Pathological Changes of Spontaneous Dual Infection of Tuberculosis and Paratuberculosis in Beef Cattle

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(Received 19 December 1983/Accepted 7 June 1984)

ABSTRACT. Four cases of spontaneous dual infection of tuberculosis and paratuberculosis in beef cattle, first noticed in Japan, were examined pathologically. All of the cattle were from the same limited area of Hokkaido island. Tuberculous lesions were found in 2 to 9 organs, including the liver, lung, kidney and mesenteric lymph nodes, and granulomas showed the same appearance as in the case of single infection. Paratuberculous lesions characterized by intracellular short acid-fast bacilli were found in the jejunum, ileum, cecum and the draining lymph nodes, but they were not severe. The mesenteric lymph nodes revealed both tuberculous and paratuberculous lesions, and in 2 of the 4 cases both types of granulomas were present in the same sections. In such sections, short acid-fast bacilli were not numerous in granulomas adjacent to tuberculous lesions.—Key words: cattle, mixed infection, paratuberculosis, tuberculosis.

INTRODUCTION

Bovine tuberculosis and paratuberculosis have been studied for a long time; however, they continue to be an important infection causing chronic granulomatous disease in cattle [4, 5, 8, 13, 14]. There still remain many unclarified points concerning the pathogenesis of granulomatous disease [2, 11].

Mixed mycobacterial infection and mixed infection with mycobacteria and other organisms revealed many different immunological and pathological characteristics from those in a single infection [2, 19, 21]. In the cases of Mycobacterium bovis (M. bovis) and Mycobacterium paratuberculosis (M. paratuberculosis), example of the spontaneous dual infection [1, 10, 15, 16] or the cross protective of the two organisms [9, 15] were reported. However, sufficient information on the histopathology of the dual infection is not yet available.

Recently an epidemic of both bovine tuberculosis and paratuberculosis broke out in an area of Hokkaido. Pathological examination of the infected cattle revealed an unusual dual infection with two types of typical tuberculous and paratuberculous granulomas.

The present paper described the macroscopic and histopathologic characteristics of the spontaneous dual infection in beef cattle.

MATERIALS AND METHODS

Animals: Four female Japanese Brown breed cattle of 2 to 11 years old, which were bred in the same area of Hokkaido where the simultaneous epidemic of tuberculosis and paratuberculosis broke out, were used. Numbers 3319 showed diarrhea and mild to moderate emaciation with a rough-coated appearance. Numbers 3385 and 3412 showed a slight rough-coated appearance and emaciation but no diarrhea. The former were diagnosed as having paratuberculosis and the latter as having tuberculosis according to clinical and immunological results, and they were killed as decreed by the law. The clinical and immunological data of the above-mentioned
cattle are summarized in Table 1. Isolation of \textit{M. bovis} and \textit{M. paratuberculosis} were routinely carried out with 1\% Ogawa’s medium and Herrold’s medium, respectively, and the results are shown in Table 2.

\textbf{Pathological examination:} After macroscopic examination, the involved organs and main organs were fixed in 10\% buffered formalin. Paraffin sections were prepared after decalcification when necessary and stained with hematoxylin and eosin (H-E) and Ziehl-Neelsen.

**RESULTS**

\textbf{Macroscopy:} Macroscopic examination of the 4 cattle revealed intestinal lesions showing various degrees of thickening of the wall, suggesting paratuberculosis and single or multiple focal caseous nodules suggesting tuberculosis, in the various organs. Distribution of the paratuberculous lesions was from jejunum to cecum in 2 cattle and from ileum to cecum in other 2 cattle. Besides lung, liver and kidney, tuberculous lesions were observed in the lymph nodes draining the lungs and those of the liver and kidneys having tuberculous lesions, and in the retropharyngeal lymph nodes as well as the mesenteric lymph nodes. Pearly nodules were found in the parietal and diaphragmatic pleura of No. 3320.

\textbf{Microscopy:} Histopathologic findings are
summarized in Table 2. In 2 cattle, both types lesions were found in the same mesenteric lymph node. In cattle No. 3319, numerous epithelioid cell granulomas and mature caseous granulomas (tuberculous granulomas) were detected (Fig. 1). The caseous granulomas had peripheral fibrotic tissues of various thickness, and a few acid-fast bacilli (suggesting *M. bovis*) were detected among the lesions. Calcification was also associated in the caseous granulomas. Epithelioid cell granulomas containing short acid-fast bacilli (suggesting *M. paratuberculosis*) were mainly found in the medullary cords and at the periphery of the lymphoid follicles. Occasionally, the follicles were replaced by such epithelioid cell granulomas. Epithelioid cell granulomas located close to the caseous granulomas tended to contain few or no short acid-fast bacilli. Some of the granulomas without the short acid-fast bacilli were infiltrated with neutrophils (Fig. 2). In the lymph nodes bearing the granulomas, plasma cells were the predominant cell type in the medullary cords.

The mesenteric lymph nodes of No. 3320 also showed mixed granulomatous changes similar to those of No. 3319 (Fig. 3 and 4).

In Nos. 3385 and 3412, some of the mesenteric lymph nodes contained only epithelioid cell granulomas with short acid-fast bacilli; these epithelioid cell granulomas were located in the medullary and peripheral sinuses of the nodes (Fig. 5). Some of the other mesenteric lymph nodes of Nos. 3385 and 3412 were almost completely replaced by the caseous granulomas. The liver of 4 cases revealed some microgranulomas consisting of macrophages, lymphocytes and plasma cells, but no acid-fast bacilli were demonstrated. Besides these microgranulomas, caseous granulomas were found in the liver of No.
3320. In No. 3320, densely disseminated severe caseous granulomas were found throughout the lungs. Acid-fast bacilli were found in the caseous necrotic area of the granulomas. The pearly nodules in the pleura had thick capsules, but no acid-fast bacilli were found. The lungs of Nos.3385 and 3412 also contained caseous granulomas; however, they were not severe.

In the intestinal lesions of 4 animals, the ileum showed the most prominent lesions (Figs. 6 and 7); the lesions in the mucosal lamina propria consisted of small epithelioid cell granulomas with occasional giant cells and diffusely accumulated macrophages and epithelioid cells. Generally, the granulomas
were small to moderate in size, and mononuclear phagocyte system (MPS) cells in this area contained a few to many short acid-fast bacilli.

DISCUSSION

Pathological findings of the dual infection of tuberculosis and paratuberculosis among beef cattle are reported. Up to now, there has no detailed report on the histopathology of such dual infection [1, 10, 15, 16]. This is the first incident of such a case in Japan.

In the present cases, typical tuberculous lesions were found in 2 to 9 organs, and *M. bovis* was isolated from some of the lesions. The paratuberculous lesions were distributed from the jejunum to the cecum and in the mesenteric lymph nodes. *M. paratuberculosis* was isolated from 2 cattle examined. Hepatic microgranulomas, which were judged to be paratuberculous lesions [4], also occurred in all the cases. Therefore, the present 4 cases were considered to represent dual infection by the macroscopic and histopathologic evidence of the two types of mycobacterial granulomas; although, bacteriological confirmation had been carried out not in all the cases.

Tuberculous and paratuberculous granulomas were found in the mesenteric lymph nodes of 4 cases. The findings of mesenteric lymph nodes of Nos. 3319 and 3320 were of particular interest. Both types of granulomas were in the same sections of the nodes: one type was of typical tuberculosis with central caseous necrosis, and the other type was epithelioid cell granulomas carrying short acid-fast bacilli. Although in some domestic and wild ruminants other than cattle *M. paratuberculosis* frequently produces caseous granulomas [12, 17, 20], the present caseous granulomas were considered to be tuberculous ones, since short acid-fast bacilli (*M. paratuberculosis*) were not detected in the necrotic area and in peripheral epithelioid cell layer of the granulomas. Epithelioid cell granulomas which were adjacent to the caseous granulomas tended to contain few or no acid-fast bacilli implicating suppression of growth inhibition of *M. paratuberculosis* might have occurred in this area, some of the epithelioid cell granulomas containing no acid-fast bacilli may be due to *M. bovis*.

M’Fadyean and Sheather [15] reported that
vaccination with *M. bovis* and *M. paratuberculosis* offered some protection against paratuberculosis; the present findings may somewhat agree with their results. Conversely, the effect of *M. paratuberculosis* infection against tuberculosis was studied by Doyle [9], and his results showed that Johne's disease live vaccine gave appreciable protection against challenge with *M. bovis* in goats. In the present dual infected cases there seems to be no apparent evidence of suppression or modification of the tuberculous lesions.

The different results in the spontaneous and experimental dual infection mentioned above may be explained by the interesting nature of mycobacteria. *M. bovis*, BCG, is generally used as a vaccine against tuberculosis and as an immunostimulator for tumors and various infections, but various kinds of suppressive effects on immunological phenomenon have also been reported experimentally [2, 18, 19, 21] in the mycobacterial infections.

The authors expected at first there might be a modification of tuberculous or paratuberculous lesions; such as disseminated mycobacterial histiocytosis showing numerous tubercle bacilli in the granulomas [6, 14] or caseation in the paratuberculous granulomas. However, no such apparent modification were observed in the present dual infection cases. There are many unclarified points concerning the mechanisms of granulomatous infection [3, 7, 11]. Continuing investigation of experimental and spontaneous granulomatous disease are required to follow-up to the present results.

ACKNOWLEDGEMENTS. The authors wish to thank Drs. Y. Namikoshi, Y. Ohmi, N. Manabe, K. Honma and K. Hirose of The Regional Livestock Hygiene Service Centers of Hokkaido for supplying the materials and providing help in the bacteriological examination. In addition, they are grateful to Drs. H. Yugi and Y. Yokomizo of National Institute of Animal Health for helpful advice and Mr. Ishikawa of Hokkaido Branch Laboratory, National Institute of Animal Health for technical assistance.

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要　約
肉牛における結核症とユーネ病の混合感染の病理学的研究：高橋亜一、吉野知男（家畜衛生試験場北海道支場）—わが国で初めて認められた牛の結核症とユーネ病の混合感染症例4例について病理組織学的検査を行った。結核病変は肝臓、腎臓、肺、腸間膜リンパ節など、症例によって2ないし9臓器に認められた。結核症の単独感染病変との差は認められなかった。ユーネ病病変は腸管と腸間膜リンパ節等に認められが、肉芽腫の拡がりは軽度ないし中等度で、病変内の小型抗酸菌の数は一般に少ないし中等量であった。2例の腸間膜リンパ節には結核およびユーネ病の2種の肉芽腫が混在していた。免疫学的、病理学的背景に大きな差を有する結核菌とユーネ菌が同一組織内に各々の病変を形成した所見は、両肉芽腫症例の病理発生を考える上できわめて興味深い。本例の病変と単独感染例の病変との間に著しい差異は認められなかった。