***Salmonella* Isolates from Cloacal Swabs and Footpads of Wild Birds in the Immediate Environment of Tokyo Bay**

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ABSTRACT. A total of 328 cloacal swabs and 163 footpads of wild birds were investigated for the presence of salmonellae. All 19 isolates from cloacal swabs were serotyped as *Salmonella* Typhimurium susceptible to all five conventional antimicrobial agents (ampicillin, chloramphenicol, streptomycin, oxytetracycline and nalidixic acid) tested. In contrast, 15 salmonellae isolated from footpads included *S. Muenhen*, *S. Virchow*, *S. Bareily* and *S. Bovismorbificans*, including *S. Typhimurium*; these non-*Salmonella* Typhimurium isolates showed multiple drug resistance.

KEY WORDS: *Salmonella*, wild bird.

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Wild birds may carry or be infected with bacteria that can adversely affect the health of livestock animals and human beings, since birds inhabit places where human beings live, migrate between waste treatment plants, grain stores and livestock pastures, making them important vehicles for the spread of zoonotic infections. Almost all *Salmonella enterica* serotypes are regarded as food-borne pathogens that can cause diarrhea in humans [10]. Therefore, to analyze the role of wild birds as reservoirs of *Salmonella* spp., we investigated the prevalence of these pathogens and analyzed their serotypes, antimicrobial susceptibility to several conventional drugs, and the genetic polymorphism of the isolated strains.

A total of 328 cloacal swabs and 163 footpad samples were collected from 55 species of wild birds that had been captured (due to falling, hunted by carnivorous animals, fallen from a nest, killed by hunters, etc.) between 2003 and 2006. The collection area was in the immediate environment of Tokyo Bay, mainly in Tokyo and Chiba Prefectures, Japan. Samples of footpads and main organs, such as the brain, lung, kidney, and liver, were also collected if the condition of the bird allowed it. The swab samples were enriched in Trypticase Soy Broth (TSB, Eiken, Japan) at 37°C for 18 to 20 hr. A portion of the enrichment culture of TSB was transferred to both Rappaport (Nissui, Japan) and HTT (Eiken, Japan) and incubated at 42°C for 24 hr, then continuously subcultured in the same selective medium for a week. The selective enrichment cultures were streaked on both ES *Salmonella* II (Eiken Kizai, Tokyo, Japan) and Chromo-*Salmonella* (CHROMO Agar, Paris) solid media, and incubated according to their directions for use. Potential salmonella colonies were confirmed by both polyvalent antisera against salmonella somatic antigens (Denka Seiken, Osaka, Japan) and salmonella common PCR, as described by Carli *et al.* [1]. The serotypes of the isolates were identified by flagellin-PCR [2, 3] and conventional serological

tests using anti-somatic antisera (O) as was each phase of anti-flagellin (H1 and H2) sera obtained from Denka Seiken Co., Ltd. To define a strain resistant to an antimicrobial agent in salmonella, the break point concentration of the drug was looked up in DANMAP 2001 (<http://www.danmap.org/>). The break point of each drug to salmonella were as follows: ampicillin: 25 µg/ml, oxytetracycline: 12.5 µg/ml, chloramphenicol: 25 µg/ml, streptomycin 25 µg/ml and nalidixic acid: 25 µg/ml.

Salmonella spp. were isolated from 19 of 328 cloacal swabs (5.8%); salmonella serotype was limited to *S. Typhimurium* (Table 1). Of 19 salmonella-isolated birds, the same salmonella were isolated in their major abdominal organs and heart in four birds classified into three species (2 of feral pigeon, Eurasian Tree Sparrow and Black-headed Gull). All four birds were flightless due to disease. All *S. Typhimurium* isolates from cloacal swabs were sensitive to the antimicrobial agents tested (ampicillin, chloramphenicol, streptomycin, oxytetracycline and nalidixic acid) and the isolates were classified into 3 groups by PFGE (picture not shown). Interestingly, 12 of 19 *S. Typhimurium* isolates did not produce H₂S on the DHL agar medium. On the other hand, salmonellae other than *S. Typhimurium* isolated from footpads were *S. Muenhen*, *S. Virchow*, *S. Bareily* and *S. Bovismorbificans* (Table 1). These non-*Salmonella* Typhimurium isolates showed multiple resistance to ampicillin, oxytetracycline, and/or streptomycin. Although three strains (from a Brown-eared bulbul, a Japanese tit and a Greater scoup) of *S. Typhimurium* from cloacal swabs possessed either *artA* [9] or a partial gene of definitive type 104 (DT104) [7], the majority of *S. Typhimurium* strains from wild birds did not appear to be classifiable as type DT104. We could not characterize the phage types of isolates; the former three strains should be examined for phage typing with DT104. Other reports suggest that type DT104 in wild birds is rare: DT40 and DT50 predominate in finches and

Table 1. Isolation of *Salmonella enterica* from cloacal swabs and footpads of wild birds between 2003 and 2006

Order of bird	Bird (common name)	Bird (nomenclature)	Cloacal swab sample			Footpad sample			
			No. tested	No. salmonella isolated	Serotype	No. tested	No. salmonella isolated	Serotype	Antibiotic resistance ^{c)}
Columbiformes	Feral Pigeon	<i>Columba livia</i>	52	2	<i>S. Typhimurium</i>	35	2* ^{b)} 1	<i>S. Typhimurium</i> <i>S. Virchow</i>	ABPC, OTC, SM
	Oriental Turtle-dove	<i>Streptopelia orientalis</i>	37			14			
	White-bellied Green pigeon	<i>Treron sieboldii</i>	5	1(1) ^{a)}	<i>S. Typhimurium</i>	2			
	Rock Dove	<i>Clombia livia domestica</i> LINNE	1			1			
Passeriformes	Eurasian Tree Sparrow	<i>Passer montanus</i>	35	2(1)	<i>S. Typhimurium</i>	18	1*	<i>S. Typhimurium</i>	
	Meadow Bunting	<i>Emberiza cioides</i>	6	1(1)	<i>S. Typhimurium</i>	2			
	White-cheeked starling	<i>Sturnus cineraceus</i>	13			8			
	Japanese White-eye	<i>Zosterops japoniceus</i>	9			5	1	<i>S. Typhimurium</i>	OTC, CP
	Brown-eared Bulbul	<i>Ixos amaurotis</i>	15	1	<i>S. Typhimurium</i>	8	1*	<i>S. Typhimurium</i>	
	Blue-and-white Flycatcher	<i>Cyanoptila cyanomelana</i>	2			2			
	Crey-capped Green finch	<i>Carduelis sinica</i>	3			1			
	White Wagtail	<i>Motacilla alba</i>	3			2			
	Japanese Robin	<i>Eriothacus akahige</i>	1			0			
	Dusky Thrush	<i>Turdus naumanni</i>	5	1(1)	<i>S. Typhimurium</i>	0			
	White Thrush	<i>Zoothera dauma</i>	1			0			
	Japanese Tit (Great Tit)	<i>Parus major</i>	4	1	<i>S. Typhimurium</i>	1	1*	<i>S. Typhimurium</i>	
	Varied Tit	<i>Parus varius</i>	2			0			
	Coal Tit	<i>Parus ater</i>	1			1			
	Barn Swallow	<i>Hirundo rustica</i>	15			10			
	Asian House Martin	<i>Delichon dasypus</i>	0			1			
	Large-billed Crow	<i>Corvus urbicamacrorhyncos</i>	10			5			
	Carion Crow	<i>Corvus corore orientails</i>	1			1			
	Azure-Winger Magpie	<i>Cyanoptica cyana</i>	3			2			
Charadriiformes	Black-headed Gull	<i>Larus ridibundus</i>	9	2(2)	<i>S. Typhimurium</i>	6	2* 1	<i>S. Typhimurium</i> <i>S. Bareilly</i>	ABPC, OTC
	Herring Gull	<i>Larus argentatus</i>	3			3	1	<i>S. Bovismobificans</i>	ABPC,OTC,CP
	Black-tailed Gull	<i>Larus crassirostris</i>	5			1			
	Eurasian Wood cock	<i>Scolopax rusticola</i>	1			0			
	Ruddy Turnstone	<i>Arenaria interpres</i>	1			1			
Anseriformes	Spot-billed Duck	<i>Anas poecilorhyncha</i>	10	4(4)	<i>S. Typhimurium</i>	1	1*	<i>S. Typhimurium</i>	
	Greater Scaup	<i>Aythya marila</i>	5	1	<i>S. Typhimurium</i>	1	1*	<i>S. Typhimurium</i>	
	Common Teal	<i>Anas crecca</i>	4			1			
	Domestic Duck	<i>Anas platyrhynchos var-domesticus</i>	1			1			
	Eurasian Wigeon	<i>Anas strepera</i>	1			0			
	Tufted Duck	<i>Aythya fuligula</i>	5			2			
	Common Pochard	<i>Aythya ferina</i>	2	1(1)	<i>S. Typhimurium</i>	0			
	Mute Swan	<i>Cygnus olor</i>	1			0			
	Eurasian Wigeon	<i>Anas penelope</i>	1			1			
Ciconiiformes	Black-crowned Night-Heron	<i>Nycticorax</i>	6	1	<i>S. Typhimurium</i>	2			
	Great Egret	<i>Egretta alba</i>	3			2			
	Intermediate Egret	<i>Egretta intermedia intermedia</i>	1			1			
	Little Egret	<i>Egretta garzetta garzetta</i>	1			0			
	Cattle Egret	<i>Bubulcus ibis coromandus</i>	1			1			
	Grey Heron	<i>Ardea cinerea jouyi</i>	4			3			
Procellariiformes	Streaked Shearwater	<i>Calonectris leucomelas</i>	8			3			
	Short-tailed Shearwater	<i>Puffinus tenuirostris</i>	5			0			
Gruiformes	Common Moorhen	<i>Gallinula chloropus, india</i>	1			0			
	Common Coot	<i>Fulica atra atra</i>	1			1			
Pelecaniformes	Great Cormorant	<i>Phalacrocorax carbo hanedae</i>	11	1(1)	<i>S. Typhimurium</i>	9	1	<i>S. Muenchen</i>	ABPC,OTC,SM
	Honsyu Ural Owl	<i>Strix uralensis hondoensis</i>	2			0			
	Brown Hawk Owl	<i>Ninox scutulate</i>	1			0			
	Japanese scops Owl	<i>Otus lempijisemitorques</i>	1			1	1	<i>S. Typhimurium</i>	
Galliformes	Ukokkei Fowl (Silky)	<i>Gallus gallus var. domesticus</i>	1			0			
	Domestic Fowl	<i>Gallus gallus domesticus</i>	4			1			
	Peach-faced lovebird	<i>Agapornis roseicollis</i>	3			3			
Cuculiformes	Oriental Cuckoo	<i>Cuculus saturatus horsfieldi</i>	1			0			
Total			328	19		163	15		

a) Number in parenthesis are number of isolates did not produce H₂S on the DHL agar medium.

b) * indicates the number of strains of the same *Salmonella* serotype that were isolated from the same bird's cloacal sample.

c) Antimicrobial abbreviations: ABPC: ampicillin, OTC: oxytetracycline, NA: nalidixic acid, CP: chloramphenicol, SM: streptomycin.

sparrows, DT41 and DT195 in gulls, and DT72 and DT99 in pigeons [6, 8]. Although phage typing was not carried out, recent *S. Typhimurium* isolates from wild cranes that migrate among East Asian countries and Siberia could be not DT104 because of the antimicrobial susceptibility

results [4]. The strains of *S. Typhimurium* phage type DT104 might not spread extensively among wild birds.

Although the behavior and feeding habits of wild birds clearly influence the likelihood of their being infected with salmonella, salmonella were isolated from almost all orders

of birds. Ducks were infected with salmonella with a notably high frequency. Only the *S. Typhimurium* serotype was isolated from the cloacal swabs, and other reports also describe the same results as ours [4–6, 8]. Although the total number of bird samples were not described, 441 of 470 salmonella isolates from wild birds were *S. Typhimurium* in Norway between 1969 and 2000 [8], all 67 salmonella isolates from wild cranes were *S. Typhimurium* in Japan [4]. On the other hand, the number of isolates was limited. *S. Muenhen* and *S. Enteritidis* were found in 7 isolates of salmonella from 82 wild birds in Spain [5]. Other than *S. Typhimurium*, *S. Muenhen* (from a Great cormorant), *S. Virchow* (from a Feral pigeon), *S. Bareilly* (from a Black-headed gull) and *S. Bovismorbificans* (from a Black-headed gull) were also isolated from the footpads. We think these salmonella might not infect birds but might be carried from farm to farm and other places when the birds migrate. Since these non-*S. Typhimurium* isolates from footpads showed multiple resistance to antimicrobial agents, the strain may have originated in an animal herd or human being, since the livestock industry often apply antimicrobial agents to treat disease, in the same way as for human medical treatment, with the result that antimicrobial-resistant strains would be selected for and amplified in the intestine.

The salmonella isolation rates in this study were slightly lower than described in other reports [5, 6]. A few reasons were considered: for example, limited species of wild birds were investigated [5], and different volumes of intestinal samples were examined. Pennycott *et al.* investigated much greater volumes of intestinal content by dissection [6].

Another reason is that the geographical conditions may be involved. However, our study revealed no changes in the salmonella-isolation rate through the seasons.

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