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

Mizuki YOSHINO1), Jun SASAKI1), Konomi KURAMOCHI1), Mitsutaka IKEZAWA2), Natsuko MUKAIZAWA3) and Masanobu GORYO1)*

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

**ABSTRACT.** In September 2012, five Bolivian squirrel monkeys housed in a zoological park died within sequential several days without obvious clinical signs. In a necropsy, 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*
Additional studies investigating the mechanisms of infection are necessary in order to preclude transmission of *P. multocida* in zoos. Animal bite have been reported [13, 15]. Therefore, from the point of view of public and animal health, extreme caution and sometimes produces serious disease in both healthy and immunocompromised human, and many cases of pasteurellosis without *P. multocida* causes a fatal outbreak in some species of animals and birds in a zoo during rainy season [17]. Moreover, infection immunocompromised conditions in examined monkeys. However, no histopathological changes were observed in spleen, lymph nodes and bone marrow, and those are related to the ambient temperature can vary greatly. In addition, windows of the cage were kept open before the outbreak, so, it is possible cholera increases in late summer and fall [9]. The outbreak reported here occurred in September, fall in Japan; during this period, [3]. Sometimes, climate change could cause an outbreak of pasteurellosis [17], and it has been reported that incidence of fowl *P. multocida*, the organisms are shed in sputum, and infection may occur from a pet licking without traumatic patients infected with *P. multocida*, the PCR assay was not performed because we could not obtain fresh samples, so it is difficult to determine exact capsular serotype. and only some reports described about capsular serotype A [1, 16]. In this investigation, neither bacteriological examination nor serotype D strain has been isolated from lesions, such as atrophic rhinitis and fowl cholera, in many domesticated and capular serotype D. Although pasteurellosis in nonhuman primates including squirrel monkeys has been reported [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 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. 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 immunocompromised 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.

### Table 1. Details of dead animals

| No. | Date of death | Body weight (g) | Age | Sex | 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<sup>b</sup> |
| 3   | 27/9/2012     | 600            | 5Y  | F   | Nothing       | Liver, Spleen, Mesenteric Ln, Blood vessels |
| 4   | 27/9/2012     | 240            | 3M  | F   | Nothing       | Liver, Lung, Kidney, Spleen, Blood vessels |
| 5   | 28/9/2012     | 680            | 9Y  | F   | Fever, Respiratory symptoms | NE<sup>c</sup> |

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

1. Arumugam, N. D., Ajam, N., Blackall, P. J., Asiah, N. M., Ramlan, M., Maria, J., Yuslan, S. and Thong, K. L. 2011. Capsular serotyping of Pasteurella multocida from various animal hosts - a comparison of phenotypic and genotypic methods. Trop. Biomed. 28: 55–63. [Medline]
2. Benjamin, S. A. and Lang, C. M. 1971. Acute pasteurellosis in owl monkeys (Aotus trivirgatus). Lab. Anim. Sci. 21: 258–262. [Medline]
3. Bronsdon, M. A. and DiGiacomo, R. F. 1993. Pasteurella multocida infections in baboons (Papio cynocephalus). Primates 34: 205–209.
4. Carter, G. R. 1962. Animal serotypes of Pasteurella multocida from human infections. Can. J. Public Health 53: 158–161. [Medline]
5. Carter, G. R. and De Alwis, M. C. L. 1989. Hemorrhagic septicaemia. pp. 131–160. In: Pasteurella and Pasteurellosis. (Adlam, C. and Rutter, J. M. eds.), Academic Press, London.
6. Chanter, N. and Rutter, J. M. 1989. Pasteurellosis in pigs and the determinants of virulence of toxigenic Pasteurella multocida. pp. 161–195. In: Pasteurella and Pasteurellosis (Adlam, C. and Rutter, J. M. eds.), Academic Press, London.
7. Duncan, M., Tell, L., Gardiner, C. H. and Montali, R. J. 1995. Lingual gongylonemiasis and pasteurellosis in Goeldi’s monkeys (Callimico goeldii). J. Zoo Wildl. Med. 26: 102–108.
8. Frederiksen, W. 1989. Pasteurellosis of man. pp. 303–320. In: Pasteurella and Pasteurellosis (Adlam, C. and Rutter, J. M. eds.), Academic Press, London.
9. Glisson, J. R., Hofacre, C. L. and Christensen, J. P. 2003. Fowl cholera. pp. 658–676. In: Diseases of Poultry, 11th ed. (Saif, Y. M., Barnes, H. J., Glisson, J. R., Fadly, A. M., McDougald, L. R. and Swayne, D. A. eds.), Iowa State University Press, Ames.
10. Gonda, H., Noda, Y., Ohishi, T., Tanigawa, Y., Sato, S., Ikenouchi, T., Yamashita, T. and Yamaguchi, I. 2001. A clinical study on patients detected Pasteurella multocida from sputum. Kansenshogaku Zasshi 75: 780–784 (in Japanese). [Medline] [CrossRef]
11. Good, R. C. and May, B. D. 1971. Respiratory pathogens in monkeys. Infect. Immun. 3: 87–93. [Medline]
12. Greenstein, E. T., Doty, R. W. and Lowy, K. 1965. An outbreak of a fulminating infectious disease in the squirrel monkey, Saimiri sciureus. Lab. Anim. Care 15: 74–80. [Medline]
13. Hubbert, W. T. and Rosen, M. N. 1970. Pasteurella multocida infections. II. Pasteurella multocida infection in man unrelated to animal bite. Am. J. Public Health Nations Health 60: 1109–1117. [Medline] [CrossRef]
14. Kalter, S. S. 1989. Infectious diseases of nonhuman primates in a zoo setting. Zoo Biol. 8: 61–76. [CrossRef]
15. Kimura, R., Hayashi, Y., Takeuchi, T., Shimizu, M., Iwata, M., Tanahashi, I. and Ito, M. 2004. Pasteurella multocida septicaemia caused by close contact with a domestic cat: case report and literature review. J. Infect. Chemother. 10: 250–252. [Medline] [CrossRef]
16. Köndgen, S., Leider, M., Lankester, F., Bethe, A., Lübke-Becker, A., Leendertz, F. H. and Ewers, C. 2011. Pasteurella multocida involved in respiratory disease of wild chimpanzees. PLoS ONE 6: e24236. [Medline] [CrossRef]
17. Okoh, A. E. J. 1980. An outbreak of pasteurellosis in Kano Zoo. J. Wildl. Dis. 16: 3–5. [Medline] [CrossRef]
18. Rimpler, R. B. and Rhoades, K. R. 1989. Pasteurella multocida infections. pp. 37–73. In: Pasteurella and Pasteurellosis. (Adlam, C. and Rutter, J. M. eds.), Academic Press, London.
19. Smith, H. C. 1954. Pasteurellosis in monkeys. J. Am. Vet. Med. Assoc. 124: 147–148. [Medline]
20. Wade, T., Booy, R., Teare, E. L. and Kroll, S. 1999. Pasteurella multocida meningitis in infancy - (a lick may be as bad as a bite). Eur. J. Pediatr. 158: 875–878. [Medline] [CrossRef]