SHORT PAPER

Eosinophilic Myositis due to *Sarcocystis hominis* in a Beef Cow

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Summary

A case of eosinophilic myositis (EM) in an 8-year-old beef cow was investigated. The animal originated from a herd in which a high incidence of the disease had been observed in slaughtered adult females over a period of 2 years. Histologically, the lesions in the muscles were characterized as granulomas with a central core of degenerate eosinophils and remnants of necrotic muscle fibres, surrounded by a rim of epithelioid cells and fibrous tissue with an infiltrate consisting predominantly of eosinophils radiating outwards. Degenerate sarcocysts with a thick (7–9 µm) wall were present in the suppurative centre of most lesions. Intact sarcocysts with similar morphology were present in adjacent muscle fibres but without an associated inflammatory reaction. By transmission electron microscopy the sarcocysts were identified as *Sarcocystis hominis*, based on the morphology of villar protrusions of the sarcocyst wall, which were broad-based and cylindrical, with a blunt distal end, and contained numerous long microfilaments. Circumstantial evidence indicated a human source of infection, human faecal material having been spread on the pasture grazed by the cattle. The findings supported a causal relationship between *S. hominis* infection and EM in cattle.

Eosinophilic myositis (EM) is a relatively rare disease in cattle and sheep, usually discovered at meat inspection after slaughter. The gross lesions of EM in cattle are well-demarcated, green, focal stripes or patches. The histopathological picture is dominated by large numbers of eosinophils (Jubb *et al.*, 1993). There is good evidence that eosinophilic myositis in cattle and sheep is caused by degenerating *Sarcocystis* spp. (Jensen *et al.*, 1986; Granstrom *et al.*, 1989; Gajadhar and Marquardt, 1992). However, sarcocysts are not easily found in the lesions and a diagnosis of muscular sarcocystosis may require multiple samples and serial sectioning of the lesions (Jensen *et al.*, 1986).

The genus *Sarcocystis* consists of cyst-forming coccidia with an obligatory two-host life cycle. Sarcocysts are usually found in cardiac and striated muscles of herbivore intermediate hosts, carnivores serving as definitive hosts. Three species of *Sarcocystis* have been described in cattle: *S. cruzi* with a canine–bovine cycle, *S. hirsuta* with a feline–bovine cycle, and *S. hominis* with a human–bovine cycle (Dubey *et al.*, 1989b). The sarcocysts of *S. cruzi* have a thin (<1 µm) wall with hair-like protrusions. Sarcocysts of *S. hominis* and *S. hirsuta* have thick (2–7 µm) cyst walls with finger-like villar protrusions and are difficult to distinguish from each other by light microscopy (Mehlhorn and Heydorn, 1978; Böttner *et al.*, 1987a,b, Dubey *et al.*, 1989b; Pena *et al.*, 2001). Sarcocystosis is widespread in cattle (Böttner *et al.*, 1987b; Van Knapen *et al.*, 1987; Vercruysse *et al.*, 1989; Pena *et al.*, 2001) but the infection is usually symptomless. EM in cattle is mainly associated with *S. cruzi* (Jensen *et al.*, 1986; Granstrom *et al.*, 1989; Gajadhar and Marquardt, 1992) and only rarely with other *Sarcocystis* species. Jensen *et al.* (1986), in a study of 33 bovine cases.
of EM, tentatively identified S. hominis in two cases. A report of EM in a cow in Finland described thick-walled sarcocysts, but the authors did not distinguish between S. hirsuta and S. hominis (Rimaila-Parnanen and Nikander, 1980). In Canada, Gajadhar et al. (1987) described a case of EM in a beef cow, associated with an unusual but unidentified species of Sarcocystis.

The present report describes cases of EM in a beef cattle herd with a high incidence of the disease in slaughtered older females. This closed herd of 280 beef cattle (including young stock) of the Blonde d’Aquitaine breed was located in the eastern part of the province of Overijssel in The Netherlands. Cows with suckled calves, together with the older groups of female replacement stock, were pastured during the summer months. Animal-driven water pumps in the pasture were used for the supply of drinking water from a bore-hole. The younger replacement animals and the fattening bulls were housed during the whole year. The ration of the fattening bulls consisted of maize-silage with concentrate supplementation. Young stock and adult cattle were fed grass-silage and hay during the winter months. Drinking water in the stable was supplied by an electric pump, which supplied water from a bore-hole with a depth of approximately 7 m. The farm possessed one dog and one cat, both of which had free access to the cattle.

Of the cows culled during the previous 2 years, 20 (ca. 30%), mostly aged >5 years, had shown macroscopic lesions of EM at meat inspection, lesions being disseminated throughout the carcasses. Lesions were not seen in fattened male animals, which were slaughtered at the age of 24–26 months.

Samples of affected muscle from the hindquarters from an 8-year-old slaughtered cow were submitted for pathological examination. The muscles had well-demarcated, oval or elongated greenish lesions, up to 1 cm in length and 2–3 mm in diameter, and often with a central core of suppuration (Fig. 1). The long axis of the lesions paralleled the muscle fibres. Samples containing visible lesions were placed in 10% buffered formalin, processed by routine methods and embedded in paraffin wax. Sections (4 μm) were cut and stained with haematoxylin and eosin (HE) and by the Weigert–van Gieson method. Small portions of formalin-fixed muscle were processed for transmission electron microscopy (TEM) as described previously (Dubey et al., 1988); semithin sections were made and stained with toluidine blue, and thin sections were stained with lead citrate and examined with an electron microscope.

Histologically, the lesions in the muscles had the characteristics of granulomas. They had a central core consisting of degenerate eosinophilic leucocytes and remnants of necrotic muscle fibres, together with phagocytic giant cells. The supplicative core was surrounded by a granulomatous reaction consisting of an inner rim of palisading elongated epithelioid cells and an outer rim of circularly oriented fibrous tissue (Fig. 2). Beyond this outer rim an infiltrate of eosinophils, admixed with smaller numbers of macrophages and lymphocytes, radiated outwards. Degenerate sarcocysts were usually present in the supplicative centre of lesions (Fig. 3). Most sarcocysts, which were cut transversely, were up to 100 μm wide. The longest section of a sarcocyst seen within a granuloma measured 1300 μm. All sarcocysts had a distinct wall, which was radially striated by palisading villar protrusions. The thickness of the sarcocyst wall, including protrusions, varied from 7 to 9 μm.

Numerous sections of adjacent muscle fibres contained intact sarcocysts, with no associated inflammatory
reaction. All sarcocysts observed were thick-walled and by light microscopy appeared to be identical (Fig. 4). Two sarcocysts examined ultrastructurally were also found to be similar. The primary sarcocyst wall consisted of a wavy parasitophorous vacuolar membrane, beneath which was an electron-dense layer 50–100 nm thick (Fig. 5). The primary sarcocyst wall was folded into perpendicular to sloping, finger-like villar protrusions, which had a broad base and were cylindrical with a blunt distal end. They were situated close to each other and were of uniform width. They were 6.8–8.1 μm long and up to 3.0 μm wide at the base, with a core containing numerous microfilaments that extended into the electron-dense ground substance. Fine granules were scattered on the microfilaments. The ground substance appeared 1–2 μm thick, depending on the plane of section (Fig. 5). Only a few metrocysts were present in sarcocysts, being located just below the ground substance (Figs 4 and 5). Bradyzoites, present in packets separated by septa (Fig. 5), were up to 9.0 μm long and up to 2.5 μm wide (Figs 5 and 6). Each possessed a conoid, a few rhoptries, and numerous micronemes located in the conoidal region. Bradyzoites also contained a few dense granules, numerous amyllopectin granules situated centrally, and a terminally located nucleus (Fig. 6).

The presence of degenerate sarcocysts in most EM granulomas examined was highly suggestive of a causal relationship between the parasite and the lesions. A single species of Sarcocystis with a thick radiating sarcocyst wall appeared to be responsible for the granuloma formation. Numerous intact sarcocysts with similar morphology were present within surrounding muscle

Fig. 3. A higher magnification of the S. hominis sarcocyst in Fig. 2. Note the pale staining (degenerate) bradyzoites and finger-like villar protrusions (vp) of the sarcocyst wall, surrounded by degenerate eosinophils and necrotic muscle fibres (m). HE. Bar, 50 μm.

Fig. 4. Cross-section of S. hominis sarcocyst in muscle. Note the finger-like villar protrusions (vp) on the sarcocyst wall (scw) and a thin membrane of ground substance (g) enclosing pale-staining metrocysts (m) at the periphery of the sarcocyst, and numerous dark-staining bradyzoites (b). Section (1 μm) stained with toluidine blue. Bar, 10 μm.

Fig. 5. S. hominis sarcocyst. Note villar protrusions (V) protruding into the host cell (arrows), ground substance layer (G), metrocysts (M), and bradyzoites (B) arranged in compartments demarcated by septa (S). TEM. Bar, 10 μm.
fibres. TEM of the sarcocyst wall would appear to be the best method of distinguishing between the two thick-walled bovine species of *Sarcocystis* (*S. hirsuta* and *S. hominis*). The villar protrusions of *S. hirsuta* sarcocysts have a narrow stalk, are expanded laterally, and are tapered distally (Böttner et al., 1987a; Dubey et al., 1989a, 1990; Odening et al., 1995). The villar protrusions of *S. hominis* have a broad base, are cylindrical, closely spaced, and have a blunt distal end (Mehlhorn et al., 1975; Dubey et al., 1988, 1989a; Odening et al., 1995; Pena et al., 2001). From the ultrastructure of the villar protrusions, it was concluded that the sarcocysts in the present case were those of *S. hominis*. Because we were unable to isolate *Sarcocystis* DNA from paraffin wax blocks, confirmation of the morphological identification of *S. hominis* by DNA analysis (Fischer and Odening, 1998) could not be carried out.

In the present study, circumstantial evidence indicated a human rather than a feline final host, and therefore *S. hominis* rather than *S. hirsuta*. It emerged that the farmer had emptied his neighbour’s cesspit, containing human faecal material, during January or February 2003. The contents had been spread over the pasture on which the cattle had grazed during the following summer. In addition, the bore-hole water used as drinking water for the housed cattle may have been contaminated by leakage from the old cesspit, groundwater being pumped up at a distance of approximately 50 m from the cesspit. The farmer and his family had, until 2000, lived in the house now occupied by their neighbour, where they had frequently enjoyed “filet americain” but had never consumed meat from cattle raised at the farm. The 10-year-old farm cat had never been fed raw steak and therefore was unlikely to have been the source of infection.

The pathogenesis of EM is not clear and EM lesions have never been found in livestock species experimentally infected with *Sarcocystis* species (Dubey et al., 1989b). Moreover, in the light of the high prevalence of *Sarcocystis* spp. infection in cattle (Boch et al., 1978; Böttner et al., 1987b; Van Knapen et al., 1987; Vercruysse et al., 1989), the incidence of EM would seem very low (Imes and Migaki, 1967). Granstrom et al. (1989) speculate that cattle with EM lesions are genetically predisposed to produce IgE in response to *Sarcocystis* bradyzoite antigen, and that EM represents an abnormal response to sarcocyst degeneration, including a host-dependent, *Sarcocystis*-specific, type-I hypersensitivity.

The ingestion of raw beef infected with *S. hominis* may cause gastrointestinal symptoms in man (Rommel and Heydorn, 1972; Aryeetey and Piekarski, 1976; Heydorn, 1977; Hiepe et al., 1979; Pena et al., 2001). Human volunteers ingesting *S. hominis* sarcocysts shed oocysts and sporocysts in their faeces; in contrast, volunteers ingesting *S. hirsuta* sarcocysts did not become ill and did not shed sporocysts (Böttner et al., 1987b; Dubey et al., 1990). Beef with lesions of eosinophilic myositis is usually condemned, although the risk of pathogenic sarcocysts being present is probably not much higher than in beef of normal appearance. However, cattle should not be exposed to human faecal material.

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**References**


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