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IMMUNOHISTOCHEMICAL STUDY OF THE INFLAMMATORY RESPONSE IN THE RUMEN AND RETICULUM OF *Calicophoron daubneyi*-INFECTED CATTLE

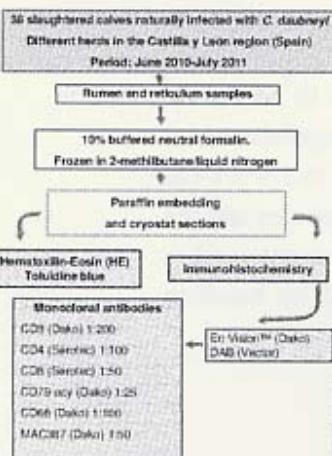
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Introduction

Calicophoron daubneyi (Digeno: Paramphistomidae) is a digestive trematode that affect ruminants considered to be the most common species found in cattle in several European countries (González-Warleta et al., 2008; Ferreras et al., 2011). Immature paramphistomes, before migration to the rumen and reticulum, may cause severe catarrhal inflammation in the proximal small intestine (mainly duodenum) (Teledo et al., 2006). The importance of changes caused by the adult worms in the forestomachs is unclear (Teledo et al., 2006). Most authors suggest that the adult paramphistomes are relatively harmless (Brown et al, 2007) but if present in very high numbers they can cause atrophy of papillae, excessive coprofication and presence of abundant eosinophils at the site of worm attachment (Rollo et al, 1994). The aim of this work was to characterize the inflammatory infiltrate present in the rumen and reticulum from cattle naturally infected with *C. daubneyi*.

Material and methods



Results

A total of 38 calves (5.79%) were parasitized and the ruminal atrium was the region showing the highest number of flukes (34.4%). Mature flukes were attached to the ruminal papillae and reticular folds by the ventral sucker (Figs. 1-3). In the lamina propria adjacent to the areas of parasite attachment numerous inflammatory cells were seen: lymphocytes, macrophages, eosinophils and mast cells (Figs. 4, 5). Foci of scattered or grouped lymphocytes, globular leucocytes, eosinophils and macrophages were found in intraepithelial and subepithelial locations in contact with the parasite (Figs. 6, 9, 14, 15). In all infected animals CD3+ pan-T lymphocytes, scattered diffusely or forming lymphoid aggregates, were seen as an element of the inflammatory infiltrate in the reticular and ruminal lamina propria mainly at the point of the parasite adhesion (Fig. 7). Furthermore, labelling of CD3 epitopes revealed grouped cells in the periphery of the lymphoid follicles and few cells inside the follicles (Fig. 8). Most CD3+ cells also expressed CD4+ helper cells (numerous in circumferential mantles around the lymphoid follicles), although sporadic CD8+ cytotoxic lymphocytes were observed (Figs. 10-12). CD4+ cells were found in intrapapillary location (Fig. 13). CD79 IgG B lymphocytes were observed constantly scattered in small groups but were also demonstrated in relation to lymphoid aggregates and follicles (Fig. 13). MAC387 and CD68 positive macrophages were found as occasional cells in the lamina propria, and as clusters in the stratified squamous epithelium at the site of parasite attachment (Figs. 14, 15).

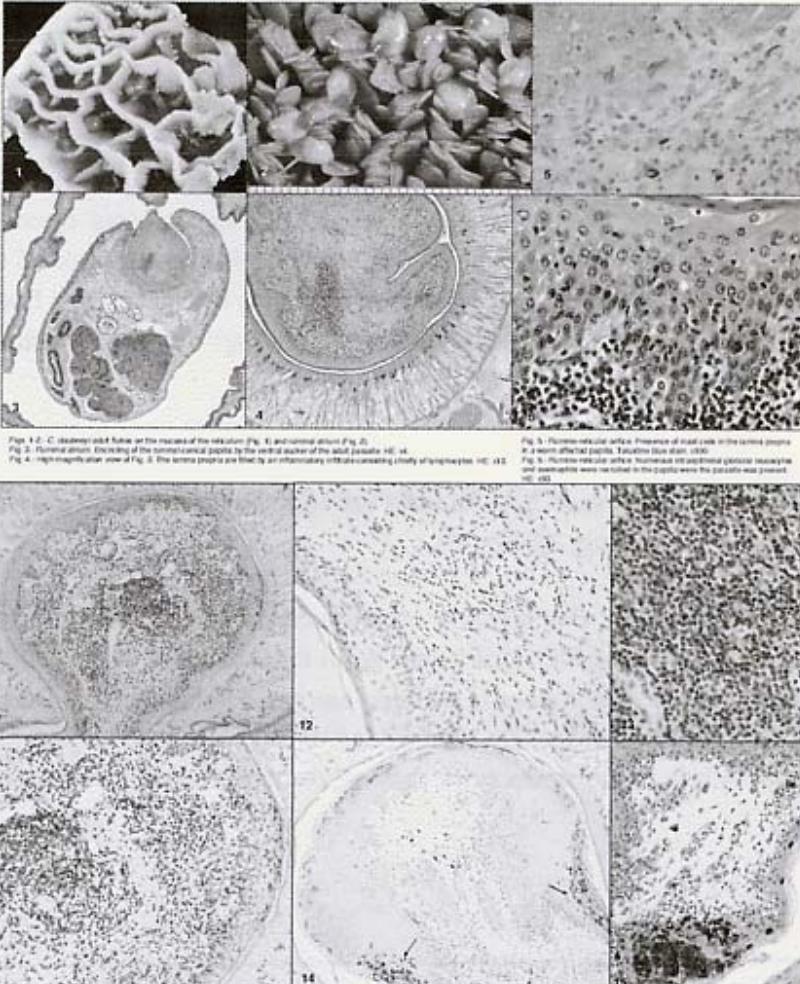


Fig. 1. Rumen-reticular surface of the papillae of the reticulum (Fig. 1) and normal atrium (Fig. 2).
Fig. 2. Rumen-reticular surface of the papillae of the reticulum mainly at the point of the parasite adhesion (Fig. 3).
Fig. 3. Higher magnification of Fig. 2. Following immunohistochemical processing positive for CD3. EnVision™ (Dako) 1:100.

Fig. 4. Higher magnification of Fig. 3. Following immunohistochemical processing positive for CD3. EnVision™ (Dako) 1:100.

Fig. 5. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 6. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 7. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 8. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 9. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 10. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 11. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 12. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 13. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 14. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Fig. 15. Atypical lymphocyte showing for CD3. The inflammatory infiltrate was composed by numerous CD3+ lymphocytes. EnVision™ (Dako) 1:100.

Discussion & Conclusions

Histopathological and immunohistochemical studies focused on characterising the inflammatory infiltrates associated with ruminal and reticular lesions in natural bovine paramphistomosis have not been carried out to date. The detection of CD3+, CD4+, CD8+ T lymphocytes, CD79 IgG B lymphocytes and MAC387+ and CD68+ macrophages suggests that all these cells participate in a local cellular immune host response against *C. daubneyi*. This immune response also includes the activation of substantial numbers of eosinophils, globular leucocytes and mucosal mast cells. In natural conditions adult flukes of *C. daubneyi* have pathogenic effect in the ruminal and reticular mucosa, mainly at the point of the parasite adhesion, and may induce a humoral and cell-mediated local immune response in cattle.

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