immunity against *Ostertagia ostertagi* infections in cattle. Vet Parasitol 174:247–256.

Li RW, Sonstegard TS, Van Tassell CP, Gasbarre LC (2007) Local inflammation as a possible mechanism of resistance to gastrointestinal nematodes in Angus heifers. Vet Parasitol 145:100–107.

Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P (2008) The immune geography of IgA induction and function. Mucosal Immunol 1:11–22.

Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3:733–744.

Marshall K, Maddox JF, Lee SH, Zhang Y, Kahn L (2009) Genetic mapping of quantitative trait loci for resistance to *Haemonchus contortus* in sheep. Anim Genet 40:262–72.

Marshall K, Mugambi JM, Nagda S, Sonstegard TS, Van Tassell SP, Baker RL, Gibson JP (2012) Quantitative trait loci for resistance to *Haemonchus contortus* artificial challenge in Red Maasai and Dorper sheep of East Africa. Anim Genetics 44:285-295.

McDermott JR, Leslie FC, D'Amato M, Thompson DG, Grencis RG, McLaughlin JT (2006) Immune control of food intake: enteroendocrine cells are regulated by CD4+ T lymphocytes during small intestinal inflammation. Gut 55:492–497.

McRae K, McEwan JC, Dodds KG, Gemmell NJ (2014) Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. Genomics 15:637.

Meeusen EN, Balic A, Bowles V (2005) Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. Vet Immunol Immunopathol 108:121–125.

Miller CM, Waghorn TS, Leathwick DM, Candy PM, Oliver AMB, Watson TG (2012) The production cost of anthelmintic resistance in lambs. Vet Parasitol 186(3–4):376–381.

Miller JE, Bishop SC, Cockett NE, McGraw RA (2006) Segregation of natural and experimental gastrointestinal nematode infection in F2 progeny of susceptible Suffolk and resistant Gulf Coast Native sheep and its usefulness in assessment of genetic variation. Vet Parasit 140:83–89.

Morris CA, Vlassoff A, Bisset S, Baker R, Watson TG, West CJ, Wheeler M (2000) Continued selection of Romney sheep for resistance or susceptibility to nematode infection: estimates of direct and correlated responses. Anim Sci 70:17–27.

Morris CA, Wheeler M, Watson TG, Hosking BC, Leathwick DM (2005) Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. N Z J Agric Res 48:1–10.

Murphy L, Eckersall PD, Bishop SC, Pettit JJ, Huntley JF, Burchmore R, Stear MJ (2010) Genetic variation among lambs in peripheral IgE activity against the larval stages of *Teladorsagia circumcincta.* Parasitol 137:1249–1260.

Nair MG, Guild KJ, Du Y, Zaph C, Yancopoulos GD, Valenzuela DM, Murphy A, Stevens S, Karow M, Artis D (2008) Goblet cell-derived resistin-like molecule beta augments CD4+ T cell production of IFNgamma and infection-induced intestinal inflammation. J Immunol 181:4709–4715.

Nakamura Y, Syouji T, Onodera T, Kawashima K, Inumaru S, Yokomizo Y (2002) Effects of recombinant bovine interferon gamma on *Strongyloides papillosus* infection in calves. J Helminthol 76:59–64.

Outteridge PM, Windon RG, Dineen JK (1985) An association between a lymphocyte antigen in sheep and the response to vaccination against the parasite *Trichostrongylus colubriformis*. Int J Parasitol 15:121–127.

Outteridge PM, Windon RG, Dineen JK, Smith EF (1986) The relationship between ovine lymphocyte antigens and faecal egg count of sheep selected for responsiveness to vaccination against *Trichostrongylus colubriformis.* Int J Parasitol 16:369–374.

Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG (2011) Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29(29):71–109.

Paludan SR (1998) Interleukin-4 and interferon-gamma: the quintessence of a mutual antagonistic relationship. Scand J Immunol 48:459–468.

Patel N, Kreider T, Urban JF, Gause WC (2009) Characterisation of effector mechanisms at the host parasite interface during the immune response to tissue-dwelling intestinal nematode parasites. Int J Parasitol 39:13–21.

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

Peck A, Mellins ED (2009) Breaking old paradigms: Th17 cells in autoimmune arthritis. Clin Immunol 132:295–304.

Pennock JL, Grencis RK (2006) The mast cell and gut nematodes: damage and defence. Chem Immunol Allergy 90:128–140.

Periasamy K, Pichler R, Poli M, Cristel S, Cetra B, Medus D, Basar M, Thiruvenkadan A.K, Ramasamy S, Ellahi M.B, Mohammed F, Teneva A, Shamsuddin M, Podesta MG, Diallo A (2014) Candidate Gene Approach for Parasite Resistance in Sheep–Variation in Immune Pathway Genes and Association with Fecal Egg Count. *PLoS ONE* 9(2):e88337. doi:10.1371/journal.pone.0088337.

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

Pernthaner A, Vlassoff A, Douch PGC, Maas D (1997) Cytokine mRNA expression and IFN-production in nematode resistant and susceptible line lambs artificially infected with gastrointestinal nematodes. Acta Parasitol 42:55–61.

Perry BD, Randolph TF (1999) Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet Parasitol 84(3–4):145–168.

Pesce JT, Liu Z, Hamed H, Alem F, Whitmire J, Lin H, Liu Q, Urban Jr JF, Gause WC (2008) Neutrophils clear bacteria associated with parasitic nematodes augmenting the development of an effective Th2- type response. J Immunol 180:464–474.

Pochanke V, Koller S, Dayer R, Hatak S, Ludewig B, Zinkernagel RM, Hengartner H, McCoy KD (2007) Identification and characterization of a novel antigen from the nematode *Nippostrongylus brasiliensis* recognized by specific IgE. Eur J Immunol 37:1275–1284.

Pulendran B (2004) Modulating Th1/Th2 responses with microbes, dendritic cells and pathogen recognition receptors. Immunol Res 29:187–196.

Rainbird MA, Macmillan D, Meeusen EN (1998) Eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunol 20:93–103.

Robinson N, Piedrafita D, Snibson K, Harrison P, Meeusen EN (2010) Immune cell kinetics in the ovine abomasal mucosa following hyperimmunization and challenge with *Haemonchus contortus*. Vet Res 41.

Rowe A, Gondro C, Emery D, Sangster N (2009) Sequential microarray to identify timing of molecular responses to *Haemonchus contortus* infection in sheep. Vet Parasitol 161:76–87.

Salle P, Jacquiet L, Gruner J, Cortet C, Sauve F, Prevot C, Grisez JP, Bergeaud LA (2012) Genome scan for QTL affecting resistance to *Haemonchus contortus* in sheep. J Anim Sci 90:4690-4705.

Sargison ND (2012) Pharmaceutical treatments of gastrointestinal nematode infections of sheep-Future of anthelmintic drugs. Vet Parasitol 189(1):79–84.

Sayers G, Good B, Hanrahan JP, Ryan M, Sweeney T (2005b) Intron 1 of the interferon gamma gene: its role in nematode resistance in Suffolk and Texel sheep breeds. Res Vet Sci 79:191–196.

Sayre BL, Harris GC (2012) Systems genetics approach reveals candidate genes for parasite resistance from quantitative trait loci studies in agricultural species. Anim Genet 43:190–8.

Schallig HDFH (2000) Immunological responses of sheep to *Haemonchus contortus*. Parasitol 120:S63–S72.

Schwaiger FW, Gostomski D, Stear MJ, Duncan JL, McKellar QA, Epplen JT, Buitkamp J (1995) An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. Int J Parasitol 25:815–822.

Shakya KP, Miller JE, Lomax LG, Burnett DD (2011) Evaluation of immune response to artificial infections of *Haemonchus contortus* in Gulf Coast Native compared with Suffolk lambs. Vet Parasitol 181 239–247.

Shaw R, Morris C, Wheeler M, Tate M, Sutherland I (2012) Salivary IgA: a suitable measure of immunity to gastrointestinal nematodes in sheep. Vet Parasitol 186:109–117.

Silva MVB, Sonstegard TS, Hanotte O (2012) Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red Maasai and Dorper sheep. Anim Genet 43:63–71.

Singh-Dang T, Walker M, Ford D, Valentine RA (2014) Nutrigenomics: the role of the nutrients in gene expression, Periodontology 2000 64:154-160.

Smith A, Madden KB, Yeung KJA, Zhao A, Elfrey J, Finkelman F, Levander O, Shea-Donohue T, Urban JF (2005) Deficiencies in selenium and/or vitamin E lower the resistance of mice to *Heligmosomoides polygyrus* infections. J Nutr 135:830–836.

Sonstegard TS, Van Tassell CP, Matukumalli LK, Harhay GP, Bosak S, Rubenfield M, Gasbarre LC (2004) Production of EST from cDNA libraries derived from immunologically activated bovine gut. In Germplasm Release.

Stear MJ, Bairden K, Innocent GT, Mitchell S, Strain S, Bishop SC (2004) The relationship between IgA activity against 4th-stage larvae and density-dependent effects on the number of 4th-stage larvae of *Teladorsagia circumcincta* in naturally infected sheep. Parasitol 129:363–369.

Stear MJ, Bishop SC, Henderson NG, Scott I (2003) A key mechanism of pathogenesis in sheep infected with the nematode *Teladorsagia circumcincta*. Anim Health Res Rev 4:45–52.

Sykes AR (2008) Manipulating host immunity to improve nematode parasite control. Parasite Immunol 30:71–77.

Taylor MD, LeGoff L, Harris A, Malone E, Allen JE, Maizels RM (2005) Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J Immunol 174:4924–4933.

Terefe G, Yacob HT, Grisez C, Prevot F, Dumas E, Bergeaud JP, Dorchies P, Hoste H, Jacquiet P (2005) *Haemonchus contortus* egg excretion and female length reduction in sheep previously infected with *Oestrus ovis* (Diptera: Oestridae) larvae. Vet Parasitol 128:271–283.

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006) TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24:179–189.

Venturina MV, Gossner AG, Hopkins J (2013) The immunology and genetics of resistance of sheep to *Teladorsagia circumcincta.* Vet Res Commun 37:171–181.

Wagland BM, Steel JW, Windon RG, Dineen JK (1984) The response of lambs to vaccination and challenge with *Trichostrongylus colubriformis*: effect of plane of nutrition on, and the inter-relationship between, immunological responsiveness and resistance. Int J Parasitol 14:39-44.

Weaver CT, Hatton RD, Mangan PR, Harrington LE (2007) IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 25:821–852.

Zhao A, Urban Jr JF, Anthony RM, Sun R, Stiltz J, Van RN, Wynn TA, Gause WC, Shea-Donohue T (2008) Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. Gastroenterology 135:217–225.

Zhu Z, Zheng T, Homer RJ, Kim Y-K, Chen NY, Cohn L, Hamid Q, Elias JA (2004) Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 304 (5677):1678–1682.