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virF-Positive *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* Found in Migratory Birds in Sweden†

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During spring and autumn migrations, 468 fecal samples from 57 different species of migratory birds were collected in Sweden. In total, *Yersinia* spp. were isolated from 12.8% of collected samples. The most commonly found species was *Yersinia enterocolitica*, which was isolated from 5.6% of all collected samples, followed by *Y. intermedia* (3.8%), *Y. frederiksenii* (3.0%), *Y. kristensenii* (0.9%), *Y. pseudotuberculosis* (0.6%), and *Y. rohdei* (0.4%). The pathogenic, *virF*-positive *Y. pseudotuberculosis* strains were recovered from three thrushes. These strains belonged to the same bioserotype, 1/O:2, but had two different profiles as determined by pulsed-field gel electrophoresis with *NotI* and *SpeI* enzymes. In addition, 10 *Y. enterocolitica* strains, all from barnacle geese, belonged to bioserotype 3/O:3, which is associated with human disease. Two of the strains were pathogenic, carrying the *virF* gene on their plasmids. All pathogenic *Y. pseudotuberculosis* and *Y. enterocolitica* strains were recovered during the spring, and as the birds were caught during active migration they likely became infected at an earlier stage of the migration, thus potentially transporting these bacterial pathogens over long geographical distances.

Yersinia pseudotuberculosis and *Y. enterocolitica* are two species that are pathogenic for both humans and animals. While they have a nearly worldwide distribution (19), outbreaks of infection with these pathogens are rare or infrequent in most developed countries, except for Japan, where several outbreaks of *Y. pseudotuberculosis* infection have been reported (15, 26), and Finland, where six large outbreaks of *Y. pseudotuberculosis* infection have recently occurred (2). The full epidemiological pathways leading to infection in humans have not yet been elucidated, but both mammals and birds are reported to be reservoirs for these bacteria (19, 20; N. H. Harcourt-Brown, Letter, Vet. Rec. **102**:315, 1978). There is a predominance of isolation of pathogenic *Y. pseudotuberculosis* from avian reservoirs over that of *Y. enterocolitica* (1, 19), and wild birds are thought to be a significant reservoir for *Y. pseudotuberculosis* (10, 11, 31). If pathogenic species of *Yersinia* are common in wild bird populations, this creates a potential for transmission of bacteria to humans and animals. Migratory birds could potentially carry bacteria along the migratory routes, allowing for dispersal of pathogenic *Yersinia* spp. over large geographical areas (7, 11, 14, 21). This could, in part, explain reports of *Y. pseudotuberculosis* and *Y. enterocolitica* infections on different continents (13, 16). The only existing data on the occurrence of *Y. pseudotuberculosis* in different migratory birds come from a survey conducted at a coastal site in Japan (13). No comparable study has been carried out to determine the occurrence of

Y. pseudotuberculosis in and the risk of yersiniosis arising from wild birds in Europe.

Y. pseudotuberculosis is a relatively homogenous species that is classified into four biotypes and over 20 different serotypes, each type including pathogenic strains. In contrast, only a few combinations of *Y. enterocolitica* bioserotypes are classified as pathogenic to humans or animals (4). Virulence properties of *Y. pseudotuberculosis* and *Y. enterocolitica* strains can be studied with a PCR assay targeting the virulence genes on the pYV plasmid (25). DNA-based molecular methods, such as pulsed-field gel electrophoresis (PFGE), provide sensitive assays for subtyping of *Y. pseudotuberculosis* and *Y. enterocolitica* strains of the same bioserotypes (8, 17, 27). The present study investigates the occurrence of different *Yersinia* species in migratory birds from Sweden, providing essential data on a public health issue. We specifically look at the occurrence of pathogenic *Y. pseudotuberculosis* and *Y. enterocolitica* in the feces of birds, as well as at the virulence properties of isolated *Yersinia* species. PFGE combined with restriction cleavage with different enzymes was used to genotype *Y. pseudotuberculosis* and *Y. enterocolitica* isolates of the same bioserotypes, enabling us to gather information on the genetic diversity of *Yersinia* spp. in wild bird populations.

MATERIALS AND METHODS

Sampling. Two locations on two different islands in the Baltic Sea in southeast Sweden were sampled: the Ottenby Bird Observatory (56°12'N, 16°24'E) on the southernmost point of Öland Island (15 March to 15 November 2000) and the coastal shore of the southern tip of Gotland Island (April 2000). Passerine birds were trapped with mistnets and Helgoland traps (5), and shorebirds were trapped with Ottenby funnel traps (5). Captured birds were banded, weighed, and measured, and their ages were determined according to differences in

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feather shapes and extents of wear (3, 28, 33). Three different approaches were used to obtain fecal samples, depending on the sizes of the trapped birds. Smaller birds were put one by one into a dark box with a clean sheet of paper at the bottom. Large birds, i.e., those with a body mass exceeding 250 g, were sampled by insertion of a sterile swab 1 to 2 cm into the cloaca. Samples from barnacle geese were taken by collecting fresh droppings from geese flocks grazing at the coastal meadows on Gotland Island. Sterile cotton swabs were used to collect fecal material from fresh goose droppings. Care was taken not to touch the surrounding grass. Samples were placed individually into a charcoal transport medium (Transwab; BioDisc, Solna, Sweden) and stored at 4 to 8°C for 2 to 7 days until cultivation.

Isolation and identification. Samples were cultivated at the laboratory of the Department of Food and Environmental Hygiene at the University of Helsinki, Finland. Before direct plating, 5 ml of phosphate-buffered saline (supplemented with 1% mannitol and 0.15% bile salts according to International Organization for Standardization protocol ISO/DIS 10273) enrichment broth was added to each tube containing fecal samples. For cold enrichment, the tubes with the supplemented saline were incubated at 4°C and studied after 7, 14, and 21 days. For alkali treatment, 0.5 ml of the sample was mixed with 4.5 ml of 0.25% KOH solution for 20 s before being streaked onto CIN agar (*Yersinia*-selective agar base; Oxoid, Basingstoke, United Kingdom). This method was used after 7, 14, and 21 days of cold enrichment. CIN agar plates were used to isolate *Yersinia*, and these plates were incubated at 30°C for 18 to 20 h. To recover *Y. pseudotuberculosis*, a further incubation of the agar plates at 22°C for 24 h was used. Up to five whole small (diameter, <1 mm) colonies with typical a bull's eye appearance (deep red centers surrounded by outer transparent zones) on each CIN agar plate were taken and streaked onto blood agar plates for pure culture. One colony from the blood agar was inoculated onto a urea agar slant (Difco, Detroit, Mich.) and incubated for 24 h at 30°C. Isolates showing urea hydrolysis were further identified by using the Api 20E test (Biomerieux, Marcy l'Etoile, France).

Putative *Y. pseudotuberculosis* isolates were further biotyped according to results of reactions with raffinose, melibiose, and citrate (35). *Y. enterocolitica* isolates were biotyped based on assessment of the following criteria: pyrazinamidase (PYZ) and Tween-esterase activity; esculin hydrolysis; salicin, xylose, and trehalose acidification; and indole production (37). *Y. pseudotuberculosis* and *Y. enterocolitica* isolates were serotyped with slide agglutination by using a commercial serum agglutination test with O:1 to O:6 and O:3, O:5, and O:9 antisera (Denka Seiken, Tokyo, Japan), respectively.

Virulence-associated tests. The virulence plasmids of *Y. pseudotuberculosis* and *Y. enterocolitica* isolates were studied with a PCR targeting the *virF* gene on the plasmids by following the modified protocol of Nakajima et al. (25). Briefly, five colonies from blood agar were suspended in 100 µl of water in one tube. DNA was extracted by boiling of the suspension for 10 min, followed by centrifugation at full speed (16,000 × g) for 3 min. Two microliters of the supernatant was used as a template in the PCR. The PCR mixture volume was 50 µl, and the mixture contained 1 U of Dynazyme DNA polymerase (Finnzymes, Espoo, Finland), 200 µM (each) deoxynucleoside triphosphates (Finnzymes), and 0.3 µM (each) primers (Pharmacia Biotech, Vantaa, Finland). PCR was performed in a 16-well PTC-150 thermal cycler (MJ Research, Watertown, Mass.). The thermal profile procedure consisted of an initial denaturation step at 94°C for 60 s followed by 29 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s, and extension at 72°C for 2 min. After completion of the cyclic reactions, a final extension step at 72°C for 5 min was added. The sizes of the amplified PCR products were determined in a 0.8% agarose gel (size, 19.5 by 20 cm; 80 V for 120 min) by comparison with DNA molecular weight marker VI (Boehringer Mannheim, Mannheim, Germany). Virulence characteristics of *Y. enterocolitica*—i.e., reactions of PYZ, esculin, salicin (37), and calcium dependence and Congo red absorption on Congo red-magnesium oxalate agar (CR-MOX) (30)—were also tested. On CR-MOX agar, the pYV-positive *Yersinia* isolates produced small orange colonies (CR-MOX positive) and negative isolates produced large colorless colonies (CR-MOX negative) when incubated at 37°C for 24 h.

PFGE. DNA extraction was performed according to the method of Niskanen et al. (27). Briefly, a single colony grown on blood agar was inoculated into 5 ml of tryptic soy broth and incubated for 18 h at room temperature. The cells from 2 ml of tryptic soy broth were washed once in 5 ml of cold PIV (10 mM Tris [pH 7.5], 1 M NaCl) and then resuspended in 750 µl of cold PIV. Next, 0.5 ml of cell suspension was mixed with 0.5 ml of 2% (wt/vol) low-melting-temperature agarose (InCert agarose; BioWhittaker Molecular Applications, Rockland, Maine) and cast in GelSyringe dispensers (New England Biolabs, Beverly, Mass.). The plugs were lysed at 37°C with shaking for 3 h in 2.5 ml of lysis solution (6 mM Tris [pH 7.5], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroyl sarcosine, 20 µg of RNase/ml, 1 mg of lysozyme/ml) and completed with a 1-h ESP (0.5 M EDTA [pH 8.0], 10% sodium

lauroyl sarcosine, 100 µg of pronase/ml) wash at 50°C. The plugs were stored at 4°C in fresh ESP solution. Before digestion, pronase was inactivated with Pefablock SC (AEBFS; Roche, Mannheim, Germany). Restriction endonuclease digestion was performed according to the manufacturer's instructions. DNA of *Y. enterocolitica* was digested with *NotI* enzyme (New England Biolabs). In addition, 10 isolates of *Y. enterocolitica* bioserotype 3/O:3 were also digested with *SpeI* enzyme (New England Biolabs). The *Y. pseudotuberculosis* isolates were digested both with *NotI* and with *SpeI* separately, and up to five different colonies from each positive bird were taken as replicates. The samples were electrophoresed at 12°C through a 1% (wt/vol) agarose gel (SeaKem Gold; FMC Bioproducts) in a 0.5× Tris-borate-EDTA buffer (Amresco, Solon, Ohio) at 200 V by using a Gene Navigator system (Pharmacia, Uppsala, Sweden) with a hexagonal electrode. Interpolation protocols with ranges from 1 to 18 s for 20 h for *NotI* and from 1 to 15 s for 18 h for *SpeI* were used. A midrange PFGE marker (New England Biolabs) was used for fragment size determination. The gels were stained for 30 min in 1 liter of running buffer containing 50 µl of ethidium bromide (10 mg/ml) and photographed under UV light with an Alpha Imager 2000 documentation system (Alpha Innotech, San Leandro, Calif.) by following standard procedures. For determination of *NotI* and *SpeI* profiles, the banding patterns were interpreted visually. Profiles were considered to be different when a one-band difference was observed.

RESULTS

We analyzed the occurrence of *Yersinia* spp. in 468 samples representing 57 different species of birds. In total, *Yersinia* spp. were isolated from 12.8% of all samples collected (Table 1). The most frequently encountered species was *Y. enterocolitica* (isolated from 5.6% of all collected fecal samples), followed by *Y. intermedia* (3.8%), *Y. frederiksenii* (3.0%), *Y. kristensenii* (0.9%), *Y. pseudotuberculosis* (0.6%), and *Y. rohdei* (0.4%). Most isolated strains appeared in the spring migration sample set; only five *Yersinia* isolates were obtained during the autumn sampling. Further, the autumn samples consisted of only non-pathogenic strains: two strains of apathogenic *Y. enterocolitica* biotype 1A, two strains of *Y. frederiksenii*, and one *Y. intermedia* strain.

The three *Y. pseudotuberculosis* strains were obtained from three birds belonging to two species of thrushes, song thrush (*Turdus philomelos*) and redwing (*Turdus iliacus*), sampled during the spring migration in April. *Y. pseudotuberculosis* strains were of bioserotype 1/O:2, and no variation in their biochemical characteristics was present (Table 2). When virulence-associated properties of the strains were examined on CR-MOX agar plates, all of the strains showed calcium dependence and Congo red absorption. Moreover, all strains were *virF*-positive on PCR. The isolates of *Y. pseudotuberculosis* from the two bird species yielded two different PFGE profiles with both the *NotI* and the *SpeI* enzymes (Fig. 1), and this difference was consistent also when multiple *Y. pseudotuberculosis* colonies from the same bird were studied, meaning that the redwing and the song thrush isolates belonged to different *Y. pseudotuberculosis* genotypes.

In bird species of which more than 10 individuals were sampled, the carriage of *Y. enterocolitica* varied from 0 to 18%, with the highest frequency observed in barnacle geese (*Branta leucopsis*) (Table 1). Of the 29 *Y. enterocolitica* isolates, 10 belonged to bioserotype 3/O:3, three belonged to 1A/O:5, and one belonged to 1A/O:3. The rest of the isolates did not belong to any known bioserotype combination (Table 2). Ten of the *Y. enterocolitica* isolates, all isolated from barnacle geese, belonged to bioserotype 3/O:3, a bioserotype associated with human disease. Two of these bioserotype 3/O:3 strains were found to be *virF* positive; thus, they carried the virulence plas-

TABLE 1. *Yersinia* spp. isolated from different bird hosts

Species	Latin name	No. tested	No. with ^a :						
			<i>Yersinia</i>	YP	YE	YI	YF	YK	YR
Barnacle goose	<i>Branta leucopsis</i>	105	42	—	19	15	12	1	1
Robin	<i>Erithacus rubecula</i>	51	—	—	—	—	—	—	—
Blackbird	<i>Turdus merula</i>	43	4	—	2	1	1	2	—
Redshank	<i>Tringa totanus</i>	30	3	—	1	2	—	—	—
Song thrush	<i>Turdus philomelos</i>	27	2	1	—	—	—	—	1
Dunlin	<i>Calidris alpina</i>	25	1	—	1	—	—	—	—
Redstart	<i>Phoenicurus phoenicurus</i>	22	2	—	1	—	1	—	—
Goldcrest	<i>Regulus regulus</i>	13	—	—	—	—	—	—	—
Pied flycatcher	<i>Ficedula hypoleuca</i>	10	—	—	—	—	—	—	—
Spotted flycatcher	<i>Muscicapa striata</i>	10	1	—	—	—	—	1	—
Willow warbler	<i>Phylloscopus trochilus</i>	10	—	—	—	—	—	—	—
Redwing	<i>Turdus iliacus</i>	10	2	2	—	—	—	—	—
Redpoll	<i>Carduelis flammea</i>	9	—	—	—	—	—	—	—
Blue tit	<i>Parus caeruleus</i>	9	—	—	—	—	—	—	—
Brent goose	<i>Branta bernicla</i>	7	—	—	—	—	—	—	—
Pied wagtail	<i>Motacilla alba</i>	6	—	—	—	—	—	—	—
Lesser whitethroat	<i>Sylvia curruca</i>	6	—	—	—	—	—	—	—
Blackcap	<i>Sylvia atricapilla</i>	5	1	—	1	—	—	—	—
Red-backed shrike	<i>Lanius collurio</i>	4	—	—	—	—	—	—	—
Garden warbler	<i>Sylvia borin</i>	4	—	—	—	—	—	—	—
Common whitethroat	<i>Sylvia communis</i>	4	—	—	—	—	—	—	—
Reed warbler	<i>Acrocephalus scirpaceus</i>	3	1	—	—	—	—	—	1
Rough-legged buzzard	<i>Buteo lagopus</i>	3	1	—	1	—	—	—	—
Jackdaw	<i>Corvus monedula</i>	3	—	—	—	—	—	—	—
Chiffchaff	<i>Phylloscopus collybita</i>	3	—	—	—	—	—	—	—
Bullfinch	<i>Pyrrhula pyrrhula</i>	3	—	—	—	—	—	—	—
Wren	<i>Troglodytes troglodytes</i>	3	—	—	—	—	—	—	—
Sparrow hawk	<i>Accipiter nisus</i>	2	—	—	—	—	—	—	—
Sedge warbler	<i>Acrocephalus schoenobaenus</i>	2	—	—	—	—	—	—	—
Tree pipit	<i>Anthus trivialis</i>	2	—	—	—	—	—	—	—
Siskin	<i>Carduelis spinus</i>	2	—	—	—	—	—	—	—
Cuckoo	<i>Cuculus canorus</i>	2	—	—	—	—	—	—	—
Bluethroat	<i>Luscinia svecica</i>	2	—	—	—	—	—	—	—
Great tit	<i>Parus major</i>	2	—	—	—	—	—	—	—
Starling	<i>Sturnus vulgaris</i>	2	—	—	—	—	—	—	—
Barred warbler	<i>Sylvia nisoria</i>	2	—	—	—	—	—	—	—
Fieldfare	<i>Turdus pilaris</i>	2	—	—	—	—	—	—	—
Marsh warbler	<i>Acrocephalus palustris</i>	1	—	—	—	—	—	—	—
Common sandpiper	<i>Actites hypoleucos</i>	1	—	—	—	—	—	—	—
Mallard	<i>Anas platyrhynchos</i>	1	—	—	—	—	—	—	—
Greylag goose	<i>Anser anser</i>	1	—	—	—	—	—	—	—
Short-eared owl	<i>Asio flammeus</i>	1	—	—	—	—	—	—	—
Long-eared owl	<i>Asio otus</i>	1	—	—	—	—	—	—	—
Canada goose	<i>Branta canadensis</i>	1	—	—	—	—	—	—	—
Scarlet rosefinch	<i>Carpodacus erythrinus</i>	1	—	—	—	—	—	—	—
Tree-creeper	<i>Certhia familiaris</i>	1	—	—	—	—	—	—	—
Mute swan	<i>Cygnus olor</i>	1	—	—	—	—	—	—	—
House martin	<i>Delichon urbica</i>	1	—	—	—	—	—	—	—
Great spotted woodpecker	<i>Dendrocopos major</i>	1	—	—	—	—	—	—	—
Ortolan bunting	<i>Emberiza hortulana</i>	1	—	—	—	—	—	—	—
Reed bunting	<i>Emberiza schoeniclus</i>	1	—	—	—	—	—	—	—
Crane	<i>Grus grus</i>	1	—	—	—	—	—	—	—
Icterine warbler	<i>Hippolais icterina</i>	1	—	—	—	—	—	—	—
Herring gull	<i>Larus argentatus</i>	1	—	—	—	—	—	—	—
Yellow wagtail	<i>Motacilla flava</i>	1	—	—	—	—	—	—	—
Grey plover	<i>Pluvialis squatarola</i>	1	—	—	—	—	—	—	—
Shelduck	<i>Tadorna tadorna</i>	1	—	—	—	—	—	—	—
Total	57 species	468	60	3	26	18	14	4	3

^a YP, *Y. pseudotuberculosis*; YE, *Y. enterocolitica*; YI, *Y. intermedia*; YF, *Y. frederiksenii*; YK, *Y. kristensenii*; YR, *Y. rohdei*; —, *Yersinia* not found.

mid. Furthermore, these isolates were PYZ negative and CR-MOX positive (Table 2). The *virF*-negative isolates of *Y. enterocolitica* bioserotype 3/O:3 were recovered from nine birds, all of which were barnacle geese. *NotI* enzyme digestion differentiated the 29 *Y. enterocolitica* isolates by producing 21

restriction patterns. The 10 isolates of bioserotype 3/O:3 corresponded to four *NotI* profiles (Fig. 1) and four *SpeI* profiles.

A total of 17 *Y. pseudotuberculosis* isolates were recovered after cold enrichment for 7, 14, or 21 days. However, when cold enrichment was combined with alkali treatment, 34 isolates

TABLE 2. Biochemical characteristics of *Y. pseudotuberculosis* and *Y. enterocolitica* strains isolated from feces of migratory birds

Biotype/serotype ^a	No. of positive birds	Salicin	Esculin	PYZ	CR-MOX ^b	virF
<i>Y. pseudotuberculosis</i>						
1/O:2	3	+	+	-	+	+
<i>Y. enterocolitica</i>						
3/O:3	2	-	-	-	+	+
3/O:3	8	-	-	-	-	-
3/NT	9	-	-	-	-	-
1A/O:3	1	+	+	+	-	-
1A/NT	3	+	+	+	-	-
1A/O:5	3	+	+	+	-	-
NT/NT	3	-	-	+	-	-

^a NT, nontypeable.

^b Isolates were tested for calcium dependence and Congo red absorption on CR-MOX agar.

were recovered. Four indistinguishable isolates of *Y. pseudotuberculosis* from one bird sample were found after direct plating. The virF-positive strains of *Y. enterocolitica* were isolated after a 1-week cold enrichment, followed by KOH treatment. Of the bioserotype 3/O:3 isolates, 1 and 11 were recovered after direct plating and cold enrichment, respectively.

DISCUSSION

The occurrence of *Yersinia* spp. among birds in this study was comparatively high, nearly 13%, and there can be no doubt that these bacteria are present in wild birds migrating through Sweden. As most of the sampled individuals were caught during active migration, the occurrence of *Yersinia* in samples from Öland and Gotland does not necessarily reflect the local situation in southeast Sweden. Depending on the time elapsed between colonization and excretion of the bacteria in feces and on the speed of migration of the birds, the samples may mirror the occurrence some distance away along the migratory route. As the birds sampled during the spring migration (at least all passerine birds caught at Ottenby Bird Observatory) were often caught just hours after reaching Sweden, colonized birds likely acquired the bacteria while on their wintering grounds or during the migratory journey. In our data set, there was a skewed ratio, with far more isolates obtained during spring migration than during autumn migration, and all strains of pathogenic *Yersinia* spp. were gathered during the spring. In interpreting results, however, one should note that the distributions of host species from which material was sampled differed between the two seasons, with some species, such as the barnacle goose, being sampled only during spring migration and others being sampled only during autumn migration, and that the number of sampled individuals was low for many species.

Most isolated *Yersinia* strains belonged to nonpathogenic species, namely *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, *Y. rohdei*, and nonpathogenic strains of *Y. enterocolitica*. These species have never been associated with human disease, they do not carry pYV, and the public health risk stemming from these bacteria is considered to be low. Nonpathogenic *Yersinia* have previously been isolated from birds, other animals, and the environment in various numbers (4, 6, 22), and differences in isolation rates can be found among different bird species in

different areas (11, 23). In our study, the only frequently colonized species was the barnacle goose, of which 38% of the sampled individuals were *Yersinia* positive (Table 1).

Apart from the nonpathogenic strains, we also managed to isolate a few pathogenic *Yersinia*—three virF-positive *Y. pseudotuberculosis* strains and two virF-positive *Y. enterocolitica* strains. The strains of *Y. pseudotuberculosis* came from thrushes, two from song thrushes and one from a redwing, and all were retrieved during spring migration in April. Individuals of these bird species, as reflected by recoveries of banded birds trapped at Ottenby Bird Observatory, spend the nonbreeding season in Western Europe, in Italy, Spain, Portugal, France, Holland, and Belgium. All *Y. pseudotuberculosis* strains were of bioserotype 1/O:2. Serotype O:2 is one of the two serotypes, the other being serotype O:1, most commonly isolated from birds in Europe, the United States, and Canada. These serotypes are typically found in healthy birds but have also been reported to cause disease in both birds and humans (6, 14, 18, 34). Though serotype O:1 has been regarded as one of the most important causes of sporadic human infection with *Y. pseudotuberculosis* in Europe and has been associated with several outbreaks of yersiniosis, serotype O:2 also has been reported to be associated with human yersiniosis in different countries in Europe (1, 29, 34). The *Y. pseudotuberculosis* strains showed similar bio-

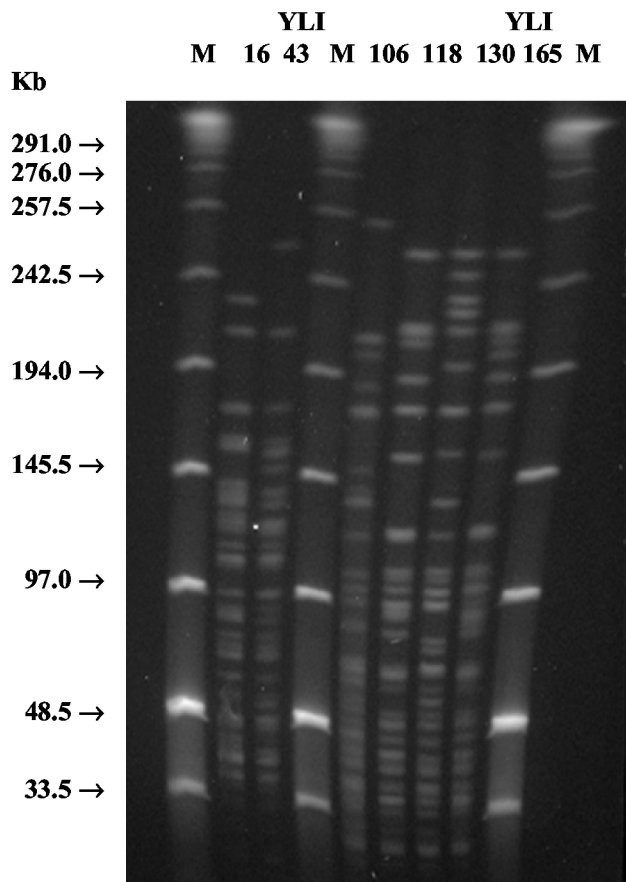


FIG. 1. Two different *NotI* profiles of *Y. pseudotuberculosis* bioserotype 1/O:2 (strains YLI16 and YLI43) and four *NotI* profiles of *Y. enterocolitica* bioserotype 3/O:3 (strains YLI106, YLI118, YLI130, and YLI165) isolated from migratory birds. M, midrange PFGE marker 1.

chemical characteristics, and they all carried the *virF* gene on their plasmids. PFGE analysis with the *NotI* and *SpeI* enzymes divided the strains on the basis of two restriction patterns (Fig. 1). Both strains from the song thrushes clustered together and were different from the strain found in the redwing, indicating different origins of colonizing *Y. pseudotuberculosis* strains.

Seven different bird species, representing five different bird families (Table 1), were found to carry *Y. enterocolitica*. In previous studies, differences in the rates of detection of *Y. pseudotuberculosis* and *Y. enterocolitica* depending on host species have been observed, with the former more frequently isolated from passerine birds and psittaciformes while the latter has typically been isolated from birds with larger body masses (7, 14, 32). Our data were consistent with this pattern; *Y. enterocolitica* was most frequently isolated from barnacle geese (Table 1). Ten of the *Y. enterocolitica* isolates, all isolated from barnacle geese, belonged to bioserotype 3/O:3, which is associated with human disease. Two of these strains were found to be *virF* positive; thus, they carried the virulence plasmid. While the other eight isolates were *virF* negative, they should still be considered to be potentially pathogenic to humans, since accidental loss of the plasmid during isolation procedures is possible. To our knowledge, this is the first documented case of the isolation of this bioserotype from birds. Bioserotype 3/O:3 is prevalent among humans in the Far East and has been recovered from pork and chicken meat samples in Japan (12). Four of the remaining isolates of *Y. enterocolitica* belonged to the nonpathogenic bioserotypes 1A/O:3 and 1A/O:5, which have never been associated with human infections. The rest of the 15 *Y. enterocolitica* isolates did not belong to any known bioserotype combination (Table 2). Pathogenic *Y. enterocolitica* strains are negative for PYZ activity, esculin hydrolysis, and salicin acidification (37). The virulence characteristics differentiated *virF*-positive *Y. enterocolitica* strains from nonpathogenic *Y. enterocolitica* 1A strains (Table 2). Twenty-one different *NotI* restriction patterns were observed with *Y. enterocolitica* isolates. The most common *NotI* profile was N9, detected in samples from seven individuals—all barnacle geese. Three birds were found to carry more than one strain of *Y. enterocolitica*.

All samples from which *Yersinia* spp. were isolated in this study came from apparently healthy individuals, which has also been the case in several other studies (6, 10, 13). *Y. pseudotuberculosis* is a pathogen frequently observed among zoo birds and domestic fowl in connection with both outbreaks and sporadic cases of infection and is recognized to cause severe clinical symptoms and sudden death in different bird species (36, 38; Harcourt-Brown, *Vet. Rec.* **102**:315, 1978). Perhaps wild birds are less affected by *Y. pseudotuberculosis* or maintain the infection at a low but latent level, developing acute clinical illness only under stressful conditions, e.g., starvation, cold temperatures, and migration.

Conventional isolation methods used in recovery of *Y. pseudotuberculosis* and *Y. enterocolitica* seem to be rather insensitive, and recovery rates are low (7, 9). Isolation of *Y. pseudotuberculosis* appears to be more efficient when sampling is from necropsy material instead of fecal material (7). *Y. pseudotuberculosis* isolates were best found after cold enrichment that was followed by KOH treatment. This has previously been shown to be the most successful method for the isolation of *Y. pseudo-*

tuberculosis from clinical and environment samples with low levels of bacteria (27). The small amount of *Y. pseudotuberculosis* in samples makes it difficult to isolate the bacterium without enrichment since it is easily overgrown by other bacteria, including *Pseudomonas*, *Citrobacter*, *Serratia*, and other *Yersinia* spp. This could explain why *Y. pseudotuberculosis* was isolated only from samples in which no other *Yersinia* species was present. In addition, cold enrichment has been found to increase the recovery of pathogenic *Y. enterocolitica* strains compared with direct plating, but it also selects for nonpathogenic *Y. enterocolitica* strains in stool cultures (24). Our figures should thus be seen as the minimum occurrence of pathogenic *Yersinia* spp. in these wild birds.

Human infection with *Y. pseudotuberculosis* is suspected to be primarily associated with consumption of food or unchlorinated water contaminated by wild animal wastes (10). In this investigation, we show that birds caught during migration in Sweden may be infected with *virF*-positive *Y. pseudotuberculosis* and *Y. enterocolitica*. Birds are also commonly colonized by nonpathogenic *Yersinia* spp. The finding of *virF*-positive *Y. pseudotuberculosis* and *Y. enterocolitica* in bird feces indicates that wild birds cannot be excluded from the epidemiological discussion of human yersiniosis. However, the low isolation rate of *virF*-positive *Y. pseudotuberculosis* and *Y. enterocolitica* suggests that birds are unlikely to be a direct source of *Yersinia* infections in humans. These results provide the first knowledge on the occurrence of these largely unknown human pathogens in migratory birds in Europe and may be regarded as one step toward unraveling their epidemiology.

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