NOTE *Public Health*

Bacteriological Survey of Feces from Feral Pigeons in Japan

Chikako TANAKA[1)], Takayuki MIYAZAWA[1)], Masahisa WATARAI[2)] and Naotaka ISHIGURO[3)]*

[1)]Laboratories of Veterinary Public Health and [2)]Veterinary Microbiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, and [3)]Laboratory of Food and Environmental Hygiene, Faculty of Applied Biological Sciences, Gifu University, Gifu 501–1193, Japan

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ABSTRACT. Some public areas in Japan such as parks and gardens can be highly contaminated with pigeon feces. We examined levels of four bacterial contaminations in fecal samples from feral pigeons in 7 prefectures. We isolated *Salmonella* Typhimurium and *S.* Cerro from 17 (3.9%) of 436 samples, as well as *Mycobacterium* spp. including *M. avium-intracellulare* complex from 29 (19.0%) of 153 samples. The polymerase chain reaction detected *Chlamydia psittaci* and *C. pecorum* in 106 (22.9%) of 463 samples, but *E. coli* O-157 was not isolated from any of the samples. Our results indicate that pigeon feces are a source of several zoonotic agents for birds, animals and humans.

KEY WORDS: feces, pigeon, salmonella.

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Pigeons (*Columba livia*) are widely distributed in urban and rural areas of Japan, and come into close contact with humans in parks, temples, shrines, public gardens and railroad stations. A recent increase in the number of pigeons has raised public health concerns [7]. Pigeons are potential reservoirs for several pathogenic microorganisms, including *Chlamydia* spp., *Salmonella* spp. and *Cryptococcus*. In Japan, *S.* Typhimurium [5, 14, 15], *C. psittaci* [3, 6, 8] and *Mycobacterium* spp. [10, 11] have been isolated from feral pigeons and the frequency of *Salmonella* spp. and *Chlamydia* spp. is particularly high [1, 2, 12]. The presence of pigeon feces in public parks and railroad stations has contributed to the spread of infectious agents in the environment. We therefore extensively surveyed four pathogens (*Salmonella* spp., *Escherichia coli* 0-157, *Mycobacterium* spp. and *Chlamydia* spp.) in pigeon feces collected from public areas in Japan from 2003 to 2004.

Fecal samples (fresh or dry composite) were collected from 38 public areas in 7 prefectures (Hokkaido, Tokyo, Kanagawa, Gifu, Aichi, Osaka and Hiroshima) in Japan. Dry samples were placed in polyethylene bags, and fresh samples were placed in 15-m*l* tubes containing 1 m*l* of phosphate-buffered saline.

To isolate *Salmonella* spp., about 0.5 to 3 g of fecal samples were cultured in Hajna tetrathionate broth (Eiken, Tokyo, Japan) and then plated on DHL agar (Eiken) and on CHROMagar Salmonella (CHROMagar Microbiology, Paris, France). *Salmonella*-like isolates on DHL or CHROMagar plates were examined using TSI (Eiken) and LIM (Eiken) media and confirmed by slide and tube agglutination with commercial antisera (Denka Seiken, Tokyo, Japan). To isolate *E. coli* O-157, about 2 to 3 g of feces was cultured in mEC broth (Kyokutou, Tokyo, Japan) with novobiocin, and examined using a PATH-STIK KIT (Celsis Lumac, B. V. Landgraaf, The Netherlands). We finally confirmed the presence of *E. coli* O-157 by slide agglutination with a specific antibody (Denka Seiken). To isolate *Mycobacterium* spp. about 1 to 3 g of fecal samples were mixed with 0.1% acriflavine and 4% NaOH, and inoculated on Ogawa slant medium (Nissui, Tokyo, Japan). Colonies on the slants were visualized by Ziehl-Neelsen staining, and *Mycobacterium* spp. were identified using the DNA-DNA hybridization kit, DDH Mycobacteria (Kyokuto) and by sequencing of 16S rRNA fragment [17]. Total DNA for the polymerase chain reaction (PCR) was extracted from 0.2 g of pigeon feces using the QIAamp DNA Stool Minikit (QIAGEN, GmbH, Germany). Mycobacterial DNA was extracted from bacterial cells using 1% sodium dodecyl sulfate and several phenol-chloroform washes. To detect *Chlamydia* spp., nested PCR amplified the major outer membrane protein (MOMP) fragment using the primers CMGP-1F, CMGP-1R, CMGP-2F and CMGP-2R provided by Dr. H Fukushi of Gifu University. The PCR for *Chlamydia* spp. started with denaturation at 94°C for 5 min, followed by 40 amplification cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec, and ending with final extension at 72°C for 7 min. The primers for the first nested PCR were CMGP-1F and CMGP-1R, and those for the second were CMGP-2F and CMGP-2R. The PCR for *Mycobacterium* spp. was proceeded as described by Springer *et al.* [17]. To amplify the DNA fragment specific for the 16S rRNA of *Mycobacterium* spp., PCR included primers 264 and 285 [17]. The PCR amplified products from *Chlamydia* spp. and *Mycobacterium* spp., were sequenced using an ABI PRISM 310 Genetic Analyzer with the BigDye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, Foster City, CA). Each bacterium was genetically identified through DNA homology analyses of a nucleotide sequence database using the BLAST algorithms.

Table 1 shows the isolation frequency of *Salmonella* spp. We identified *S.* Cerro (7 strains), *S.* Typhimurium subsero-

* CORRESPONDENCE TO: ISHIGURO, N., Laboratory of Food and Environmental Hygiene, Faculty of Applied Biological Sciences Gifu University, Gifu, 501–1193, Japan.

Table 1. Detection frequency of organisms from pigeon feces

Prefecture	No. of sites	Detection frequency[a)]			
		Salmonella spp.	*Mycobacterium* spp.	*E. coli* 0–157	*Chlamydia* spp.[b)]
Hokkaido	6	6/30[c)]	0/25	0/29	28/35[i)]
Tokyo	7	1/16[c)]	0/25	0/12	14/23[i)]
Kanagawa	2	0/3	2/2[g)]	0/1	1/4[i)]
Gifu	16	9/276[d,e,f)]	24/56[g,h)]	0/24	41/290[i,g)]
Aichi	4	1/8[c)]	1/33[g)]	1 0/14	10/81[i,k)]
Osaka	2	0/29	2/11[g)]	0/27	11/29[i)]
Hiroshima	1	0/1	0/1	0/1	1/1[i)]
Total	38	17/436 (3.9%)	29/153 (19.0%)	0/108	106/463 (22.9%)

a) No. of positive samples / No. of samples examined. b) Genetical detection by PCR. c) *S.* Typhimurium, d) *S.* Cerro, e) Untypable strain in O4 group, f) Untypable strain in O8 group, g) *M. avium-intracellulare* complex, h) *M. terrae, M. gordonae, M. hiberniae, M. szulgai, M. porcinum, M. malmonse* or *M. selatum*, i) *C. psittaci*, g) *C. pecorum* (from two samples), k) *C. pecorum* (from one sample).

Table 2. Longitudinal investigation of *Salmonella* and *Chlamydia* spp. at city C in Gifu Prefecture

Species	Detection frequency					
	Sep. 2003	Nov. 2003	Dec. 2003	Apr. 2004	Jul. 2004	Total
Salmonella spp.	5 / 9[a)] (55.6%)	0 / 14 (1.6%)	3 /182[a,b)] (3.0%)	1 / 33[c)] (3.6%)	0 / 12 0%	9 / 250 (3.6%)
Chlamydia spp.	11 / 16[d,e)] (68.8%)	11 / 11[d)] (100%)	19 / 187[d)] (10.2%)	0 / 50 0%	0 / 26 (14.1%)	41 / 290 (14.1%)

a) *S.* Cerro, b) One untypable strain in O4 group, c) Untypable strain in O8 group, d) *Chlamydia psittaci*, e) *C. pecorum* (from two samples).

var Copenhagen (8 strains), and untypable strains in serogroups O4 (1 strain) and O8 (1 strain) from 17 isolates (3.9%) in 436 fecal samples. *S.* Typhimurium strains were isolated from railroad stations and public parks in Hokkaido (6/30: 20.0%), from railroad stations in Tokyo (1/16: 6.3%) and from Buddhist temples in Aichi Prefecture (1/81: 1.2%). *S.* Cerro strains were isolated from railroad stations and public parks in city C in Gifu Prefecture during a longitudinal survey from September 2003 to July 2004, (Table 2). The isolation frequency of *S.* Cerro was 55.6% in September 2003 and 1.1% in December 2003.

All 108 fecal samples examined were negative for *E. coli* O-157 (Table 1).

We found that 19.0% (29/153) of the fecal samples contained *Mycobacterium* spp. The following *Mycobacterium* spp. were identified by DNA sequencing and homology analyses: *M. avium-intracellulare* complex (MAC, 10 strains), *M. terrae* (9 strains), *M. gordonae* (4 strains), *M. szulgai* (2 strains), *M. hiberniae* (2 strains), *M. porcinum* (1 strain), and 1 unidentifiable strain (*M. malmonse* or *M. selatum*). The MAC strains were detected in the samples from Kanagawa, Gifu, Aichi and Osaka Prefectures (Table 1). Several *Mycobacterium* strains were predominant in fecal samples from Gifu Prefecture, but the prevalence did not differ between dry and fresh fecal samples from any area. We identified *Chlamydia* spp in 106 (22.9%) of 463 fecal samples by nested PCR. *C. psittaci* (103 strains) and *C. pecorum* (3 strains) were confirmed by sequencing the MOMP fragment (Table 1). *C. psittaci* was detected in the samples from all 7 prefectures examined. Only three PCR products were identified as *C. pecorum*, and these were from Gifu and Aichi Prefectures. *C. psittaci* was frequently detected in the fecal samples from a longitudinal survey of city C in Gifu Prefecture (Table 2). The high frequency of *C. psittaci* continued until Dec. 2003, but had decreased by Apr. 2004.

An important agent of food poisoning in humans is *S.* Typhimurium subserovar Copenhagen [9] found in the present study. This organism has frequently been isolated from pigeons in Japan [14, 15]. *S.* Cerro has been isolated from chickens in Japan [16], although the serovar has been rarely detected in pigeons. We found *S.* Cerro isolates twice during the survey period. This serovar might spread among pigeons in city C over a short period. Although food poisoning caused by *S.* Cerro was not recorded in city C during 2003, the spread of *S.* Cerro among pigeons should be a health concern for human health, because this serovar has been isolated from the case of food poisoning in Japan [9]. The risk of secondary infection with *Salmonella* spp. can be reduced by removing piles of pigeon feces. In the present study, MAC strains were isolated from pigeon feces obtained from Kanagawa, Gifu, Aichi and Osaka Prefectures. MAC can cause disease in humans and other animals [4, 13] and dry dust from pigeon feces contaminated with such strains in public places might be a source of infection for birds and humans.

We detected *C. psittaci* in all prefectures examined in the present study. The isolation frequency of this organism in fecal samples from the same public park or railroad station differed even when the sampling sites were very close (data not shown). These results suggest that *C. psittaci* infection of pigeons is rare or arises only as a result of very close contact, for example, in nests where the risk of infection with various fecal pathogens increases. The present results suggest that pigeon feces represent a source of several zoonotic agents. We believe that continuous surveys can estimate, and thus help to minimize the risk of humans contracting diseases from pigeons.

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