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Short communication

Pathology, immunohistochemistry, and ultrastructural findings associated with neurological sarcocystosis in cattle

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ABSTRACT

Paraffin-embedded blocks of brain of a nine months old bull calf that died of neurological signs in 1982 in Germany were restudied. Numerous schizonts and merozoites were found associated with extensive but focal necrosis and severe meningoencephalitis. Developing stages of schizonts as well as free merozoites were identified. The schizonts were primarily in perivascular areas. Ultrastructurally, schizonts were seen both in capillaries and in extravascular space. Merozoites were often concentrated in adventitial layers of capillaries. Schizonts divided by endopolygeny, the nucleus became multi-lobed, and at the terminal stage nuclear lobes were incorporated into budding merozoites. Individual merozoites were seen in neurons, astrocytes, oligodendrocytes, leukocytes, and vascular endothelial cells. Occasionally merozoites were present in the nucleus of mononuclear cells. Individual merozoites were ovoid, 3–5 × 2–3 μm in size, and contained a prominent nucleus, numerous micronemes, a conoid, but no rhoptries. Schizonts and merozoites did not react to polyclonal rabbit *Neospora caninum*, *Toxoplasma gondii*, and *Sarcocystis neurona* antibodies but did react to *Sarcocystis cruzi* antibodies. Because of morphological characteristics and the type of lesions, the parasite was likely due to an unidentified *Sarcocystis* species, different from *S. cruzi*.

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1. Introduction

Sarcocystis infections are common in cattle worldwide. Clinical sarcocystosis in cattle is relatively rare. Five or more species of *Sarcocystis* have been reported in cattle, *Sarcocystis cruzi, Sarcocystis hirsuta, Sarcocystis rommeli, Sarcocystis heydorni, Sarcocystis hominis*, and possibly a sixth species, *Sarcocystis bovifelis* (Dubey et al., 2015, 2016; Gjerde, 2016). Of these *Sarcocystis* species, *S. cruzi* is considered the most pathogenic (Dubey et al., 2016). Clinical sarcocystosis in cattle has been reported from Canada, USA, England, Norway, Ireland, and Australia (reviewed by Dubey et al., 2016). Here, we have reevaluated the case of acute sarcocystosis in a nine month old bull from Germany reported by Takla (1984); *S. cruzi* was considered the likely cause based on histological examination.

2. Materials and methods

In November 1986 Dr. Takla sent to one of us (JPD) two paraffin blocks from this calf for further diagnosis. After initial examination, JPD communicated to Dr. Takla in 1987 that the infection resembled Equine Protozoal Myeloencephalitis (EPM), later determined to be *Sarcocystis neurona* infection (Dubey et al., 2015). No further details are available because Dr. Takla passed away in 2012.

Numerous histological sections were cut from the two blocks of paraffin sent by Dr. Takla. Histological sections were examined after staining with hematoxylin and eosin.

For transmission electron microscopy (TEM), the areas with lesion (by matching with H and E sections) were deparaffinized in 1% w/v osmium tetroxide in xylene and embedded in LX-112 epoxy resin (Van den Berg Weermans and Dingemans, 1984). 60–90 nm silver gold sections were cut on a Reichert/AO Ultracut ultramicrotome with a Diatome diamond knife and mounted onto 200 mesh formvar-coated copper grids. Grids were stained with 4% uranyl acetate and 3% lead citrate and imaged at 80 kV with a Hitachi HT-7700 transmission electron microscope.

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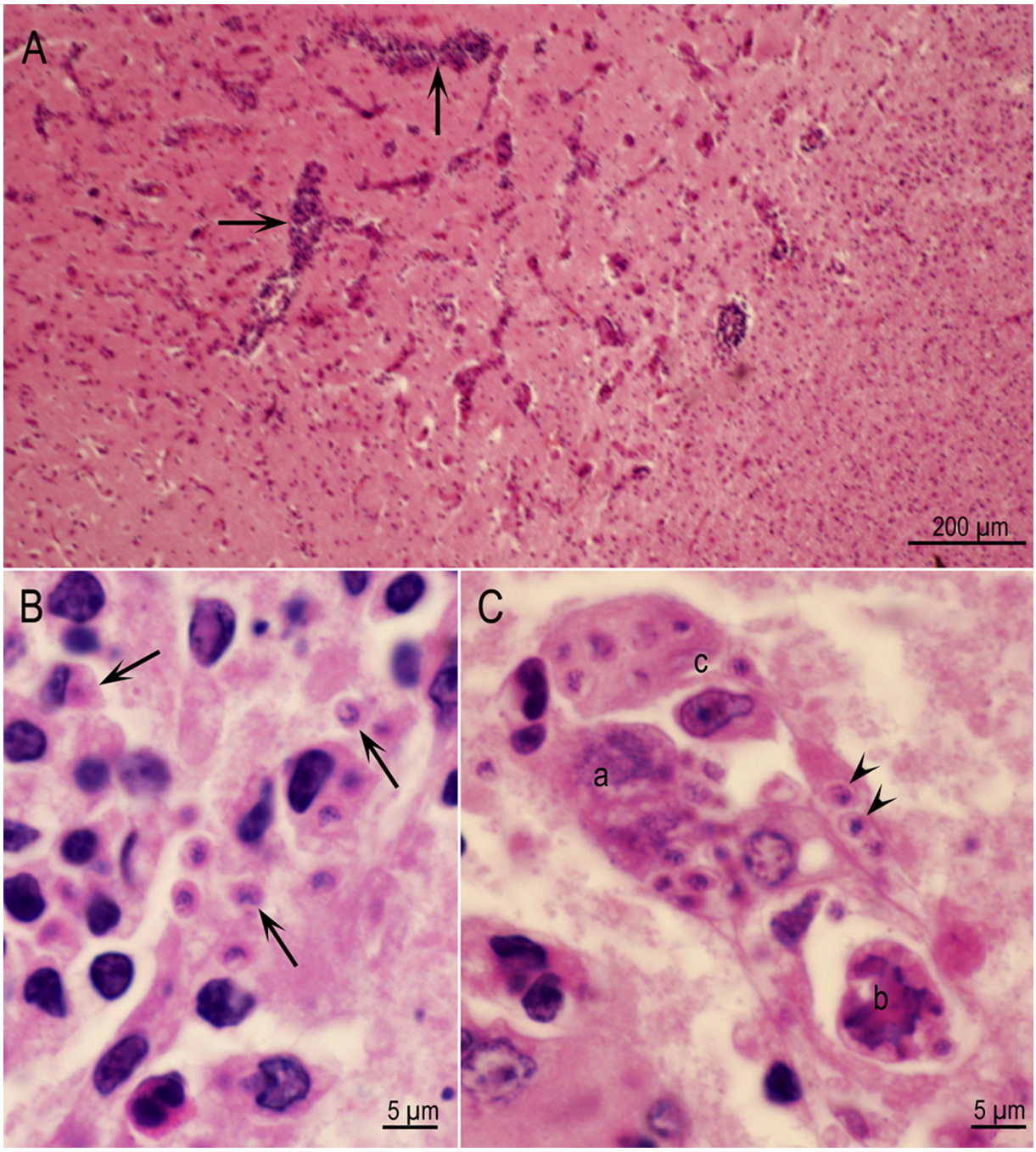

Fig. 1. Section of cerebrum of the calf. Hematoxylin and eosin stain. (A) Focal encephalitis characterized by neovascularization, perivascular cuffings (arrows), and necrosis.The adjoining tissue is unaffected. (B) Numerous free and intracellular merozoites among inflammatory cells in the perivascular areas (arrows). (C) Schizonts in a capillary. Note immature schizonts (a,b), mature schizont (c) and individual merozoites (arrowheads) in the perivascular area.

For immunohistochemical staining, deparaffinized histological sections were reacted with polyclonal rabbit antibodies to *Toxoplasma gondii* and *Neospora caninum* (Lindsay and Dubey, 1989), *S. neurona* (Dubey et al., 1999; Dubey and Hamir, 2000), and *S. cruzi* (Granstrom et al., 1990, 1991) using the previously described procedure (Dubey, 2010). Appropriate controls were used. The *S. cruzi* polyclonal antibodies were prepared by injecting rabbits with saline extract of bradyzoites from an experimentally infected cow; this antibody is not species specific, and even reacts with *T. gondii* (Granstrom et al., 1990, 1991). However, it is known to react with all *Sarcocystis* species that have been tested (Dubey et al., 2016).

3. Results

In both paraffin blocks there was a solitary lesion characterized by intense perivasculitis involving most capillaries in the affected area (Fig. 1A). Schizonts and intracellular and free merozoites were found only in the area with lesion (Fig. 1B, C). Only merozoites, not schizonts, were found in the perivascular infiltrate consisting of mostly mononuclear cells (Fig. 1B). Schizonts were located both in and out of blood vessels (Fig. 1C). Examples of developing stages of schizonts are shown in Fig. 2A–E. Most of these were in neural parenchyma. Fully mature merozoites were seen