NOTE

Caseous Granulomas in Bovine Paratuberculosis

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ABSTRACT. Caseous granulomas were found in the mesenteric lymph nodes of an Aberdeen-Angus cow, 5 years old, having paratuberculosis. They were found together with epithelioid cell granulomas, and both of them contained numerous acid-fast bacilli. The intestine showed extensive paratuberculous lesions with numerous acid-fast bacilli. There was no caseation but focal neutrophil infiltration in the intestinal granulomas. Mycobacterium paratuberculosis was isolated from both the mesenteric lymph nodes and the intestine. No tuberculous bacilli were detected, and tuberculin and Johnin tests were negative.—KEY WORDS: cattle, paratuberculosis,

Paratuberculosis is a very important mycobacterial granulomatous disease in domestic and wild ruminants [1, 3]. The disease showed some variation in pathology, while the typing of the disease has not been well defined as in leprosy [2, 12]. It is generally considered that bovine paratuberculosis granulomas are not associated with caseous necrosis [2, 3, 11, 12], which is commonly observed in tuberculosis [5]; however, in other ruminants including domestic sheep [6, 10], goat [4, 7, 9] and wild ruminants [3, 14], caseous granulomas of various degrees have been observed.

We recently encountered a case of spontaneous bovine paratuberculosis with caseous granulomas. This brief report describes the histopathological characteristics of the lesion and their distinction from bovine tuberculous lesions.

The affected cow was a female Aberdeen-Angus breed, 5 years old, which was imported from Canada at one year old. Clinically the cow showed diarrhea in January, 1981, and thus was kept isolated, after clinical diagnosis of amyloidosis by a veterinary practitioner. The cow was treated with anti-biotics, a cardiac, astringent and nutrient, but the clinical condition was not improved. Complement fixation tests for paratuberculosis were carried out four times during the period from March to June, 1980, and all testes were positive at 1:40. In June, short acid-fast bacilli were detected in clumps in the Ziehl-Neelsen stained fecal smears, and a final diagnosis of paratuberculosis was made. The cow was killed immediately according to the law.

At necropsy, the cow showed roughcoated appearance and marked emaciation. The duodenum and the upper part of the jejunum were mildly edematous. The intestinal mucosa from the middle portion of the jejunum to the cecum was markedly thickened and corrugated. The colonic and rectal mucosa showed edema and petechia. The mesenteric lymph nodes was enlarged. The cut surface of some nodes showed many caseous necrotic foci of various sizes. The inguinal lymph nodes also showed caseous necrotic changes.

For histopathological examinations the intestine, mesenteric, inguinal and cervical lymph nodes, tonsil and other main organs were collected. The tissue blocks were fixed
in 10% neutral buffered formalin, and embedded in paraffin and the sections were stained with hematoxylin and eosin (H-E) and Ziehl-Neelsen.

Histopathologically, the mesenteric lymph nodes showed very intensive granulomatous changes. Epithelioid cell granulomas were distributed mainly in the peripheral sinuses and the paracortical area. In addition, caseous granulomas (tuberculoid granulomas) having a central necrotic area and surrounded by an epithelioid cell-macrophage layer were observed (Fig. 1). The epithelioid cells and macrophages were irregular in shape and size and frequently showed fibroblastic appearance. The peripheral connective tissue layer was not prominent. Numerous short acid-fast bacilli were demonstrated in the epithelioid cell granulomas and caseous ones. Many short acid-fast bacilli were present also in the central necrotic area (Fig. 2). A considerable number of plasma cells were found in the medullary sinus.

Epithelioid cell and caseous granulomas were found in the inguinal lymph nodes. The cervical lymph nodes contained epithelioid cell granulomas. A large number of epithelioid cell granulomas extended into the lamina propria mucosae and tunica submucosa of the jejunum, ileum (Fig. 3), cecum and colon. The structure of the crypt glands was distorted by intensive granuloma formation (Figs. 3 and 4). Focal infiltration with neutrophils was infrequently found in the intestinal granulomas (Figs. 3 and 4). Numerous acid-fast bacilli were demonstrated in the granulomas but not in the focal infiltration with neutrophils (Fig. 5).

Microgranulomas consisting of some epithelioid cells and surrounding lymphoid cells were found in the hepatic lobules (Fig. 6).

Mycobacterium paratuberculosis (M. paratuberculosis) was isolated from the intestine and mesenteric lymph node with Herrold’s medium, but no other mycobacteria were isolated with 10% Ogawa’s medium.
It is generally recognized that in bovine paratuberculosis, caseous necrosis, mineralization and ulceration as in tuberculosis are always absent [2, 3, 10, 11]. This characteristic is helpful in distinguishing paratuberculosis from tuberculosis in cattle [2, 3]. In contrast, the necrotic changes commonly associated with paratuberculous granulomas are found in domestic goats [4, 7, 9], sheep [6, 10] and wild ruminants [3, 14].

Nevertheless, in the present study we regarded the caseous granulomas in the mesenteric and inguinal lymph nodes as lesions of paratuberculosis because of the presence of numerous short acid-fast bacilli indicating _M. paratuberculosis_, in the granulomas and even in the central caseation area, and the isolation of _M. paratuberculosis_ from the nodes and intestine. Recently we reported an unusual case of dual infection with tuberculosis and paratuberculosis in cattle [7]. In dually infected mesenteric lymph nodes, in contrast to the present case, the tuberculous granulomas contained a few or no acid-fast bacilli. The possibility of the association with tuberculous infection in the present case may be ruled out by the negative result of the careful bacteriological examination for tuberculous bacilli in the mesenteric lymph nodes and intestine and the negative results in tuberculin tests. Isolation of bacteria of other types was not done; therefore, we could not exclude the association of the granulomas with other bacterial species than mycobacteria in the lymph node lesions. The histopathological findings, such as predominance of monocellular phagocytes, minimum granulocytic infiltration and the absence of sulfur granules or grains in the granulomas, however, strongly suggest the mycobacterial origin of the granulomas.

It is well known that the focal neutrophil infiltration in epithelioid cell granulomas is an early changes of the caseation process of tuberculous granulomas [5]. Therefore, neu-
trophil infiltration in the intestinal granulomas was considered to be related to the caseous changes in mesenteric nodes.

In various kinds of granulomas, development of delayed-type hypersensitivity (DTH) to the intruder produces necrosis [1]. In the present case, however, the Johnin test, which indicates DTH against *M. paratuberculosis*, gave a negative reaction. In the course of infection with *M. paratuberculosis*, the animal may undergo various stages; cell-mediated response, humoral response and anergy [3]. We suspected that the caseous necrosis and focal neutrophil infiltration in the present case were produced in the early stage when the cell-mediated immune response was strongly generated. The cattle might have then become anergic by the time of the test.

There are many unclear points concerning the pathogenesis and immunological states of paratuberculosis [2]. One clear point, however, is that caseous granulomas are rarely produced in bovine paratuberculosis.

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REFERENCES
要約

乾酪性肉芽腫を伴った牛ヨーネ病の1例（短報）：百済英一・吉野知男（農林水産省家畜衛生試験場，北海道支場）—典型的腸管変変を有したヨーネ病感染牛の腸間膜リンパ節に、定型的な類上皮細胞肉芽腫とともに結核に類似した乾酪性肉芽腫が多数認められた。多数の抗酸菌がすべての肉芽腫内に認められた。ヨーネ菌が腸管および腸間膜リンパ節から分離されたが、結核菌は分離されなかった。ヨーネ病CF抗体価に1:40、ヨーネン反応およびツベルクリン反応は陰性であった。