Haemonchus contortus-SHEEP RELATIONSHIP: A REVIEW.

Relación *Haemonchus contortus*-Ovino: Una Revisión.

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ABSTRACT

Haemonchosis, caused by the abomasal nematode *Haemonchus contortus*, is among the most relevant parasitic diseases of small ruminants all over the world. The infections are responsible for anemic and bad digestion/absorption syndromes causing the death of severely infected animals in acute cases and the reduction of production scores in the chronic forms of the disease. The keys for the appearance of the disease include the biological behavior of the helminth and mechanisms of pathogenesis, besides the host's response. The updated knowledge of these aspects would result in a higher efficiency of diagnostic and control methods, thus reducing the risks of appearance of the disease. In the present review, several aspects of the sheep - *H. contortus* relationship are analyzed with the aim of reducing the impact of this parasitosis on livestock health and productivity.

Key words: Haemonchosis, *Haemonchus contortus*, pathophysiology, immune response, sheep.

RESUMEN

La hemoncosis, causada por el nematodo del abomaso de rumiantes *Haemonchus contortus*, constituye una de las enfermedades parasitarias más notables del ganado ovino en todo el mundo. Las infecciones provocan síndromes anémicos y de mala digestión/absorción que pueden causar la muerte en los casos agudos y disminución de la producción en las formas crónicas. Las claves principales para la aparición de esta enfermedad son el comportamiento biológico del helminto, su patogenia, además de la respuesta del hospedador. El conocimiento actualizado de estos aspectos permitirá una mayor eficiencia de los métodos de diagnóstico y control del proceso y, como consecuencia, la disminución de los riesgos de aparición de esta enfermedad. La presente revisión analiza y discute algunos aspectos de la relación ganado ovino - *H. contortus* de interés para disminuir el impacto de esta parasitosis sobre la salud y producción ganaderas.

Palabras clave: Hemoncosis, *Haemonchus contortus*, fisiopatología, respuesta inmunitaria, ovinos.

INTRODUCTION

Sheep are an important source of high quality food products for humans (meat, milk). Gastrointestinal nematodoses are among the most destructive pathologies affecting ovine production, given the high prevalence in flocks and the serious consequences of infection. In lambs, the acute form of gastrointestinal nematode infection causes death; the more frequent, chronic form leads to notable reductions in productivity.

Sheep haemonchosis is a parasitic disease caused by *Haemonchus contortus*, a nematode species that during its adult stage lives in the host's abomasa. *H. contortus* is widely distributed in all geographic areas where small ruminants are bred. Clinical signs of the infection include anemia, digestion-absorption syndromes and, in many cases, the death of animals. The severity of the disease depends on a variety of factors, including the number of helminths infecting an animal (intensity of the infection), isolated-related parasite virulence, host age, animal breed, and the nutritional and immunological status of the infected sheep. All these factors can be aggravated by local environmental conditions. Therefore, in order to ascertain the factors favoring the appearance and severity of the disease in small ruminants, it is necessary to gain an insight into the relationship between sheep and *H. contortus*, covering biological aspects of the parasite helminth as well as

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the importance of the physiological and immune responses of infected animals.

***Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898**

The taxonomic position of the nematode is as follows:

Kingdom: Animalia; Phylum: Nemathelmintes; Class: Secernentea; Order: Strongylida; Suborder: Trichostrongylina; Superfamily: Trichostrongyloidea; Family: Haemonchidae; Subfamily: Haemonchinae; Genus: *Haemonchus*; Species: *H.contortus* [32, 47, 60, 123].

The genus *Haemonchus* apparently originated in Africa, with an initial diversification in antelopes and subsequent colonization and development in other wild ruminants. There were independent colonizations in domestic ruminants. Later, human migrations enabled the spread of *Haemonchus* to wild and domestic ruminants on other continents and *H.contortus* in sheep [2, 50, 70]. In addition, eleven more species have been described in the genus: *H. similis* (Travassos, 1914); *H. longistipes* (Railliet y Henry, 1909); *H. placei* (Place, 1893) Ransom 1911; *H. bedfordi* (LeRoux, 1929); *H. mitchelli* (LeRoux, 1929); *H. vegliali* (LeRoux, 1929); *H. lawrencei* (Sandground, 1933); *H. okapiae* (van den Berghe, 1937); *H. krugeri* (Ortlepp, 1964); *H. horaki* [71]; *H. dinnik* [50, 55, 70]. Moreover, hybrids of *H. contortus* and *H. placei* have arisen [69].

Haemonchus contortus has a tooth or lancet in its poorly developed oral cavity to perforate the gastric mucosa and suck blood. Ingested blood gives the helminth a reddish coloration; the females resemble a bicolor braid or barber pole by having off-white ovaries coiled around the reddish intestine [97]. They have two cervical lateral papillae in the anterior end, and male helminths show a well-developed copulatory bourse characterized by the asymmetrical dorsal lobe; there are spines near the distal end of both spicules [50,55,70]. Females generally have vulvar pouches [70]. Male helminths measure on average 1310 µm and females 1850 µm [70]. Females are extremely prolific -5000 eggs/day/female [69]- and the eggs (70-45 µm), of strongyle type, are excreted into the environment in the morula phase (8-16 cells) in the feces of the infected host.

H.contortus has a direct life cycle, part of which occurs in the external environment, where the infective larvae (L3) develop. Under optimal humidity and temperature conditions, development from egg to L3 in the environment takes place in 6-7 days. The sheep acquire the infection by ingesting L3 in the grass. Ingested larvae unsheathe and penetrate the glands of the gastric mucosa where they molt to fourth stage larvae (L4) [28, 86, 110]. At this point, due to a variety of factors (adverse macro- and micro-environmental conditions, immune response of the host, parasite genetic constitution, among others), a phenomenon called “hypobiosis” occurs, in which L4 diminishes its metabolism, halts its development and remains inside the gastric gland. Hypobiosis, as a rule, takes place in winter in temperate areas and during the dry season in arid areas of the world [19, 41]. Once development resumes, L4 leaves the mucosa and, in the gastric lumen, molts to young adult, which copulates when it reaches maturity. The prepatent period varies between 15 and 18 days in normal conditions [61, 91, 110].

Pathogenesis

Several factors are involved in the pathogenesis of haemonchosis. In terms of the development of disease, the most important factors are parasite virulence and host-response. The main pathogenic mechanisms of *H. contortus* are a direct lesion on the gastric mucosa and hematophagy. The effects of pathogenic mechanisms during intra-host parasite development and the subsequent response of infected ruminants provoke morpho-functional changes, particularly in the abomasum. Also, variations appear in some blood parameters, resulting in the appearance of both anemic and impaired digestion-absorption syndromes.

H.contortus adult parasites can ingest 0.05 ml of blood/helminth/day [95], causing notable blood loss [130] with a reduction of packed cell volume (PCV). This parameter has, in fact, been used as a marker of parasite virulence [33, 113] and indirect estimation of parasite burden in haemonchosis [34, 129].

The fall in PCV, a common finding in sheep haemonchosis [3, 38, 46, 84, 87, 111, 127, 128], is visible from day 4 post infection (PI), coinciding with the L4 exit from the mucosa. The decline accelerates with the beginning of the patent period (3 weeks PI), due to the combined effect of young and adult parasites’ increased blood demand, the efficiency of now well-developed lancet in the worms’ oral cavity, and blood loss to the gastrointestinal tract caused by infection-related hemorrhagic gastritis [52, 95, 111]. Minimum values are reached in the 7th week PI [63].

The fall in PCV values coincides (3 weeks PI) with a fall in hemoglobin concentration (5.3 -7.7 mg/dL) [63]. This is related to the hematophagy of the worms, as well as to blood loss through the intestine [95] and erythrocyte lysis caused by hemolytic factors excreted by the parasite [36, 37].

A reduction of plasma protein concentrations has been found in haemonchosis [4, 45, 128, 131] due to blood loss [130] and hemorrhagic gastritis. In addition, leakage of proteins to the gastric lumen occurs as a result of the disruption of intercellular unions and increased permeability [8, 39, 95], epithelial cells loss, tissue reparation, increase in mucus production, and increased requirements for protein synthesis by the immune system [106].

Moreover, many factors prevent such protein losses from being replaced through feeding. Infected animals have lower food intake [126], due to anemia [100, 111, 129]; gastrin reduces food passage through the gastrointestinal tract [39, 106]; bad digestion syndrome, caused by the increase of abomasal

pH value, which prevents pepsin synthesis [49, 81, 103, 106], reduces aminoacid and small peptides absorption [105, 106].

This pH increase in the abomasum comes from a decrease in the production and excretion of hydrochloric acid (HCl) by the parietal cells of the gastric mucosa, generated by their loss and/or a decrease in their number. Lowered numbers of parietal cells is due to tissue lesion, cellular infiltration caused by the presence of the parasitic stages or their secretion-excretion products released into the medium, and cellular replacement by immature nonfunctional cells [49, 81, 102, 103, 106].

Moreover, it has been observed that the parasites release substances such as ammonium. It increases the pH around the parasites so as to avoid the action of gastric acids or pepsin on its cuticle [49, 80, 106], so there is an inhibition in the transformation of pepsinogen, produced in the principal cells of the gastric glands, to pepsin. As a result, pepsinogen accumulates in these glands [62, 102, 113]. Pepsinogen is then released by the gastric glands. From there, it passes into the bloodstream due to the increase in mucosa permeability caused by the lesion, the inflammatory response, and the dissociation of mucosa intercellular unions, increasing its concentration in the sanguineous plasma [77, 81, 103, 113].

The development of parasites and their secretion-excretion products stimulate the liberation of substances such as acetylcholine, histamine, epithelial growth factor and gastrin, which in turn stimulate the principal cells to produce and release more pepsinogen [40, 77]. In experiments in sheep and goats, it has been demonstrated that the levels of serum pepsinogen in infections with *H. contortus* show a significant increase from days 4 to 14 PI, with average values of 741 mUTyr, later diminishing to pre-infection values by 40 PI [31, 38, 39, 103].

While these pathogenic actions directly affect the health of the animals, they also indirectly affect ovine yield, although this loss may be unnoticed if the parasitism presents a sub clinical course. Thus, the nematode infection of the abomasum has a notable effect on live weight [18, 84, 111, 125]. This has also been observed in weight and carcass conversion [127, 128]. This indirect effect is mainly due to a decrease in nutrient use [127] resulting from many factors, such as a lack of appetite and a decrease in the voluntary feed intake observed in the parasitized animal [40, 92, 117, 125], irreversible loss of proteins in the gastric lumen by hematophagy, hemorrhagic gastritis and loss of plasma proteins through greater mucosa permeability [39, 95, 100] and lowered digestibility [22, 23, 24, 39, 105, 106].

The symptoms of haemonchosis include lack of appetite [65, 124], lethargy, loss of weight [18], reduction in milk and wool production [21, 85], presence of pale mucosa, edemas, diminution of PCV [12, 63, 91], hemoglobin, plasma proteins [45] and increase in the number of circulating eosinophils in peripheral blood [3, 7] and serum pepsinogen and gastrin [40, 102, 103, 105, 106]. The final stages of the disease may be accompanied by emaciation, and death may result [123].

In the necropsy, the macroscopic lesions observed are: emaciation, pale mucosa, edemas in body cavities [91], degradation of the fat deposits [123], hypertrophy of local lymph nodes [30, 88], edema of the abomasal mucosa with petechial hemorrhages, presence of nodules and the observation of adult parasites [110]. In the microscopic evaluation, cellular infiltration, dilatation of the gastric glands, ulcers, edema, hemorrhage and an increase in the number of mastocytes and eosinophils [4, 5, 11, 14, 17, 54, 89] are observed.

Host immune response

Host immune response to haemonchosis is complex and shows cellular, humoral, and inflammatory mechanisms [75, 78] that can vary depending on the parasitic phases present [11]. The parasitic antigens interact with innate immune system cells (macrophages, dendritic cells, natural killer (NK), basophils, among others), which release cytokines, mainly IL-4, that provide instructions to T and B cells of the acquired immune system to generate a specific response [35, 51, 72, 75].

Infection with helminths induces the liberation of cytokines associated with T helper 2 (Th2) response, accompanied by eosinophilia, mastocytosis and the production of IgE. The cells that recognize parasitic antigens send molecular signals that induce T helper (Th) cells to secrete interleukins (4, 5, 10, 12). Interleukins stimulate B cells to produce and secrete antibodies (IgE, IgG, IgA); these antibodies bind to antigens. The complex formed is recognized by mastocytes, eosinophils and neutrophils, which release the content of their granules (histamine, leukotrienes, prostaglandins and other mediators). This induces an inflammatory process, increases mucus production, and provokes the contraction of smooth musculature, causing the expulsion of the parasite or its death. [5, 64, 72, 78, 81, 132].

The increase of complement receptors on the eosinophils' surface and the complement protein deposits on the helminth surface cause cell degranulation and a direct effect on the parasite as well as an indirect effect through the complement classic pathway [79].

Many studies on natural or experimental infections with *H. contortus* have reported cellular infiltrations in the mucosa and regional lymph nodes , increases in T CD4+, T CD8+, T gamma delta ($\gamma\delta$) and B lymphocytes, eosinophils and mastocytes [5, 11, 17, 75]. Also, a rise in immunoglobulins has been observed during infections with increases in IgE, IgG, IgA [3, 5, 15, 25, 26, 27, 29, 30, 44, 45, 46, 132].

CD4+ lymphocytes are effector and regulator cells, performing collaborative functions [104] which increase in number after *H. contortus* infection in the abomasum mucosa and regional lymph nodes, a fact demonstrated by their hypertrophy and CD4+ relative reduction in peripheral blood [11]. This sug-

gests an important role in lambs' resistance to haemonchosis [43] and partly reflects how, as a result of a nonspecific recruitment [11] induced by pathogenic actions of abomasal larvae and adults, cells migrate towards infected tissues [90].

There is no evidence that CD4+ cells directly limit parasitic establishment, survival and fecundity. It is more probable that they contribute with the production of cytokines which extend and regulate the differentiation, proliferation and recruitment of effector cells and antibody producers [43].

This role for CD4+ cells is supported by research with lambs resistant to gastrointestinal nematodes. The lambs were given successive doses of monoclonal anti-CD4+ antibodies, a treatment that severely affected resistance to infection. This group showed significantly greater amounts of eggs per grams (EPG) in faeces and parasitic burdens [59]. Furthermore, there was a significant reduction in mastocyte and eosinophil numbers in mucosa and lower levels of specific anti-*H. contortus* antibodies [43]. Also, an increase in the regional lymph nodes weight was observed in infected animals [11, 30].

CD8+ lymphocytes are considered effector and regulatory cells of the immune system, carrying out suppressing and cytotoxic functions [104]. In *H. contortus* infections their role is less important, as observed by the lack of effect of CD8+ reduction on HPG, parasitic burdens in lambs, and cell infiltration in abomasal mucosa during the infection [43].

The $\gamma\delta$ lymphocytes express on their surface a TcR receiver (T lymphocytes specific) made up of $\gamma\delta$ heterodimers, and CD3. They do not express CD4, CD8, or surface immunoglobulins and were previously called non B non T lymphocytes. These $\gamma\delta$ lymphocytes are found in much larger proportions in the peripheral blood of ruminants than in other species such as humans or mice [22, 107, 121]. The location of the $\gamma\delta$ lymphocytes in the epithelia, intestines, and the reproductive and respiratory tracts indicate their importance as a forward line of defense against pathogens [121]. The increases in these cells in the abomasal mucosa after the *H. contortus* infection suggest that they may play some role in the immune response to this nematode [11].

B lymphocytes mediate the humoral immune response and are in charge of antibody production, expressing on their surface when they are mature: CD19, PAN B antigens (CD21, CD24) and surface immunoglobulins, in addition to major complex antigens of type II histocompatibility and complement receptors [1]. Balic et al. [11] did not detect effects of the infection on the B lymphocyte population occurring in lymph nodes. However, these authors did observe a reduction in the values of fluorescence intensity in the surface immunoglobulins, which is probably attributable either to the reduction of surface molecules in activated B cells in the final differentiation to plasma cells, or to an increase in immature cells [75].

T lymphocytes are mediators of cellular immunity and can be used to measure cellular specific immune reactions [15]. This quantification can be made by determining the proliferation of mononuclear cells exposed to parasitic antigens *in vitro*. Several authors have reported proliferation of lymphocytes from peripheral blood or lymph nodes in infected animals, induced by larval and adult stages of nematode antigens [42, 98, 119, 120].

Alunda et al. [3] observed an elevation of the cellular response in the infected lambs, with maximum values of stimulation indices (S.I.) of 11, against mononuclear cells of the experimental *H. contortus* antigen-infected animals, although this increase was observed in late patency of the infection. Other authors have shown increases in S.I. of lymphocytes with values of 2.5 [100] and 10 [76]. The use of isolated lymphocytes from regional lymph nodes has served to measure local cellular response, obtaining from low or non-differentiable [30, 56] to significant values, as shown by McClure et al. [76] of 8 S.I.

Mastocytes are markers of helminth infection [35]. They are generated in the bone marrow from a haematopoyetic precursory cell that expresses CD34 on its surface, similar to eosinophils and basophils, and must mature in the target organ [35]. Mastocytes play an effector role in resistance against gastrointestinal nematodes [42], and their proliferation, recruitment and differentiation are regulated by cytokines released by lymphocytes CD4+ [43].

The hyperplasia of mastocytes in the abomasum mucosa is associated mainly with the presence of adult parasites and is greater in re-infections [10, 42], requiring a continuous stimulation on the part of parasitic antigens [4, 10]. The increase in its number suggests a local inflammatory response, implied in the protective response against re-infection [115] and is influenced by animals' age, being more evident in adults [96, 126]. Mastocytes release, during infection, granules content, composed of proteoglycans, basic proteins and monoamines [53] which are found in host mucous secretions [14] after their degranulation and are associated with larvae expulsion [10]. The degranulated mastocytes present in the mucosa are called globule leukocytes [53, 74].

Negative correlations have been registered between the values of EPG and mastocytes [4] and between these cells and the parasitic burden [42]. Also, negative correlations of the HPG with globule leukocytes have been shown [4, 5, 43]. Between mastocytes and globule leukocytes, a very positive and highly significant correlation has been observed [5].

Eosinophils are antiparasitic effector cells [20, 114], whose main function is as a defense against non-phagocytable organisms, particularly helminths [13]. For this reason, they are considered markers of nematode infections [35, 81]. The IL-4 and IL-5 release by CD4+ and $\gamma\delta$ lymphocytes triggers eosinophils activation and an increase in their production by the bone marrow [79]. Eosinophils migrate to the target organs through the bloodstream [10, 54, 79, 132], which causes eosinophilia, a characteristic of helminth infections [64, 81].

During nematode infection, eosinophils present a direct cytotoxic effect, in particular due to the granule protein release and the superoxide anion production, killing larval stages [10] and damaging the host tissue [64, 81]. The increased expression of complement receptors on the cells surface, along with the abundant protein deposits of the same on the surface of the parasite, would cause degranulation and death [79]. A greater amount of eosinophils in the tissue suggests that they might be involved in larvae development prevention or in the rapid expulsion phenomenon [17].

In experimental infections, eosinophilia appears from the 5th PI day, reaching a peak between the second and third PI week, and soon declines between the fourth and fifth PI week [4, 7, 10, 42, 115]. This demonstrates an active modulation which generally happens when the infection becomes patent [64]. Cell quantifications in the infected abomasum mucosa have shown significantly increases in eosinophils in the infected animals in comparison to control groups [11, 42, 99, 126].

In several studies, negative correlations have been observed between the eosinophil number in abomasal mucosa and both EPG [5] and parasitic burden [43], although in other cases this has not been observed [4, 42]. In the case of the circulating eosinophils, significant associations have not been reported [4, 114].

With regard to humoral immune response, it has been shown in different papers that *H. contortus* infection causes an increase in specific immunoglobulins, slightly in primary infections and strongly in re-infections [25, 26, 44, 45, 46].

These immunoglobulin increases are associated with the immediate hypersensitivity response, by means of the binding of the IgE to parasitic antigens with the subsequent degranulation of the effector cells. The result is the expulsion or death, thus regulating the parasitic burden [5]. The specific local response of IgA and IgG is consistently associated with reduction of the helminth size and parasitic fecundity [5, 112], by metabolic enzyme neutralization, interfering with the feeding [100] and metabolism of *H. contortus* [116], although participation of the immediate hypersensitivity should not be ruled out [42].

In primary infections, lambs infected with *H. contortus* did not show any serum- IgG specific response with values of optical density (O.D.) in basal levels similar to those of the non infected group [15, 25, 26, 29, 30, 42, 45, 68, 98]. Alunda et al. [3], observed a slight increase in the values of IgG after the primary infection of 0.46 O.D. Other authors have observed increases in the serum levels of IgG in primary infections with O.D. values of 0.353 [87], 0.55 [101] and 0.6 [126].

In the case of IgA levels, basal levels similar to pre-infection values have been reported [45], although in other cases a slight increase in O.D. values of 0.68 [116] and 1.0 [128] has been observed. In re-infections, significant increases in IgA in serum [46] have been observed. For the IgE, the O.D. of the primarily infected group remains near 0.2 [68, 126]. It seems that humoral responses regulate parasite size and fecundity, while hypersensitivity reactions regulate parasite burden [5].

The immunoprofilaxis of haemonchosis has evolved from the use of vaccines with irradiated larvae [122] to the characterization of parasite antigens, recognized or not by the infected animal. For the last twenty years, vaccines have been tried, using proteins isolated from the microvilli of the digestive tract of *H.contortus* (Contortin) [83], cuticle [16], membranes (H11) [6, 66, 84, 109], infecting larvae cuticle [57], glycoprotein complexes containing galactose [H-gal-GP] [58, 108], helminth soluble extracts (p26/23) [3, 29, 30] and secretory-excretory (E/S) products [9, 99, 126]. These antigens, according to their exposure to the host immune system have been classified as natural and hidden [82, 93, 94]. Studies carried out by the aforementioned authors have reported marked reductions (40 to 80.14%) in the parasitic burdens of vaccinated animals.

In spite of the efficacy of these vaccinations, they are neither economical nor practical, because they use natural antigens. The obvious solution would be to purify a protective antigen and produce it on a large scale by recombinant DNA technology [109]. Up to now, vaccinations with recombinant proteins expressed in *Escherichia coli* have been carried out but they have not induced a significant protective immune response. Work is still being done in the development of different expression systems to address this problem [67].

H. contortus immune evasion

Parasites are exposed to host immune system attack, which is why they must develop efficient immune evasion strategies [48]. Among the mechanisms used by *H. contortus* to diminish the host's local response efficacy are: location in the abomasum lumen and parasite mobility. In addition, the helminths produce a number of inhibitors of proteases as well as immunomodulator components that block host effector mechanisms [81, 130].

Thus, cystatins of *H. contortus* are inhibitors of proteases involved in the process of antigen presentation, reducing the T lymphocytes response. Moreover, they modulate cytokine response, by reducing the co-stimulator molecule expression on macrophage surface, thus contributing to an anti-inflammatory microenvironment induction with a strong diminution of cellular proliferation [48]. Type C lectin, identified in *H. contortus* and other nematodes, inhibits leukocyte adhesion to endothelial cells by competing with selectin and their subsequent migration to infected tissue, reducing the inflammation [73]. It has been determined that another E/S protein of adult *H. contortus* called calreticulin, in addition to inhibiting blood clothing and facilitating haematophagy, binds with and inhibits complement C1q component, which facilitates helminth survival [118].

CONCLUSIONS

In the *Haemonchus contortus* – ovine livestock relationship, there are numerous elements that may or may not favor the appearance of haemonchosis, whose the clinical manifestation is not only caused by parasite pathogenesis, but also by different host responses to infection. This complex situation requires the planning of different control strategies in order to reduce parasite action and stimulate host resistance, and thus increase the productivity of ovine flocks.

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