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SARCOCYSTIS INFECTION AND MYOCARDIAL PATHOLOGICAL CHANGES IN CATTLE FROM SOUTH-EASTERN NORWAY

By

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BRATBERG, BJØRN and THOR LANDSVERK: *Sarcocystis infection and myocardial pathological changes in cattle from south-eastern Norway*. Acta vet. scand. 1980, 21, 395—401. — The incidence of myocardial sarcocystis infection and of myocardial pathological changes was recorded in samples of 79 healthy cattle obtained from an abattoir. The incidence rate of thin-walled cysts of *S. cruzi* was 81.0 %, while mixed infection with thick-walled cysts of *S. hominis* and/or *S. hirsuta* was found in 5.0 %. Focal interstitial myocarditis was found in 31.6 % of the samples. The sarcocystis infection and the interstitial mononuclear cell infiltrates were positively associated ($P < 0.05$). Intimal proliferations of musculo-elastic or fibro-elastic tissues in the intramural coronary arteries were found in 75.0 % of the cattle older than 3½ years of age, and in 45.7 % of the cattle less than 3½ years old. No association of the arterial lesions and the sarcocystis infection was demonstrated.

sarcocystis; myocarditis; arteriosclerosis;
cattle.

Cattle are reported to be intermediate hosts for 3 species of sarcocystis: *S. cruzi* (syn. *S. bovicanis*), *S. hirsuta* (syn. *S. bovis-felis*) and *S. hominis* (syn. *S. bovihominis*) (*Dubey 1976, Mehlhorn & Heydorn 1978, Markus 1978*). One species only, *S. cruzi*, has been reported to be pathogenic for cattle (*Fayer & Johnson 1973*). Spontaneous disease in cattle associated with sarcocystis infection has recently been described from several countries (*Meads 1976, Frelief et al. 1977, 1979, Landsverk 1979*).

Sarcocystis are muscle parasites commonly encountered in Norwegian cattle, but the significance of this infection is not known. The purpose of the investigation was to estimate the

incidence, infection rate and sarcocyst type according to cyst wall criteria in a sample of Norwegian cattle, and to determine a possible covariation with the occurrence of myocardial lesions.

MATERIALS AND METHODS

Specimens of the hearts of 79 apparently healthy cattle were sampled at an abattoir (Fellesslakteriet, Oslo) during the period from December 1978 to February 1979. Forty-four of the cattle were 3½ years of age or older (Group I), while 35 were from 1½ to 3½ years old (Group II). The age estimates were based on incisor eruptions. Five specimens were selected from each heart: 2 from each ventricle and 1 from the papillary muscles of the left ventricle. The specimens were fixed in 10 % neutral buffered formalin, processed routinely, and stained with haematoxylin and eosin (HE), selected sections being stained with elastin van Gieson (EVG). The frequency of sarcocysts was quantitated according to the average number of cysts observed in 5 fields of view in the same section, using a light microscope objective lens X 4. The size and the wall thickness of the sarcocysts were measured with an ocular micrometer. For statistical evaluation the chi-square test was used.

RESULTS

The hearts examined did not have any gross abnormalities. Histological lesions consisted of focal infiltrates of mononuclear cells between myocardial fibres and sometimes in perivascular locations. The cells in the infiltrates were mainly lymphocytes

Figure 1. Mature cyst of *S. cruzi* with thin capsule (arrows). HE × 1450.

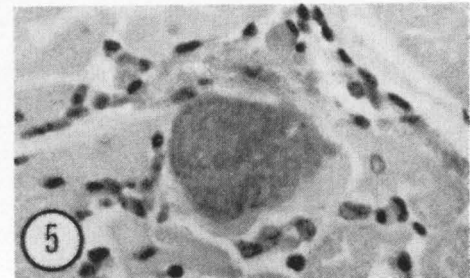
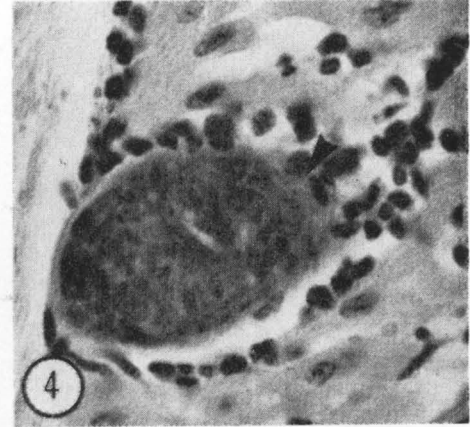
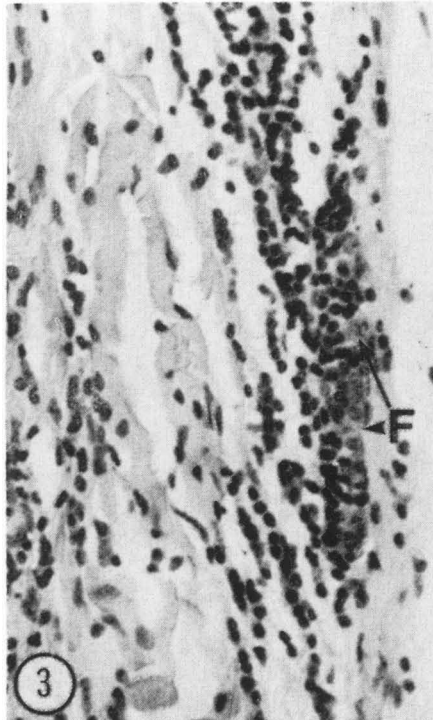
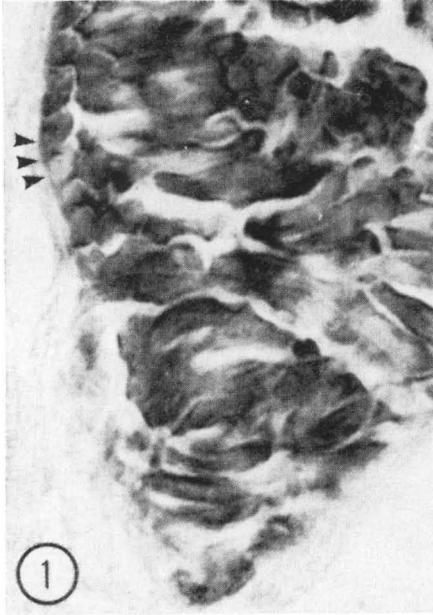
Figure 2. Sarcocyst with thick capsule of *S. hominis* (syn. *S. bovi-hominis*) or *S. hirsuta* (syn. *S. bovifelis*). Prominent radial striations. HE × 1450.

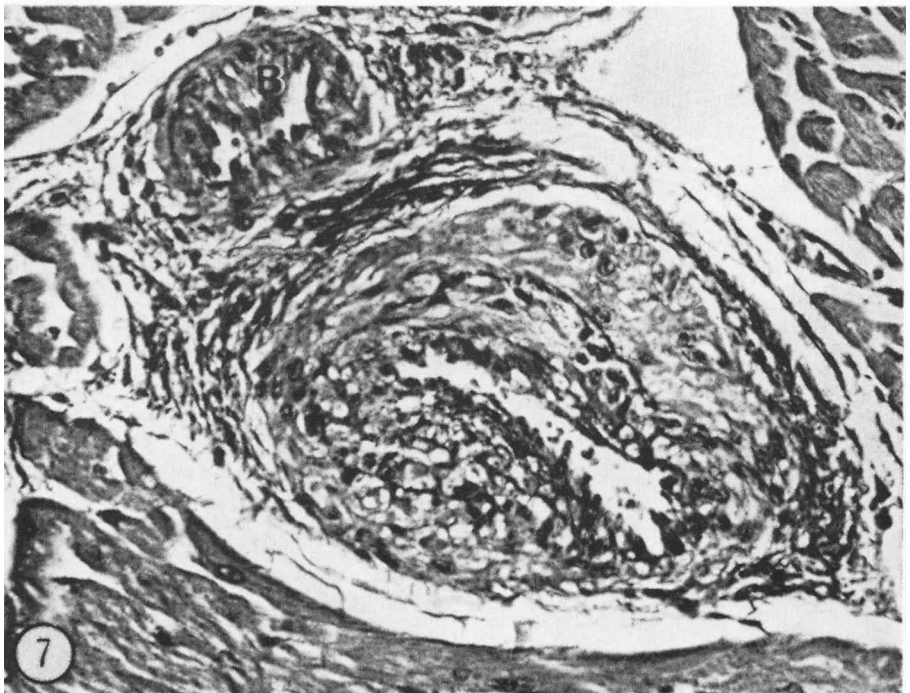
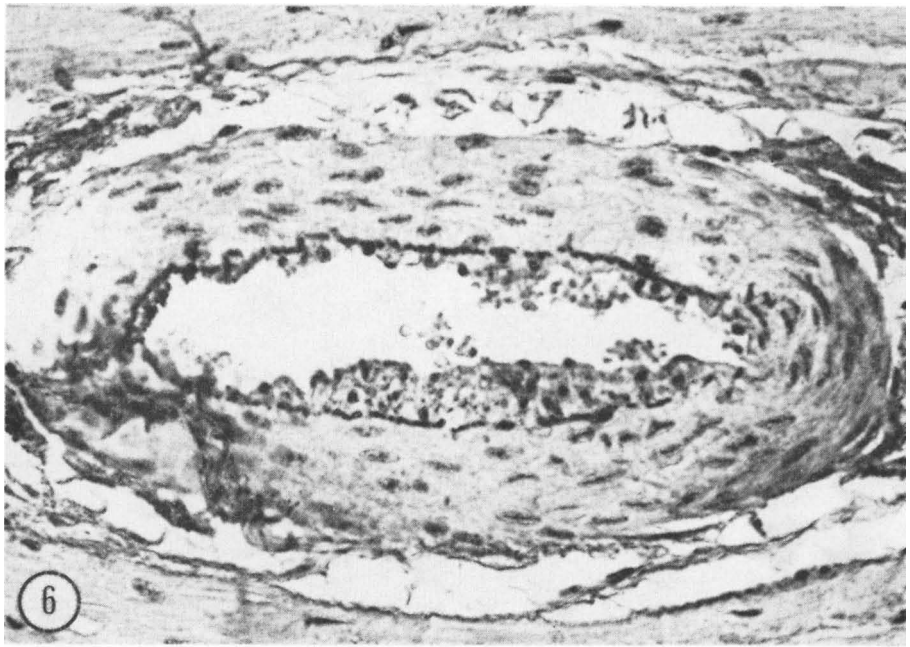
Figure 3. Inflammatory focus. Necrosis of myofibrils and heavy mononuclear cell infiltration. F = a probable nerve fibre showing degeneration. HE × 360.

Figure 4. Thin-walled cyst surrounded by mononuclear cells and a few neutrophils. An area of the cyst wall appears degenerated (arrow). HE × 550.

Figure 5. Degenerated sarcocyst with sparse inflammatory reaction. HE × 550.

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and macrophages. Occasionally neutrophils and a few eosinophils were mixed with the mononuclear cells. Necrosis of myocardial fibres was slight and inconstant (Fig. 3). Interstitial inflammatory cell infiltrates were found in 25 samples (31.6 %). Initial arterial lesions were characterized by intimal thickening and proliferations (Fig. 6). The thickened intima consisted of musculo-elastic and fibro-elastic tissues. There was disruption and splitting of the internal elastic membrane. Thickening and vacuolization of the media were found in some instances (Fig. 7). The intimal thickening caused a reduction of the lumen, but in no instances was complete occlusion observed. Arterial lesions were found in 49 samples (62.0 %) with a significantly higher frequency in Group I (75.0 %) than in Group II (45.7 %) ($P < 0.05$). The arterial lesions were consistently most frequent in the sections from the papillary muscles of the left ventricle ($P < 0.05$). The vascular lesions did not show any association with the occurrence of sarcocystis ($P > 0.05$). Sarcocystis appeared randomly distributed in the sections, usually without inflammatory reaction. Infrequently cysts with degenerated wall structures (Fig. 4) or fully degenerated cysts could be found surrounded by a sparse collection of inflammatory cells (Fig. 5). Sarcocystis species were found in 64 samples (81.0 %). All the samples positive for sarcocystis contained thin-walled cysts of *S. cruzi* (Fig. 1), while mixed infection with thick-walled cysts of *S. hominis* or *S. hirsuta* (Fig. 2) were found in 4 samples (5.0 %). There were no differences between the 2 age groups.

Evaluation of the histological method with respect to the detection of cysts was performed by comparing the first and the 4 consecutive sections. In 44 samples the infection rate score was 1 in the first section, and the infection was confirmed in 43 samples in 1 or more of the 4 consecutive sections. Quantitation of sarcocysts and interstitial infiltrates is presented in

Figure 6. Intramural coronary artery. Intimal proliferation of smooth muscle cells. The internal elastic membrane seems mostly intact. EVG \times 425.

Figure 7. Intramural coronary artery. Thickening of the intima by smooth muscle cells. Disruption and reduplication of the internal elastic membrane. Vacuolization of the intima and the media is prominent. Branch artery (B) showing vacuolization of the media. EVG \times 425.

Table 1. Quantitation of myocardial sarcocystis infection and interstitial infiltrates.

Severity of infection*	Number of samples	Interstitial infiltrates**	
		+	++
0	15	1	0
1	54	12	6
2	8	2	2
3	2	0	2

* Grading of sarcocysts present:

1 Sarcocystis present in sections.

2 1—5 sarcocysts per field of view.

3 6—10 sarcocysts per field of view.

** Grading of interstitial infiltrates:

+ small.

++ large.

Table 1. The sarcocystis infection and the occurrence of inflammatory infiltrates exhibited a positive association ($P < 0.05$). The cyst wall proper of mature thin-walled cysts measured 0.5—1 μm . The cyst wall of thick-walled cysts had distinctive radial striations. The wall proper measured 5—6 μm . Mean cyst size of the thin-walled cysts was 180 μm by 60 μm .

DISCUSSION

The incidence of myocardial sarcocystosis in our material corresponds with the results of various reports, although different techniques have been used (*de Kruijf et al.* 1974, *Boch et al.* 1978). Thin-walled cysts in a German report (*Boch et al.*) were found in 65.5 % of the samples by a digestion technique. The percentage of thick-walled cysts representing *S. hirsuta* and *S. hominis* was found to be 34.5 % and 63.3 %, respectively, as compared to a total of 5.0 % in the present material. The cyst wall criteria used are in accordance with *Mehlhorn & Heydorn* (1978) for thin-walled cysts of *S. cruzi* and the thick-walled cysts of *S. hominis* and *S. hirsuta*.

The results of the histological examination in the present investigation have not been evaluated against the results of digestion methods with respect to the incidence of sarcocysts. Enzymatic digestion of the tissues is claimed to be the most

reliable method (Erber 1977), but histology may also be applied with a considerably degree of repeatability.

The incidence of interstitial myocarditis in the present investigation was unexpectedly high. Except for sarcocysts no parasites were seen in the sections, but other agents capable of provoking interstitial myocarditis can not be excluded. In experimentally induced sarcocystis infection Johnson *et al.* (1975) reported moderate to severe mononuclear cell infiltration in the heart on post-infection days 26 to 54, and in naturally occurring sarcocystosis Frelier *et al.* (1979) found infiltrating macrophages and lymphocytes in the heart.

Landsverk (1979) observed focal mononuclear cell infiltrations and immature and mature sarcocysts in the myocardium in spontaneous sarcocystosis. In our material the sarcocystis infection and the inflammatory infiltrates were positively associated, but this does not necessarily imply a causal relationship or any clinical importance. According to Frelier *et al.* (1979) the number of sarcocysts observed in the tissues had no relationship to the severity of the disease, although, experimentally, clinical symptoms were related to the number of sporocysts ingested.

The nature of the infection and the inflammatory response to the development phases of sarcocysts seem to be not fully elucidated. Unlike the degenerated or degenerating cysts, intact sarcocysts are infrequently surrounded by inflammatory cells, but the inflammatory reaction to the mature sarcocysts might not exclusively be a sequence to cyst wall rupture or degeneration, as described by Jubb & Kennedy (1970).

The vascular lesions observed were in accordance with sclerotic arterial changes described by Ratcliffe & Redfield (1972) in man and Neumann & Klopfer (1974) in cattle. The latter authors found a considerably higher frequency of arteriosclerotic changes in the cow group and a low frequency in calves, based on 2 sections from the big papillary muscle of the left ventricle of each animal. A higher prevalence rate of arteriosclerotic lesions could probably have been found if 2 sections instead of 1 from the papillary muscles in each animal in our material had been used. Further, there is an age-dependent increase in the number of cattle affected with arteriosclerotic changes, but the causes remain obscure. However, vascular parasites like sarcocysts might, during multiplication phases, possibly repre-

sent a contributory or enhancing factor with respect to the observed pathological changes in the intramural coronary arteries. Sarcocystis schizonts have been associated with vascular lesions, in both cattle and sheep (*Leek et al.* 1977, *Landsverk*).

In conclusion, this study shows a high incidence of sarcocysts with *S. cruzi* as the dominant species. The occurrence of focal interstitial myocarditis is stated. Homologous arterial lesions are found in a high frequency, with different levels in the 2 age groups. A correspondence exists between the occurrence of sarcocysts and interstitial myocarditis, and lack of correspondence is found between the sarcocysts and the arteriosclerotic lesions.

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SAMMENDRAG

Sarcocystis-infeksjon og patologiske forandringer i myocard hos storfe fra Sør-Øst Norge.

Insidensen av *sarcocystis*-infeksjon og patologiske forandringer i myocard ble undersøkt i et slaktehusmateriale fra i alt 79 friske storfe. Frekvensen av cyster med tynn vegg som er karakteristisk for *S. cruzi*, ble påvist hos 81,0 %. Blandingsinfeksjon av *S. hominis* og *S. hirsuta* som begge har tykk cystevegg, ble funnet hos 5,0 %. Fokal interstitiell myocarditt ble påvist i 31,6 % av prøvene. *Sarcocystis*-infeksjonen og forekomsten av interstitiell myocarditt var positivt assosiert.

I koronar-arteriene ble det påvist intimafortykkelser som følge av proliferasjon av glatt muskulatur, bindevev og elastiske fibriller. Hos storfe eldre enn 3½ år ble det funnet karforandringer hos 75,0 %, og hos storfe yngre enn 3½ år hos 45,7 %. Det syntes ikke å være sammenheng mellom *sarcocystis*-infeksjonen og forekomsten av karforandringer.

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