

REVIEW ARTICLE

Sheep and goat immune responses to nose bot infestation: a review

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Abstract. *Oestrus ovis* L. (Diptera: Oestridae) is a cosmopolitan agent of myiasis in sheep and goats. The parasitic phase begins after adult females deposit first-stage larvae (L1) into the nostrils of hosts; these larvae develop into L2 and L3 in the nasal and sinus horn cavities. Sneezing and nasal discharges are the major clinical signs in infected animals. The pathogenesis of *O. ovis* infection is caused by: (a) the trauma resulting from the mechanical action of spines and hooks during larval movement on mucosal membranes, and, more importantly, (b) an allergenic reaction provoked by molecules excreted/secreted by larvae, of which salivary antigens are those mainly recognized by the host's immune system. The recruitment of immune reactive cells increases gradually from the nasal to sinus cavities in infected hosts. Mast cells, eosinophils, macrophages and lymphocytes are always more numerous in infected than non-infected animals. Humoral (antibody) systemic response of immunoglobulin G (IgG) usually reaches seroconversion 2–4 weeks post-first infection and the highest levels are observed during the development of L2 and L3 larvae. Local antibody responses include specific IgG, which has been found to negatively correlate with larval survival and development. Hypersensitivity reaction, immunomodulation, immunization trials and mixed infections of *O. ovis* and helminths are discussed.

Key words. *Oestrus ovis*, goats, host–parasite relationships, immune responses, oestrosis, sheep.

Introduction

Oestrus ovis causes a cavitary myiasis (oestrosis) in sheep and goats. This parasitic disease affects the well-being and performance of infected animals, resulting in significant economic losses (El-Tahawy *et al.*, 2010). Myiasis is defined as 'the infestation of live vertebrate animals with dipterous larvae, which, at least for a certain period, feed on the host's dead or living tissue, liquid body substances, or ingested food' (Zumpt, 1965). Active *O. ovis* infection begins when female flies deposit tiny larvae on or in the nostrils of the host; these larvae then feed on mucosa and secrete mucous to support their growth and development (Cepeda-Palacios *et al.*, 1999;

Tabouret *et al.*, 2003a). When larvae become mature, they leave the host to pupariate into the soil and adults emerge ready for mating, larval incubation and larviposition activities (Cepeda-Palacios & Scholl, 2000a). The main clinical signs of infection in the host are sneezing and nasal discharges (Dorchies *et al.*, 1992; Dorchies & Alzieu, 1997). The pathogenesis of oestrosis is related to the traumatic effects caused by the cuticular spines and oral hooks during larval migration, but is mainly caused by molecules secreted and excreted by larvae that induce a hypersensitivity immune reaction (Jacquiet *et al.*, 2005). Such stimuli are responsible for pathological damage and local systemic immunostimulation (Jagannath *et al.*, 1989; Nguyen *et al.*, 1999a, 1999b; Dorchies *et al.*, 2006).

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Earlier studies carried out from the late 1980s outlined the involvement of the host immune system in this myiasis (Marchenko & Marchenko, 1989; Abella, 1990; Dolbois-Charrier & Dorchie, 1993). Since these reports, research has focused on elucidating how ovine and caprine immune systems attack the larvae and how the larvae modulate these immune reactions in order to survive and develop inside the host. Immune responses against *O. ovis* have been studied over the last two decades (Dorchies & Yilma, 1996; Duranton *et al.*, 1999; Suárez *et al.*, 2005; Angulo-Valadez *et al.*, 2009a, 2009b) and knowledge of the relationships between *O. ovis* and sheep and goats has progressed considerably. However, the approaches and methods used by oestrosis researchers have been diverse and published data need further analysis and consensus. Therefore, the main objectives of this paper are to review current knowledge on immune responses in *O. ovis*-infected hosts and to provide new insights into the biological implications of this myiasis in sheep and goat husbandry.

Lifecycle

Oestrus ovis adult females deposit first-stage larvae (L1) directly on or into the nostrils of sheep and goats. The larvae quickly colonize the nasal cavities, nasal septum, turbinates and ethmoid bone. Thereafter, L1s moult to the second stage (L2) and migrate to the frontal sinus horn cavities. Later, L2s moult to the third stage (L3). Second instars are in an intermediate growing stage and demand a high-protein diet to feed their developing metabolism, which they obtain mainly from mucus and seroproteins by way of trypsin-like enzymes (Tabouret *et al.*, 2003a). By contrast, L3s must store energy reserves for the free phases of the lifecycle (pupariation, fly emergence, mating and larviposition).

Under environmentally favourable conditions, the durations of the first, second and third stages are <10–25 days, 7–15 days and 13–18 days, respectively (Cepeda-Palacios, 2002). However, the hypobiotic period of L1 may be extended for several months in cold weather (Yilma & Dorchie, 1991). When the larvae have attained their maximal growth, they are expelled by the sneezing of the host onto the ground where they pupate (Cepeda-Palacios *et al.*, 1999). Intrapuparial metamorphosis occurs over 19–27 days, after which flies emerge; female flies carry a fully developed egg complement and male flies emerge with mature spermatozoa ready for fertilization (Cepeda-Palacios & Scholl, 2000a, 2000b; Cepeda-Palacios *et al.*, 2001). Egg incubation in gravid females takes about 12 days, after which the infective activity of female flies on new hosts begins (Cepeda-Palacios & Scholl, 1999).

Clinical signs

Infected animals demonstrate two clinical phases, which may overlap: the fly strike induced by larvipositing females occurs first, and subsequent sinusitis develops later (Dorchies *et al.*, 1992; Dorchies & Alzieu, 1997). The nasal discharges and

sneezing which characterize both phases constitute the major clinical signs of *O. ovis* infection in sheep and goats. Nasal discharges can be serous during fly strike and mucous may be muco-purulent and/or purulent, and is occasionally tinged with blood (Butterfield, 1900; Dorchies *et al.*, 1996). Eventually, the nasal passages become obstructed by mucus and dust. In this condition, hosts are forced to breathe by mouth, which reduces grazing and rumination time and commonly results in negative nutritional effects such as general malnutrition and low performance. Additional clinical signs of oestrosis include cough, ataxia, weakness, vertigo and epistaxis, as well as adenocarcinoma and interstitial pneumonia, which may, in turn, result from secondary respiratory infections (Dorchies, 1997). Interestingly, in slaughterhouse studies some sheep or goat hosts have shown no clinical signs despite huge parasite burdens in the nasal and sinus cavities found at necropsy. By contrast, some individuals have demonstrated strong clinical evidence of infection although only a few or no *O. ovis* larvae were found inside them. The absence of clinical findings in a host with a large parasite burden is likely to represent a strategy of immune tolerance on the part of the host in order to avoid self-damage, whereas the absence of larvae in a heavily symptomatic host probably implies that the larvae abandoned the host a few days before the necropsy examination (Jacquet *et al.*, 2005). However, these contradictory findings suggest that variations in genetic resistance and susceptibility exist in sheep (Angulo-Valadez *et al.*, 2008). In addition, genetic and/or antigenic variations in *O. ovis* larvae may influence clinical manifestations in their hosts. At necropsy, rhinitis and sinusitis are generally present in the host, depending on the stage of larval development. In sheep and goats, the lesions are generally more severe immediately after L2 and L3 moults. It is likely that decaying exuviae contribute to stimulating the immune response to the higher level needed to fulfil the larvae's new nutritional requirements for growth. It is significant that goats appear to be more resistant to *O. ovis* infection than sheep and display less evident clinical signs. Because the clinical signs are more evident and parasite burden is higher in sheep than in goats, it is likely that goats were infected first by *O. ovis*, which then migrated to sheep. Consequently, goats have been presumed to be better adapted than sheep to *O. ovis* (Dorchies *et al.*, 1999). In addition, clinical manifestations are closely related to the chronobiology of *O. ovis* (i.e. fly strike season, larval hypobiotic or developmental periods) in specific geographic zones, which are strongly influenced by climatic conditions (i.e. Dorchies *et al.*, 1996; Scala *et al.*, 2002; Papadopoulos *et al.*, 2010).

Pathogenesis

The pathogenesis of *O. ovis* infection is induced partially by spines on the larval cuticle and oral hooks, but mainly by biomolecules (enzymes and antigens) excreted or secreted by the larvae onto the mucosa (Dorchies *et al.*, 2006). The larvae migrate inside the host with the aid of their ventral cuticle spines and oral hooks, which cause mechanical mucosal irritation. The importance of these structures is critical

in first-instar larvae, the cuticle spines of which are numerous compared with those on L2 and L3. These spines and oral hooks allow for larval attachment, permit quick L1 displacement on the nasal mucosa and contribute to the rhinitis process. By contrast, L2 and L3 larvae lose their dorsal spines, indicating that spine-facilitated traits are not important for either the developing stages or for pathogenesis.

The severity of lesions observed in the ethmoid bone and sinus is, partially a consequence of the strong trophic activity of L2, but mainly of L3 larvae (Angulo-Valadez *et al.*, 2009b). The biomolecules excreted/secreted by the larvae and those in the larval salivary gland comprise a complex array of enzymes necessary to degrade mucus and plasma proteins for larval feeding and nutrition (Frugère *et al.*, 2000; Angulo-Valadez *et al.*, 2007a). Thus, when larval development increases, not only does protein production in excreted/secreted products increase, but proteolytic activity is augmented (Tabouret *et al.*, 2003a). Therefore, pathogenesis is amplified in the ethmoid and sinus mucosae when L2 and L3 are present. Specifically, larval enzymes (mainly serine proteases) are able to degrade Type I (extracellular matrix) and Type IV (basal blade) collagens, mucins, albumin and immunoglobulins (Tabouret *et al.*, 2003a; Dorchies *et al.*, 2006). In this regard, microhistological studies have characterized ultrastructural pathology changes caused by *O. ovis*. This infection induces hyperplasia and metaplasia in the epithelium (Tabouret *et al.*, 2003b), confirming (by increases in the Ki-67 nuclear epitope) an increase in the number of epithelial cells in the nasal and sinus cavities in infected sheep to twice the number found in non-infected sheep (Nguyen *et al.*, 1998). Moreover, in experimentally infected lambs, cells were around two, eight and 40 times as numerous in the nasal turbinate-septum, ethmoid and sinus, respectively, than in control lambs (Tabouret *et al.*, 2003b). These findings suggest the existence of a highly regenerative process resulting from *O. ovis* infection in sheep. In addition, epithelial cells showed reductions in cilia and became dissociated, rounded and degenerated. In the lamina propria and chorion, multifocal irregularities were observed, which led to an increased influx of the plasma molecules used for food by *O. ovis* larvae. It would appear that the monomers (i.e. amino acids, carbohydrates) produced from the degradation of polyglucosides and polypeptides were needed for larval physiological functions.

Antigens also contribute to the pathogenesis in the host. Cuticle and excreted/secreted antigens have been identified in immunoblots and larval salivary antigens in particular have been found to strongly stimulate the immune system of sheep and goats (Innocenti *et al.*, 1997; Tabouret *et al.*, 2001a; Angulo-Valadez *et al.*, 2007b, 2009a). The immune signalling and responses (cellular and humoral) derive from the recognition and processing of larval molecules at the mucosal level.

Antigens

Immunoblots have revealed several antigenic bands using cuticle-associated proteins, crude (somatic) extracts, excreted/secreted products and salivary gland contents. However, a protein complex of 28 kDa (four to five protein bands of 20–29 kDa) from salivary gland content is the most antigenic

fraction recognized by systemic immunoglobulin G (IgG) of infected sheep and goats (Innocenti *et al.*, 1995; Tabouret *et al.*, 2001a; Angulo-Valadez *et al.*, 2007a), which indicates that sheep and goat immune systems probably recognize similar antigens. Protein (or gene) sequences have yet to be characterized and their possible functions in host–parasite interactions remain to be determined. In addition, systemic IgG reacts with larval cuticle proteins, of which a free polypeptide of 56 kDa was found to be the strongest antigenic (Innocenti *et al.*, 1997).

Cellular immune response

Mast cells

Mast cells are resident in tissues and play a crucial role during the inflammatory process. They degranulate and release a large number of proinflammatory, immunoregulatory and tissue mediators after activation induced by binding the allergen (antigen) to the IgE antibodies bound to the high-affinity IgE receptor FcεRI (Roitt *et al.*, 2002). In addition, mast cells liberate enzymes to attack a parasite. *Oestrus ovis* induces huge recruitment and degranulation of mast cells. Following a single L1 experimental infection in lambs, mast cells in the nasal cavity were twice as numerous in the experimental animals as in the controls; after multiple infections serous mast cells and globule leukocytes increased by 11 and five to seven times, respectively (Dolbois-Charrier & Dorchies, 1993). Similarly, in naturally infected ewes, mast cells were as twice as high in number as in non-infected sheep (Nguyen *et al.*, 1996, 1999a).

Histologically, these mast cells were seen to be localized mainly in the subepithelial chorion, as well as in the interglandular chorion and submucosa (Jacob *et al.*, 2002, 2004a, 2004b). In these reports, numbers of mast cells were found to sharply increase from the septum to the ethmoid sinus; again, this was related to larval development and infection sites. From the earlier results, the effect of reactive cells on infecting larvae is clear. Jacquiet *et al.* (2005) demonstrated that corticoid treatment of experimentally infected sheep resulted in an increase in the rate of surviving larvae compared with controls; thus, mast cells may limit the larval population inside the host. It is supposed that these reactive cells liberate hydrolytic enzymes which may act on the cuticle of the parasite. More recently, the activity of local mast cells was confirmed by the identification of sheep mast cell protease (SMCP) in the mucosae of infected lambs (Dorchies *et al.*, 2006).

Eosinophils

Eosinophils are produced in the bone marrow and migrate by peripheral blood until they reach the mucosal surface where parasitic infections occur. These cells come into close contact with the parasite, where eosinophil degranulation is induced by complement C3 or IgG (which act as ‘bridges’ between parasite tissue and eosinophils) to release cytotoxic proteins that are capable of inducing not only parasite damage, but also mucosal damage (Roitt *et al.*, 2002). Eosinophil recruitment

patterns in the mucosa (anatomically and histologically) of *O. ovis*-infected sheep are similar to those observed for mast cells, but involve higher numbers of cells (Yacob *et al.*, 2002, 2004a, 2004b; Tabouret *et al.*, 2003b; Jacquiet *et al.*, 2005). For example, Dolbois-Charrier & Dorchies (1993) reported that eosinophil counts were 34 times greater (in the subepithelial chorion) in infected lambs than in non-infected lambs. In naturally infected sheep, eosinophils counts were multiplied by 17, 29 and 58 times in the septum, turbinate and sinus, respectively, and were mainly found in the subepithelial chorion (Nguyen *et al.*, 1996, 1999b). However, no relationship between the number of eosinophils in mucosae and larval burden or development was apparent. In addition, the kinetics of blood eosinophilia have been studied in experimentally infected lambs (Dorchies *et al.*, 1997; Yacob *et al.*, 2002, 2004b, 2006; Jacquiet *et al.*, 2005; Terefe *et al.*, 2005). In general, blood eosinophils reach a peak following the first and second *O. ovis* infections. Later, this number remains high, but does not show dramatic increases. If no new re-infections occur, eosinophilia returns to near pre-infection levels (Yacob *et al.*, 2004b). This phenomenon indicates that the immune system is strongly stimulated during the first seasonal infections. From then on, a re-infected host is self-protected against its own tissue damage because unnecessary eosinophil recruitment and activation at infection sites is avoided.

Macrophages

Macrophages are mononuclear phagocytes that participate as regulators of chronic inflammation (cytokine production), as cleaners of dead cell material and as efficient antigen-producing cells in the immune system (Roitt *et al.*, 2002). Tabouret *et al.* (2003b) demonstrated the involvement of macrophages in lambs experimentally infected with *O. ovis*. Immunohistochemistry assays were performed on mucosal tissue sections using anti-CD68 monoclonal antibody (Clone Ki-M6 human monocyte/macrophage). CD68⁺ (macrophages) cells were significantly more numerous in all mucosal locations (mainly in the frontal sinus) in infected than in non-infected animals, except in the turbinate region. Therefore, the substantial recruitment of macrophages to sites of *O. ovis* infection implies intense activity in engulfing, digesting and presenting epitopes (antigens) to T helper lymphocytes. Antigen presentation must occur in association with major histocompatibility complex class II (MCH-II) molecules in order to establish intercellular contact with T cell receptors (TCR) of T helper lymphocytes.

Again, this activity was intense in the nasal sinus, where macrophages also participate by removing cellular debris from the damage induced by the infection. All physiopathological evidence indicates that a Th₂ response is developed against *O. ovis* (Jacquiet *et al.*, 2005). However, this remains to be demonstrated (in the cytokine profile) during infection.

T and B lymphocytes

The active recruitment of mononuclear cells in the mucosa of sheep following *O. ovis* infection has been demonstrated

by immunohistochemistry techniques (Nguyen *et al.*, 1999a; Tabouret *et al.*, 2003b). Anti-CD antibodies to B lymphocytes [monoclonal Ab specific for human CD20-like (clone BLA36) and T lymphocytes (rabbit anti-human CD3 antibody)] showed differences between infected and control lambs. The CD20⁺ and CD3⁺ counts increased gradually from the turbinates to the sinus, and CD3⁺ counts were observed in the chorion, especially in the ethmoid and sinus mucosa. Furthermore, plasma cells were localized (by histochemistry) near the secretory glands in the subepithelial region. Increased numbers of T lymphocytes and localization in the chorion represent clear evidence that most antigen recognition occurs in antigen-presenting cells in the sinus cavities and indicates that adaptive immunity is being developed. In addition, the recruitment of B lymphocytes and plasma cells indicates that specific anti-*O. ovis* immunoglobulins are being produced and adaptive immunity has developed. According to previous results, these particular antibodies will mainly arrive at the sinus mucosa, where actively feeding L3 larvae will use them as a source of protein.

Humoral immune response

Systemic antibody responses

The humoral antibody response of sheep or goats against *O. ovis* involves the production of parasite-specific local and systemic immunoglobulins (Bautista-Garfias *et al.*, 1988; Sánchez-Andrade *et al.*, 2005; Angulo-Valadez *et al.*, 2009a). The precise action of these immunoglobulins against *O. ovis* is unknown. However, positive interactions in systemic immunoglobulin responses have been useful in determining antibody kinetics, antigen recognition and oestrosis serodiagnosis.

Immunoglobulin G. In repetitively infected experimental animals, seroconversion is usually reached 2–4 weeks post-first infection (Tabouret *et al.*, 2001a; Yacob *et al.*, 2002; Angulo-Valadez *et al.*, 2007a). However, when only L1 or very low numbers of larvae are present, the positive IgG threshold can be detected after several weeks in both ovine and caprine hosts (Terefe *et al.*, 2005; Angulo-Valadez *et al.*, 2009a). It is known that L1 are more discrete, smaller and less nutrient-demanding than L2 or L3 larvae and, as a consequence, they stimulate the immune system less (Dorchies *et al.*, 1999). Nevertheless, specific IgG levels were found to sharply increase and positively interact with developing L2 and/or L3 larvae in sheep and goats (Dorchies & Yilma, 1996; Suárez *et al.*, 2005; Angulo-Valadez *et al.*, 2009a; Romero *et al.*, 2010). Therefore, the IgG response in naturally infected sheep or goats depends on the chronobiology of *O. ovis* larvae located in a specific area, as well as on particular local conditions (Scala *et al.*, 2002). Likewise, specific IgG was found to have almost disappeared 160 days after the administration of an effective anti-parasite treatment (Dorchies *et al.*, 2003).

Immunoglobulin M. IgM is produced during the early phase of an infection (Roitt *et al.*, 2002). Using L2 crude extracts as antigens in an enzyme-linked immunosorbent assay (ELISA), systemic production of IgM in infected sheep was positively correlated with the number of *O. ovis* first instars hosted (Suárez *et al.*, 2005). Therefore, this immunoglobulin seems to be useful to estimate the presence of quiescent L1 larvae in studies of the chronobiology of oestrosis in temperate or oceanic climates and is of practical use in determining the efficacy of chemotherapies under field conditions (Romero *et al.*, 2010). In addition, L1 larvae in IgM-positive sheep were responsible for lymphoproliferative values in blood (Scala *et al.*, 2006). This finding may indicate the early detection of infection and subsequent antigen presentation by the host immune system.

Immunoglobulin A. Systemic involvement of IgA has been found in very low levels in *O. ovis*-infected sheep, as well as in non-infected animals, showing no relationship with oestrosis infection (Jacquiet *et al.*, 2005; Suárez *et al.*, 2005). It would be interesting to determine if IgA plasma cells in blood are related to oestrosis as IgA participation may be important at the mucosal surface level.

Local antibody responses

Infected lambs secrete specific nasal mucous IgG and IgA in response to *O. ovis* infection. Local IgG production has been found to be clearly associated with *O. ovis* infection (Tabouret *et al.*, 2003b; Jacquiet *et al.*, 2005). Moreover, adult ewes naturally infected with *O. ovis* showed high local IgG responses that were negatively correlated with larval survival and development inside the host (Angulo-Valadez *et al.*, 2008). Our interpretation of these data is that local antibodies probably play crucial roles in the opsonization of the larvae or the neutralization of larval enzymes needed for nutrition that finally affect the biology of the parasite. Such correlations were observed in some sheep families but not in others, which suggests that some genetic control of resistance and susceptibility mediated by IgG antibodies may be involved. By contrast, local IgA responses need further investigation. Tabouret *et al.* (2003b) reported significant differences in the production of local IgA between infected and non-infected lambs, whereas Jacquiet *et al.* (2005) and Angulo-Valadez *et al.* (unpublished data) did not find differences in local IgA production in either controls or experimentally and naturally infected sheep.

By contrast, antibody (IgG, IgA) detection in the milk of naturally infected animals may represent a valuable alternative (needle-free) method of diagnosing caprine oestrosis; this is currently being investigated with promising results (Angulo-Valadez *et al.*, unpublished data).

Hypersensitivity phenomenon

Three main factors are associated with hypersensitivity phenomenon Type I: mast cells; eosinophils, and immunoglobulin

subtype E (Roitt *et al.*, 2002). In sheep and goats, significant differences in the recruitment of mast cells and eosinophils between *O. ovis*-infected and non-infected animals support this hypothesis (Jagannath *et al.*, 1989; Abella, 1990; Dolbois-Charrier & Dorchies, 1993; Nguyen *et al.*, 1999b; Yacob *et al.*, 2004b). However, evidence of IgE involvement remains to be determined. Mast cells and eosinophils are the major mediator-secreting cells during a hypersensitivity reaction (Roitt *et al.*, 2002). For example, preformed mediators released from the granules of mast cells, such as histamine, eosinophils/monocyte chemotactic factors and Th2 type cytokines [i.e. interleukin-4 (IL-4), IL-5, eotaxin] among others, drive eosinophils and primed T cells into the inflamed site. Jacquiet *et al.* (2005) demonstrated an immediate hypersensitivity reaction in *O. ovis*-infected sheep: 30 min after intradermal injections of excreted/secreted larval products, animals developed extensive skin reactions (10–16 mm). Conversely, control sheep did not react at 30 min. Both infected and control sheep showed substantial skin reactions at 3 h, but no skin reactions were observed at 72 h after intradermal injections.

As previously mentioned, eosinophils and mast cells were localized mainly in the chorion layer, just beneath the nasal epithelium, which means that reactive cells migrate to be in close contact with the parasite or antigens. Therefore, it has been hypothesized that eosinophils are responsible for limiting parasite populations and mast cells are responsible for sustaining the hypersensitivity phenomenon at the site of tissue damage (Dorchies *et al.*, 1998). In addition, because these cells are very numerous on the ethmoid and frontal sinuses, which are inhabited by advanced stages of *O. ovis* instars, strong proinflammatory stimulation by L2 and L3 larvae has been suggested (Nguyen *et al.*, 1999b; Dorchies *et al.*, 2006).

Immunomodulation by *O. ovis*

Host–*O. ovis* relationships include dynamic and complex strategies of resistance and defence (Dorchies *et al.*, 1999). *Oestrus ovis* is considered a parasite with proinflammatory activity; this is especially true of L2 and L3 larvae, which need sufficient amounts of nutrients from the mucosa and plasma to grow and develop. However, when host immune conditions are adverse (i.e. after immunization), larvae stop developing or diminish their rate of development (Frugère *et al.*, 2000; Angulo-Valadez *et al.*, 2007b). Various experiments in this area have yielded unexpected and contradictory results, reflecting the great complexity of this parasite–host relationship. In general, *in vitro* results suggest a host immunomodulation by the three *O. ovis* instars, as we will describe.

Immunostimulation

The increase in *in vitro* lymphocyte proliferation in lambs experimentally infected with L2 or L3 larvae suggests an immunostimulation by L2 and L3 inside the host (Dolbois-Charrier & Dorchies, 1993). By contrast, *in vitro* assays have demonstrated that *O. ovis* larval extracts, mainly L2

somatic extracts, induce nitric oxide (NO) production by macrophages (Duranton *et al.*, 1999). Interestingly, products excreted/secreted by larvae were also able to induce NO production in murine RAW 264.7 macrophages (Tabouret *et al.*, 2001b). Induction of NO in these tumoral cells was principally linked to a 29-kDa protein secreted by the larval salivary glands. The authors pointed out that NO plays a multi-functional role in inflammation that benefits the parasite. Nitric oxide induces tissue damage, vasodilatation and plasma protein leakage. Thus, although an NO burst may be harmful to the host, the plasma protein leakage and hypersecretion induced by excreted/secreted larval products may be a source of food and nutrients for larvae (Tabouret *et al.*, 2001b, 2003b).

Immunosuppression

Dolbois-Charrier & Dorchies (1993) reported for the first time that *in vitro* lymphocyte proliferation decreased and mitotic activity was depleted to levels comparable with those in non-infected lambs on days 19 and 33 post-infection, respectively. Further research showed that L1 somatic extracts inhibited the *in vitro* NO production of ovine macrophages, suggesting that L1 larvae may regulate the inflammatory reaction at a certain level (Duranton *et al.*, 1999) and that immunosuppression may be orchestrated by L1 larvae. For L1 larvae, modulating the immune response is important as an overstimulation of the immune response may lead to a 'self-cure' phenomenon such as that which occurs in gastrointestinal parasite infections. It is important to consider that most larval mortality occurs during the L1 stage because larvae are very small (1 mm in length) and their localization in the nasal cavities puts them at high risk for becoming trapped in dense mucus, asphyxied and expelled from the host. Therefore, L1 larvae need to develop biological strategies to modulate the immune response by regulating macrophage NO production and lymphocyte proliferation in order to survive inside the host. Moreover, Jacquet *et al.* (2005) observed that lymphocytes in previously infected sheep did not respond to the specific antigen stimulation (excreted/secreted products of L2 and L3) after a third infection. Consequently, lymphocyte responsiveness diminished with the number of exposures, suggesting immunosuppressive activity by the parasite. These results give some insight into why animals do not develop immune resistance after natural repeated exposures under field conditions. However, several issues require further explanation, including, for example, the characterization of *O. ovis*-secreted biomolecules that might mimic cytokines or cell receptors to control cell signalling in order to modulate immune response. It is interesting that after an adaptive immune response developed against *O. ovis*, excreted/secreted larval proteases are able to cleave the heavy chain of IgG (Tabouret *et al.*, 2003a). The cleavage of IgG together with the production of F(ab)'₂ and a pFc' fragments may lead to an incapacity for antibody-dependent cell cytotoxicity by eosinophils (Tabouret *et al.*, 2003a). This suggests a likely immune evasion whereby degraded antibodies may be eventually used as a source of amino acids for larval nutrition and metabolism.

Immunization and vaccine development

Marchenko & Marchenko (1989) demonstrated the participation of the host's immune system in the regulation of *O. ovis* larval populations. After the administration of an immunosuppressive treatment (chlorambucil, 1 mg/kg daily) in lambs, the rate of larval recovery was 63%, compared with 26–36% in untreated controls. In addition, longlasting corticoid (triamcinolone acetonid i.m., 80 mg/sheep) treatment in lambs resulted not only in a higher rate of larval establishment, but also in quicker development of established larvae than in untreated lambs (Jacquet *et al.*, 2005). Both experiments indicated that enhanced immune responses may have a detrimental effect on *O. ovis* larvae. Later, immunization trials against *O. ovis* were performed in sheep using excreted/secreted products and digestive tract protein extracts of third instars (Frugère *et al.*, 2000; Angulo-Valadez *et al.*, 2007b). In the first experiment, it was supposed that immunization with antigens that are normally in permanent contact (excreted/secreted products) with nasal mucosae would promote a strong immune response against *O. ovis*. Necropsy showed that a strong antibody response has been generated and size and growth were significantly lower in immunized lambs than in controls, but there was no effect on larval establishment rate, which suggests that the immunization of sheep has an inhibitory effect on larval growth. In general, larval antigens were recognized by the immune system, but it seems that immunity was not fully developed. This leads us to hypothesize that some excreted/secreted antigens may be 'decoy' antigens for the host immune system and may exert a 'non-substantial' effect after sheep immunization. In the second experiment, hidden protein antigens were obtained from the larval intestine. It was assumed that immunized animals would develop humoral (antibody) responses against such antigens (i.e. intestinal enzymes) that are normally not in contact with the host. Then, specific antibodies might affect both larval food digestion and nutrient absorption processes. Retardation of larval growth and development was achieved, but survival rate was not affected. It is important to acknowledge that although immune responses in treated animals did not influence the larval survival rate, the growth and development of *O. ovis* were significantly reduced (Cepeda-Palacios *et al.*, 2000). The authors concluded that a reduction of 40% in the weight of mature larvae would result in a 38% decrease in the adult population. Clearly, more experiments with new vaccination strategies using the nasal mucosa route, with single antigens or cocktails of antigens and other adjuvant formulations, should be evaluated in order to establish whether the viability of the adult population can be affected significantly.

Oestrus ovis and concomitant gastrointestinal nematode infections

Mixed infections by dipteran larvae and helminths are quite common in ruminants. The adverse effects of *O. ovis* infection on the outcome of subsequent gastrointestinal parasites in sheep and goats were first observed by Dorchies *et al.* (1997). Study of the relationship between *O. ovis* and helminth co-infections revealed that antagonistic interactions between *O. ovis*

larvae and either of the Strongylidae nematodes *Trichostrongylus colubriformis* or *Haemonchus contortus* exist. In fact, the succession of infections is not considered a major modulating factor for the expression of negative effects. Indeed, parasitic and pathological changes have been related to increases in mast cells, globule leukocytes and eosinophils in the respiratory and digestive tracts. Whereas *O. ovis* larvae were not affected in number or development, the number, length, egg excretion and fecundity of *T. colubriformis* and *H. contortus* were negatively associated with concomitant *O. ovis* infection. It seems that *O. ovis* larvae induce 'at distance' inflammatory reactions in the whole mucosal system. These downregulating effects on nematodes might be eliminated after antiparasitic treatment against *O. ovis* (Yacob *et al.*, 2002, 2004a, 2006, 2008; Terefe *et al.*, 2005).

Conclusions

Proinflammatory immune reactions are characteristic of *O. ovis* infection and involve huge recruitments of cells (mast cells, eosinophils, macrophages, T and B lymphocytes) and the secretion of immunoglobulins that suggest a type Th2 immune response. Interestingly, the most important inflammatory reaction is localized in the sinus horn cavities because this is where the largest larval growth is attained by L2 and L3. It seems that, conveniently, proinflammatory reactions are stimulated by larvae in synchrony with the rhythms of larval population development, as evidenced by the observation that batches of larvae were chronologically regulated after an L1 experimental infection. We suppose that such an equilibrium strategy on the part of the parasite is intended to avoid overcrowding that could result in a hyperimmunostimulation and lead to a 'self-curing' phenomenon in the host, similar to that observed in gastrointestinal helminth parasitoses. The evidence discussed herein indicates that *O. ovis* uses immunosuppressive strategies such as the depletion of the number of lymphocytes and the degradation of immunoglobulins to evade defensive attacks from the host. This may be a reason why poor acquired immunity develops over the lifespan of sheep and goats after several natural exposures to *O. ovis*. However, it is clear that immune innate and adaptive systems participate actively in the regulation of the *O. ovis* larval population. Efforts should focus on gaining knowledge of molecular and cellular signalling in order to facilitate understanding of the main immunological factors involved in larval establishment and development. Certainly, biological strategies of defence and attack cycles in sheep and goats, and parasites, suggest a long and successful host–*O. ovis* co-evolution. Finally, the biotechnological goals of future investigations are to develop immunoprophylactic and diagnostic tools as alternative methods of control of *O. ovis* populations in the field.

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