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

Introduction
Calicophoron daubneyi (Dinophyceae: Paramphistomatidae) is a digenetic trematode that affect ruminants considere to be the most common species found in cattle in several European countries (González-Varleto et al., 2008; Fortuna et al., 2011). Immune paramphistomiasis, before migration in the rumen and reticulum, may cause severe enteritis in the proximal small intestine (mainly duodenum) (Bosio et al., 2008). The importance of changes caused by the adult worms is unclear (Tierso et al., 2006). Most authors suggest that the adult paramphistomes are relatively harmless (Brown et al., 2007) but if present in very large numbers they can cause atrophy of papilla, erosive inflammation and presence of abundant eosinophils at the site of worms attachment (Rosa et al., 1999).

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
A total of 38 calves (57%) were studied and the rumen and reticulum was the region showing the highest density of flukes (94%). Mature flukes were attached to the ruminal papillae and reticular folds by the ventral sucker (Figs. 1, 2). In the lamina propria adjacent to the anus of parasitic worm, numerous inflammatory cells were seen lymphocytes, macrophages, eosinophils and mast cells (Figs. 3, 4). Foci of scattered or grouped lymphocytes, globular leukocytes, eosinophils and mast cells were found in intrapapillary and subpapillary locations in contact with the parasites (Figs. 5, 6). In all infected animals CD4+ and CD8+ lymphocytes, scattered diffusely or forming lymphoid aggregates, were seen as an element of the inflammatory infiltrate in the reticulum and lamina propria mainly at the point of the parasite adhesion (Figs. 7, 8). Furthermore, labeling of CD3+ and CD68+ positive macrophages were found in the vicinity of the lymphoid follicles and foci cells in the lamina propria. In the lamina propria, although sporadic CD8+ cytotoxic lymphocytes were observed (Figs. 9, 10), CD4+ cells were found in intrapapillary foci (Fig. 11). CD4+ and CD8+ lymphocytes were observed scattered in small groups but were also demonstrated in relation to lymphoid aggregations and follicles (Fig. 12). MHCII 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. 13, 14).

Discussion & Conclusions
Histopathological and immunohistological studies focused on characterizing the inflammatory infiltrate associated with ruminal and reticular flukes in natural bovine paramphistomiasis have not been carried out to date. The detection of CD3+, CD4+, CD8+, and CD68+ lymphocytes and MHCII+ 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 leukocytes and mucosal mast cells. In natural conditions adult flukes of C. daubneyi have pathogenic effect in the ruminal and reticular mucosa, mainly at the site of the parasite adhesion, and may induce a humoral and cellular-mediated local immune response in cattle.

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References


