

Molecular Epidemiology of *Salmonella enterica* Serovar Typhimurium Isolates Determined by Pulsed-Field Gel Electrophoresis: Comparison of Isolates from Avian Wildlife, Domestic Animals, and the Environment in Norway

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The molecular epidemiology of 142 isolates of *Salmonella enterica* serovar Typhimurium from avian wildlife, domestic animals, and the environment in Norway was investigated using pulsed-field gel electrophoresis (PFGE) and computerized numerical analysis of the data. The bacterial isolates comprised 79 isolates from wild-living birds, including 46 small passerines and 26 gulls, and 63 isolates of nonavian origin, including 50 domestic animals and 13 environmental samples. Thirteen main clusters were discernible at the 90% similarity level. Most of the isolates (83%) were grouped into three main clusters. These were further divided into 20 subclusters at the 95% similarity level. Isolates from passerines, gulls, and pigeons dominated within five subclusters, whereas isolates from domestic animals and the environment belonged to many different subclusters with no predominance. The results support earlier results that passerines constitute an important source of infection to humans in Norway, whereas it is suggested that gulls and pigeons, based on PFGE analysis, represent only a minor source of human serovar Typhimurium infections. Passerines, gulls, and pigeons may also constitute a source of infection of domestic animals and feed plants or vice versa. Three isolates from cattle and a grain source, of which two were multiresistant, were confirmed as serovar Typhimurium phage type DT 104. These represent the first reported phage type DT 104 isolates from other sources than humans in Norway.

There is strong evidence that *Salmonella enterica* serovar Typhimurium has established reservoirs in wild-living birds and hedgehogs in Norway (15, 27). Wild-living birds and hedgehogs may function as effective spreaders of *Salmonella* bacteria to humans and to different animal species through contamination of the environment (15, 23). In Norway, sporadic indigenous cases and a national outbreak of human salmonellosis, caused by serovar Typhimurium, have been related to infections in small passerines (22, 23). In a waterborne serovar Typhimurium infection outbreak in northwestern Norway in 1999, gulls were suggested to be the most likely source of infection (1; T. Refsum, G. Kapperud, and G. Holstad, submitted for publication). Moreover, two human outbreaks in 1996 and 2000 have been associated with infected hedgehog populations (15). It is therefore important to gain further knowledge of the epidemiology of *Salmonella* bacteria in wild-living species, in particular of the endemically distributed serovar Typhimurium.

Most previous studies are based on analytical epidemiological investigations and traditional phenotypic analysis, such as biotyping, serotyping, antimicrobial susceptibility, and phage typing. However, the limitations associated with several of these techniques have stimulated interest in DNA-based typing methods, such as pulsed-field gel electrophoresis (PFGE) (3, 5). This method has proven to be highly discriminatory and

comparable or superior to other techniques (3) and has been useful in epidemiological investigations of, e.g., serovar Typhimurium outbreaks (5, 26, 31). In Norway, several serovar Typhimurium outbreaks in humans have been investigated by this method (16). The PFGE method might also be useful in surveillance of variants of particular interest, such as the multiresistant serovar Typhimurium definitive phage type DT 104. Fingerprinting by PFGE may be a valuable tool in epidemiological investigation and surveillance by relating isolates from different sources to a common origin.

The aim of this study was to investigate the molecular epidemiology of serovar Typhimurium isolates from wild-living birds, domestic animals, and the environment by using genomic fingerprinting by PFGE.

MATERIALS AND METHODS

Isolate characteristics. A total of 142 isolates of serovar Typhimurium from avian wildlife, domestic animals, and other sources in Norway were investigated (Table 1). The 79 isolates from wild-living birds comprised 46 isolates of serovar Typhimurium O:4,12 randomly selected from a total of 350 small passerine isolates, using a random-number generator (9), and all available isolates of the serovar Typhimurium variants O:4,12 and O:4,5,12 from other wild-living bird species. The isolates were obtained from birds from all over the country. All isolates originated from birds received for postmortem examination at the National Veterinary Institute during 1969 to 2000 (27) or from surveys of wild-living birds conducted during 1997 to 2001 (T. Refsum, T. Vikøren, K. Handeland, G. Holstad, and G. Kapperud, submitted for publication; Refsum et al., submitted), except for two isolates from a crow and a gull collected in a follow-up investigation of a human outbreak in southeastern Norway in 1996 (15).

A total of 63 isolates of serovar Typhimurium isolated from sources other than wild-living birds from 1990 to 2001 were included (Table 1). All but four of the nonavian isolates originated from southern Norway. All available epidemiolog-

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TABLE 1. The 142 isolates of serovar Typhimurium from avian wildlife, domestic animals, and the environment according to origin, year of isolation, county, and PFGE cluster^a

Origin	Yr of isolation	Serotype	County	PFGE cluster
Black-headed gull, <i>Larus ridibundus</i>	1994	O:4,5,12	Hedmark	A1
Dairy cattle	1995	O:4,5,12	Rogaland	A1
Dairy cattle	1997	O:4,5,12	Rogaland	A1
City pigeon, <i>Columba livia</i>	1993	O:4,12	Hordaland	A1
City pigeon, <i>Columba livia</i>	1993	O:4,5,12	Oslo	A1
Domestic pigeon	1994	O:4,12	Møre og Romsdal	A1
Domestic pigeon	1994	O:4,12	Rogaland	A1
Domestic pigeon	1994	O:4,12	Rogaland	A1
Domestic pigeon	1995	O:4,12	Rogaland	A1
Domestic pigeon	1998	O:4,12	Hedmark	A1
Domestic pigeon	1999	O:4,12	Hordaland	A1
Domestic pigeon	2000	O:4,12	Rogaland	A1
Feed plant	1995	O:4,12	Østfold	A1
Fish feed	1994	O:4,12	Rogaland	A1
Horse	1994	O:4,5,12	Vestfold	A1
Horse	1994	O:4,5,12	Oslo	A1
Swine	1994	O:4,5,12	Hedmark	A1
Swine	1994	O:4,5,12	Rogaland	A1
Swine feed	1994	O:4,5,12	Oslo	A1
Bullfinch, <i>Pyrrhula pyrrhula</i>	1969	O:4,12	Oslo	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1972	O:4,12	Vest-Agder	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1982	O:4,12	Oppland	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1982	O:4,12	Nord-Trøndelag	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1988	O:4,12	Oslo	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1988	O:4,12	Møre og Romsdal	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1992	O:4,12	Oslo	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1999	O:4,12	Østfold	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1999	O:4,12	Nordland	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	1999	O:4,12	Nord-Trøndelag	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	2000	O:4,12	Nordland	A2
Bullfinch, <i>Pyrrhula pyrrhula</i>	2000	O:4,12	Troms	A2
Cat	1998	O:4,12	Østfold	A2
Cat	2000	O:4,12	Nordland	A2
Dairy cattle	1995	O:4,12	Rogaland	A2
Common redpoll, <i>Carduelis flammea</i>	2000	O:4,12	Nordland	A2
Eurasian siskin, <i>Carduelis spinus</i>	1974	O:4,12	Akershus	A2
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Hedmark	A2
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Telemark	A2
Fur animal feed	1997	O:4,12	Unknown	A2
Geese, breeding	1995	O:4,12	Østfold	A2
Great tit, <i>Parus major</i>	1975	O:4,12	Akershus	A2
Great tit, <i>Parus major</i>	1994	O:4,12	Vest-Agder	A2
Greenfinch, <i>Carduelis chloris</i>	1969	O:4,12	Oslo	A2
Greenfinch, <i>Carduelis chloris</i>	1969	O:4,12	Vestfold	A2
Greenfinch, <i>Carduelis chloris</i>	1969	O:4,12	Oslo	A2
Greenfinch, <i>Carduelis chloris</i>	1972	O:4,12	Østfold	A2
Greenfinch, <i>Carduelis chloris</i>	1972	O:4,12	Akershus	A2
Eurasian tree sparrow, <i>Passer montanus</i>	1982	O:4,12	Akershus	A2
Poultry	1995	O:4,12	Rogaland	A2
Poultry	1995	O:4,12	Oppland	A2
Swine	1995	O:4,12	Rogaland	A2
Swine	1995	O:4,12	Østfold	A2
Swine	1995	O:4,12	Østfold	A2
Swine	1996	O:4,12	Rogaland	A2
Swine	1999	O:4,12	Østfold	A2
Black-headed gull, <i>Larus ridibundus</i>	1977	O:4,12	Rogaland	B1
Bullfinch, <i>Pyrrhula pyrrhula</i>	1972	O:4,12	Oslo	B1
Canada goose, <i>Branta canadensis</i>	1989	O:4,12	Oslo	B1
Common guillemot, <i>Uria aalge</i>	1997	O:4,12	Oslo	B1
Mew gull, <i>Larus canis</i>	1997	O:4,12	Oslo	B1
Mew gull, <i>Larus canis</i>	2000	O:4,12	Hordaland	B1
Mew gull, <i>Larus canis</i>	2001	O:4,12	Hordaland	B1
Feed plant	1998	O:4,12	Vestfold	B1
Feed plant	1995	O:4,5,12	Oslo	B1
Great black-backed gull, <i>Larus marinus</i>	2001	O:4,5,12	Møre og Romsdal	B1
Herring gull, <i>Larus argentatus</i>	1999	O:4,12	Nordland	B1
Herring gull, <i>Larus argentatus</i>	2000	O:4,5,12	Oslo	B1
Herring gull, <i>Larus argentatus</i>	2000	O:4,12	Hordaland	B1
Herring gull, <i>Larus argentatus</i>	2000	O:4,12	Buskerud	B1
Duck, breeding	1995	O:4,5,12	Telemark	B2
Abattoir	1999	O:4,12	Hedmark	B3

Continued on following page

TABLE 1—Continued

Origin	Yr of isolation	Serotype	County	PFGE cluster
Blue tit, <i>Cyanistes caeruleus</i>	1989	O:4,12	Hordaland	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1972	O:4,12	Hedmark	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1972	O:4,12	Akershus	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1982	O:4,12	Nordland	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1982	O:4,12	Nordland	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1988	O:4,12	Telemark	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1990	O:4,12	Nordland	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1998	O:4,12	Vest-Agder	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1998	O:4,12	Sør-Trøndelag	B3
Bullfinch, <i>Pyrrhula pyrrhula</i>	1999	O:4,12	Buskerud	B3
Cat	1998	O:4,12	Nord-Trøndelag	B3
Cat	2000	O:4,12	Rogaland	B3
Dairy cattle	2000	O:4,12	Sør-Trøndelag	B3
Common redpoll, <i>Carduelis flammea</i>	1999	O:4,12	Sør-Trøndelag	B3
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Nord-Trøndelag	B3
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Oslo	B3
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Sogn og Fjordane	B3
Eurasian siskin, <i>Carduelis spinus</i>	1999	O:4,12	Østfold	B3
Great tit, <i>Parus major</i>	1988	O:4,12	Oslo	B3
House sparrow, <i>Passer domesticus</i>	1999	O:4,12	Nord-Trøndelag	B3
House sparrow, <i>Passer domesticus</i>	2000	O:4,12	Nord-Trøndelag	B3
Red fox	1995	O:4,12	Oppland	B3
Greenfinch, <i>Carduelis chloris</i>	1969	O:4,12	Oslo	C
Dairy cattle	1991	O:4,12	Buskerud	D
Bullfinch, <i>Pyrrhula pyrrhula</i>	1971	O:4,12	Hedmark	E
Turkey	2000	O:4,12	Østfold	F
Black-headed gull, <i>Larus ridibundus</i>	1984	O:4,5,12	Sør-Trøndelag	G1
Black-headed gull, <i>Larus ridibundus</i>	1993	O:4,5,12	Oslo	G1
Black-headed gull, <i>Larus ridibundus</i>	1994	O:4,5,12	Hedmark	G1
Black-headed gull, <i>Larus ridibundus</i>	1997	O:4,12	Oslo	G1
Dairy cattle	1995	O:4,5,12	Nord-Trøndelag	G1
Dairy cattle	1996	O:4,5,12	Vest-Agder	G1
Dairy cattle	1997	O:4,5,12	Østfold	G1
Dairy cattle	1999	O:4,5,12	Rogaland	G1
Dairy cattle ^b	1992	O:4,12	Hordaland	G1
City pigeon, <i>Columbia livia</i>	1993	O:4,5,12	Oslo	G1
Mew gull, <i>Larus canis</i>	1997	O:4,5,12	Oslo	G1
Mew gull, <i>Larus canis</i>	2000	O:4,5,12	Hordaland	G1
Feed plant	1997	O:4,5,12	Østfold	G1
Great black-backed gull, <i>Larus marinus</i>	2000	O:4,5,12	Hordaland	G1
Herring gull, <i>Larus argentatus</i>	1999	O:4,5,12	Sogn og Fjordane	G1
Herring gull, <i>Larus argentatus</i>	2000	O:4,5,12	Hordaland	G1
Herring gull, <i>Larus argentatus</i>	2000	O:4,5,12	Hordaland	G1
Herring gull, <i>Larus argentatus</i>	2000	O:4,5,12	Akershus	G1
Horse	1999	O:4,5,12	Rogaland	G1
Horse	2000	O:4,12	Sør-Trøndelag	G1
Lesser black-backed gull, <i>Larus fuscus</i>	2000	O:4,5,12	Oslo	G1
Magpie, <i>Pica pica</i>	1993	O:4,5,12	Oslo	G1
Passerine bird ^p	1992	O:4,12	Hordaland	G1
Swine	2000	O:4,12	Nordland	G1
Domestic pigeon	1999	O:4,12	Aust-Agder	G2
Feed plant	1997	O:4,12	Vestfold	H1
Wheat-based feed	1995	O:4,5,12	Unknown	H1
Domestic pigeon	2001	O:4,12	Hordaland	H2
Domestic pigeon	2001	O:4,12	Sogn og Fjordane	H2
Dairy cattle	1997	O:4,5,12	Møre og Romsdal	H3
Dairy cattle	2001	O:4,5,12	Rogaland	H3 ^c
Grain for human consumption	1997	O:4,5,12	Oslo	H3 ^c
Ostrich	1996	O:4,5,12	Hordaland	I
Dairy cattle	1996	O:4,5,12	Hordaland	J1
Gull feathers sample from water supply	1999	O:4,5,12	Møre og Romsdal	J1
Herring gull, <i>Larus argentatus</i> (adult)	1999	O:4,5,12	Møre og Romsdal	J1
Herring gull, <i>Larus argentatus</i> (chick)	1999	O:4,5,12	Møre og Romsdal	J1
Herring gull, <i>Larus argentatus</i> (chick)	1999	O:4,5,12	Møre og Romsdal	J1
Sheep	1998	O:4,5,12	Hordaland	J2
Gull, unknown species	1997	O:4,5,12	Østfold	K
Hooded crow, <i>Corvus corone</i>	1997	O:4,5,12	Østfold	K
Poultry	1993	O:4,5,12	Akershus	L
Swine	1994	O:4,5,12	Oppland	L
Swine	1995	O:4,5,12	Hedmark	L
Feed plant	1998	O:4,5,12	Oslo	M

^a The genus and species of the wild-living birds are specified. The cluster capital letters and subcluster suffixes correspond to the dendrogram in Fig. 1.^b Serovar Typhimurium was isolated from cattle and from a small passerine bird found dead within the same cowshed.^c Serovar Typhimurium DT 104 isolate, multiresistant to ACSSuT.

ically unrelated isolates from domestic animals were examined. If more than one isolate was obtained from the same herd or production unit, only one of the isolates was included. The total number comprised 50 isolates from cattle ($n = 13$), swine ($n = 10$), horses ($n = 4$), cats ($n = 4$), sheep ($n = 1$), poultry ($n = 3$), waterfowl ($n = 2$), turkey ($n = 1$), ostrich ($n = 1$), red fox ($n = 1$), and domestic pigeon ($n = 10$) (Table 1). In addition, 13 environmental isolates, recovered from a contaminated water supply ($n = 1$), from an abattoir ($n = 1$), and from feed ($n = 10$) and food ($n = 1$) plants were investigated. The isolates were obtained from the National Veterinary Institute and the National Salmonella Reference Laboratory at the Norwegian Institute of Public Health.

Phenotypic and genotypic characterization. All isolates included in the present study were analyzed by PFGE. DNA preparation, restriction enzyme digestion (*Xba*I), and PFGE were performed as previously described (17). Lambda DNA (Sigma, St. Louis, Mo.) served as a molecular size standard in all PFGE investigations. After electrophoresis, PFGE gels were stained with ethidium bromide and photographed with GelDoc 2000 using Quantity One software (Bio-Rad, Hercules, Calif.). Thirty-one of the passerine isolates included in our study have previously been phage typed (27) by the typing scheme of Callow (8), as extended by Anderson et al. (2). In addition, three isolates from cattle and a grain source, which showed a PFGE profile characteristic for phage type DT 104 (16), were phage typed and subjected to antibiotic susceptibility testing as described by Leegaard et al. (24).

Computerized numerical analysis of PFGE data. Images saved in TIFF format were transferred to the GelCompar II software (Applied Maths, Kortrijk, Belgium) for computer analysis. Similarity between fingerprints was determined on the basis of the Dice coefficient. A band position tolerance of 2% was used for analysis of PFGE patterns. Dendrograms were generated by the unweighted pair group method with arithmetic averages. Capital letters (A to M) were used to designate the main cluster lineages of serovar Typhimurium isolates in the dendrogram, while subclusters were given numerical suffixes.

RESULTS

In our study, 13 main clusters (designated A to M) were discernible at the 90% similarity level (Fig. 1). Most of the isolates (83%) were grouped into three main clusters: A (39%; $n = 55$) B (27%; $n = 38$), and G (18%; $n = 25$) (Fig. 1; Table 1). The main clusters could be further divided into 20 subclusters at the 95% similarity level. Most of the wild-bird isolates (90%) belonged to only five of these subclusters (Fig. 1 and 2), whereas isolates from domestic animals and other sources belonged to many different subclusters, with no predominance of isolates in distinct subclusters.

The A2 and B3 subclusters comprised 42 (91%) of the passerine isolates. No isolates from other wild-bird species belonged to these subclusters. Subcluster A2 also harbored 12 isolates from swine, poultry, cats, cows, breeding geese, and fur animal feed, whereas B3 contained five isolates originating from domestic cats, cows, red foxes, and an abattoir. All isolates within subclusters A2 and B3 belonged to the antigenic variant serovar Typhimurium O:4,12. Both subclusters contained passerine isolates originating from all over the country. Of the 31 phage-typed passerine isolates, all 16 isolates belonging to phage type DT 40 fell into subcluster A2, whereas all 12 isolates of phage type U277 fell into subcluster B3 together with two DT 99 isolates and one isolate classified as RDNC (routine dilution no conformity).

In parallel to passerine bird isolates, 20 (77%) of the isolates from gulls belonged to two subclusters, B1 and G1. Subcluster B1 was made up predominantly of isolates from gulls ($n = 9$) although sporadic isolates from other wild-living birds and two feed plants also fell into this subcluster. Subcluster G1 included 12 isolates from gulls in addition to 13 isolates from other wild-living birds, cattle, horse, swine, and a feed plant.

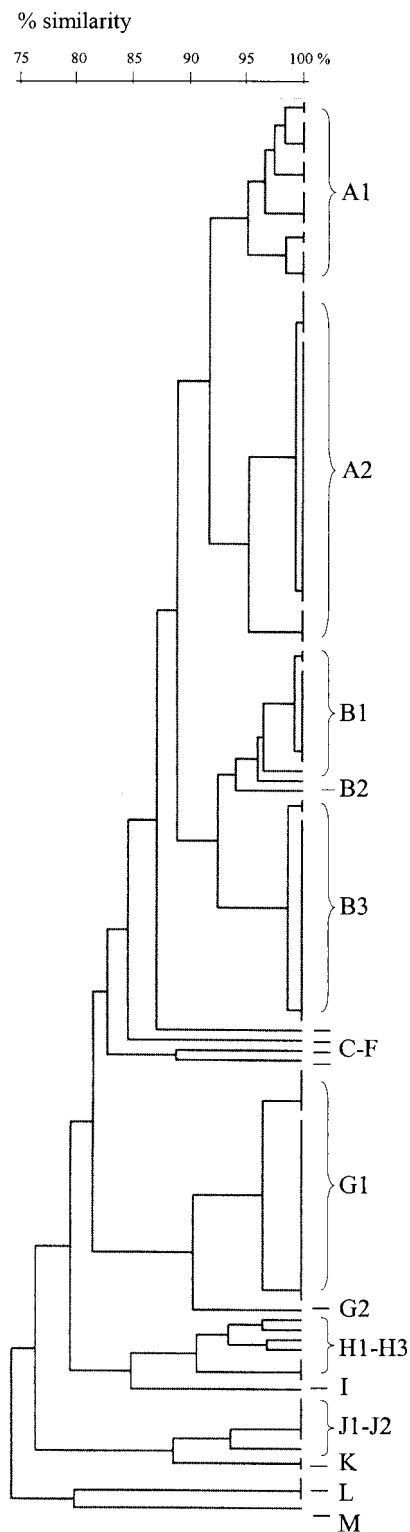


FIG. 1. Dendrogram based on PFGE macrorestriction profiles of 142 isolates from avian wildlife, domestic animals, and the environment in Norway. The 13 clusters (90% similarity level) are designated by capital letters (A to M), while the subclusters (95% similarity level) are assigned numerical suffixes.

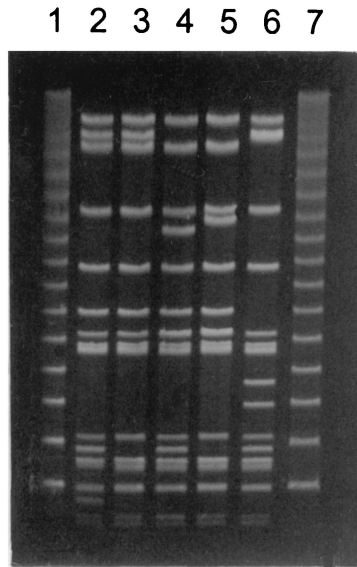


FIG. 2. PFGE of serovar Typhimurium isolates from avian wildlife in Norway. Representatives of the five most prevalent subclusters and typical origin are shown. Lanes: 1 and 7, molecular weight marker, lambda ladder; 2, A1 (pigeon); 3, A2 (small passerine); 4, B1 (gull); 5, B3 (small passerine); 6, G1 (gull).

Both subcluster B1 and G1 clones were geographically widespread.

The A1 subcluster ($n = 19$) was made up predominantly of isolates from pigeons, including seven domestic pigeons and two wild-living city pigeons. This subcluster also included isolates from horses, cattle, swine, a black-headed gull, and animal feed plants. Both antigenic variants of serovar Typhimurium were represented in the cluster. All the A1 isolates originated from southern Norway.

The other main clusters (C to F and H to M) were composed of the remaining 24 isolates (17%) (Table 1). The three isolates within subcluster H3, which originated from cattle and from grain meant for human consumption, showed a PFGE pattern previously found to be characteristic of phage type DT 104 (15). Phage typing confirmed that they belonged to this type, and two of them expressed a multiresistant pattern typical for DT 104 strains (ACSSuT: resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline).

Subcluster J1 contained isolates from gulls and a gull feather sample collected from a contaminated surface drinking-water supply in northwestern Norway, as well as an isolate from a cow in western Norway. Two isolates from a gull and a crow in southeastern Norway fell into subcluster K. Subcluster L comprised two isolates from swine and one isolate from poultry. This subcluster PFGE L pattern was not obtained from any of the isolates from wild birds.

DISCUSSION

The high similarity found between isolates from wild-living birds indicates a close genetic relationship between avian serovar Typhimurium isolates compared to that of isolates from humans and other sources. Previous investigations reporting

clonal relationships of serovar Typhimurium from humans (16) and various other sources (26) showed that isolates of this serovariant were clustered within a window of similarity of 70%. In spite of the tight genetic relationship, it was possible to divide the majority of the wild avian isolates into five subclusters. With four exceptions, isolates from small passerines, gulls, and pigeons fell into different subclusters. The PFGE results thus indicate little crossover of *Salmonella* bacteria among those birds. However, it is also possible that this observation represents different host preferences of the genotypes. The three subclusters made up predominantly of isolates from gulls and pigeons also contained isolates from other wild-living bird species. It is impossible to determine whether or not this represents transmission from gull and pigeon reservoirs.

Previous investigations including a case-control study and plasmid profile and phage type analyses (21, 22, 23) have shown an epidemiological link between small passerines and humans. Recently, Heir et al. (16) have estimated that the clones within subclusters A2 and B3 (designated F1 and F3, respectively, by Heir et al.) were responsible for 32% of the sporadic, domestic human cases of serovar Typhimurium O:4,12 infection during 1996 to 1999. Thus, our PFGE results reinforce previous investigations suggesting that wild passerine birds are an important source of human serovar Typhimurium infections.

The pigeon patterns, of which the majority belonged to the heterogeneous A1 subcluster, were detected only twice in strains isolated from Norwegian patients by Heir et al. (16) (designated F9). Their study included a representative collection of 102 isolates of both foreign and domestic origins. Our results might thus support previous studies based on biochemical and phage type analyses, which suggest that the zoonotic infection hazard is of limited significance (14, 28, 33, 36, 37).

The most common gull profiles (B1 and G1) were also seen only twice among the human isolates studied by Heir et al. (designated F6 and L1, respectively, by Heir et al.) (16). It has been suggested that *Salmonella*-carrying gulls are of little significance as a health hazard to domestic animals and humans (11, 13). Nevertheless, gulls washing and roosting in drinking-water supplies constitute a potential human health hazard, especially if the water is consumed without prior disinfection (6, 12). Gulls were considered the most likely source of infection in a waterborne human outbreak in northwestern Norway in 1999 (1). In the present study, isolates from gulls, sampled in the municipality where the outbreak occurred, were found to be identical to the outbreak clone (J1) (Fig. 1; Table 1). However, in the follow-up survey of gulls along the Norwegian coast (Refsum, Holstad, and Kapperud, submitted), we failed to detect this clone elsewhere.

Among sporadic human isolates, the J1 profile (termed E5 by Heir et al. [16]) was observed throughout 1996 to 1999 but was geographically restricted to only two counties in western Norway. Both the J1 and L clones were implicated in an outbreak in Bergen and in two neighboring municipalities during autumn 2000 (16, 30). The L clone caused previous outbreak in southeastern Norway in 1996 (16, 34). Based on findings of the J1 and K clones in extensively infected hedgehog populations within the areas of the outbreaks in 1996 and 2000 (15), one might suggest that hedgehogs constitute the primary reservoir

of these clones and that opportunistic birds like gulls might have been infected from hedgehogs.

The five subclusters which included most of the avian wild-life isolates also contained isolates from several domestic animal species and from feed plant samples. The PFGE patterns of isolates from cats and passerines were identical (A2 and B3), supporting an epidemiological link reported previously in a Swedish study, using phage type analysis (32). Sparrows frequently visit animal sheds and barns in Norway during the winter, and it is thus not surprising that carrier birds could represent a potential health hazard to domestic animals. Previously, we have demonstrated that passerines, including sparrows, maintain a reservoir of serovar Typhimurium O:4,12 (Refsum, Vikøren, et al., submitted). The present results also support the assumption that serovar Typhimurium may be transmitted from gulls to domestic animals or vice versa. Transmission of different salmonella serovars from gulls to cattle and sheep through contaminated drinking water and pastures has previously been suggested (10, 20, 29, 35). Since gulls and pigeons often visit feed and food plants, it is not unreasonable to consider wild birds a potential source of contamination of the factory environment or vice versa.

In Norway, only a few domestically acquired cases of human salmonellosis caused by the multiresistant isolates of serovar Typhimurium DT 104 have been reported (25), and the sources of infection in these cases remain unknown. The two isolates of multiresistant serovar Typhimurium DT 104 reported in this study represent the first known isolates from sources other than humans in Norway. Multiresistant serovar Typhimurium DT 104 has not yet been detected in wild-living birds in Norway. None of the avian isolates in our study exhibited a *Xba*I PFGE profile identical to the DT 104 prevailing in Europe (4). However, this phage type has previously been detected in passerines, pigeons, and gulls in other countries (7, 18, 19), although information on antibiotic resistance is lacking in these studies.

The L pattern demonstrated in isolates from poultry and swine in our study was also seen in a human outbreak in 1993, but it has not been possible to establish an epidemiological link, and the primary source of infection still remains unknown (Norwegian Institute of Public Health, unpublished data).

Interestingly, we detected a relationship between the PFGE patterns in our study and the phage types previously obtained from analysis of passerine isolates of serovar Typhimurium O:4,12 (27). Characteristic PFGE patterns of different phage types have previously been demonstrated, including serovar Typhimurium DT 104 (4, 5, 7, 16). In this study, PFGE patterns proved to be stable over time, a finding supported by others (5). The patterns of the passerine isolates within the most common subclusters were identical, in spite of a range of 20 to 30 years in their date of collection. This suggests that PFGE is a robust method, not only for discriminative short-term outbreak investigations but also for showing epidemiological significance and applicability in long-term surveillance of serovar Typhimurium epidemiology.

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