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Short communication

Toxoplasmosis in Bennett's wallabies (*Macropus rufogriseus*) in Spain

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ABSTRACT

Toxoplasmosis is one of the more common parasitic zoonoses world-wide. In this study, an epizootic of toxoplasmosis among captive Bennett's wallabies (*Macropus rufogriseus*) from different locations is reported. By means of light microscopy, *Toxoplasma gondii*-like tachyzoites were observed associated to interstitial pneumonia, non-suppurative myocarditis, cholangiohepatitis and severe gastroenteritis. The protozoa stained positively with a *T. gondii* antibody and ultrastructurally were similar to *T. gondii*. Strikingly, tachyzoites appeared sometimes in an intranuclear location within granulocyte-like cells. Feral cats or reactivation of a latent infection are discussed as the possible sources of infection. As far as we know, this is the first confirmed report of toxoplasmosis in Bennett's wallabies in Spain and Europe, and may constitute a risk of infection for humans since new alimentary habits are being imposed in our countries.

1. Introduction

Toxoplasmosis is a common parasitic disease world-wide of clinical and veterinary importance, being not only a major cause of congenital disease and abortion in human and domestic animals but also a life-threatening opportunistic infection in immunodepressed individuals such as transplant recipients and AIDS patients, where it is the single major cause of cerebral mass lesions (Luft and Remington, 1992; Ambroise-Thomas and Pelloux, 1993; Dubey, 1997; Tenter et al., 2000). In animals, *Toxoplasma gondii* infects most of homeothermic organisms (Tenter et al., 2000; Coppens and Joiner, 2001), even though susceptibility to clinical disease varies: rats, cattle, horses and Old World monkeys are fairly resistant to clinical disease, while Australian marsupials and New World monkeys are particularly susceptible to severe and often fatal clinical disease (Adkesson et al., 2007). Thereby wallabies and other macropods, which represent an alternative source of meat in some countries, are considered among the most susceptible species to this apicomplexa parasite and toxoplasmosis has been reported in several species of marsupials throughout the world (Dubey et al., 1988; Adkesson et al., 2007; Basso et al., 2007).

In this work, two cases of massive toxoplasmosis in captive Bennett's wallabies (*Macropus rufogriseus*) submitted to our laboratory are reported for the first time in Europe.

2. Materials and methods

A dead adult male Bennett's wallaby was submitted to the Diagnostic Pathology Service of the School of Veterinary Sciences of the University of Santiago de Compostela from a private zoo in Lugo (Northwestern Spain). Five wallabies had arrived to this zoo few weeks before and they had not been quarantined. Three of these animals began to show non-specific symptoms such as apathy, depression and emaciation and, after a few days, finally they died. The other partners did not show

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apparent clinical signs of disease around the date. Necropsy examination was performed on one dead specimen of wallaby and tissues samples were fixed in 10% formalin and sections were processed routinely, sectioned, stained with both hematoxylin and eosin (H&E) and Giemsa, and examined by means of light microscopy. Brain samples could not be taken since the specimen was decapitated previously to be sent. On the other hand, biopsies of ulcerated oral and palatine mucosa of other dead wallaby were brought to our laboratory, sent by a private citizen who lived in a different area 100 km far away. Samples from this second specimen were processed in the same way. Immunohistochemistry was conducted by an immunoperoxidase method, using antibodies against *T. gondii* and *Neospora caninum*, gently provided by Dr. Pumarola (UAB, Spain). Pieces of ulcerated areas of stomach from the first specimen previously fixed in 10% formalin were diced to obtain 1 mm^3 cubes and routinely prepared for transmission electron microscopy (TEM). The samples were fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in epoxy resin. Afterwards, 50 nm sections were contrasted with lead citrate and uranyl acetate and examinated under a JEOL electron microscope.

3. Results

The carcass of the dead wallaby showed evident cachexia and loss of condition. The main macroscopical changes were pleural and pericardial serosanguinuous effusion, congestion of the lungs, petechia in several organs, manifest gastric ulcers (Fig. 1A) accompanied by

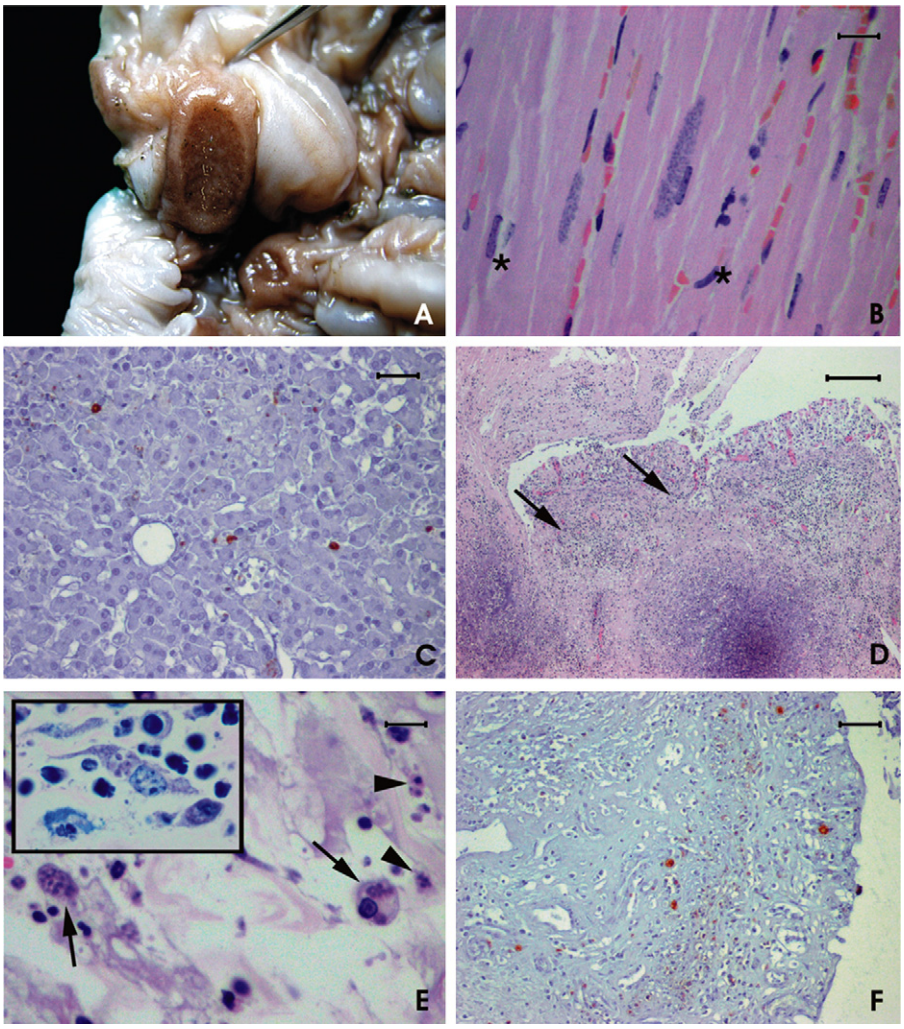

Fig. 1. Lesions and *T. gondii* forms in sections of tissues of the first wallaby. (A) Two crateriform well-delimitated ulcers in the gastric mucosa. (B) Histological view of a gastric ulcer, with loss of epithelium and presence of abundant necrotic debris and inflammatory cells (asterisks). H&E stain. Bar = 120 μm. (C) Mononuclear infiltration and cellular debris in the gastric mucosa. Note the tachyzoites either within the mononuclear cells (arrows) or free in the tissue (arrowheads). H&E stain. Bar = 6 μm. Inset: a group of tachyzoites contained in their parasitophorous vacuole within a fibroblast-like cell. Giemsa stain. Bar = 6 μm. (D) Several *T. gondii* tissue cysts in the myocardium (arrows). H&E stain. Bar = 20 μm. (E) Mild focal hepatic necrosis with the presence of individual tachyzoites. IHC staining with *T. gondii* antibody. Bar = 30 μm. (F) Many intralesional parasitic forms in a gastric ulcer. IHC staining with *T. gondii* antibody. Bar = 30 μm.