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Occurrence of *Yersinia enterocolitica* in the Tokyo Tama Zoo

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ABSTRACT: *Yersinia enterocolitica* serogroups O5A and O8 were isolated from fecal samples of one colony of Japanese macaques (*Macaca fuscata*) from the Tokyo Tama Zoo (Japan). Serogroup O5A was detected in brown bear (*Ursus arctos*) prior to the isolation from the macaques. Serogroup O5A organisms also were isolated from the Japanese macaques' breeding area. Serogroup O5A and O8 isolates were not pathogenic. Serogroup O8 isolates did not possess the O7 or O19 antigens.

Key words: *Yersinia enterocolitica*, Japanese macaques (*Macaca fuscata*), brown bear (*Ursus arctos*), zoo animals, survey.

Free-flying wild birds and feral rodents inhabit zoological gardens in addition to zoo animals. Isolation of *Yersinia enterocolitica* from wild birds and feral rodents has been reported (Kaneko and Hashimoto, 1981; Kapperud and Olsvik, 1982; Kato et al., 1985). It is known that zoo animals may be infected with *Y. enterocolitica* (Poelma et al., 1977; Mingrone and Fantasia, 1988). However, there have been no systematic studies to describe the ecology of these bacteria in zoological gardens. Therefore, the present study was initiated to determine the prevalence of *Y. enterocolitica* in animals of the Tokyo Tama Zoo (Japan).

From August 1985 to November 1986, fecal samples were examined from 310 Japanese macaques (*Macaca fuscata*), 56 lions (*Panthera leo*), 41 hares (24 *Lepus brachyurus* and 17 *Lepus timidus ainu*), 35 chimpanzees (*Pan troglodytes*), 24 brown bear (*Ursus arctos*), 13 Afgan pikas (*Ochotona rufescens*), nine gorillas (*Gorilla gorilla gorilla*), nine foxes (*Vulpes vulpes*) and four wattled cranes (*Bugeranus carunculatus*) collected in the Tokyo Tama Zoo (Hino, Tokyo 191, Japan). All these animals appeared healthy. Fecal samples were collected in the morning. In addition to the samples of the zoo animals, intestinal contents were collected from 196 feral animals consisting of 80 domestic pigeons (*Columba livia domestica*), 60 house sparrows (*Passer montanus*), 49 Old World wood mice (*Apodemus speciosus*) and seven Norway rats (*Rattus norvegicus*). Six samples of animal diets, seven water and four soil samples inside a Japanese macaques' breeding area, and four water samples outside the macaques' breeding area also were tested.

Feces and intestinal contents were incubated in phosphate-buffered solution containing M/15 Na_2PO_4 and M/15 KH_2PO_4 (PBS) at pH 7.6, and water and soil were incubated in PBS (pH 7.6) containing 10% heat-sterilized dog feces at 4 C for 3 to 4 wk for enrichment. After alkali treatment according to the method of Auliso et al. (1980), 0.1 ml of sample suspension was spread on the cefsulodin irgasan novobiocin (CIN) agar plates of Devenish and Schiemann (1981). These plates were incubated at 25 C for 48 hr. Red colonies on the CIN agar plates were submitted for identification. The methods of identification were the same as those of Kato et al. (1985). Biotyping of the isolated strains was performed according to the schema of Wauters (1973). Serotyping of the isolated strains was accomplished by using slide agglutination with commercial rabbit anti-*Y. enterocolitica* O1, O2, O3, O5, O8 and O9 sera (Denkaseiken Co., Nihonbashi, Tokyo, Japan) prepared according to the method of Winblad et al. (1966).

Preparation of rabbit anti-*Y. enterocolitica* O7 and O19 is described as follows. Strains used for immunization and absorption were *Y. enterocolitica* O7,8, O8,19, and O8 strains donated by G. Wauters

(Brussels University, Brussels, Belgium). The O7,8 and O8,19 strains were grown on trypticase soy agar (BBL Microbiology Systems, Becton Dickensson and Co., Cockeysville, Maryland, USA) at 25 C for 48 hr. The organisms grown were suspended in saline solution and autoclaved for 2 hr. First, 0.5 ml, second, 1 ml, and third and thereafter, 2 ml of the suspension containing 1 mg/ml of autoclaved bacteria (wet weight) were injected into the ear veins of Japanese white rabbits (*Oryctolagus cuniculus domesticus*) at 4- or 5-day intervals for 3 or 4 wk. After the tube agglutination titer was at 1:1,000, immune sera were obtained and were absorbed by the O8 strain.

Thirty-six O5A, 56 O8 and 16 untypeable strains that were isolated were examined for temperature dependent autoagglutination and temperature dependent calcium requirement; 56 O8 strains also were examined by a mouse lethality test and 9 O8 strains by colonization on the murine intestines according to a previous report by Tanaka et al. (1987).

Prevalence data on *Y. enterocolitica* in the zoo is presented in Table 1. The O5A organisms were isolated first from brown bears, and subsequently from Japanese macaques. The O5A organisms were isolated from pond water and soil, as well as from sewage outside of the Japanese macaques' breeding area. The O8 organisms were isolated only from the Japanese macaques (Table 1). The O8 strains isolated were not agglutinated with anti-O7 and O19 sera. They were esculin positive. The organisms that could not be typed also were isolated first from a brown bear and then from Japanese macaques as well as from feral mice (*Apodemus speciosus*) and the water of a pond outside Japanese macaques' breeding area. All of O5A, O8 and strains that could not be typed were negative with temperature dependent autoagglutination test, temperature dependent calcium requirement test, mouse lethality test, and the murine intestinal colonization test.

Yersinia enterocolitica was not isolated from chimpanzees, gorillas, lions, Afgan pikas, hares, red foxes, wattled cranes, Norway rats, house sparrows, domestic pigeons, diets of Japanese macaques, horse meat or chicken heads.

Isolation of O5A organisms from nonhuman primates is reported in The Netherlands by Poelma et al. (1977). In our study, the O5A organisms were detected in a colony of 50 Japanese macaques over a 10-mo period and they were spread among the colony. Furthermore, the O5A organisms were detected in the pond water and the soil in the macaques' breeding area. This suggests that a possible source of the O5A organisms for the macaques was the water and the soil. The O5A organisms were not isolated from diets of the Japanese macaques. The O5A and organisms that could not be typed were detected in brown bears prior to the isolation from the macaques. The brown bears' cage was in an independent house located about 50 m from the macaques' breeding area. The animal keeper worked in the brown bears' quarters first and then went to the macaques' breeding area each morning. It is unknown whether or not the O5A and organisms that could not be typed were introduced from the brown bear to Japanese macaques by the keeper through contaminated shoes, since his shoes were not submitted for bacterial culture. The fact that the O5A organisms were detected in sewage from the macaques' breeding area suggested that the O5A organisms would contaminate the environment in the other areas of the zoo.

Avirulent O8 organisms were isolated from Japanese macaques over a 4-mo period. Because this serogroup was not isolated from the diet or the environment, its origin and mode of transmission were not determined. Although the O5A and O8 organisms were not isolated from the feral rodents and birds that were examined, O5A organisms have been isolated from these animals in previous studies (Kaneko and Hashimoto, 1981; Kato et al., 1985). Untypeable organisms were isolated from

TABLE 1. Prevalence of *Yersinia enterocolitica* in the Tokyo Tama Zoo (Japan).

O serogroup	Biovar	Specimen	Prevalence[a]								
			1985		1986						
			August	December	March	May	June	July	August	September	November
O5A	1	Zoo animal									
		Brown bear[b] (*Ursus arctos*)	2/4	1/4	1/4				1/4	0/4	1/4
		Japanese macaque (*Macaca fuscata*)	0/42	34/42	22/50	14/37	7/31	2/9	4/29	1/30	0/40
		Environment inside Japanese macaques' breeding area									
		Diets for Japanese macaques				0/1		0/1			
		Water of a small pond			0/1	1/1	0/1	0/1	0/1	0/1	0/1
		Soil				1/1	1/1		0/1	0/1	
		Environment outside Japanese macaques' breeding area									
		Sewage from the pond			1/1						
O8	1	Zoo animal									
		Japanese macaque	0/42	0/42	21/50	24/37	11/31	0/9	0/29	0/30	0/40
Untypeable	1	Zoo animal									
		Japanese macque	0/42	0/42	7/50	9/37	0/31	0/9	0/29	2/30	2/40
		Brown bear	0/4	1/4	0/4				0/4	1/4	1/4
		Feral animal									
		Large Japanese field mouse (*Apodemus speciosus*)	0/7	0/15	1/12	0/15					
		Environment outside Japanese macaques' breeding area									
		Water of main pond in the zoo							1/1	0/1	0/1

[a] Each value indicates number of positive/number of examined.
[b] The same bear yielded the O5A organisms in August 1985, March and August 1986, and the organisms that could not be typed in December 1985, September and November 1986.

Japanese field mice. The relationship between zoo animals and feral rodents in the ecology of *Y. enterocolitica* warrants future investigation.

Since disease was not observed in the animals infected with *Y. enterocolitica*, it was unknown whether or not these bacteria were associated with disease in zoo animals. Because the O5A or O8 strains were not found in macaques at the beginning of the study, but suddenly appeared and then disappeared at the end of this investigation, these organisms might not be members of the normal intestinal flora of the Japanese macaques, although they were avirulent.

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