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Pathological Changes in Captive Monkeys with Spontaneous Yersiniosis due to Infection by *Yersinia enterocolitica* serovar O8

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Summary

An outbreak of fatal yersiniosis due to infection with *Yersinia enterocolitica* serovar O8 is documented in two species of captive monkey. Five of 50 squirrel monkeys (*Saimiri sciureus*) and one of two agile gibbons (*Hylobates agilis*) died following several days of diarrhoea. Necropsy examination revealed necrotizing enterocolitis and multifocal necrosis or abscesses in various organs. Microscopically, these lesions comprised multifocal necrosis with bacterial colonies, neutrophils and accumulation of nuclear debris. Occasional lesions included macrophages and abscess formation. Immunohistochemically, the bacteria were identified as *Y. enterocolitica* O8. In addition, *Y. enterocolitica* serotype O8 was isolated from animal organs in pure culture. This is the first report of fatal cases of infection with *Y. enterocolitica* serovar O8 in animals.

Keywords: outbreak; squirrel monkey; *Yersinia enterocolitica* serovar O8; yersiniosis

Introduction

Yersiniosis is a zoonotic disease caused by *Yersinia enterocolitica* or *Yersinia pseudotuberculosis* and appears as enteritis and sometimes septicaemia in man and animals. Latent infection by these species occurs in free-living wild mice and wild birds and bacteria are excreted in their faeces. Contaminated food and water are common sources for the introduction of pathogens (Fukushima *et al*., 1988; Sunahara *et al*., 2000; Han *et al*., 2003). These pathogens have a very wide host range and have been detected in more than 110 species of animal worldwide, including mammals, birds and reptiles (Shayegani *et al*., 1986; Kwaga and Iversen, 1993; Greene, 1998). Infection is almost always latent and there have been sporadic reports of occurrences in animals such as non-human primates, rabbits, guinea pigs, chinchillas, livestock and birds, with the disease appearing as enteritis, mesenteric lymphadenitis, multiple nodules with necrosis, abscess formation and septicaemia (Mair, 1973; Baskin *et al*., 1977; Harcourt-Brown, 1978; Hanssen, 1982; Parsons, 1991; Seimiya *et al*., 2005). *Y. enterocolitica* is found more frequently than *Y. pseudotuberculosis*, but actual cases are limited to a few species, such as non-human primates and chinchillas, and the lesions are similar to those of *Y. pseudotuberculosis*.

Y. enterocolitica has over 60 serotypes, but only a limited number of these (i.e. serotypes O3, O4/32, O5/27, O8, O9, O13a, O13b, O18, O20 and O21) are pathogenic in man. Of these the most frequently detected pathogenic serotypes are O3, O5/27, O8 and O9. Serotypes O3, O5/27 and O9 are distributed worldwide and many cases have been reported in man and animals, while serotype O8 is limited to North America and is thus referred to as the North American strain (Schiemann, 1989). Serotype O8 has been isolated mainly from human patients (Bissett, 1976; Winblad, 1979; Shayegani *et al*., 1983) and a few healthy foxes (*Urocyon cinereoargenteus*) and porcupines (Shayegani *et al*., 1986) and is supposedly confined to North America. However, isolation of this serotype from human patients and healthy wild rodents has been reported sporadically in Japan since 1990 (Ichinohe *et al*., 1991; Iinuma *et al*., 1992;

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Hayashidani *et al*., 1995; Sakai *et al*., 2005) and it has recently been reported in Europe (Schubert *et al*., 2003; Rastawicki *et al*., 2009). However, in animals, fatal or non-fatal cases caused by this serotype have not been reported. Serotypes O3, O5/27 and O9 have relatively low pathogenicity and mainly cause diarrhoea, but serotype O8 is highly pathogenic in man and may cause severe septicaemia (Schiemann *et al*., 1981). In mice experimentally infected with serotypes O3, O5/27 and O9, infection can be subclinical, but in infection with O8 almost 100% of individuals become moribund with septicaemia (Carter *et al*., 1973; Maruyama *et al*., 1979).

Non-human primates appear to be sensitive to *Y. enterocolitica* and *Y. pseudotuberculosis* and many fatal cases of yersiniosis have been reported worldwide (Mair *et al*., 1970; McClure *et al*., 1971; Baggs *et al*., 1976; Poelma *et al*., 1977; Vandamme *et al*., 1978; Buhles *et al*., 1981; MacArthur and Wood, 1983; Skavlen *et al*., 1985; Plesler and Claros, 1992; Sasaki *et al*., 1996; Kageyama *et al*., 2002). In Japan, squirrel monkeys (*Saimiri sciureus*) are reported to be highly sensitive to *Y. pseudotuberculosis*, and outbreaks occur every year in zoos (Iwata *et al*., 2008). There have also been reports of non-human primate infection with pathogenic *Y. enterocolitica* elsewhere, but no such infections have yet been reported in Japan. This is the first report of animal fatalities due to *Y. enterocolitica* serotype O8. The aim of the present study was to elucidate the pathological features of an outbreak of *Y. enterocolitica* O8 infection in captive monkeys in Japan.

Materials and Methods

Case History

In December 2002, five of 50 squirrel monkeys housed at a zoo in Japan died following several days of diarrhoea. At that point, 14 of the remaining 45 monkeys had diarrhoea. The outbreak was controlled by treatment with antibiotics. However, after the end of the outbreak, in April 2003, one of two agile gibbons (*Hylobates agilis*) showed similar clinical signs and was given antibiotics for 3 days, but the symptoms worsened and the animal died on the fourth day. The squirrel monkeys were fed fruits, such as apples and bananas, and commercial monkey food, and were housed in an indoor-outdoor enclosure. Other monkeys were housed in outdoor cages about 50 m from the squirrel monkey enclosure.

Animals and Pathological Examination

The subjects of this report were five squirrel monkeys and one agile gibbon (Table 1). All squirrel monkeys were juveniles aged less than 1 year (four males and one female). The agile gibbon was a 14-year-old female. A complete necropsy examination was performed on each dead animal (except one squirrel monkey) as soon as possible after death in the Laboratory of Veterinary Pathology of Azabu University. For microscopical examination, specimens of various tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3 μm) were stained with haematoxylin and eosin (HE) and Gram stain (by the method of Brown–Hopps).

Table 1
Details of animals investigated

Case no.	*Species*	*Date of death*	*Body weight*	*Age*	*Clinical signs*
1	SM 1	8/12/2002	180 g	Juvenile	NA
2	SM 2	22/12/2002	288 g	Juvenile	Diarrhoea, mandibular swelling
3	SM 3	23/12/2002	NA	Juvenile	Diarrhoea
4	SM 4	28/12/2002	272 g	Juvenile	Depression
5	SM 5	29/12/2002	250 g	Juvenile	Mandibular swelling
6	Agile gibbon	4/4/2003	5 kg	Adult	Diarrhoea, depression

SM, squirrel monkey; NA, data not available.

Immunohistochemistry (IHC) was performed using a set of commercial rabbit anti-*Y. enterocolitica* sera specific for O1-2, O3, O5, O8 and O9 and a set of anti-*Y. pseudotuberculosis* sera specific for O1, O2, O3, O4, O5 and O6 (Denka-Seiken Co., Tokyo). Secondary reactions were performed with a peroxidase-conjugated Histofine-Simplestain kit (Simplestain MAX-PO; Nichirei, Tokyo). 3, 3′-diaminobenzidine and H_2O_2 was used to 'visualize' the reaction products. Slides were counterstained with Mayer's haematoxylin.

Bacteriological Culture and Molecular Typing

Bacteriological features and the results of molecular typing of isolates have been described previously (Iwata *et al*., 2005). Briefly, organs (liver, spleen, lung, intestine and mandibular abscesses) or faecal samples collected from the five dead squirrel monkeys and one dead agile gibbon, and 98 faecal samples (45 from squirrel monkeys, 20 from other monkeys of 18 different species and 33 from black rats [*Rattus rattus*] captured around the monkey cages) were examined for the presence of *Yersinia* spp. by culture on irgasan-novobiocin (IN) agar plates. *Y. enterocolitica* serovar O8 was then isolated based on biochemical characteristics and a slide agglutination test. In addition, isolates were examined using the molecular typing method based on pulsed field gel electrophoresis (PFGE), ribotyping and restriction endonuclease analysis of virulence plasmid DNA (REAP) (Iwata *et al*., 2005).