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NOTE

Pathology

Outbreak of pasteurellosis in captive Bolivian squirrel monkeys (*Saimiri boliviensis*)

Mizuki YOSHINO[1)], Jun SASAKI[1)], Konomi KURAMOCHI[1)], Mitsutaka IKEZAWA[2)], Natsuko MUKAIZAWA[3)] and Masanobu GORYO[1)]*

[1)]Department of Veterinary Pathology, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan
[2)] National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
[3)] Ikeda Zoo, Kyoyama, Kita-ku, Okayama 700-0015, Japan

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ABSTRACT. In September 2012, five Bolivian squirrel monkeys housed in a zoological park died within sequential several days without obvious clinical signs. In a necrospy, one monkey presented swelling of the kidney with multifocal white nodules in the parenchyma, and other two had pulmonary congestion. Histopathologically, multifocal bacterial colonies of gram-negative coccobacillus were found in the sinusoid of the liver in all monkeys examined (Nos.1–4). Additionally, purulent pyelonephritis, pneumonia and disseminated small bacterial colonies in blood vessels were observed. Immunohistochemically, the bacterial colonies from two monkeys were positive for *P. multocida* capsular serotype D. Based on these findings, these monkeys were diagnosed as septicemia caused by acute *P. multocida* infection.

KEY WORDS: outbreak, pasteurellosis, septicemia, squirrel monkey

Pasteurellosis is a zoonotic disease commonly caused by *Pasteurella multocida*. *P. multocida* can present as a commensal in the upper respiratory tract of mammals and birds, and can cause either primary or secondary disease processes in a variety of domestic and wild mammals, birds and humans [18]. These diseases include fowl cholera in poultry [9], atrophic rhinitis in pigs [6] and bovine hemorrhagic septicemia [5]. *P. multocida* has been classified into five serogroups (A, B, D, E and F) based on capsular antigens and 16 serotypes by somatic antigens [18].

Nonhuman primates appear to be predisposed to *P. multocida* infection, and some cases of pasteurellosis have been reported worldwide. *P. multocida* has been reported to be associated with respiratory tract disease, such as bronchitis and pneumonia [3, 11, 16], septicemia [7, 12] and several systemic suppurative diseases [2] in captive and wild nonhuman primates. To our knowledge, all *P. multocida* strains isolated from nonhuman primates have been classified into serogroup A [1, 16]. However, little is known about its pathogenicity and capsular serotypes characteristic of *P. multocida* isolated from nonhuman primates. The aim of this report was to describe the pathological features of captive monkeys suffered from an outbreak of *P. multocida* capsular serotype D infection in Japan.

In September 2012, five of nine Bolivian squirrel monkeys (*Saimiri boliviensis*) (animals 1–5, Table1) housed at a zoological park in Japan died within 3 consecutive days without obvious clinical signs. Animals 1 (20 years old) and 2 (15 months old) were found dead in the morning of the first day. On the next day, animals 3 (5 years old) and 4 (3 months old) were found to lay down on the floor and died after treatment with antibiotics and fluid therapy. Animal 5 (9 years old) showed depression, elevated body temperature and respiratory symptoms on the first day and was administered antibiotics and fluid therapy for two days, but the symptoms worsened and the animal died on the third day. Before these events, these monkeys were fed fruits and commercial monkey food, and housed in an indoor-outdoor enclosure. Windows, which were connected to the outside, were kept open when the outbreak occurred. Necropsy on these animals was performed in a zoo. Tissue samples were collected from the liver, lung, heart, intestine and mesenteric lymph nodes from animals 1–4, and additional tissues including the spleen, kidney, pancreas and brain were collected from animals 3 and 4. The tissue samples from No.5 were not available. Samples were fixed in 10% formalin, embedded in paraffin wax and sectioned into 4 μm thick. Sections were stained with hematoxylin and eosin (HE) and Gram's stain. Additionally, tissue sections from the lung and liver were stained with periodic acid Schiff (PAS) and Grocott methenamine silver stain. Immunohistochemistry (IHC) tests were performed on the liver from animals 1 and 2 using a set of rabbit anti-*P. multocida*

*Correspondence to: Goryo, M., Department of Veterinary Pathology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan. e-mail: goryo@iwate-u.ac.jp

Table 1. Datails of dead animals

No.	Date of death	Body weight (g)	Age	Sex [a)]	Clinical signs	Bacterial colonies in tissues
1	26/9/2012	640	20Y	F	Nothing	Liver
2	26/9/2012	440	1Y 3M	F	Nothing	Liver, Mesenteric Ln [b)]
3	27/9/2012	600	5Y	F	Nothing	Liver, Spleen, Mesenteric Ln, Blood vessels
4	27/9/2012	240	3M	M	Nothing	Liver, Lung, Kidney, Spleen, Blood vessels
5	28/9/2012	680	9Y	F	Fever, Respiratory symptoms	NE [c)]

a) F: female M: male. b) Ln: lymph node. c) NE: not examined.

sera specific to A, B, D, E and F serotypes (National Institute of Animal Health, Tsukuba, Japan). We used the liver tissue injected with *P. multocida* capsular serotypes A,B,D,E and F isolated in National Institute of Animal Health as a positive control.

At necropsy, animal 4 showed swelling and fading of both kidneys with multiple white nodules in the parenchyma, and animals 1 and 2 had pulmonary congestion. Other findings included enlargement of the heart (animal 1) and ascites (animal 4) that was thought to be due to fluid therapy. Necropsy findings of Nos. 3 and 5 were not remarkable.

Histopathologically, all monkeys examined had multifocal bacterial colonies in the sinusoid of the liver (Fig. 1). These were associated with foci of necrosis and accompanied by inflammatory infiltration including neutrophils and macrophages in animals 2 and 3, but were rarely associated with inflammatory reaction in animals 1 and 4. Hepatocytes around the bacterial colonies diffusely enlarged with cytoplasmic vacuoles. Gram staining revealed that the bacterial colonies were composed of gram-negative coccobacillus. Animal 4 had fibrinopurulent pneumonia and purulent pyelonephritis with multiple bacterial colonies. Additionally, bacterial colonies without inflammatory reaction were seen in the spleen and mesenteric lymph nodes (animals 2–4) (Fig. 2). Similar colonies were also seen in the blood vessels of the pancreas and brain (animals 3 and 4). The tissues of each animal that have bacterial colonies are summarized in Table 1. Other lesions included pulmonary edema and congestion (animals 1–3), slight pericholangitis (animals 1 and 2) and follicular hyperplasia of the lymph node (animals 1–3). The other significant pathogens were not observed in PAS and Grocott methenamine silver stain. The bacterial colonies were immunolabeled only for *P. multocida* D (Fig. 3).

All Bolivian squirrel monkeys investigated histopathologically in the present study exhibited disseminated bacterial embolism with systemic suppurative and necrotizing disease involving the liver, lungs and kidney. These bacterial colonies were characterized immunohistochemically as *P. multocida* capsular serotype D. Similar lesions have been reported in animals infected with *P. multocida* [2, 7, 12], and on the basis of these findings, this outbreak was considered to be related to septicemia associated with *P. multocida* infection. There is scarce information on the capsular serotype of *P. multocida* isolated from nonhuman primates, and only some reports described about capsular serotype A [1, 16]. In this investigation, neither bacteriological examination nor PCR assay was not performed because we could not obtain fresh samples, so it is difficult to determine exact capsular serotype. However, serotype D strain has been isolated from lesions, such as atrophic rhinitis and fowl cholera, in many domesticated and wild animal species [4, 6, 9], and it is one of the most common serotype isolated from human infections [4]. Thus, this outbreak was likely to be associated with *P. multocida* capsular serotype D. Although pasteurellosis in nonhuman primates including squirrel monkeys has been reported [2, 3, 7, 12, 19], the occurrence of mass outbreak appears to be rare. To our knowledge, this is the first report about the outbreak of pasteurellosis in Bolivian squirrel monkeys in Japan caused by *P. multocida* capsular serotype D.

P. multocida infection is usually considered to occur through respiratory route via direct contact to infectious secretions or inhalation of aerosols, by uptake of contaminated water or food, or through wound or bite [8, 9]. *P. multocida* is able to survive for relatively long periods in moist conditions [5], and wild rats and birds are considered to be carriers of this organism [9]. In human patients infected with *P. multocida*, the organisms are shed in sputum, and infection may occur from a pet licking without traumatic contacts [10, 20]. For these reasons, the possibility of sputum-mediated transmission among nonhuman primates cannot be ruled out.

Pasteurellosis in nonhuman primates often occurs when local and systemic defense mechanisms are impaired. Predisposing factors include stress induced by shipment, crowding or concurrent illness including parasitism or viral infection [7, 12, 16, 19]. In addition, nonhuman primates have developed *P. multocida* infections secondary to surgical procedures or chronic catheterization [3]. Sometimes, climate change could cause an outbreak of pasteurellosis [17], and it has been reported that incidence of fowl cholera increases in late summer and fall [9]. The outbreak reported here occurred in September, fall in Japan; during this period, the ambient temperature can vary greatly. In addition, windows of the cage were kept open before the outbreak, so, it is possible that asymptomatic carriage for long periods followed by cold stress may have triggered the development of pasteurellosis. However, no histopathological changes were observed in spleen, lymph nodes and bone marrow, and those are related to the immunocompromised conditions in examined monkeys.

Various species of nonhuman primate can be infected with *P. multocida* in zoos [14], and a report suggested *P. multocida* causes a fatal outbreak in some species of animals and birds in a zoo during rainy season [17]. Moreover, *P. multocida* infection sometimes produces serious disease in both healthy and immunecompromised human, and many cases of pasteurellosis without animal bite have been reported [13, 15]. Therefore, from the point of view of public and animal health, extreme caution and additional studies investigating the mechanisms of infection are necessary in order to preclude transmission of *P. multocida* in zoos.

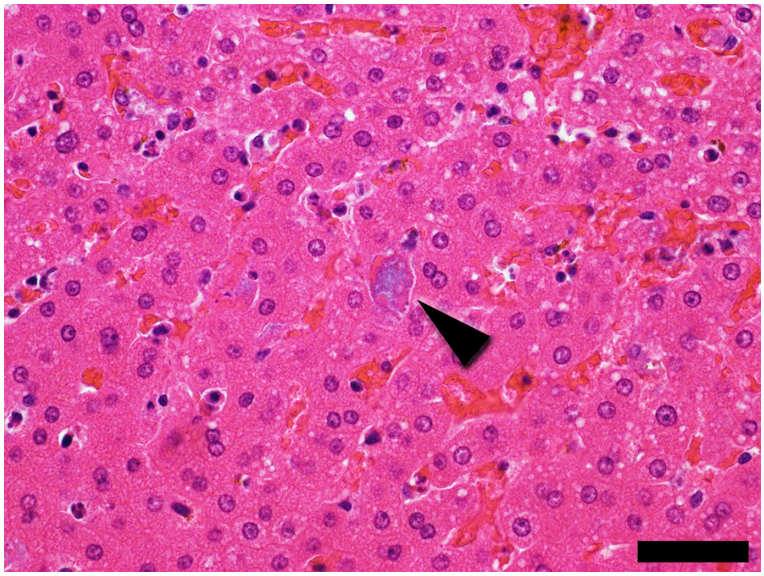

Fig. 1. Multifocal bacterial colony (arrowhead) in the sinusoid of the liver. Animal 2. HE. Bar, 50 μm.

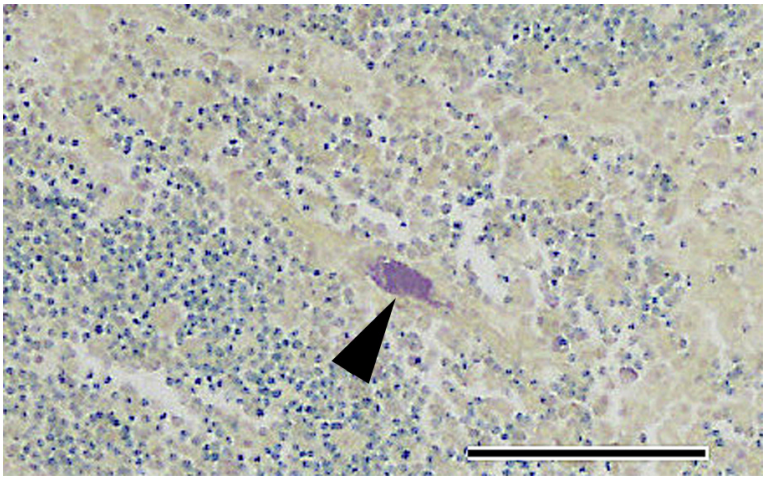

Fig. 2. Gram-negative bacterial colony (arrowhead) in the mesenteric lymph node. Animal 2. Gram's stain. Bar, 100 μm.

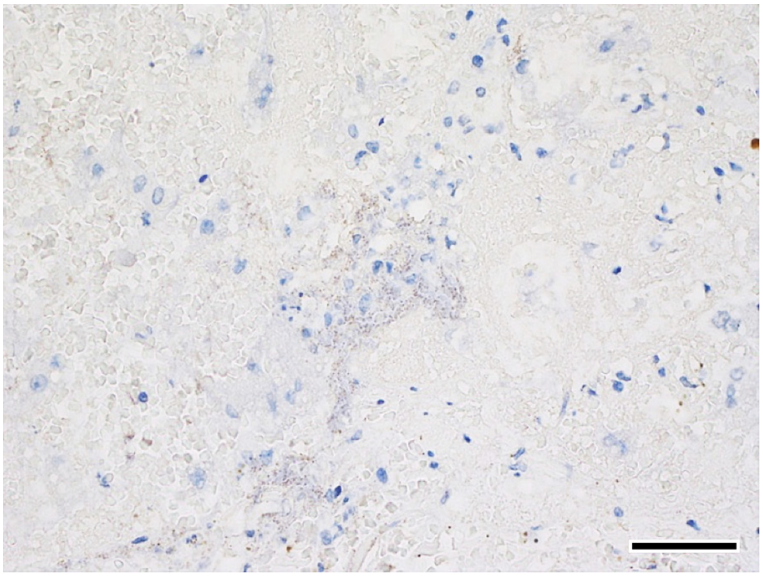

Fig. 3. Bacterial colonies in the liver immunolabeled for *P. multocida* D. IHC. Bar, 50 μm.

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