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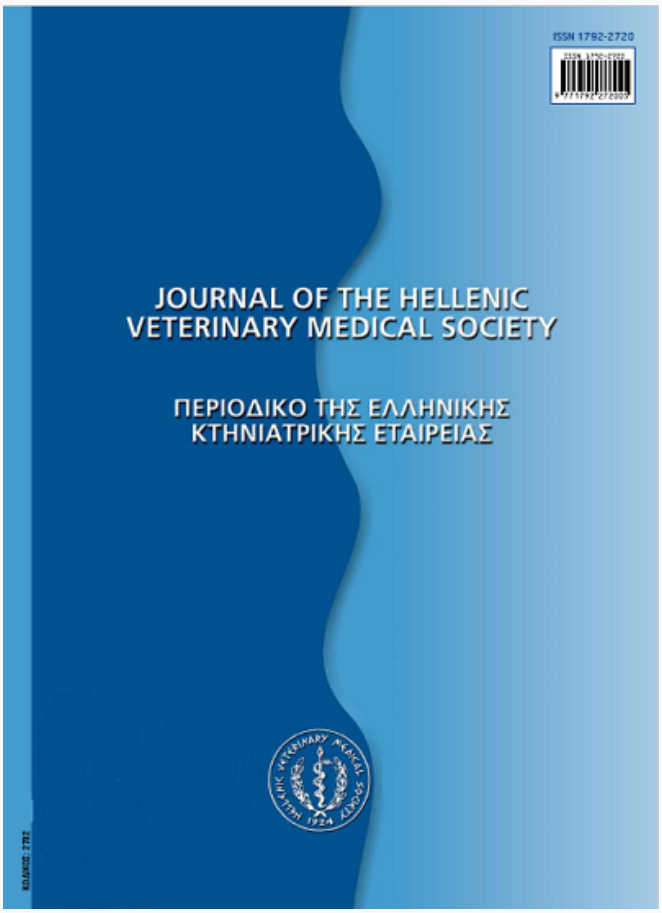

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Review Article
Ανασκόπηση

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 Τ 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 Τ 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 compli-

cates the management of parasitic diseases in small ruminants (Gasbarre et al., 2009; Kim et al., 2013). Taken together the increasing incidence of anthelmintic 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 activa-

tion 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.

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