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Severe meningeal fibrinoid vasculitis associated with *Theileria taurotragi* infection in two short-horned Zebu cattle.

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Abstract The Authors describe a severe vasculitis with fibrinoid necrosis of the meningeal arteries observed in two brains of indigenous short-horn zebu (*Bos indicus*) cattle, with Bovine cerebral theileriosis (BCT) caused by a tick-transmitted hemoprotozoan, *Theileria taurotragi,* from Northern Tanzania. In the Author's opinion the role of *Theileria taurotragi* infection in the angiocentric and angiodestructive detected features remains to be evaluated. A possible immunopathologic cancerous mechanism, secondary to the lymphoid deregulation, could be involved. This report suggests further studies to better characterize the lymphoid cells involvement in the pathogenesis of the meningeal vascular lesions by *Theileria taurotragi*.

Key words: East-African short-horned Zebu, Brain, histopathology, immunopathology *Theileria*.

Bovine cerebral theileriosis (BCT) is a parasitic infection of cattle caused by *Theileria parva* or *Theileria taurotragi,* rarely by *Theileria annulata* (Lawrence et al., 2004). Suspected cases of BCT have been reported from Maasai herders in Northern Tanzania since the mid-1980's (Field et al., 1988; Nsengwa, 1993). This disease usually occurs in young animals and pathologically it is characterized by severe congestion and hemorrhages in the meninges and in the brain, particularly involving the ventricles associated with subacute-chronic areas of malacia. Microscopically, the obstruction of arteries and arterioles with a large numbers of parasitized lymphoblasts is the most prominent finding. The blood vessels are frequently thrombosed and necrotic, with perivascular hemorrhages and malacic areas in the surrounding parenchyma (Lawrence et al., 2004).

This paper describes two atypical cases of cerebral theileriosis observed in East-African short-horned Zebu, from Northern Tanzania (Arusha Region, Ngorongoro District, Endulen ward), which presented at post-mortem and histological examination an unusual fibrinoid vasculitis of the meningeal arteries.

The brains of two female East-African short-horn Zebu cattle aging respectively 3 and 6 years, collected between 2001 and 2003, in Tanzania were submitted to histopathological investigations during a research project on Ormilo disease disorder (Catalano et al., 2015). Coronal slices were embedded in paraffin wax, sectioned at 5µm and stained with hematoxylin and eosin (HE).

The indirect immunoperoxidase method (streptavidin biotin- peroxidase - Vectastain ABC Kit, Vector Laboratories, Inc. Burlingame, CA) was used for immunolabeling selected brain sections using one monoclonal primary antibody,anti-CD79 (clone HM57-Monoclonal Anti-Human CD79 αcy, clone HM57, code No.

M7051,Dako, DK-2600 Glostrup, Denmark) and one polyclonal primary antibody antihuman CD3 (Polyclonal Rabbit Anti-Human CD3, code No. A0452; Dako, DK-2600 Glostrup, Denmark) for the detection of B lymphocytes and T lymphocytes, respectively. Tissue sections were rehydrated and treated with 3% hydrogen peroxide in water for 30 min to eliminate endogenous peroxidase activity. Antigen retrieval was performed by microwave irradiation for 30 minutes in 10 mM citrate buffer at pH6 and pH 2.5 for the detection of B and T lymphocytes, respectively. Slides were incubated with primary antibodies overnight at 4°degrees. Dilutions were 1: 100 for anti-CD3 and 1: 25 for anti-CD79. The level of cellular staining was evaluated in a semi-quantitative scoring system [negative (-); ＜10% (+); 10-25% (++); 25-50% (+++); ＞50% (++++)] by examination of 10 random fields (x 400).

Brain tissues from the same animals were also subjected to PCR and reverse line blot hybridization to detect and identify *Theileria* and *Babesia* spp. as previously reported (Catalano et al., 2015). PCR investigations to exclude the infection by Ovine herpesvirus 2 and Alcephaline herpesvirus 1 were also performed according to the literature (Flach et al., 2002).

Histological investigations revealed the classical lesions attributed to *Theileria* spp. infection (congestion of the meningeal vessels, hemorrhages and plasmorrhages in and around the ventricles, multifocal to disseminated necrotic areas, oedema, vascular proliferation and axonal degeneration). Severe accumulation of mononuclear cells in the cerebral and meningeal blood vessels was the most significant lesion. Morphologically, large lymphoblastic cells predominated, including sometimes small and medium sized lymphocytes, rare plasma cells and macrophages. Most lymphocytic cells showed a variable number of the schizonts in the cytoplasm.

An uncommon severe vasculitis characterized by fibrinoid necrosis was diffusely present in the meningeal arteries of both brains (Fig. 1a). Large and small lymphocytes infiltrated the tunica adventitia and media of the arteries and arterioles of the meninges sometimes invading the surrounding parenchyma. A fibrinoid degeneration of infiltrated mural tissues and smooth muscle elements of vessel walls was considered to be the defining characteristic of this vasculitis.

Immunohistochemistry performed to better characterize the infiltrating lymphocytes revealed only a very few number of mononuclear cells accumulated into or around the CD3 positive arteries. However, the level of cellular staining was always less than 10% of cells counted in 10 random fields (x400). Most cells expressed no detectable markers.

DNA extracts from the brain tissues were positive to PCR and revealed the presence of *T. taurotragi* as the unique infectious agent (Catalano et al., 2015).

Macroscopical and histological lesions are compatible with those reported in BCT caused by *Theileria* species (Lawrence *et al.* 2004), except for the presence of a severe vasculitis, which represents an unusual lesion. Herpesvirus infection could result with fibrinoid vasculitis, but in the present cases Ovine herpesvirus 2 and Alcephaline herpesvirus 1 DNA were not detected.

The observed intravascular and perivascular mononuclear cells, only rarely expressing CD3 and none CD79 markers, indicate a lymphoblastic stage of development. The pathogenetic role of these cells remain to be clarified, even if the range of *Theileria* spp. infected cells differ significantly between species. In fact, *T. parva* is capable of infecting and transforming only cells of lymphocyte origin, but both B and T cells could be parasitized, whereas *T. annulata* can infect also monocytes (Dobbelaere and Heussler, 1999). No detailed reports are available about *T. taurotragi* infected cells.

It is interesting to note that in both brains, the accumulation of large and small lymphocytes into the blood vessels was associated with perivascular cuffs mainly composed by CD3 positive cells (Figs. 1b, 1c) and, severe vasculitis with concentric fibrinoid necrosis of the vessels walls.

Pathogenesis of the BCT is still unclear. Possibilities of crossing the CNS barriers by the parasite and causing auto immune disorders, characterized by intra vascular agglutination of the parasitized blood cells in the cerebral capillaries with consequential embolism/thrombosis could not be ruled out. This vasocentric and vasoinvasive lesion is reminiscent of lymphomatoid granulomatosis (LG), a slowly progressive diffuse or nodular angiocentric and angiodestructive lymphoproliferative disorder that most commonly involves pulmonary parenchyma, but can rarely involve the skin, kidney and central nervous system (Colby et al., 1994; Valli, 2007). This disorder is characterized by a heterogeneous population of lymphoid cells, often admixed with macrophages, and large atypical cells forming both nodular and diffuse interstitial infiltrates, with transmural invasion of blood vessel walls by atypical lymphoid cells, angiodestruction, and necrosis (Valli et al., 2002). It is thought to be a distinctive form of angioinvasive lymphoma (Valli et al., 2002; Valli, 2007). The pathogenesis of LG remain unclear and the immunophenotypic features are controversial even if the disease seems to progress from an inflammatory condition with cytotoxic T cells as key cells, to malignant transformation (Morice et al., 2002). Human LG is an angiocentric proliferation of B cells surrounded by a mixed inflammatory cell population composed especially by reactive T-cell (Lundell et al., 2008). It is uncertain if the human disorder is analogous in animals, in fact, in most cases reported in veterinary pathology, a proliferation of T cells or both B- and T-cell lineages was demonstrated, similarly to those described in the present research, but in contrast to human cases. In animals LG has been associated with malignant T-cell type lymphocytes proliferation and is considered a T-

cell angiotropic lymphoma (Valli et al., 2002). In human LG, B neoplastic cells can activate T cells resulting in the elaboration of cytokines that can recruit the cytolytic lymphocytes responsible of the development of necrotic areas, characterizing the host response to these tumors. Because of the T cells activation in the cases of vasculitis here reported, the authors speculate similar immunologic mechanisms to induce the host response and to cause the generation of the necrotic foci in BCT too.

T. parva is reported to cause lymphocyte proliferation particularly in vitro, but massive death of lymphocyte (necrosis) is observed in the lymphoid tissue, leading us to consider this infection a lympho-destructive disease (Mbassa et al., 2006). No specific information is available regarding the pathogenesis of *T. taurotragi* infection. Moreover as recently reported by Tretina *et al.* (2015) some species of the genus *Theileria*, including *T. parva* and *T. annulata*, infect leukocytes inducing phenotype changes like cancer, mostly immortalization, hyperproliferation, and dissemination, even if the parasite proteins directly responsible for these changes remain unknown. A similar cancer like immunologic mechanism in *T. taurotragi* infection cannot be excluded.

A detailed evaluation of non-B non-T cells accumulating into and around the blood vessels, using specific antibodies, will be helpful in clarifying the characteristics of these lymphoid/myeloid precursors potentially involved in the pathogenetic mechanism of *T. taurotragi* cerebral infection.

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Figure captions Figure 1a. Vascular occlusion (*), severe perivascular non suppurative inflammation, vasculitis and fibrinoid necrosis (#) of a meningeal artery. Hematoxylin and eosin (HE). **Figure 1b.** Perivascular cuffs mainly composed by CD3 positive cells (arrows). **Figure 1c**. Focal CD79 immunopositivity of inflammatory cells located at the periphery of the cuffing (circle). The intravascular mononuclear cells are negative for both lymphoid markers. Immunohistochemistry for the detection of B and T lymphocytes counterstained with hematoxylin. Streptavidin biotin- peroxidase method. (a,b,c bar = 50 μm)

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