

Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*

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Abstract – Histopathological changes and the distributions of T and B lymphocytes and IgG producing plasma cells were recorded in the abomasum and abomasal lymph nodes of goats 3, 7 and 21 weeks post-infection (wpi) after an experimental infection with *H. contortus*. The low rate of worm recovery by 3 wpi (5.6%) might have been due to larvae death as suggested by the presence of granulomas in the abomasal mucosa at 3 and 7 wpi, or simply due to a poor larval establishment. Marked increase in the secretion of mucus by mucous cells together with an abundant infiltration of eosinophils, mast cells, CD3⁺ T lymphocytes, CD79a⁺ B cells, IgG⁺ plasma cells and globule leukocytes were recorded in the abomasal mucosa, especially at 7 wpi. Except for the globule leukocytes, this reaction decreased substantially by week 21, suggesting this cell type may have been involved in rejection of adult nematodes. The abomasal lymph nodes showed marked hyperplasia, particularly of CD79a⁺ B cells and IgG⁺ plasma cells in all infected goats. These reactions may have been responsible for the reduction in the number of worms found in the abomasum between 3 and 7 wpi.

***Haemonchus contortus* / goat / histopathological lesions / immune response**

Résumé – Étude anatomopathologique et immunohistochimique de la caillette et des ganglions lymphatiques de la caillette chez des chèvres infestées expérimentalement par *Haemonchus contortus*. Les changements histopathologiques et la distribution des lymphocytes T et B et des plasmocytes producteurs d'IgG ont été observés dans la caillette et dans les ganglions lymphatiques de la caillette de chèvres infestées expérimentalement par *H. contortus* à 3, 7 et 21 semaines

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post-infestation (SPI). Le faible taux d'établissement des vers à 3 SPI (5,6 %) peut être dû à la mort des larves, comme le suggère la présence de granulomes dans la muqueuse de la caillette à 3 et 7 SPI, ou peut-être simplement à une faible installation des larves. Une augmentation marquée de la sécrétion de mucus par les cellules à mucus ainsi qu'une infiltration abondante d'éosinophiles, de mastocytes, de lymphocytes T CD3⁺, de cellules B CD79a⁺, de plasmocytes IgG⁺ et de leucocytes globuleux ont été observées dans la muqueuse de la caillette, en particulier à 7 SPI. À la 21^e semaine, à l'exception des leucocytes globuleux, cette réaction a nettement diminué, suggérant que ce type de cellule peut avoir contribué au rejet de nématodes adultes. Les ganglions lymphatiques de la caillette ont révélé une hyperplasie prononcée, en particulier des cellules B CD79a⁺ et des plasmocytes IgG⁺ chez toutes les chèvres infestées. Ces réactions peuvent avoir provoqué la réduction du nombre des helminthes observée dans la caillette entre 3 et 7 SPI.

***Haemonchus contortus* / chèvre / lésions histopathologiques / réponse immune**

1. INTRODUCTION

Haemonchus contortus is a common parasite of sheep and goats in areas with a warm climate, where it is economically important due to the loss of milk, meat and wool production and frequent death in young animals. There is an increasing interest in the production of vaccines against gastrointestinal nematodes due to the emergence of anthelmintic resistance [35] and the concern about chemical residues in animal products. Alternative strategies for the control of gastrointestinal nematode infections in ruminants include the selection of genetically resistant breeds [7, 26] and the development of effective vaccines [20, 33]. The knowledge of the immune mechanisms involved in an effective response against gastrointestinal nematodes is of interest in order to develop effective strategies of immunisation [18, 19].

It is well documented that some breeds of sheep are genetically resistant to *H. contortus* infection [7] and numerous studies have been carried out to analyse different aspects of the host immune response and mechanisms of resistance. Thus, it has been shown that genetic resistance is immunologically mediated by proliferation of mucosal mast cells, globule leukocytes and eosinophils [7, 9, 34] as well as specific antibody responses [9, 10, 13, 32], which was repre-

sented mainly by IgG1 and IgA in resistant breeds [11]. The local cellular immune response appears to play a crucial role in the effective host response. Proliferation of CD4⁺ T lymphocytes appears necessary for the generation of mucosal mast cell hyperplasia, tissue eosinophilia and specific antibody production [3, 12].

In contrast, little is known about the pathogenesis and host response against gastrointestinal nematode in goats. Few studies have investigated the host response to *Trichostrongylus vitrinus* and *Teladorsagia circumcincta* [16, 21] and some experimental studies have been carried out to analyse the effects of *H. contortus* infection on milk production [14], and the gross and histopathological changes induced from 4 to 21 days post-infection [1]. Recently, clinical, parasitological and serum parasite-specific IgG responses were examined in goats after primary and secondary *H. contortus* infections [6]. However, the local humoral and cellular immune response together with the histopathological changes have not been evaluated in goats.

The aim of this work was to analyse the histopathological changes and the distribution of T and B lymphocytes and IgG in the abomasum and abomasal lymph nodes of goats experimentally infected with *H. contortus* L3 at 3, 7 and 21 weeks post-infection.

2. MATERIALS AND METHODS

2.1. Experimental design and histopathology

Sixteen 5-month-old male Serrana goats, recently weaned, were obtained from a *Haemonchus* free farm. They were assessed to be free of gastrointestinal nematodes by measurement of faecal eggs counts and drenched with fendendazole (Panacur, Hoechst Roussel Vet, Barcelona, Spain). Goats were housed indoors under worm-free conditions and fed goat pellet and lucerne chaff. They were divided into six groups (Tab. I), one of the groups (group F) being used as uninfected controls. Goats of the remaining five groups were infected orally with *Haemonchus contortus* larvae (L3) of ovine origin provided by the Central Veterinary Laboratory, Weybridge, UK. Group A, C and E were infected with 20,000 L3, whereas group B and D received 40,000 L3. Goats were killed by intravenous injection of a mixture of embitramide and mebezonio iodure (T61, Hoechst Roussel Vet, Barcelona, Spain); goats of groups A and B were killed at 3 week-post-infection (wpi), those of groups C and D at 7 wpi, and those of group E at 21 wpi. One goat from group F was killed respectively at week 3, 7 and 21 pi.

2.2. Worm counts

At necropsy, the abomasum was isolated and its contents, mucosal washings and scrapings were collected and processed for worm counting according to the Manual of Veterinary Parasitological Laboratory Techniques [2].

2.3. Histopathology

Infected and control goats were necropsied and tissue samples were collected from the abomasum (cardiac, fundic and pyloric

areas), abomasal lymph nodes, fixed in 10% buffered formalin and embedded in paraffin wax. Tissue sections (4 μ m) were stained with haematoxylin and eosin, Periodic acid Schiff (PAS) and Toluidine blue for the histopathological study.

2.4. Immunohistochemistry

The avidin-biotin-peroxidase complex (ABC) method [23] was used. Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide 0.3% in methanol for 30 min. Sections for the detection of CD3 and λ -IgG were incubated with 0.1% pronase (Sigma Chemical, St Louis, MO, USA) for 10 min, whereas those for the detection of CD79a were incubated in 0.01 M buffer citrate (pH 6.0) in a microwave oven (100 °C) for 7 min. After three rinses in phosphate buffered saline (PBS) 0.01 M, pH 7.2, tissue sections were incubated with 5% normal goat serum for 30 min at room temperature (20-25 °C). A rabbit anti-human CD3 polyclonal antibody (pAb) diluted 1 in 200 in phosphate buffered saline (PBS) pH 7.2, a rabbit anti-human IgG-lambda light chain pAb diluted 1 in 2000 in PBS, or a mouse anti-human CD79a monoclonal antibody (mAb) diluted 1 in 15 in PBS, were applied overnight at 4 °C as primary antibodies. These antibodies were obtained from Dako, Glostrup (Denmark). The anti-CD3 pAb was previously shown to cross-react with caprine T lymphocytes [29], the CD79a with caprine B cells [23] and the λ -IgG with caprine plasma cells [22]. After three 10 min rinses in PBS, a biotinylated goat anti-rabbit immunoglobulin G diluted 1 in 200 in PBS (Vector Laboratories, Burlingame, CA, USA) was applied for 30 min as secondary reagent for primary pAb, and a biotinylated goat anti-mouse immunoglobulin G diluted 1 in 50 in PBS (Dako) was applied for 30 min as secondary reagent for the anti-CD79a mAb. An ABC complex (Vector) diluted 1 in 50 in PBS was then applied for 1 hour at room

temperature. Then, tissue sections were incubated in 3-3'-diaminobenzidine tetrahydrochloride (Sigma Chemical) diluted to 0.035% in Tris-buffered saline containing hydrogen peroxide 0.01%, for 1 min. Finally, sections were rinsed in tap water, counterstained with Mayer's haematoxylin, dehydrated and mounted. Tissue sections in which the specific primary antibodies were replaced by PBS, rabbit or mouse non-immune sera were used as negative controls. Abomasal lymph node tissue sections were used as positive controls.

2.5. Cell counting

Immunoreactive cells were counted in three sections from each goat (10 fields of 0.06 mm² per section randomly selected in the abomasal mucosa of the fundus) using a ×40 objective and a ×10 ocular lens with a graticule. Similarly, eosinophils and globule leukocytes were counted in haematoxylin and eosin stained tissue sections, and mast cells in Toluidine stained sections. Results were given as number of cells per field of 0.06 mm²: ±: 0-3, +: 3-10, ++: 10-20, +++: 20-30, ++++: more than 30 cells. Histopathological changes in the abomasal mucosa and abomasal lymph nodes were evaluated separately by two pathologists.

3. RESULTS

3.1. Gross changes and worm burdens

The abomasal mucosa was oedematous and congestive and showed petechial haemorrhages in all infected goats except at 21 wpi. These changes were more severe in fundic than in cardiac and pyloric areas. Gastric lymph nodes were oedematous and markedly enlarged in all infected goats.

The number of worms recovered in the abomasal contents is given in Table I. The worm counts revealed a low establishment by 3 wpi (8.4% and 4%) for groups A and B, respectively. A reduction in the number of worm burdens was found by 7 and 21 wpi, although the groups were too small to perform a statistical analysis.

Histopathological changes. In all infected goats, except at 21 wpi, the lamina propria and submucosa of the abomasum showed moderate oedema and congestion. In control goats, the PAS staining revealed mucous secretion in the apical zones of the cytoplasm of epithelial cells located in the gastric areas, pits and in the neck of gastric glands. Infected goats presented marked (3 wpi), very marked (7 wpi) or moderate (21 wpi) increase of the PAS staining, which occupied the majority of the cytoplasm of mucous cells of the isthmus and neck of gastric glands, and the apical zones of the cytoplasm of cells located in the base of the glands.

Table I. Design of experimental infection with *H. contortus* and results of worm counts in the abomasal contents.

Group	Number of goats	week killed post-infection	Infective Dose (L3)	Worm burdens (mean ± SD)	Establishment rate (%)
A	3	3	20,000	1,674.7 ± 791.7	8.4
B	3	3	40,000	1,609.3 ± 227.7	4.02
C	2	7	20,000	296.0 ± 124.8	1.5
D	3	7	40,000	412.0 ± 22.6	1
E	2	21	20,000	49.0 ± 5.7	0.25
F	3	3/7/21	0	0	

Degenerated larvae were found among the abomasal glands, mainly in deep areas of the lamina propria in goats killed at 3 wpi. These larvae were composed of a cuticle surrounding with homogeneous, basophilic material. In most cases they were also surrounded by inflammatory infiltrate composed mainly of lymphocytes (Fig. 1). At 3 wpi, granulomas with a necrotic centre (Fig. 2) surrounded by some macrophages, neutrophils, numerous eosinophils and lymphocytes were present deep in areas of the mucosa. At 7 wpi, larvae were not observed within the abomasal mucosa and the centre of the granulomas was less necrotic. Granulomas were more common in goats infected with 40,000 L3. Numerous globule leukocytes were observed among epithelial cells of the abomasal glands (Fig. 3), particularly at 7 and 21 wpi (Tab. II). The number of mast cells in the abomasal mucosa, although increased at 3 and 7 wpi, was lower than the numbers of globule leukocytes.

A heavy inflammatory infiltrate composed with eosinophils, lymphocytes and plasma cells was observed in the lamina propria, mainly in deep areas, and to a lesser degree in the submucosa at 3 and 7 wpi. This infiltrate was moderate by 21 wpi (Tab. II). Occasional lymphoid follicles were found either in the submucosa or lamina propria of both infected and control goats, although in the former they were larger and often contained a germinal centre.

The abomasal lymph nodes of all infected goats showed marked hyperplasia of the follicles, with large germinal centres and a thin corona of mature lymphocytes. Paracortical areas and medullary cords were also enlarged in all infected goats compared to control ones. In infected goats, medullary cords showed marked hyperplasia due to the large numbers of plasma cells and lymphocytes. Numerous eosinophils were observed in the medullary and peritrabecular sinuses, particularly at 7 wpi.

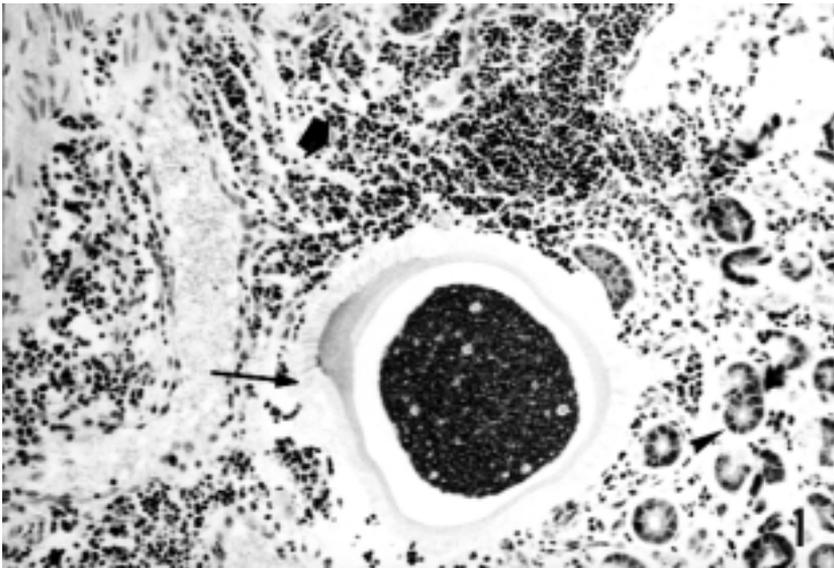


Figure 1. Group A. Deep areas of the abomasal lamina propria showing a degenerated larvae (thin arrow) with amorphous basophilic material, surrounded by a heavy inflammatory infiltration (thick arrow) at 3 wpi. Abomasal glands (arrowhead). Haematoxylin and eosin. Original magnification $\times 200$.

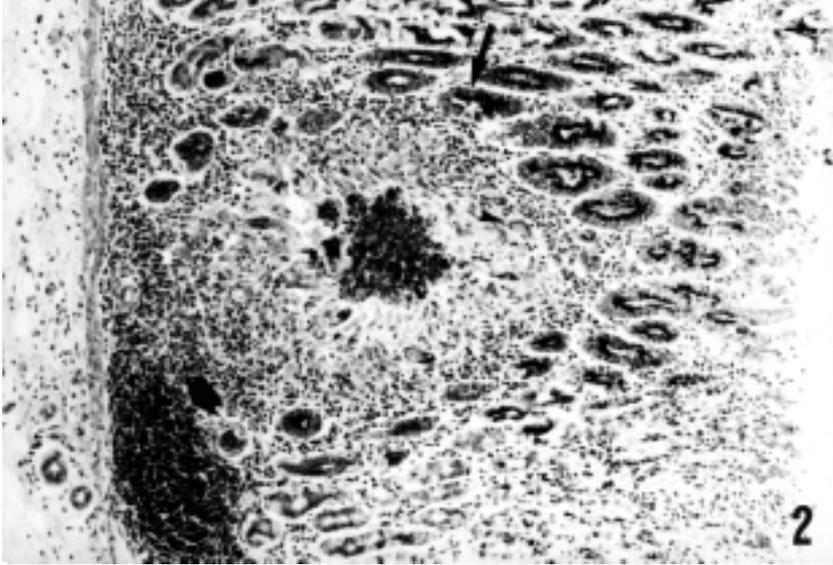


Figure 2. Group A. A granuloma composed of a necrotic centre surrounded by some multinucleate giant cells (arrowhead) and abundant inflammatory infiltrate (thick arrow) is observed in the abomasal lamina propria at 3 wpi. Abomasal glands show strong staining for mucus (thin arrow). Periodic Acid Schiff. Original magnification $\times 100$.

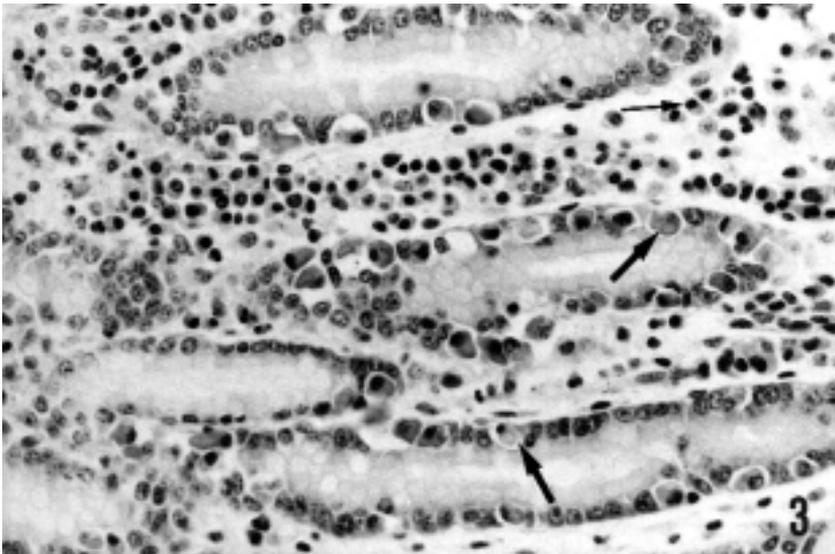


Figure 3. Group D. Detail of abomasal glands surrounded by a heavy infiltrate of lymphocytes and plasma cells (thin arrow). Note the presence of numerous globule leukocytes showing marked tropism for abomasal glands (arrows). Haematoxylin and eosin. Original magnification $\times 400$.

Table II. Cell counting in abomasal mucosa.

Group	<i>Eosinophils</i>	<i>Mast cells</i>	<i>Globule leukocytes</i>	<i>CD3</i>	<i>CD79a</i>	<i>IgG</i>
A	++	±	±	+++	+++	++
B	+++	+	+	++++	+++	++
C	+++	+	++	++++	++++	+++
D	+++	+	++	++++	++++	+++
E	+	+	++	+	+	+
F	±	±	±	±	±	±

Number of cells per field of 0.06 mm²: ±: 0-3, +: 3-10, ++: 10-20, +++: 20-30, ++++: more than 30.

3.2. Immunohistochemical study

Results of the immunohistochemical study are summarised in Table II. The CD3 pAb, reacted with numerous lymphocytes contained in the inflammatory infiltrate of the abomasal lamina propria (Fig. 4), with fewer lymphocytes in the submucosa, and isolated within abomasal glands. The infil-

trate of CD3⁺ lymphocytes in abomasal mucosa was mild (control group), moderate (21 wpi) and severe (3 and 7 wpi). Numerous CD3⁺ T lymphocytes were found at the periphery of degenerated larvae and granulomas observed in the abomasal mucosa. The majority of lymphocytes within the paracortical areas of lymph nodes of both control and infected goats were CD3⁺,

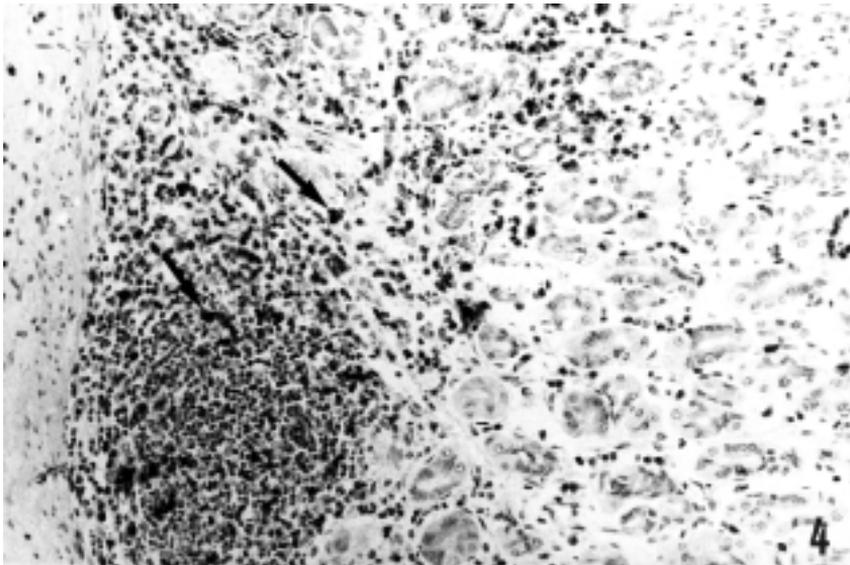


Figure 4. Group D. CD3⁺ T lymphocytes (arrows) are infiltrating the lamina propria diffusely, and a few of them are located within a lymphoid follicle. Avidin-biotin-peroxidase complex (ABC) technique. Original magnification ×200.

whereas they were sparse in lymphoid follicles and moderate number in the medullary cords.

The CD79a mAb displayed a cytoplasmic immunoreactivity in lymphocytes, located either in the abomasal infiltrate or in isolated lymphoid follicles (Fig. 5), and plasma cells. The CD79a⁺ infiltrate was very low in control, moderate at 21 wpi and severe at 3 and 7 wpi. This mAb also showed cross reactivity with smooth muscle and with chief cells of the abomasal glands. In the abomasal lymph nodes, the CD79a mAb stained the majority of lymphoid cells in the follicles, both in germinal centres and corona. Numerous plasma cells located in medullary cords showed an intense cytoplasmic immunoreactivity for this mAb in all infected goats.

In the abomasal mucosa IgG⁺ plasma cells and lymphocytes were located in the lamina propria, both in superficial and deep areas (Fig. 6), mainly at 3 and 7 wpi. In abomasal lymph nodes numerous IgG⁺ plasma

cells were observed in the medullary cords of all infected goats.

4. DISCUSSION

Abomasal changes were observed mainly in gastric folds at 3 and 7 wpi, agreeing with the findings reported previously in sheep and goats experimentally infected with *H. contortus* [1, 15, 28, 30]. At 3 wpi, larvae were observed within deep areas of the abomasal mucosa, a location previously reported in goats infected with *H. contortus* of ovine origin, whereas in sheep larvae showed tropism for superficial areas of the mucosa [1].

Goats are considered more susceptible than sheep to gastrointestinal nematodes [17, 25]. However, in relation to the dose of larvae given, few worms were recovered from the present goats by 3 wpi, which could have been due to poor larval establishment. The ovine origin of the larvae may explain

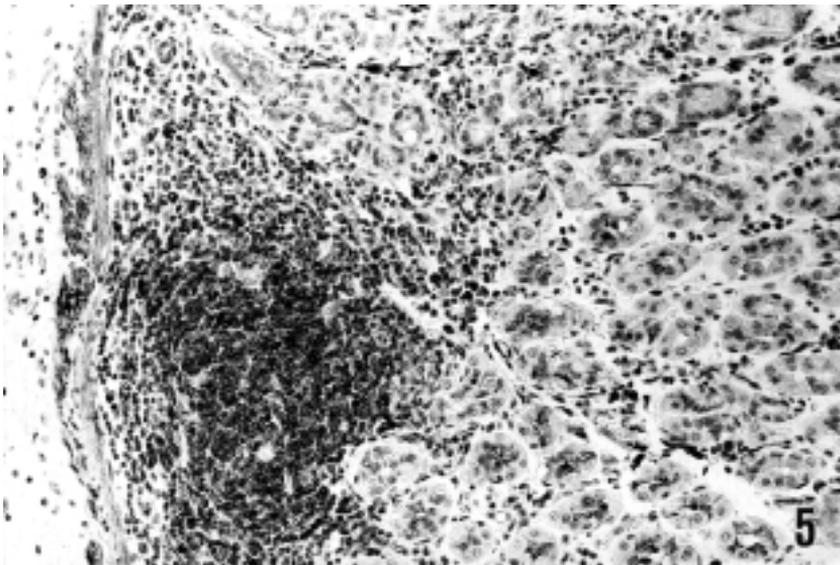


Figure 5. Group D. CD79a. Similar area of Figure 4 showing numerous CD79a⁺ lymphoid cells located mainly in a lymphoid follicle. Avidin-biotin-peroxidase complex (ABC) technique. Original magnification $\times 200$.

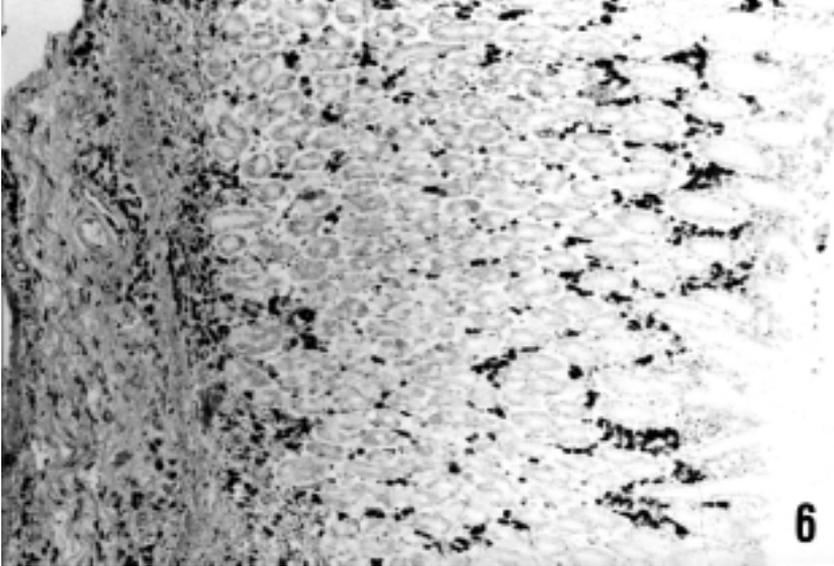


Figure 6. Group C. IgG. Numerous IgG⁺ plasma cells are located in the lamina propria and submucosa. Avidin-biotin-peroxidase complex (ABC) technique. Original magnification $\times 200$.

this result since sheep and goats-derived lines of gastrointestinal nematodes with different establishment rate and pathogenicity were reported [8, 27]. On the other hand, the host response may have induced death of some larvae within the abomasal mucosa, as suggested the presence of degenerated larvae surrounded by lymphocytes and eosinophils by 3 wpi, and granulomas by 3 and 7 wpi. These granulomas have not been reported in sheep and goats infected with *H. contortus*, although whitish spots or plaques were described in goats at 21 days post-infection [1]. Further studies at early stages post-infection and with reinfected goats are of interest to better investigate the relationship between presence of abomasal granulomas and the mechanism of protection against *H. contortus* infection.

The decline in worm burdens between 3 and 7 wpi suggests that the host response may play a role in the reduction of worms. Alternatively, some worms may have been lost through old age or other factors. In

sheep a variety of defensive mechanisms have been described in the host response against *H. contortus*, including mucous cell hyperplasia, mast cell, eosinophil, lymphocyte and globule leukocyte infiltrates, and humoral immune responses [12, 30]. The increase staining for mucous secretion in abomasal glands was marked at 3, very marked at 7 wpi, and moderate at 21 wpi, when the number of worms recorded was low. Similar findings have been reported in goats [1] and adult sheep, whereas in young lambs without resistance to *H. contortus*, a decrease in detectable mucus was found [30].

The abomasal infiltrate of eosinophils was heavy at 3 and 7 wpi, coinciding with previous finding in goats [1, 28] and sheep [12, 15, 29] infected with *H. contortus* and goats infected with *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* [21]. Eosinophils have been related with mechanisms of resistance against *H. contortus* in sheep, since they were absent in young

lambs and susceptible breeds, but abundant in resistant breeds [12, 30].

The preponderance of globule leukocytes over mast cells in the abomasal infiltrate has also been reported previously in goats infected with *T. vitrinus* and *T. circumcincta* [21], however, in other studies this cell type was not reported in goats infected with *H. contortus* [1, 28]. In sheep this cell type is considered of importance in the mechanisms of resistance to *H. contortus* infection since it secreted leukotrienes and molecules that inhibited larval migration [5]. Moreover, marked proliferation occurred in resistant sheep, whereas susceptible ones or young lambs did not show increase in this cell type [7, 12, 30]. The results of the present study suggest that globule leukocytes may play an important role in the response against adult *H. contortus* in goats, and in the response to reinfections since they remained for a long time in the abomasal mucosa. The increase of mast cells in the abomasal infiltrate of infected goats in respect to control ones is similar to the results reported in goats infected with *H. contortus* [1], *T. vitrinus* and *T. circumcincta* [16]. However, the number of mast cells did not change significantly in responders and non-responders goats infected with *T. vitrinus* and *T. circumcincta* [21].

The heavy abomasal infiltrate of CD3⁺ and CD79a⁺ lymphocytes and IgG⁺ plasma cells found at 3 and 7 wpi coincides with the heavier lymphoid infiltrate in goats compared with sheep infected with *H. contortus* [1]. In sheep, CD4⁺ T lymphocytes have been shown to play a central role in immunity to *H. contortus* infection [12, 24]. The results of the present study suggest that T cells may play equivalent role in goats. Numerous studies reported an important role of IgG in the response against *H. contortus* in sheep [4, 10, 11, 13, 18, 31, 32]. In Nigerian West African Dwarf goats, serum specific IgG was raised after primary infection with *H. contortus*, but no significant increase was found after secondary infection [6]. The heavy infiltrate of CD79a⁺ lymphocytes and

IgG⁺ plasma cells in abomasal mucosa and abomasal lymph nodes of the goats of the present study suggest that IgG appears to play a role in the local response against *H. contortus*.

Further studies are being carried out in reinfected goats to evaluate the role of T cell subsets and antibodies (IgA, IgM and IgG subclasses) to improve the understanding of the immune response of goats against this nematode.

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