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

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

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

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42 Bovine cerebral theileriosis (BCT) is a parasitic infection of cattle caused by *Theileria parva* or *Theileria*  
43 *taurotragi*, rarely by *Theileria annulata* (Lawrence et al., 2004). Suspected cases of BCT have been reported  
44 from Maasai herders in Northern Tanzania since the mid-1980's (Field et al., 1988; Nsengwa, 1993). This  
45 disease usually occurs in young animals and pathologically it is characterized by severe congestion and  
46 hemorrhages in the meninges and in the brain, particularly involving the ventricles associated with subacute-  
47 chronic areas of malacia. Microscopically, the obstruction of arteries and arterioles with a large numbers of  
48 parasitized lymphoblasts is the most prominent finding. The blood vessels are frequently thrombosed and  
49 necrotic, with perivascular hemorrhages and malacic areas in the surrounding parenchyma (Lawrence et al.,  
50 2004).

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

54 The brains of two female East-African short-horn Zebu cattle aging respectively 3 and 6 years, collected between  
55 2001 and 2003, in Tanzania were submitted to histopathological investigations during a research project on  
56 Ormilo disease disorder (Catalano et al., 2015). Coronal slices were embedded in paraffin wax, sectioned at 5µm  
57 and stained with hematoxylin and eosin (HE).

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

61 M7051, Dako, DK-2600 Glostrup, Denmark) and one polyclonal primary antibody antihuman CD3 (Polyclonal  
62 Rabbit Anti-Human CD3, code No. A0452; Dako, DK-2600 Glostrup, Denmark) for the detection of B  
63 lymphocytes and T lymphocytes, respectively. Tissue sections were rehydrated and treated with 3% hydrogen  
64 peroxide in water for 30 min to eliminate endogenous peroxidase activity. Antigen retrieval was performed by  
65 microwave irradiation for 30 minutes in 10 mM citrate buffer at pH6 and pH 2.5 for the detection of B and T  
66 lymphocytes, respectively. Slides were incubated with primary antibodies overnight at 4°degrees. Dilutions were  
67 1: 100 for anti-CD3 and 1: 25 for anti-CD79. The level of cellular staining was evaluated in a semi-quantitative  
68 scoring system [negative (-); < 10% (+); 10-25% (++); 25-50% (+++); > 50% (++++)] by examination of 10  
69 random fields (x 400).

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

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

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

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

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

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

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

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

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

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

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

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

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

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