Prevalence and genetic characteristics of Salmonella in free-living birds in Poland

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Abstract

Background: Salmonella species are widespread in the environment, and occur in cattle, pigs, and birds, including poultry and free-living birds. In this study, we determined the occurrence of Salmonella in different wild bird species in Poland, focusing on five Salmonella serovars monitored in poultry by the European Union: Salmonella serovars Enteritidis, Typhimurium, Infantis, Virchow, and Hadar. We characterized their phenotypic and genetic variations.

Results: Sixty-four Salmonella isolates were collected from 235 cloacal swabs, 699 fecal samples, and 66 tissue samples (6.4% of 1000 samples) taken from 40 different species of wild birds in Poland between September 2011 and August 2013. The largest numbers of isolates were collected from Eurasian siskin and greenfinch: 33.3% positive samples for both. The collected strains belonged to one of three Salmonella subspecies: enterica (81.25%), salamae (17.19%), or houtenae (1.56%). Eighteen strains belonged to Salmonella ser. Typhimurium (28.13%), one to ser. Infantis (1.56%), one to ser. Virchow (1.56%), and one to ser. Hadar (1.56%). All isolates contained spiA, mrgA, invA, sipB, prgA, spaN, orgA, tolC, ironN, sitC, ipfC, sifA, sopB, and pefA among the isolated strains was determined. We categorized all the Salmonella ser. Typhimurium strains with enterobacterial repetitive intergenic consensus (ERIC)-PCR.

Conclusions: Our results confirm that some wild avian species are reservoirs for Salmonella serotypes, especially Salmonella Typhimurium.


have also been documented in gulls, crows, vultures, and parrots [6,7]. *Salmonella* is an environmentally persistent pathogen that can survive and proliferate in diverse environments, including in animals that form part of the human food chain [8]. The molecular characterization of *Salmonella* serovars isolated from poultry, food, and the environment has been reported (e.g., virulence genes and the homology of strains) [9-12]. In contrast, there are few reports of the characterization of strains isolated from wild birds throughout the world. The aim of this study was to isolate and characterize *Salmonella* strains from selected free-living bird species in Poland.

**Methods**

During the period from September 2011 to August 2013, 1000 samples were collected: 235 cloacal swabs from four species of aquatic wild birds, and 699 fecal samples and 66 tissue samples from 36 different species of free-living birds (Table 1). Birds found dead and feces were collected by ornithologists from live and dead individuals in six different regions of Poland during the following bird ringing seasons:

- winter and early spring in the Wroclaw city center, suburbs, and parks, in the Lower Silesia region, the Baltic coast, and two wildlife rescue centers;
- summer and early autumn in the Rakutowskie Lake of Kuyavian–Pomeranian Voivodeship (northern Poland) and in the Sudetic Mountains (southern Poland).

The ornithologists ringed the birds with the consent of the General Directorate of Environmental Protection, Poland (nos. 253/2012 and 259/2013).

Cloacal swabs from mallard ducks and black coots were obtained during the hunting season by two hunting associations in accordance with local hunting laws, special permission (with the consent of the Regional Directorate of Environmental Protection, Wroclaw, Poland, no. WPN. 6205.67.2012.MK.1), and hunting programs. Samples from great cormorants were obtained during the annual population cull in Poland. All cloacal swabs from mallards, black coots, and great cormorants were collected in the lakes of the Lower Silesia region between August 15, 2012, and December 12, 2012. Cloacal swabs were collected from velvet scoters that were found dead in fishing nets on the Baltic coast in late winter and early spring.

The species of birds were grouped by their preferred habitats and/or behavior and were divided into waterfowl, songbirds, and birds kept in rescue centers, as well as migratory, partially migratory, or resident species (Table 1). The research was conducted with the consent of the 2nd Local Ethical Committee for Animal Experiments (Wroclaw, Poland; no. 41/2011).

**Bacterial isolation**

All the samples were analyzed for *Salmonella* strains, which were isolated using the International Organization for Standardization Procedure PN-EN ISO 6579: 2003/A1: 2007. The samples were pre-enriched in nonselective buffered peptone water (Merck, Darmstadt, Germany) for 20 h at 37°C. After incubation, enriched modified semisolid Rappaport–Vassiliadis medium (Merck) was inoculated with the samples and incubated for 24 h at 41.5°C. The cultures were differentiated on solid xylose–lysine–deoxycholate agar (Merck) and on MacConkey agar (Merck), incubated for 24 h at 37°C. Three colonies per plate with the characteristics of *Salmonella* spp. were then spread onto nutrient agar (Merck) and incubated for 24 h at 37°C. The colonies were then identified biochemically with the API 20E system (BioMerieux, Marcy l’Etoile, France). All isolates were stored in Microbank vials (Microbank, Pro-Lab Diagnostics, Round Rock, TX, USA) at −70°C for further analysis.

**DNA extraction**

After the cells were incubated overnight at 37°C on nutrient agar (Merck), the bacterial genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s instructions. The DNA was quantified spectrophotometrically (BioPhotometer, Eppendorf, Wesseling-Berzdorf, Germany) and stored at −20°C.

**Salmonella identification with PCR**

The genus *Salmonella* was confirmed with multiplex PCR. *Salmonella* was identified at the genus level with the invA gene and at the subspecies level with the same multiplex PCR. The primer sequences used for amplification are summarized in Table 2. *Salmonella* was identified at the genus and subspecies levels according to Lee et al. [13].

**Salmonella serotyping**

*Salmonella* isolates were serotyped using single-factor antisera (Sifin, Berlin, Germany), according to the White–Kauffman–Le Minor scheme, focusing particularly on the five serovars mentioned above, which are monitored in poultry by the EU.

**Enterobacterial repetitive intergenic consensus (ERIC)-PCR**

The genetic diversity of the isolated *Salmonella* ser. Typhimurium strains was analyzed with ERIC-PCR, using a protocol and primers (ERIC-R: 5′-ATGTAAG CTCTGAGGATTCA-3′; ERIC-F: 5′-AAGTAAGT GACTGAGGATGAGCG-3′) targeting the palindromic
| No. | Origin                  | Type of material | Total amount of tested individuals | Positive samples (%) | Environmental data **/* *** |
|-----|-------------------------|------------------|------------------------------------|----------------------|-----------------------------|
| 1   | Mallard duck *Anas platyrhynchos* | cloacal swabs     | 121 (d)                            | 8 (6.61)             | 1/A                         |
| 2   | Great cormorant *Phalacrocorax carbo* | cloacal swab     | 77 (d)                             | 8 (10.39)            | 1/A                         |
| 3   | Velvet scoter *Melanitta fusca* | cloacal swab     | 30 (d)                             | 0 (0.00)             | 7/A                         |
| 4   | Black coot *Fulica atra* | cloacal swab     | 7 (d)                              | 0 (0.00)             | 1/B                         |
| 5   | Mute swan *Cygnus olor* | feces            | 27 (a)                             | 0 (0.00)             | 1.2/A                       |
| 6   | Whooper swan *Cygnus cygnus* | feces            | 6 (a)                              | 0 (0.00)             | 1.2/A                       |
| 7   | Great tit *Parus major* | feces/tissue     | 109 (92a/17d)                      | 10 (9.17)            | 3,4,5,6/B                   |
| 8   | Blue tit *Cyanistes caeruleus* | feces/tissue    | 43 (36a/7d)                        | 1(2,32)              | 3,4,5,6/C                   |
| 9   | Eurasian tree sparrow *Passer montanus* | feces/tissue | 53 (48a/5d)                        | 2 (3,77)             | 3,4,5,6/C                   |
| 10  | Redpoll *Carduelis cabaret* | feces            | 57 (a)                             | 1(1.75)              | 6/ A                        |
| 11  | Eurasian siskin *Carduelis spinus* | feces/tissue   | 48 (39a/9d)                        | 16 (33,3)            | 3,4,5,6/A                   |
| 12  | Common chiffchaff *Phylloscopus collybita* | feces        | 45 (a)                             | 0 (0.00)             | 5,6/A                       |
| 13  | Bluethroat *Luscinia svecica* | feces            | 43 (a)                             | 0 (0.00)             | 3,4,5,6/A                   |
| 14  | European robin *Erithacus rubecula* | feces          | 36 (a)                             | 0 (0.00)             | 5,6/A                       |
| 15  | Common reed bunting *Emberiza schoeniclus* | feces      | 35 (a)                             | 0 (0.00)             | 3,4,5,6/A                   |
| 16  | Eurasian blackcap *Sylvia atricapilla* | feces         | 35 (a)                             | 0 (0.00)             | 3,4,5,6/B                   |
| 17  | Greenfinch *Carduelis chloris* | feces/tissue   | 30 (20a/10d)                       | 10 (33,3)            | 3,4,5,6/C some populations A |
| 18  | Pied flycatcher *Ficedula hypoleuca* | feces          | 19 (a)                             | 0 (0.00)             | 6/ A                        |
| 19  | Hedge sparrow *Prunella modularis* | feces          | 17 (a)                             | 0 (0.00)             | 5,6/ B                      |
| 20  | Barn swallow *Hirundo rustica* | feces          | 17 (a)                             | 0 (0.00)             | 3,4,5,6/A                   |
| 21  | Common starling *Sturnus vulgaris* | feces/tissue   | 16 (13a/3d)                        | 3 (18,75)            | 3,4,5,6                     |
| 22  | Eurasian reed warbler *Acrocephalus scirpaceus* | feces      | 15 (a)                             | 0 (0.00)             | 5,6/A                       |
| 23  | Fieldfare *Turdus pilaris* | feces          | 13(a)                              | 0 (0.00)             | 5,6/A                       |
| 24  | Yellow wagtail *Motacilla flava* | feces          | 13 (a)                             | 0 (0.00)             | 3,4,5,6/ A                  |
| 25  | Blackbird *Turdus melura* | feces/tissue   | 11 (10a/1d)                        | 1 (9.09)             | 3,4,5,6/B                   |
| 26  | Common chaffinch *Fringilla coelebs* | feces       | 9(a)                               | 0 (0.00)             | 3,4,5,6/B                   |
| 27  | Whitethroat *Sylvia borin* | feces          | 9 (a)                              | 0 (0.00)             | 5,6/A                       |
| 28  | Yellow- hammer *Emberiza cinniella* | feces         | 7 (a)                              | 0 (0.00)             | 3,4,5,6/B                   |
| 29  | Lesser whitethroat *Sylvia curruca* | feces       | 7 (a)                              | 0 (0.00)             | 5,6/A                       |
| 30  | Long-tailed tits *Aegithalos caudatus* | feces     | 6 (a)                              | 0 (0.00)             | 6/B                         |
| 31  | Hooded crow *Corvus cornix* | tissue        | 6 (d)                              | 0 (0.00)             | 2/B                         |
| 32  | Rook *Corvus frugilegus* | feces/tissue   | 6 (3a/3d)                          | 1 (16,66)            | 2/A                         |
| 33  | Common wood pigeon *Columba palumbus* | feces/tissue | 6 (2a/4d)                          | 1 (16,67)            | 2/A                         |
| 34  | Common swift *Apus apus* | feces/tissue   | 5 (4a/1d)                          | 1 (20,00)            | 3,4,5,6/A                   |
| 35  | Willow warbler *Phylloscopus trochilus* | feces   | 5(a)                               | 0 (0.00)             | 6/A                         |
| 36  | Willow tit *Poecile montanus* | feces        | 5 (a)                              | 0 (0.00)             | 3,4,5,6/ B                  |
| 37  | Eurasian marsh harrier *Circus aeruginosus* | feces      | 1(a)                               | 1 (100,00)           | 8/A                         |
sequences of ERIC with the method described by Versalovic et al. [14].

PCR detection of virulence genes

The virulence genotyping of Salmonella ser. Typhimurium (18 strains), Salmonella ser. Hadar (one strain), Salmonella ser. Infantis (one strain) was performed with the multiplex PCR described by Skyberg et al. [9]. The primers used in this experiment are listed in Table 3.

Positive controls

Two strains, Salmonella ser. Typhimurium (ATCC # 14028) and Salmonella ser. Hadar (laboratory strain), previously shown to contain all the genes tested (Salmonella species, subspecies and virulence genes), served as positive control strains. Identity of Salmonella ser. Hadar strain was verified by sequencing.

Results

Isolation and identification

Salmonella species were isolated from 64 (6.4%) of the 1000 samples collected (Tables 1 and 4). Most of the positive isolates came from the Eurasian siskin (Carduelis spinus) (16/48, 33.33%) and the greenfinch (Carduelis chloris; 10/30, 33.33%). Positive samples were also collected from 13 other species, including the great cormorant (Phalacrocorax carbo; 8/77, 10.39%), great tit (Parus major; 10/109, 9.17%), and mallard duck (Anas platyrhynchos; 8/121, 6.61%). A positive sample was also obtained from a Eurasian marsh harrier (Circus aeruginosus; 1/1, 100.00%). This last sample was collected from the bird during its second day at a wildlife rescue center in Lower Silesia before antibiotic treatment was commenced (Table 1).

The collected Salmonella strains all belonged to one of three subspecies: enterica (81.25%), salamae (17.19%), or houtenae (1.56%). S. enterica subsp. enterica was isolated from the vast majority of bird species, but S. enterica subsp. salamae was collected from four species of birds (Eurasian tree sparrow, great cormorant, great tit, and common swift). Only one strain, isolated from a mallard duck, was S. enterica subsp. houtenae (Table 4).

Among the Salmonella strains collected, four of the five serovars of Salmonella that are constantly monitored by the EU in poultry were found in free-living birds. Eighteen strains belonged to ser. Typhimurium (28.13%), one to ser. Infantis (1.56%), one to ser. Virchow (1.56%), and one to ser. Hadar (1.56%). No Salmonella ser. Enteritidis was isolated from any sample collected from free-living birds.

ERIC-PCR categorized the 18 Salmonella ser. Typhimurium strains obtained from free-living birds into different profiles. One strain remained as nonhomologous to any other strain. The Salmonella ser. Typhimurium strains showed no correlation with bird species (e.g., isolates from Eurasian siskin nos. 22, 42, and 16 differed), but similarity was observed among the strains isolated from the same environmental areas (strain nos. 60, 12, 2, 18, and 37 were similar). The first cluster included strains collected in two regions: Wroclaw city center and suburbs. The Salmonella ser. Typhimurium isolates collected from dead birds also displayed genetic diversity (Figure 1).

All the isolated Salmonella ser. Typhimurium strains contained the spiA, msgA, invA, lpfC, and sifA genes; 94.45% isolates also contained the sitC and sopB genes. None of the Salmonella ser. Typhimurium strains contained the cdtB gene. The presence of other genes was investigated. The genes in the Salmonella ser. Typhimurium strains were highly variable. The one Salmonella ser. Hadar strain contained all the tested genes, except spvB and pefA; the one Salmonella ser. Infantis strain contained all the tested genes, except spvB, pefA, and cdtB; and the one Salmonella ser. Virchow contained all the tested genes, except spvB, pefA, cdtB, and

| Table 1 Salmonella isolates obtained from free-living birds (Continued) |
|----------------|----------------|----------------|----------------|
| 38 | Sparrowhawk Accipiter nisus | feces | 1(a) | 0 (0.00) | 8/B |
| 39 | Common buzzard Buteo buteo | feces | 1(a) | 0 (0.00) | 9/B |
| 40 | Golden eagle Aquila chrysaetos | feces | 3(a) | 0 (0.00) | 9/C |

A, dead individuals; a, alive individuals; None of the strains belonged to ser. Typhimurium strains obtained from free-living birds into different profiles. One strain remained as nonhomologous to any other strain. The Salmonella ser. Typhimurium strains showed no correlation with bird species (e.g., isolates from Eurasian siskin nos. 22, 42, and 16 differed), but similarity was observed among the strains isolated from the same environmental areas (strain nos. 60, 12, 2, 18, and 37 were similar). The first cluster included strains collected in two regions: Wroclaw city center and suburbs. The Salmonella ser. Typhimurium isolates collected from dead birds also displayed genetic diversity (Figure 1).

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| Genes | Function of gene | Sequence of nucleotides | Size |
|-------|-----------------|-------------------------|------|
| STM   | encodes a putative inner membrane protein, specific for S. enterica subsp I | F-GGTGGCCTCAGATGATTCCCG R-CGCACTTGTAGCGAGCGGCCG | 137 bp |
| stn   | encodes Salmonella enterotoxin and is specific for S. enterica | F-CGATCCCTTTTCCGCCTATC R-GGCAGATGAGACGCTTAAG | 179 bp |
| invA  | invasion protein, for simultaneous identification of Salmonella at the genus level | F-ACAGTGCTGCTTACAGCTGAAAT R-AGAGACTGTGACTGATCGATAAT | 244 bp |
| gatD  | encodes the galactitol-1-phosphate dehydrogenase (gatD), contributes to acid production from galactitol | F-GGCAGATATTATTCCTATTAC R-CATTCCCCGGCTATTACCGTAT | 501 bp |
| mdcA  | encodes the alpha subunit of the enzyme that contributes to malonate utilization | F-GGATGTACTCTCCATCCCCAGT R-CGTAGCGAGCATCTGGATATCTTT | 728 bp |
| fljB  | encodes phase 2 flagellin, enables differentiation between monophasic and diphasic subspecies | F-GACTCCATCCAGGCTGAATCAC R-CGGCTTTGCTGGCATTTAG | 848 bp |
The prevalence of virulence genes in the *Salmonella* ser. Typhimurium strains varied among the live and dead free-living birds (Figure 1).

**Discussion**

The results of this study confirm that *Salmonella* ser. Typhimurium, one of the most frequently reported serotypes in human salmonellosis in the EU, occurs among free-living birds. Three other serotypes monitored in poultry flocks by the EU, Hadar, Virchow, and Infantis, were also present among the free-living bird populations.

Free-living birds are considered to be potential carriers of these bacteria and to play a role in the ecology and circulation of several zoonotic pathogens [4-7].

In Central Europe, only a few reports of salmonellosis in wild birds have been published, in the 1990s [7,15]. In Poland, all similar research has been conducted in the small northern region of the country, and there is a dearth of wide epidemiological studies in this field [16,17].

*Salmonella* infection may occur as a visible illness or be asymptomatic, depending upon the bird species. It

| Genes | Function of gene | Sequence of nucleotides | Size |
|-------|------------------|-------------------------|------|
| spvB  | Growth within host | F-CTATCAGCCGACGAGCGAGCTATTGT | 717 bp |
| spiA  | Survival within macrophage | F-CGAAGGCGTGTGTTATGAGGTTGAGT | 550 bp |
| pagC  | Survival within macrophage | F-CGCTTTTCTGTGAGTATGATGAG | 454 bp |
| cdtB  | Host recognition/invasion | F-ACCACTTTCGCTCTCAGGTTGTTGGTTGAGT | 268 bp |
| msgA  | Survival within macrophage | F-GCCAGGCAGCGCTGAAATCCATTGGGTTA | 189 bp |
| invA  | Host recognition/invasion | F-CTGGCGGTGTTTGTCTCTTCTATT | 1070 bp |
| sipB  | Entry into nonphagocytic cells | F-GAAGGCGGTTTGATGAGTTGAGT | 875 bp |
| pigH  | Host recognition/invasion | F-GCCGCGAGCAGCTGACTGAGTTGTTGTC | 756 bp |
| span  | Entry into nonphagocytic cells | F-AAAACCGCGGATTACCTGGTATTGAGT | 504 bp |
| orgA  | Host recognition/invasion | F-TTGTGCAAGCTCATGCGAGGTA | 255 bp |
| tolC  | Host recognition/invasion | F-TACCCAGGGCAAAAGAGGCCTATC | 161 bp |
| iron  | Iron acquisition | F-CTGCGGTGTTATGCTTGGTGTTG | 1205 bp |
| sirC  | Iron acquisition | F-CAGTATATGCTCAACCGCGATGTTGGTGCT | 768 bp |
| lpfC  | Host recognition/invasion | F-GCCGCCGTGAAAGCGCTGTTG | 641 bp |
| sipA  | Filamentous structure formation | F-TTGTGCAAAGGCTGTTGCCCT | 449 bp |
| sopB  | Host recognition/invasion | F-CGGAGCGGCGAAGCGA | 220 bp |
| pefA  | Host recognition/invasion | F-GCGCGTGCCATGGCGA | 157 bp |
may also result from exposure to an environment that has been contaminated by infected humans or livestock [15,18,19]. Migratory birds, in particular, are potential reservoirs for bacterial agents [20]. Many wild passerines have been documented as carriers of Salmonella strains, and their involvement in the transmission of Salmonella to mammals and people has been suggested [21,22]. In this study, most of the positive samples came from garden bird species: Eurasian siskins and greenfinches. These results are compatible with the findings of Hughes et al. [23], who reported that Salmonella caused mortality in wild birds, particularly garden birds, in the United Kingdom. Lawson et al. [24] also reported that house sparrows and greenfinches are particularly susceptible to salmonellosis. Consistent with our results, it has also been documented that the Salmonella serovar most commonly isolated from free-living birds is ser. Typhimurium. This result suggests a high incidence of Salmonella exposure near bird feeders during winter and its transmission to birds. It can be inferred that the risk of transmission from the feces of infected wild passerines to uninfected birds is high, especially in urban areas with many bird feeders. As reported by Hamer et al. [25] and later noted by Borrelli et al. [26], the key features of the urban environment that promote the transmission of pathogens include increased host contact rates, susceptibility to infection, high rates of pathogen introduction, pollution and stress (which reduce the host immune function), and warmer microclimates with reduced seasonality (which allow the environmental persistence of some pathogens). These factors may explain the increased frequency of salmonellosis we observed in birds between February and April during a prolonged winter in Poland in 2013 (data not shown in the table). In the United Kingdom, Hughes et al. [23] reported similar

### Table 4 Species, subspecies, and serotypes of Salmonella isolates collected

| Species             | Subspecies | Serotype              | Origin                  | Number of isolates |
|---------------------|------------|-----------------------|-------------------------|--------------------|
| Salmonella enterica | enterica   | Typhimurium 4,12:i:1,2| Eurasian siskin         | 7                  |
|                     |            |                       | Greenfinch              | 3                  |
|                     |            |                       | Mallard duck            | 3                  |
|                     |            |                       | Redpoll                 | 1                  |
|                     |            |                       | Common wood pigeon      | 1                  |
|                     |            |                       | Blue tit                | 1                  |
|                     |            |                       | Great tit               | 1                  |
|                     |            |                       | Blackbird               | 1                  |
|                     | Infantis   | 6,7:r:1,5             | Common starling         | 1                  |
|                     | Virchow    | 6,7:r:1,2             | Common starling         | 1                  |
|                     | Hadar      | 6,8:z:1,2             | Mallard duck            | 1                  |
|                     | others     |                       | Eurasian siskin         | 8                  |
|                     |            |                       | Great cormorant         | 7                  |
|                     |            |                       | Mallard duck            | 4                  |
|                     |            |                       | Common starling         | 1                  |
|                     |            |                       | Greenfinch              | 7                  |
|                     |            |                       | Great tit               | 1                  |
|                     |            |                       | Rook                    | 1                  |
|                     |            |                       | Eurasian marsh harrier  | 1                  |
|                     |            |                       | Eurasian tree sparrow   | 1                  |
|                     | salamae    |                       | Eurasiar siskins        | 8                  |
|                     | others     |                       | Great cormorant         | 1                  |
|                     |            |                       | Great ttit              | 8                  |
|                     |            |                       | Common swift            | 1                  |
|                     | houtenae   |                       | Mallard duck            | 1                  |
|                     | others     |                       |                         |                    |
peaks of *Salmonella* isolation in January and February. Kapperud et al. [18] documented the seasonality of salmonellosis outbreaks, simultaneously in people and wild passerines, in Norway in 1998, which appeared in both groups between January and April. It is also possible that salmonellosis outbreaks in free-living birds during this time of year are associated with the feeding of birds by people. Supplemental feeding creates high densities of birds, high concentrations of feces, and stress arising from social interactions, which may also increase the prevalence of some bacterial species among wild birds [25]. It has been suggested that certain strains of *Salmonella ser.* Typhimurium are associated with different groups of wild birds [19,23,27-30]. This is supported by the recovery of this serotype from mallard ducks and great cormorants in this study.

Daoust and Prescott [31] reported that salmonellosis can cause sporadic mortality, particularly among birds around feeders, but also in young birds in large breeding colonies. These results prompted us to check the prevalence of selected virulence genes (encoding virulence factors) that are also capable of causing human infections [9,10,12]. In this study, we have demonstrated the great variability in the virulence genes present in isolated *Salmonella* strains in both dead and live birds, and among birds of the same species.

Similar results for the prevalence of virulence genes have been reported by other researchers. Skyberg et al. [9] recorded that the same 17 virulence genes were widespread in many *Salmonella* serovars isolated from both sick and healthy birds. Similar findings were recorded by Mezal et al. [11] among environmental samples, including dust, water, and other materials from poultry houses. Our study confirms the presence of the same virulence genes, which might play important roles in the bacterial invasion and survival in the host of *Salmonella* isolates collected from different species of free-living birds, as in human clinical isolates. These findings suggest that like poultry flocks, poultry houses, and the environments around poultry farms, wild birds might be a source of *Salmonella* strains that are pathogenic to people. We also found evidence that the genetic heterogeneity of some *Salmonella* serovars (e.g., *ser.* Typhimurium) is changeable, but is greater among different species of birds that spend their lives in similar geographical localities. Chrzastek et al. [32] also demonstrated a correlation between genetic homogeneity and the geographical origin of the host, but with *Pasteurella multocida* strains collected from poultry in different regions in Poland. Our results confirm the genetic similarity of *Salmonella ser.* Typhimurium strains isolated from wild birds in the area of Wroclaw.
Conclusions

Salmonella species are present in populations of free-living bird species, especially in birds sampled in urbanized areas. Some wild avian species are reservoirs for Salmonella serotypes, especially Salmonella serovar Typhimurium. Most of the positive samples came from the Eurasian siskin and the greenfinch. The Salmonella isolates presented the same virulence genes as in human clinical isolates. This suggests a potential risk for people feeding infected wild birds.

Availability of supporting data

The study was conducted with the special consent mentioned in the text above (see Methods). All dead birds (except game birds) were found already dead and brought to the clinic. Game birds were hunted and collected by hunters in accordance with local hunting laws. Samples of great cormorants were obtained during the annual population cull in Poland, in accordance with the annual specifications of the Regional Directorate of Environmental Protection.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

MK (Krawiec): main researcher, collected the samples, performed the microbiological analyses and molecular biological analyses, wrote the paper. MK (Kuczkoński): coordinated the molecular biological analyses. AGK: scientific consultation. AW: principal investigator, scientific coordinator of the research and data analysis, collaborated in writing the paper. All authors read and approved the final manuscript.

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