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SHORT COMMUNICATIONS

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Toxoplasmic Encephalitis in a Free-ranging Rocky Mountain Bighorn Sheep from Washington

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ABSTRACT: A 4-mo-old free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) from the Hells Canyon area (Washington, USA) was diagnosed with encephalitis associated with *Toxoplasma gondii* infection. The sheep had concurrent pneumonic pastuerellosis and resided in a geographic area with endemic *Pastuerella*-associated pneumonia and mortality in bighorn sheep. The brain had multifocal necrotizing and nonsuppurative encephalitis with intralesional protozoa. The protozoa were identified as *T. gondii* by immunohistochemistry. To our knowledge, this is the first report of *T. gondii* infection in a Rocky Mountain bighorn sheep.

Key words: Bighorn sheep, case report, encephalitis, *Ovis canadensis canadensis*, *Toxoplasma gondii*.

The protozoan *Toxoplasma gondii* has a widespread host range and worldwide distribution (Dubey and Beattie, 1988). Infection results in fatal disseminated disease, localized disease (usually localized to the nervous system or reproductive tract), or inapparent infection depending on the susceptibility of the host (Innes, 1997). In domestic sheep, natural *T. gondii* infection during pregnancy can cause reproductive failure or neonatal mortality. To date, there is no documented report of post-neonatal clinical toxoplasmosis in sheep (Dubey and Beattie, 1988). The present communication describes localized encephalitis associated with *T. gondii* in a juvenile free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) from the Hells Canyon area (Washington, USA).

A 4-mo-old female Rocky Mountain bighorn lamb was submitted for necropsy to the Washington Animal Disease Diagnostic Laboratory (Washington State University, Pullman, Washington, USA) in September 1997. The sheep was found dead in its resident range on the Washington border of the Hells Canyon of the Snake River (46°5′N, 117°5′W). The lamb and its dam were being monitored by the Idaho Department of Game and Fish (Lewiston, Idaho, USA) by radio collar as part of an on-going study of mortality of Hells Canyon bighorn sheep following a large die-off in 1995–96 associated with pneumonic pasteurellosis (Cassirer et al., 1997).

Necropsy examination revealed severe fibrinohemorrhagic bronchopneumonia and pleuritis involving the cranioventral 60% of both lungs. The heart had multiple ecchymoses. Both external ear canals contained moderate quantities of yellow-brown greasy exudate and *Psoroptes* spp. ear mites. No gross lesions were seen in the brain. Multiple tissues were fixed in 10% neutral buffered formalin, paraffin-embedded, and 5 μm sections stained with hematoxylin and eosin (H & E) for routine histopathology. Tissues examined histologically included brain, lung, tongue, liver, spleen, kidney, skeletal muscle (rear limb, front limb and diaphragm), heart, small and large intestine, abomasum, and mesenteric and tracheobronchial lymph node.

Histologic examination of the lung revealed necrotizing and fibrinous bronchopneumonia compatible with pneumonic pasteurellosis. Twelve sections of brain were examined and included multiple sec-

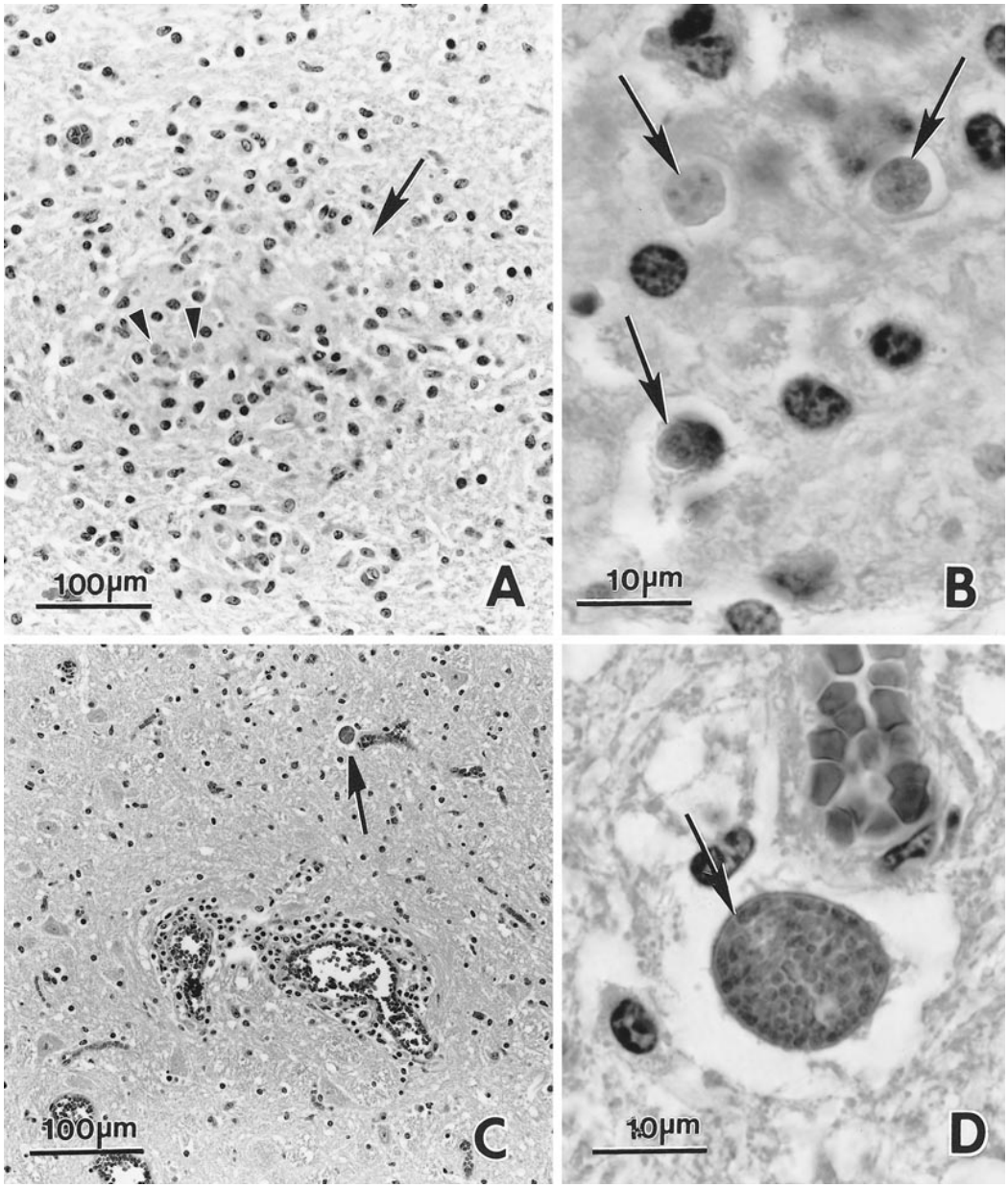

FIGURE 1. Lesions and *Toxoplasma gondii* in the brain of the bighorn sheep. All H & E. A. Focus of necrosis with gliosis and mononuclear inflammatory cell infiltration (arrow); two small protozoan cysts within the lesion (arrowheads). B. Higher magnification of Figure 1A showing three protozoan tissue cysts (arrows). C. Perivascular infiltration by mononuclear inflammatory cells. Protozoan tissue cyst in adjacent neuropil (arrow). D. Higher magnification of tissue cyst (arrow) in Figure 1C.

tions from cerebral cortex, midbrain, brainstem, and cerebellum. Multiple sections of cerebral cortex and midbrain had random foci of neuropil necrosis and gliosis in the white matter and gray matter (Fig. 1A). Some necrotic foci contained small tissue cysts ranging from 5–10 µm in diameter (Fig. 1B). Numerous arterioles were rimmed by lymphocytes and histiocytes in cuffs 1–3 cell layers thick (Fig.

1C). Another group of tissue cyst-like organisms was seen adjacent to a blood vessel (Fig. 1D). Protozoan organisms or focal necrotizing lesions were not seen in other tissues than the brain.

To identify the protozoa, formalin-fixed paraffin-embedded tissue sections were tested by immunohistochemistry using avidin-biotin-complex (ABC) immunoperoxidase procedures as described previously (Lindsay and Dubey, 1989; Long et al., 1998). Electron microscopy was not done because tissue preservation was poor and only paraffin-embedded tissues were available for ultrastructural examination. Primary antibodies for immunohistochemistry were all polyclonal and included anti-*Neospora caninum* (G270 goat serum, 1:6,000, VMRD Inc., Pullman, Washington, USA), anti-*T. gondii* (9070-004 rabbit serum, 1:20,000, Biogenesis Inc., Kingston, New Hampshire, USA), and anti-*Sarcocystis neurona* (UCD-1 rabbit serum, 1:2000, generously donated by B. Barr, University of California-Davis, Davis, California, USA). All immunostaining was done using an automated capillary action immunostainer (TechMate, Vantana Medical Systems Inc., Tuscon, Arizona, USA) using commercially available ABC-immunoperoxidase kits (Vectastain Elite, Vector Laboratories, Burlingame, California, USA). Positive control tissue included (1) brain from a cat experimentally infected with *T. gondii* (2) brain from a mouse experimentally infected with *N. caninum* and (3) brain from a horse naturally-infected with *S. neurona.* Negative controls were done on all test tissues and consisted of substitution of primary antiserum with a similar dilution of species matched normal serum. The protozoan organisms in the brain of the bighorn sheep were strongly immunoreactive with *T. gondii* antiserum and not immunoreactive with *N. caninum* and *S. neurona* antisera. Immunohistochemistry revealed more *T. gondii* within the brain than evident from hematoxylin and eosin stained sections.

To our knowledge, this is the first report of toxoplasmic encephalitis in a free-ranging Rocky Mountain bighorn sheep and represents a new host record for *T. gondii.* The sheep also had concurrent pneumonic pasteurellosis, which was the most severe lesion in this sheep and likely directly resulted in death. How the bronchopneumonia contributed to the *T. gondii* encephalitis is not known, and whether or not the *T. gondii* encephalitis contributed to clinical disease and death in the sheep is speculative. Regardless, the disease caused by *T. gondii* in the brain was significant because inflammatory lesions were present in multiple brain regions, and lesions were acute and associated with ongoing tissue necrosis indicating an active infection. The presence of small tissue cysts, together with free tachyzoites associated with active tissue necrosis suggested recently acquired infection.

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