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A Survey for *Yersinia pseudotuberculosis* in Migratory Birds in Coastal Japan

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ABSTRACT: *Yersinia pseudotuberculosis* was isolated from three specimens of two species of birds, the black-faced bunting (*Emberiza spodocephala*) and pied wagtail (*Motacilla alba*), of 528 specimens of birds examined from coastal regions in Japan. The two isolated strains of *Y. pseudotuberculosis* were identified as serovar 4b and serovar 3. This is the first isolation of *Y. pseudotuberculosis* from birds in Japan. *Yersinia enterocolitica* was isolated from three specimens of the pied wagtail, one specimen of the reed bunting (*Emberiza schoeniclus*) and one specimen of the rustic bunting (*Emberiza rustica*). *Yersinia frederiksenii* was isolated from two specimens of the gray-rumped sandpiper (*Heteroscelus brevipes*). *Yersinia intermedia* was isolated from one specimen of the pied wagtail.

Key words: *Yersinia pseudotuberculosis*, *Yersinia frederiksenii*, *Yersinia intermedia*, migratory birds, pied wagtail, *Motacilla alba*, survey.

In Europe and North America, free-living birds are thought to be significant reservoirs in the maintenance and dissemination of *Yersinia pseudotuberculosis* because of their great mobility and high prevalence of infection (carriers) (Hubbert, 1972; Mair, 1973). Alternatively, *Y. pseudotuberculosis* has not been isolated from free-living birds in Japan, although they have been surveyed previously (Kato et al., 1985). The present study was initiated to determine if *Y. pseudotuberculosis* could be isolated from free-living birds in Japan.

From August 1987 to August 1988, feces were collected by cloacal swab from 528 free-living adult birds representing 15 species (Table 1) when the birds were captured for an ongoing banding study. Samples were obtained at three estuary localities near Ichikawa City (35°44'N, 139°54'E) and Kisarazu City (35°21'N, 139°55'E) in Chiba Prefecture and Kamisu

Town in Ibaragi Prefecture (35°52'N, 140°38'E), and a lagoon near Toyosaka City in Niigata Prefecture (38°14'N, 139°34'E), Japan.

Approximately 0.1 g of fecal material was immediately inoculated into 3 ml of phosphate buffered solution containing 15 M Na₂PO₄ and 15 M KH₂PO₄ at pH 7.6, and incubated at 4 C for 3 wk for *Yersinia* enrichment. After alkali treatment according to the method of Auliso et al. (1980), 0.1 ml of sample suspension was spread on Cefsulodin-Irgasan-Novobiocin agar plates of Devenish and Schiemann (1981) and salmonella-shigella agar (Eiken Chemical Company, Hongo, Tokyo, Japan). These plates were incubated at 25 C for 48 hr. Suspected colonies on these plates were submitted for identification. The method of biochemical identification was the same as Kato et al. (1985). Serotyping of *Y. pseudotuberculosis* strains isolated was accomplished using slide agglutination with the rabbit O antisera against serovars 1a, 1b, 2a, 2b, 3, 4a, 4b, 5a, 5b and 6 prepared according to the method of Tsubokura et al. (1970). Serotyping of the *Y. enterocolitica* strains was accomplished using slide agglutination with commercial rabbit antisera against O1, O2, O3, O5, O8 and O9 (Denkaseiken Company, Nihonbashi, Tokyo, Japan) prepared according to the method of Winblad et al. (1966).

The data on isolations of *Yersinia* sp. are shown in Table 1. *Yersinia* spp. was isolated from five of 15 species of birds examined. *Yersinia enterocolitica* was isolated from five birds, *Yersinia frederiksenii* from two birds, *Yersinia intermedia* from one bird and *Y. pseudotuberculosis* from three birds. Two of three *Y. pseudotuberculosis* isolated strains were identified as

TABLE 1. Isolation of *Yersinia* spp. from free-living birds in Japan.

Bird species	Locality	Number of birds examined	Number of birds yielding respective species of <i>Yersinia</i> ^a			
			<i>Y. enterocolitica</i>	<i>Y. frederiksenii</i>	<i>Y. intermedia</i>	<i>Y. pseudotuberculosis</i>
Black-faced bunting (<i>Emberiza spodocephala</i>)	Toyosaka	38	0	0	0	2 (4b, 4b) ^b
Reed bunting (<i>Emberiza schoeniclus</i>)	Toyosaka	35	1 (NG)	0	0	0
Rustic bunting (<i>Emberiza rustica</i>)	Toyosaka	45	1 (NG)	0	0	0
Gray-rumped sandpiper (<i>Heteroscelus brevipes</i>)	Kisarazu	95	0	2	0	0
Pied wagtail (<i>Motacilla alba</i>)	Ichikawa	73	3 (O5, NG, NG)	0	1	1 (3)

^a *Yersinia* sp. was not isolated from 23 Schrenk's reed warblers (*Acrocephalus bistrigiceps*), 15 Japanese reed buntings (*Emberiza yessoensis*), 14 Japanese marsh warblers (*Megalurus pryoti*), 12 oriental great reed warblers (*Acrocephalus orientalis*), 10 fan-tailed warblers (*Cisticola juncidis*) collected at Kamisu Town, and 66 little terns (*Sterna albigrons*), 27 terek sandpipers (*Xenus cinereus*), 43 dunlins (*Calidris alpina*), 12 turnstones (*Arenaria interpres*), and 20 red-necked stints (*Calidris ruficollis*) collected at Kisarazu City.

^b Serogroups of isolated strains are in parentheses. NG means not identified with antisera against *Y. enterocolitica* O1, O2, O3, O5, O8 and O9.

serovar 4b, the other was serovar 3. All of the species of birds that we examined were insectivorous except for the little tern (*Sterna albigrons*) which is piscivorous.

The present isolations of *Y. pseudotuberculosis* from birds are the first reported in Japan. *Yersinia pseudotuberculosis* 4b organisms were isolated from two black-faced buntings (*Emberiza spodocephala*). These organisms have been isolated previously from human patients, dogs, cats (Fukushima et al., 1987), pigs (Tsubokura et al., 1970) and a small wild rodent (Kaneke and Hashimoto, 1981). These have been isolated not only in Japan, but from other regions of the world. In Japan, the black-faced buntings migrate between southern Sakhalin or northern Hokkaido Island and Honshu, Shikoku or Kyushu Island. Therefore, the birds with *Y. pseudotuberculosis* 4b might have been infected with the organism in Japan. Kato et al. (1985) reported no isolations of *Y. pseudotuberculosis* from 500 wild birds living in mountain regions of Japan. The two *Y. pseudotuberculosis*-positive species examined were living in coastal regions. It

was not clear if these birds were especially susceptible to serovar 4b organisms since both birds were the same species and captured at the same localities.

Black-faced buntings are partially and pied wagtails (*Motacilla alba*) are entirely insectivorous. Furthermore, all of the *Y. enterocolitica*-, *Y. frederiksenii*- or *Y. intermedia*-positive birds were insectivorous. It is unknown, however, whether or not these organisms originate from insects or other items, since *Yersinia* sp. was not isolated from 176 specimens representing nine species of other birds including the Schrenk's reed warbler (*Acrocephalus bistrigiceps*), oriental great reed warbler (*Acrocephalus orientalis*), Japanese reed bunting (*Emberiza yessoensis*), Japanese marsh warbler (*Megalurus pryoti*), fan-tailed warbler (*Cisticola juncidis*), terek sandpiper (*Xenus cinereus*), dunlin (*Calidris alpina*), red-necked stint (*Calidris ruficollis*) and turnstone (*Arenaria interpres*).

Since clinical disease was not found in the birds examined in the present study, importance of *Yersinia* spp. as a disease of

Japanese birds was still unclear. However, these birds apparently can serve as a reservoir for this infection.

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