Spontaneous Yersiniosis Due to *Yersinia pseudotuberculosis* Serotype 7 in a Squirrel Monkey

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**ABSTRACT.** A captive male Bolivian squirrel monkey (*Saimiri boliviensis*) of less than 1 year of age died following diarrhea and debilitation on the day of death. At necropsy, necrotizing enteritis accompanied with enlarged Peyer’s patches, solitary lymphatic follicles and mesenteric lymph nodes, and multiple yellowish-white nodules in the spleen and liver were found. Histopathologically, these lesions were necrotizing inflammation containing Gram-negative bacilli. *Yersinia pseudotuberculosis* serotype 7 was isolated from the spleen and liver. The *virF* gene, which is an essential virulent plasmid (*pYV*) in pathogenic *Y. pseudotuberculosis* isolates, and the *ypmA* gene, which is a superantigenic toxin, were detected in the isolates. This is the first report of a fatal case of *Yersinia pseudotuberculosis* 7 infection in the world.

**KEY WORDS:** pathogenic Yersinia, squirrel monkey, *Yersinia pseudotuberculosis* 7 infection.

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Yersiniosis refers to infections caused by either *Yersinia enterocolitica* or *Yersinia pseudotuberculosis*, which appear as enteritis and sometimes as septicemia in humans and animals [12]. *Y. pseudotuberculosis* is detected in many animal species including wild animals, livestock, and companion animals. Many cases of yersiniosis have been reported in Japan, especially in nonhuman primates with high sensitivity to *Y. pseudotuberculosis* in zoos [8, 10, 19]. This trend is also seen overseas [2, 11, 15, 17]. *Y. pseudotuberculosis* is a Gram-negative bacillus and it has been classified into serotypes O:1 to O:15 based on O-antigen, and 7 pathogenic serotypes, i.e., 1 to 6 and 10. Other serotypes including 7 are derived from the environment, and are known to be non-pathogenic [7, 14]. Most of the strains from Europe belong to serotypes 1a and 3 [18]. In contrast, *Y. pseudotuberculosis* strains belonging to three serotypes (4b, 5a, and 5b) have been isolated from human patients in Japan, and 5 serotypes (1b, 2b, 3, 4b, and 6) have been influenced by animals [3, 5, 16, 18]. The pathogenicity of *Y. pseudotuberculosis* is associated with several virulence factors. Pathogenic strains of *Y. pseudotuberculosis* have a 70-kb virulence plasmid (*pYV*). Additionally, a high-pathogenicity island (*HPI*), *Y. pseudotuberculosis*-derived mitogen (*YPM*), which is a superantigenic toxin, and invasin, which allows efficient entry into mammalian cells, are known to play important roles in causing severe systemic infection [9].

In this study, we report the first instance ever of a fatal case of *Yersinia pseudotuberculosis* 7 infection. In June 2003, a captive male Bolivian squirrel monkey (*Saimiri boliviensis*) of less than 1 year of age was found moribund and soon died. The monkey had diarrhea (mucous and bloody stool) on the day of death, and its weight was 380 g. It was the only such case to occur, and was subjected to necropsy. For histopathological examination, specimens of various tissues and organs were fixed in neutral buffered 10% formalin and embedded in paraffin wax. Sections (approximately 3 mm) were cut and stained with hematoxylin-eosin (HE) and Gram stain (Brown-Hopps method). Immunohistochemical examination was done using a commercial rabbit anti-*Y. pseudotuberculosis* 1–6 sera (Denka-Seiken, Co., Tokyo, Japan) and an indirect method. Bacteriological examination of the spleen and liver was also done.

At necropsy, there were bloody ascites, swelling of Peyer’s patches, mesenteric lymph nodes, and enlargement of the spleen and liver with multi-focal yellowish-white nodules. Pseudomembranous enterocolitis was associated with the Peyer’s patches and solitary lymphatic nodules and mucosa of the small intestine, and these were sometimes accompanied by hemorrhages. No other irregularities were seen in the lung, kidney, or heart.

Histopathologically, nodules seen in the liver and spleen were foci of necrosis accompanied by infiltration of neutrophils and macrophages containing Gram-negative bacilli (Figs. 1, 2, and 3). Lesions in both the small and large intestine were characterized by small and large foci of necrosis with ulceration and erosion of the mucosa, mainly Peyer’s patches and solitary lymphatic nodules (Fig. 4). Occasionally the lesions extended to the submucosa. There was also desquamation of the mucosal epithelium and congestion, haemorrhage and accumulations of necrotic debris and numerous bacterial colonies in the lesions. Severe neutrophil infiltration was apparent. The mesenteric lymph nodes were markedly expanded by the influx of edema fluid and by large numbers of neutrophils and macrophages mixed with bacterial colonies. Necrotic foci with neutrophils and...
lymphocytes were seen in the kidneys, but there were no bacterial colonies. Other findings included swelling and vacuolar degeneration of hepatocytes, myocardial degeneration, and pulmonary edema. Bacterial colonies seen in lesions were immunohistochemically negative to slightly positive for anti-*Y. pseudotuberculosis* 1–6 sera.

Only *Y. pseudotuberculosis* 7 was isolated from the spleen and liver. Microbiological features of isolates were described in a previous report [9]. Briefly, serotyping of isolates was performed by slide agglutination with rabbit immune sera and polymerase chain reaction (PCR) as described by Bogdanovich *et al.* (2003) [1]. The presence of the virulence genes *virF, inv, ypm (ypmA, ypmB, and ypmC)* and *irp2* were confirmed by PCR. The *virF* and *irp2* genes were used as markers for the presence of pYV and HPI, respectively. An isolated strain had *virF, inv* and *ypmA*, but not *ypmB, ypmC*, or *irp2*.

This animal had localized necrotizing enteritis in the lymphoid organs and enlarged spleen and liver accompanied with multi-focal necrosis with intralesional Gram-negative bacilli. These lesions were typical of those seen in other squirrel monkeys infected with *Y. pseudotuberculosis*. A diagnosis of yersiniosis was confirmed by isolation of *Y. pseudotuberculosis* in pure culture and PCR from the spleen and liver. On the basis of these findings, this case was diagnosed as yersiniosis due to *Yersinia pseudotuberculosis* serotype 7, and we concluded that the monkey died of sepsis caused by *Yersinia pseudotuberculosis* serotype 7.

To our knowledge, this is the first reported fatal case due to *Y. pseudotuberculosis* serotype 7 infection. This serotype has been isolated from healthy dogs, moles, and wild mice, but did not have pathogenic plasmids, and it was thought to be a non-pathogenic strain [4, 6, 13]. The PCR analysis demonstrated that this strain also had pYV and *ypmA* genes.
The pathological findings of this case were not different from those of other serotypes. Also, this case did not show immunological deterioration or ateliosis. These results suggest that the strain serotype 7 isolated in the present study has the same degree of pathogenicity as other pathogenic serotypes.

In Japan, *Yersinia pseudotuberculosis* has been isolated from human patients and from various animals including wild animals, livestock, and companion animals, and various serotypes have been isolated. Because there have been no reports about *Y. pseudotuberculosis* serotype 7 isolated from human patients and animals, additional pathological and epidemiological studies are necessary, and we should pay attention to the possibility of fatal infection in humans and other animals by serotype 7.

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REFERENCES