Brief 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 month 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 indentified. 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, where 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 x 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 *Sarcocystis cruzi*.

Keywords: Sarcocystosis, Cattle, Meningoencephalitis, Neurons, Schizonts

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., 2015a, b; Gjerde, 2015). Of these *Sarcocystis* species, *S. cruzi* is considered the most pathogenic (Dubey et al., 2015a). Clinical sarcocystosis in cattle has been reported from Canada, USA, England, Norway, Ireland, and Australia (reviewed by Dubey et al., 2015a). 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., 2015b). 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.

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., 2015a).

3. Results

In both paraffin blocks there was a solitary lesion characterized by intense perivascularitis 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 in mononuclear cells (Fig. 2F). Individual merozoites were mostly globular, 2-3.5 µm long, and had a vesicular nucleus (Figs. 1, 2).

Immunohistochemically, organisms stained with antibodies to *S. cruzi*, but not *T. gondii* *S. neurona* and *N. caninum* antibodies (Fig. 2G, H).

Ultrastructurally, schizonts were seen both in capillaries and in extravascular space. Merozoites were often concentrated in adventitial layers of capillaries. Schizonts divided by

endopolygeny, where the nucleus became multi lobed and at the terminal stage nuclear lobes were incorporated into budding merozoites (Fig. 3A). Individual merozoites were seen in astrocytes, oligodendrocytes, leukocytes, and vascular endothelial cells. Occasionally merozoites were present in the nucleus of mononuclear cells (Fig. 3B). Individual merozoites were ovoid, and contained a prominent nucleus, numerous micronemes, a conoid, but no rhoptries (Fig. 3B). They were 3-5 x 2-3 μ m in size.

4. Discussion

As stated earlier there are several outbreaks of clinical sarcocystosis in adult cattle (reviewed in Dubey et al., 2015a). There are few cases of severe meningoencephalitis associated with a *S. cruzi*-like infection in cattle, simulating rabies (Table 1). Data are available from single cases where only brains were studied histologically. In the 2 cases from South Africa (Van der Lugt et al., 1994), the schizonts were much larger in size (Table 1) than reported in other cattle naturally infected with *S. cruzi*. There are at least three generations of *S. cruzi* schizonts in experimentally cattle (Dubey et al., 2015a). The first generation schizonts measure up to 41 μ m in diameter, occur 7-26 days post inoculation (p.i.), and are found predominantly in arteries and arterioles of mesenteric and intestine. The second generation schizonts are approximately half the size of first generation, and capillaries of blood vessels in many organs. A third generation occurs in leukocytes, and detected 19-46 days p.i. (Dubey et al., 2015a). The diagnosis in the case from England (Gunning et al., 2000) was based on immunohistochemical staining, and details are missing. The encephalic case from Canada (Dubey et al., 1987) had a grossly visible area of necrosis in the cerebellum, but testing was limited to histological examination. A similar case found in a steer from Montana, USA (Dubey et al., 2015a) but tissues from the animal are lost (JPD, own observations).

The case of neurological sarcocystosis reported by Takla (1984) and reevaluated here is distinct from all reports of sarcocystosis in cattle. The meningoencephalitis was most severe, lesions were focal, and *Sarcocystis* stages were confined to lesions. There were numerous free merozoites present than ever reported in cattle experimentally or naturally infected with *S. cruzi*. The parasitization was more intense in the adventitial layers of capillaries than in the endothelium. Schizonts were seen frequently extravascularly, and merozoites were seen in many types of host cells, including astrocytes, neurons, and leukocytes. Gliosis, often reported in cases of neural sarcocystosis, was not observed in the present case. Necrosis is not a predominant feature of lesions associated with *S. cruzi* infections, both natural and experimental cases.

The characteristic of lesions observed in the present case simulates *Sarcocystis neurona* associated EPM in horses and other animals (Dubey et al., 2015b). Dr. Takla had sent the paraffin blocks to JPD for differential diagnosis. An immunohistochemical test for EPM was not developed until 1999 (Dubey et al., 1999) and thus the decision was not made until now. There was no reactivity to antibodies to *S. neurona*, *N. caninum*, and *T. gondii*. The parasite reacted with *S. cruzi* antibodies, but as stated earlier this antibody is not species specific. However, staining with *S. cruzi* antibodies were very helpful in recognition of merozoites, particularly in neurons. Merozoites in sections are small (mostly 2-3µm) and difficult to recognize. During the immunohistochemical procedure, merozoites become larger in size and thus are easily recognized. Ultrastructurally, parasites in the present case were identified in several host cells, including neural cells. Most merozoites were globular, and morphologically resembled tachyzoites of *T. gondii* in sections. Epidemiologically, *S. neurona* is not known to occur in Europe. Collectively, data suggest that another *S. cruzi*-like parasite may cause encephalitis in cattle.

Conflict of interest

None.

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Figure Legend

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 meozoites 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.

Fig.2. Extravascular schizonts and meozoites in section of cerebrum of the calf. Note variable thickness of the schizonts wall (arrows). A-F, hematoxylin and eosin stain. G and H, immunohistochemical staining with rabbit polyclonal *Sarcocystis cruzi* antibodies. (A) Immature schizont (arrow) with a faintly stained lobulated nucleus (arrow heads). (B) Elongated immature schizont with more advanced lobulation of nucleus. (C) Immature schizont (arrow) with lobulated nucleus (arrowheads). (D) Immature schizont, presumably in a neuron. Arrow points to the host cell nucleus (E, F). Immature schizont with formation

of merozoites. Arrowheads point to free merozoites. (G) A neuron with 4 merozoites (arrowheads). (H) Schizont with a rosette of merozoites.

Fig.3. Transmission electron micrographs of schizont and merozoites in cerebrum of the calf. (A)

Immature schizont . Arrow points to lobes of nucleus being incorporated in budding

merozoites (arrowheads). (B) Extracellular merozoite (arrowhead) and a meozoite within

the nucleus of a mononuclear cell. (C) A merozoite with a conoid (co), a nucleus (nu), and

numerous micronemes (mn) but no rhopties.

Table 1Neurological sarcocystosis documented in cattle^a

Country	History	Main lesions	Schizonts	References
South Africa	One 2-year beef heifer on pasture died suddenly after convulsions and recumbency.	Necrosis, vasculitis, gliosis	Large sized (52x22 µm) schizonts in arteries and arterioles. No extravascular parasites.	Van der Lugt et al. (1994)
	11 of the 15 calves developed nystagmus and opisthotonus with 5 days. One 2-4 months old calf died and necropsied.	Necrosis, vasculitis, gliosis	Large sized schizonts in arteries and arterioles. No extravascular parasites. Results confirmed by TEM.	
Canada	18 months old Hereford steer on pasture developed ataxia, recumbency and blindness.	1 cm grossly visible malacia in cerebellum. Meningoencephalitis throughout the brain.	Small-sized (20x15 µm) schizonts in capillaries and in unidentified cells. Extracellular 3.0 x 1.5 µm merozoites.	Dubey et al. (1987)
England	Limousin x Friesian heifer developed hind limb ataxia.	Meningoencephalitis in brain and spinal cord.	Protozoa identified <i>Sarcocystis</i> based on immunohistochemistry. No details of the parasite were described.	Gunning et al. (2000)
Germany	One 9-month old calf	Meningoencephalitis	Schizont structure described in detail	Takla (1984); present study

^a Single cases studied histologically.

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