NOTE *Pathology*

Spontaneous Tyzzer's Disease with the Central Nerve Involvement in a Newborn Common Marmoset

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ABSTRACT. A new-born (8-day-old) male marmoset (*Callithrix jacchus*) was found dead in a zoo. The littermate and parents had no clinical abnormalities. By gross observations at necropsy, there were moderate to severe multiple necrotic foci in the liver and heart. Histopathological examinations also revealed mild focal necrosis with neutrophilic infiltration in the cerebral cortex. By Giemsa stained sections, intracytoplasmic bundles of large bacilli were observed in the hepatocytes, intestinal epithelial cells, cardiac myocytes and neuronal cells around the necrotic lesions. Immunohistochemically, these bacilli were intensely positive for rabbit sera against *Clostridium piliforme*, RT and MSK strains. Although Tyzzer's disease has been rarely reported in primates, the central nervous system (CNS) lesions by *Clostridium piliforme* infections are very unusual.

KEY WORDS: *Clostridium piliforme*, encephalitis, marmoset, myocarditis, Tyzzer's disease.

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Tyzzer's disease is caused by the infection of *Clostridium piliforme* (*C. piliforme*) and has been reported in various animal species including laboratory, domestic, zoo and wild animals [1, 2, 4–15]. Spontaneous Tyzzer's disease in primates has been rarely reported [11], including common marmoset (*Callithrix jacchus*). The disease is pathologically characterized by severe multifocal liver necrosis, enteritis and occasional myocarditis [1, 2, 5, 6, 8–14]. Diagnoses for the disease are usually based on the detection of characteristic intracytoplasmic bacilli at the margin of the necrotic lesions by histopathological examinations [1, 2, 4–14]. In addition, immunohistochemical analysis using specific antibodies for *C. piliforme* [5, 6, 9] and polymerase chain reaction assay [2, 3, 5–7, 9] are useful diagnostic tools for the disease.

The bacilli have the ability to infect various organs including nervous tissues, while the central nervous system (CNS) involvement is very rare in Tyzzer's disease, except for experimental direct injections to the CNS [10]. Spontaneous encephalitis of Tyzzer's disease has been only recognized in the gerbils [15], and recently, Mete *et al.* [7] also reported spontaneous encephalitis by *C. piliforme* in a weaver bird. Here, we present the pathological features of spontaneous Tyzzer's disease with the CNS lesion in a newborn common marmoset.

Among two common marmosets born in the Akita Omoriyama Zoo, one male marmoset was found dead at 8 days after birth. The littermate and parents showed no clinical abnormalities. A complete necropsy was performed on the same day. Grossly, the liver was swollen, and there were multifocal necrotic foci with hemorrhage (Fig. 1). In the heart, there were similar necrotic foci with petechial hemorrhage. Similar petechial hemorrhage was also found in the lungs, kidneys and occipital subcutaneous regions. There were no gross lesions in the brain. Tissue samples from the brain, lungs, heart, liver, spleen, kidneys, pancreas, adrenal glands, thymus, stomach, intestines and mesenteric lymph nodes were fixed in 10% formalin. The formalin fixed tissues were embedded in paraffin after dehydration, and paraffin sections of 4 to 6 μm thick were stained with hematoxylin and eosin (HE). Some selected sections were also stained with Giemsa and Gram stains. Immunohistochemistry using rabbit antiserum against *C. piliforme* RT and MSK strains was also performed as described previously [5, 6, 9]. These rabbit antisera for *C. piliforme* have been produced by Dr Seiji Kawamura (Department of Experimental Animals, Faculty of Agriculture, the University of Tokyo).

Histological examinations revealed marked necrotic and inflammatory lesions in the liver, heart and brain. In the liver, there were severe multiple necroses with moderate infiltration of neutrophils, macrophages and a few lymphocytes (Fig. 2). Moderate periportal infiltration of lymphocytes, plasma cells and macrophages was also observed. There were vacuolar hepatocytes around the necrotic foci, and some of these degenerating hepatocytes contained intracytoplasmic bundles of large bacilli (Fig. 3). These bacilli were positively stained by Giemsa (Fig. 4), but those were negative for Gram stain. Immunohistochemically, these bacilli were intensely positive for *C. piliforme* (Fig. 5). In the submucosa

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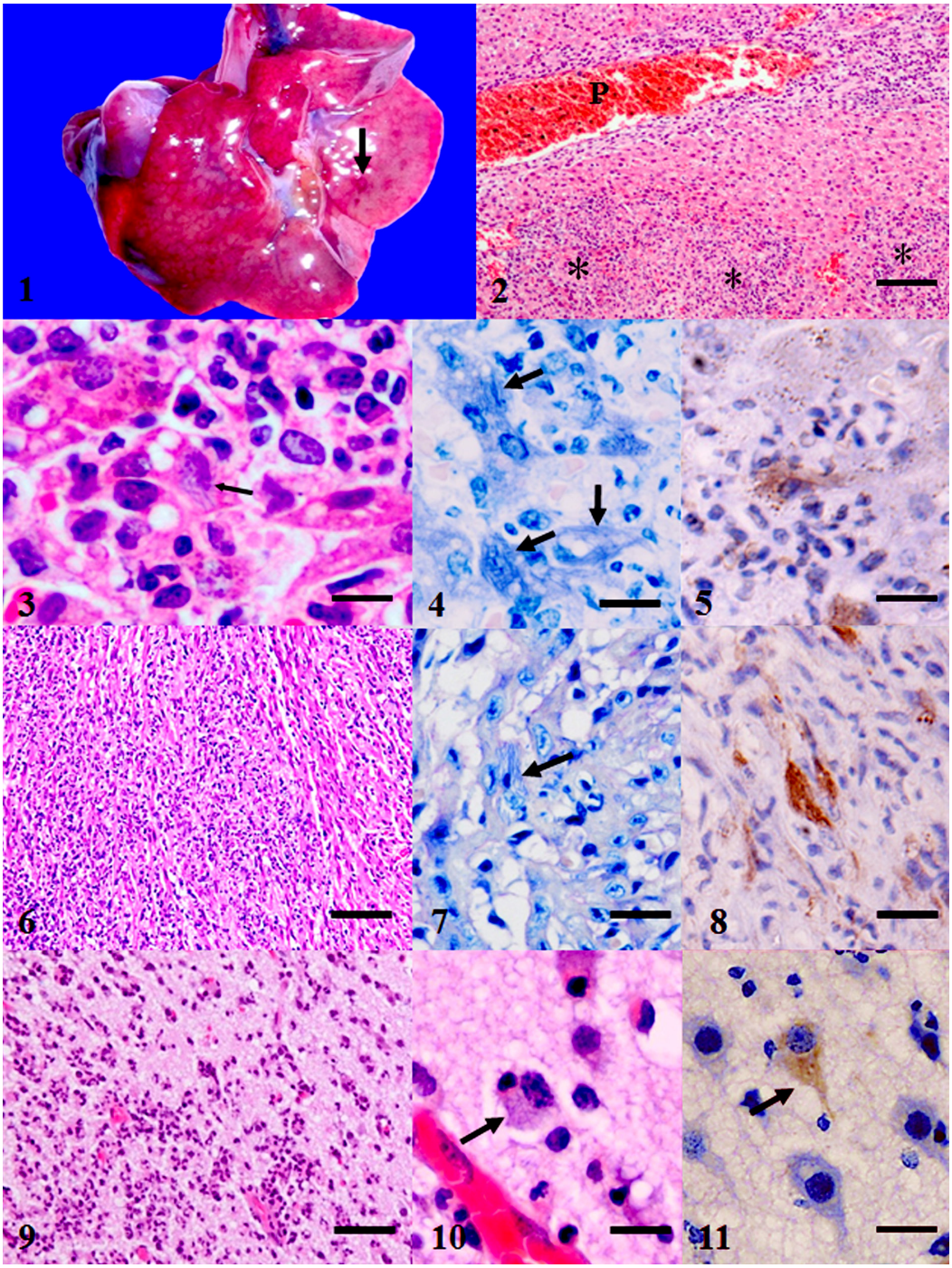

Fig. 1. Gross view of the liver at necropsy. Multifocal pale necrotic foci (arrow) with hemorrhage.
Fig. 2. Multiple necrotic foci (asterisks) with severe inflammation in the liver. Moderate infiltration of mononuclear cells around the portal vein (p). HE stain. Bar=200 μm.
Fig. 3. Intracytoplasmic bundles of large bacilli (arrow) in a hepatocyte around a necrotic focus. HE stain. Bar=20 μm.
Fig. 4. Intracytoplasmic bundles of large bacilli (arrows) in hepatocytes. Giemsa stain. Bar=20 μm.
Fig. 5. The bacilli in the hepatocytes are immunopositive for *C. piliforme* RT strains. Immunostaining with hematoxylin counterstain. Bar=20 μm.
Fig. 6. Multiple necrotic foci (asterisks) with severe inflammation in the heart. HE stain. Bar=100 μm.
Fig. 7. Intracytoplasmic bundles of large bacilli (arrow) within the cardiac myocytes are positively stained with Giemsa. Bar=20 μm.
Fig. 8. The bacilli in the cardiac myocytes were positive for *C. piliforme* RT strains. Immunostain. Bar=20 μm.
Fig. 9. Necrotic focus (asterisk) with inflammation in the cerebral cortex. HE stain. Bar=100 μm.
Fig. 10. A neuronal cell in the cerebral lesion contains intracytoplasmic bundles of large bacilli. HE stain. Bar=12 μm.
Fig. 11. The bacilli within neuronal cells are immunopositive for *C. piliforme* RT stain. Immunostain. Bar=12 μm.

of the ileum, cecum and colon, there was mild infiltration of neutrophils and macrophages with mild lymphoid follicular hyperplasia. There were moderate diffuse degenerative and necrotic lesions in the mucosal epithelium of the cecum and colon. Giemsa-positive intracytoplasmic bacilli were very rarely observed in the remaining epithelial cells. These organisms were also immunopositive for *C. piliforme*. In the mesenteric lymph nodes, there were a few Giemsa-positive bacilli within sinus macrophages.

In the heart, there were multiple necrotic foci with moderate to severe infiltration of neutrophils and a few macrophages (Fig. 6). The cardiac myocytes at the periphery of the necrotic foci were mildly swollen and sometimes contained intracytoplasmic bundles of large bacilli. These bacilli were also intensely positive with Giemsa stain (Fig. 7) and were immunopositive for *C. piliforme* (Fig. 8). In the brain, symmetric necrotic foci were also found in the cerebral cortex (Fig. 9). Neutrophils and a few macrophages infiltrated around the necrotic foci. Some neuronal cells in the lesions contained intracytoplasmic bundles of large bacilli (Fig. 10). Immunohistochemically, these bacilli were intensely immunopositive for *C. piliforme* (Fig. 11). There were no histological lesions in the pancreas, lungs, kidneys, spleen, adrenal glands and thymus. The diagnosis of Tyzzer's disease in this case is based on the characteristic lesions and the presence of intracytoplasmic bacilli in the liver, large intestines, heart and brain. The positive results of immunohistochemistry for *C. piliforme* strongly support the diagnosis.

Tyzzer's diseases usually occur in immunosuppressed or neonatal animals. Complications of other infectious [5, 8] or toxic [2] diseases were also reported. Thymic atrophy [5, 6, 12] and lymphoid depletion in the spleen or lymph nodes [12, 14] were frequently observed in the affected animals. In the present case, there were no significant lesions indicating immunosuppression or some other infections. The mode of infection in the present case remains unclear. The infection of *C. piliforme* most commonly occurs via an oral route by bacterial spores [4]. Although the large intestines are most possible primary entry site ingesting of the infection, the intestinal lesions were very mild. Macrophages residing in the intestinal mucosa may phagocytize the bacilli soon after the entry, and the organisms may be already transmitted with macrophages to the other organs, such as the liver and heart.

The CNS lesions by *C. piliforme* have been reported in laboratory animals intracerebrally inoculated with the bacilli [10] and very rarely in spontaneous cases [7, 15]. Experimentally infected mice died between the third and eighth day after intracerebral injection, and the bacilli were found in the CNS lesions [10]. In spontaneous Tyzzer's disease, on the contrary, the CNS involvement seemed to be unusual [7] and may appear only at some unusual status or the latest stage of the disease. The affected animals may die due to severe necrotizing hepatitis or myocarditis before the development of the CNS lesions. Therefore, the CNS lesions have been very unusual in spontaneous Tyzzer's disease, even if the bacilli have enough ability to infect and grow within the brain cells, including neuronal cells. Besides, Mete *et al.* [7] described that in a weaver bird suffering from encephalitis by *C. piliforme*, there were no bacilli in the intestines with crypt enteritis and liver with any pathologic lesions. Thus, they supposed the bacilli could cause enteritis leading directory to bacteremia and encephalitis. To know the detailed pathogenesis of CNS lesions in Tyzzer's disease, further case studies or some adequate experimental models will be needed.

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