Chronic *Sarcocystis* Infections in Slaughtered Cattle

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ABSTRACT. Parasitological, histopathological and immunohistochemical examinations were carried out on three slaughtered cattle with many nodules in all the striated muscles. At necropsy, many yellowish green rice-grain sized nodules including cheesy contents were observed in all the striated muscles. Histopathologically the nodules were granuloma principally consisting of eosinophiles. No *Sarcocystis* cysts nor bradyzoites were found in the nodules, but intact sarcocysts were found in the normal tissues surrounding the nodules. The central necrotic focus of nodules showed intense positive responses against anti-*Sarcocystis cruzi* rabbit serum by immunohistochemical examination. From the above findings the slaughtered cattle were diagnosed as chronic sarcocystiasis.—KEY WORDS: cattle, diagnosis, pathology, *Sarcocystis*.

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The pathogenicity of *Sarcocystis* to livestock differs due to the developmental stage of the parasite. *Sarcocystis* tachyzoites develop acute infection, whereas bradyzoites cause chronic infection [20].

The pathogenesis of the acute infection has been shown experimentally by inoculation of sporocysts to cattle and pigs [2, 7–9, 24, 29] and the clinical acute infections have been also reported [4–6, 10, 11, 14, 21]. The pathogenesis of the chronic infection has been considered to be due to degenerated cysts in the muscle, but the factors correlated to the pathogenesis remain unknown.

In Japan no chronic *Sarcocystis* infections have been confirmed in slaughtered cattle although the acute infection has been produced experimentally. The present study shows three cases of chronic sarcocystiasis of slaughtered cattle first diagnosed in Japan by the immunohistochemical methods.

MATERIALS AND METHODS

Materials: Muscle blocks were obtained from three 5 year old dairy cattle with many yellowish green nodules in all the striated muscles, slaughtered at a few abattoirs in Saitama Prefecture from April, 1984 to March, 1992. Anti-*Sarcocystis cruzi* and anti-*Toxoplasma gondii* rabbit sera were primarily used for the immunohistochemical examination.

Parasitological method: A total of 13 muscle blocks, 2 × 5 × 0.5 cm in size, with many nodules were dissected out of the heart, diaphragm, tongue and masseter of slaughtered cattle and examined for sarcocysts under a binocular dissecting microscope [28].

Pathological method: Muscle blocks were fixed in 10% formalin, sectioned and stained with hematoxylin and eosin by the routine method.

Immunohistochemical method (Immunoperoxidase technique): Muscle blocks were fixed in 10% formalin and sectioned by the routine method. The subsequent technical process was carried out following Haritani's [15].

S. cruzi antigen was detected with the ABC (avidin-biotin peroxidase complex) Elite Kit (Vestastain, U.S.A.). Briefly, histological sections on slide glasses were deparaffinized, passed through a descending ethanol series and distilled water, and dried in air. After immersed in 50% ethanol for 10 sec and then in 3% H_2O_2 methanol for 30 min, sections were rinsed in phosphate buffered saline (PBS) for 5 min, maintained in 0.1% pronase PBS at 37°C for 30 min and then rinsed again in PBS three times for 5 min each. Sections on slides were coated with normal serum in a moist chamber for 20 min. After the serum was removed, the primary serum was put on the sections, which were allowed to stand for 30 min and then rinsed with PBS three times for 5 min each. The biotinized secondary antibody was then layered on sections for 30 min, and thereafter sections were rinsed with PBS as described above. Subsequently, ABC solution was put on sections for 30 min and then rinsed with PBS. Thereafter, sections were stained with peroxidase diaminobenzidine, and rinsed with running water for 5 min and further slightly with distilled water. Sections were then stained with methyl green for the nucleus, dehydrated in 95% and absolute ethanol, and mounted. The preparations were examined for *S. cruzi* antigen under a light microscope.

RESULTS

Macroscopical findings: All the striated muscles such as the heart, diaphragm, tongue and masseter contained rice-grain sized, yellowish green nodules which were fairly hard and had viscous cheesy contents (Figs. 1 & 2). No marked pathological changes were seen in other organs.

Parasitological findings: No cysts nor bradyzoites of *S. cruzi* were seen in any of the nodules whereas in the other normal area of muscle than the nodules were found intact *S. cruzi* cysts with the thin wall (Fig. 3).

Histopathological findings: Nodules were granulomatous and infiltrated with eosinophiles. The central necrotic focus of nodules was surrounded by the epithelial cells,

Fig. 1. Cardiac muscle with yellowish green, rice-grain sized nodules.

Fig. 2. Masseter with the same nodules as shown in Fig. 1.

Fig. 3. Intact *S. cruzi* cyst in cardiac muscle. HE stain, × 400.

Fig. 4. Histopathological findings of a nodule in cardiac muscle. HE stain, × 40.

multinuclear giant cells, lymphocytes and eosinophiles, and further the connective tissue multiplied around the nodules (Fig. 4). In the central part of granuloma cyst-like structures were occasionally observed (Fig. 5).

Immunohistochemical findings: Cyst-like structures and the tissues surrounding them showed intense positive response to anti-*S. cruzi* rabbit serum by ABC immunological staining technique (Figs. 6 & 7). When anti-*Toxoplasma gondii* rabbit serum as the primary serum or no primary serum was applied, no positive response was observed (Fig. 8).

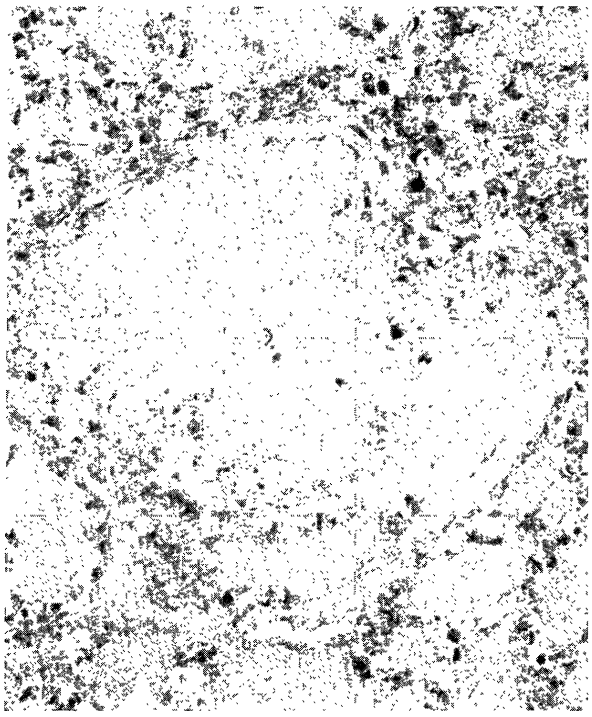

Fig. 5. Cyst-like structure in a necrotic focus of nodule in cardiac muscle. HE stain, × 400.

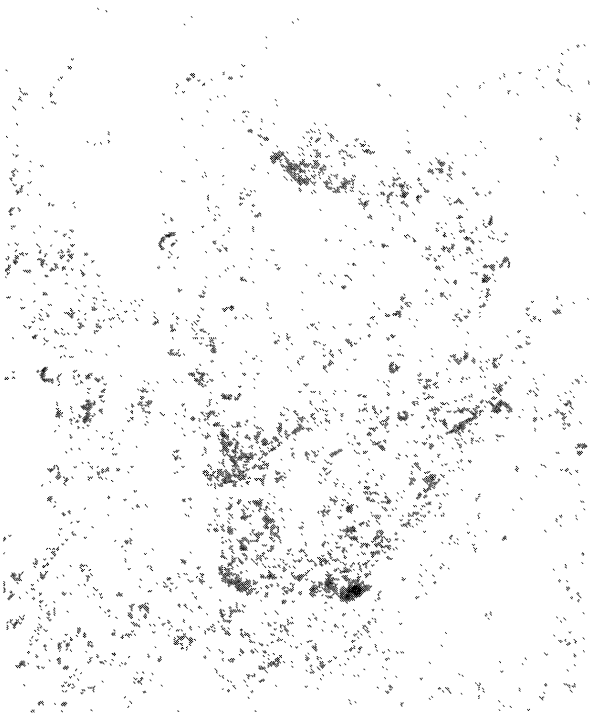

Fig. 7. Necrotic focus of a nodule showing intense positive response to anti-*S. cruzi* rabbit serum (ABC technique). × 400.

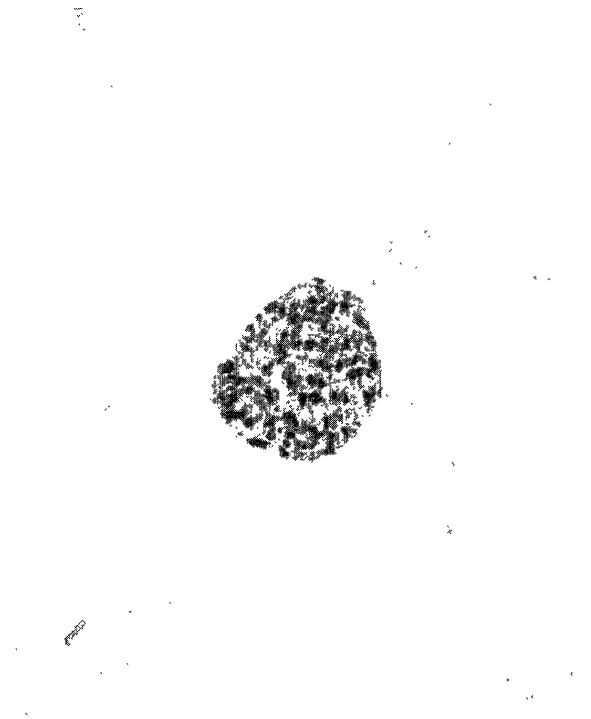

Fig. 6. Intense positive response of a live *S. cruzi* cyst to anti-*S. cruzi* rabbit serum (ABC technique). × 400.

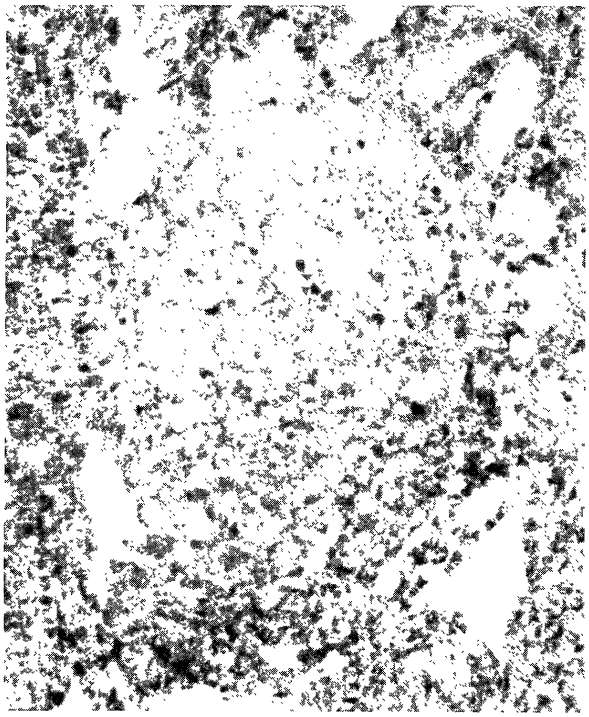

Fig. 8. Immunological staining of a necrotic focus without applying primary serum (ABC technique). × 400.

DISCUSSION

Sarcocystis infections of the intermediate host such as cattle and pigs can be divided into the acute and the chronic diseases. The pathogenesis of the acute infection has been experimentally confirmed [2, 7–9, 18, 23, 29–31] and spontaneous acute *Sarcocystis* infections have been also reported [4–6, 10, 11, 14, 21].

No clinical chronic infections of cattle have been reported in Japan and, in other countries also, a few studies have been made of the chronic infection [1, 3, 12, 17, 24, 25].

Pathogenesis of the chronic infection seems to be correlated with the destruction of degenerated cysts in the muscle although it has not been confirmed experimentally because it is difficult and takes a long period of time to develop the clinical disease by experimental inoculation. This was also shown in the present study by the fact that all the cows with chronic sarcocystosis were five years old.

Although degenerated cysts have been considered to produce the chronic diseases in host, all the cows examined in the present study were parasitized by live *Sarcocystis* cysts together with parasitic nodules which were immunohistopathologically identified to be induced by degenerated cysts. This would show that in the field, small numbers of *Sarcocystis* sporocysts will be successively ingested by host animals and successfully developed to mature cysts in the muscle. This is suggested by the facts that *Sarcocystis* infection induces immunity of intermediate hosts against challenge infection [31], and that the immunity is generally concomitant and IgM level induced by the infection is lowered until sarcocysts mature [13]. Consequently early developed cysts will die and induce nodule formation.

The correlation between *Sarcocystis* infection and eosinophilic myelitis has been suggested in some reports [1, 3, 17, 26, 27]. In the United States, 5% of the slaughtered cattle condemned as eosinophilic myelitis was caused by *Sarcocystis* infection [17], but in Japan the correlation between both clinical diseases has been denied [19, 24, 25].

The present study confirmed the incidence of chronic *Sarcocystis* infection in slaughtered cattle by the immunohistochemical and the routine histopathological methods, and both methods were useful for the diagnosis of chronic *Sarcocystis* infections in slaughtered cattle. In Japan the prevalence of *Sarcocystis* infections is around 100% in slaughtered cattle more than five years old [16, 22, 28], so chronic *Sarcocystis* infections detected in slaughtered cattle will progressively increase in frequency by applying the immunodiagnostic methods.

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