Salmonella infection in birds kept in parks and pet shops in Tehran, Iran

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Abstract

Salmonellosis is one of the most important zoonotic diseases worldwide. *Salmonella* infections in wild birds are reported frequently. The objectives of this study were to isolate *Salmonella* serovars from a large collection of samples obtained from pet birds in Tehran, Iran, and then to determine the serotypes and antimicrobial susceptibility profile of the isolates. Between October 2007 and August 2008, 668 samples from 24 different species were collected from birds kept in parks and pet shops of Tehran. Samples contained cloacal swabs from large birds, freshly-dropped feces from small birds and, infrequently, carcasses. Multiple samples from the same bird were pooled and considered as an individual sample. All samples were cultured for the isolation and identification of *Salmonella* serovars according to standard procedures. Serotyping was performed by slide agglutination test to determine the O and H antigens of the isolates. The antimicrobial susceptibility of the isolates was determined to a panel of 30 antimicrobial agents using the agar disc diffusion method. In total, 19 *Salmonella* isolates (2.8%) were identified. Samples that were positive for *Salmonella* originated from canaries (10 out of 62, 16.1%), pigeons (5 out of 139, 3.6%), psittacines (3 out of 130, 2.3%), and eagles (1 out of 2, 50%). All *Salmonella* isolates were susceptible to danofloxacin, norfloxacin, levofloxacin, amikacin, gentamicin, and tobramycin. Resistance to other antibacterial agents was variable and ranged from 0-57.9%. There were 17 resistance patterns among the isolates. Serotyping identified nine isolates (47.3%) as serogroup B, six isolates (31.5%) as serogroup C, and four isolates (21%) as serogroup D. The findings of this study showed the presence of *Salmonella* infection among captive birds. Due to the close contact between these type of birds and humans, these findings present an important risk for public health.

Introduction

Salmonellosis is one of the most important zoonotic diseases worldwide (Gast, 2008). In addition to the risks it provides for public health, *Salmonella* infections impose economical losses to both the public healthcare system and the poultry industry (Collard *et al*., 2008). More than 2,600 serovars of *Salmonella* have been identified, some of which are responsible for human illness, as well as diseases in a wide variety of animals (Gast, 2008).

Salmonella infections in wild birds occur frequently, are a cause of mortality in birds, and can be transferred to humans and domestic animals. Immunosuppressed people are susceptible to the more common *Salmonella* Typhimurium carried by some pet birds (Fudge, 2001). It appears that the prevalence of *Salmonella* in wild birds has been increased significantly in recent years and there have been several studies on this issue to determine and evaluate this risk (Tizzard, 2004). However, compared with research performed in poultry, studies on *Salmonella* infections in wild birds have been sparse to date.

The exposure of wild birds to a contaminated environment may create infection accidentally. This occurs commonly in domestic pigeons and colonial water birds. *Salmonella* can be easily transmitted to other animals by contaminated birds' feces, since they often gather in very large numbers at feeders. Sources of stress, including food shortage, poor husbandry with overcrowding and lack of aviary maintenance, breeding, poor weather conditions, and the introduction of new birds, may cause the development of salmonellosis and death (Fudge, 2001; Tizzard, 2004). These infected birds may transmit infections to humans, either directly as a result of handling, or indirectly. Small passerines, canaries, and finches are social birds often bred and housed in flock aviaries. Some species like finches, siskins, and sparrows often seek food on the ground that may be contaminated by droppings from infected birds. These birds also probably encounter a higher risk, since they often spend a relatively long duration at the feeding site. Due to the zoonotic nature of *Salmonella*, it is very important that pet bird owners are trained to practice good hygiene.

In Iran, few studies have examined *Salmonella* infections in pet birds or those that live in proximity to humans, such as in gardens. In this study, we investigated the prevalence of *Salmonella* infections in wild birds from parks in Tehran and pet shops, the *Salmonella* serotypes involved, and the drug resistance patterns of the *Salmonella* isolates.

Materials and Methods

Sampling and bacteriological procedures

A total of 668 samples from 24 different species (of five orders) were collected from birds kept in Tehran parks and pet shops during October 2007 - August 2008 and were investigated for the presence of *Salmonella*. Samples included the cloacal swabs from large birds, freshly-dropped feces from small birds and, infrequently, carcasses. Multiple samples from the same bird were pooled and considered as an individual sample. All samples were cultured for the isolation and identification of *Salmonella* according to standard procedures that have been previously described (Waltman *et al.*, 1998). Briefly, the selective enrichment of samples in selenite F at 41°C for 24 h was followed by sub-cultivation on *Salmonella*-*Shigella* and MacConkey agar at 37°C for 24 h. Then, the suspect colonies were selected, isolated and further characterized by biochemical identification. Positive samples were kept at -70°C and in liquid nitrogen for future use.

Determination of serogroups and serotypes

The slide agglutination test was carried out using *Salmonella* somatic O poly A-S antisera (ProLab, England), as previously described (Waltman *et al.,* 1998). Each suspect *Salmonella* culture was mixed with a drop of polyvalent antisera and incubated for up to 2 min at room temperature. Positive reactors were then tested separately with different somatic O monovalent (O2, O4, O5, O7, O8, O9, O12) and flagellar H monovalent (H2, H6, HL, Hgm) antisera (ProLab, England) to determine the serogroups and serotypes of the isolates. Controls were run simultaneously in parallel in all tests. All negative results were re-tested by the tube agglutination test (Waltman *et al*., 1998).

Drug susceptibility test

The susceptibility of the *Salmonella* isolates to a panel of antimicrobial agents was determined by the agar disk diffusion method and the interpretation of results was performed according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2000). The antimicrobial agents that were tested and their concentrations (μg) were as follows: ciprofloxacin (5), danofloxacin (10), ofloxacin (5), norfloxacin (10), enrofloxacin (5), levofloxacin (5), nalidixic acid (30), flumequine (30), cephalothin (30), ceftazidime (30), ceftriaxone (30), cefixime (5), ampicillin (10), amoxi-clav (30), carbenicillin (100), piperacillin (100), kanamycin (30), neomycin (30), streptomycin (10), amikacin (30), gentamicin (10), tobramycin (10), Fosbac® (200), fosfomycin (200), lincospectin (15/200), chloramphenicol (30), florfenicol (30), furazolidone (100), tetracycline (30), and trimethoprim-sulfamethoxazole (1.25/23.75). Fosbac® and fosfomycin disks were provided from Bedson Co. (Buenos Aires, Argentina). All other antibacterial disks were purchased from Padtan Teb Co. (Tehran, Iran). The ATCC reference strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, ATCC 27853, and *E. coli* ATCC 35218, were used for quality control purposes. In this study, the *Salmonella* isolates with intermediate susceptibility classification were considered not to be resistant to that drug. Multidrug resistance (MDR) was defined as resistance to more than one drug.

Results and Discussion

Out of 668 samples tested, 19 *Salmonella* isolates (2.8%) were identified. Samples that were positive for *Salmonella* originated from canaries (10 out of 62, 16.1%), pigeons (5 out of 139, 3.6%), psittacines (3 out 130, 2.3%), and eagles (1 out of 2, 50%). All *Salmonella* isolates were susceptible to danofloxacin, norfloxacin, levofloxacin, amikacin, gentamicin, and tobramycin (Table 1). Resistance to other antibacterial agents was variable and ranged from 0%–57.9% (Table 1). Isolates were resistant to at least one and to a maximum of 11 agents (Table 2). No isolate was resistant to more than 11 agents. There were 17 resistance patterns among the isolates. Out of 19 *Salmonella* isolates, 16 (84.2%) belonged to 16 different resistance patterns, whereas the remaining three isolates (15.8%) were not resistant to any agent. Serotyping identified nine isolates (47.3%) as serogroup B, six isolates (31.5%) as serogroup C. The last four isolates (21%) belonged to serogroup D, and these were all shown to be *Salmonella* Enteritidis.

There is much evidence on the involvement of domestic and companion animals in direct transmission of pathogens to humans, whereas the reservoir for most zoonoses is wildlife (Kruse *et al.*, 2004). In the case of *Salmonella*, researchers have shown the presence of this important zoonotic agent in wild birds. In 2001, Kirk *et al.* isolated *Salmonella* spp. from 2.5% of a cohort of 892 birds tested in California. Kobayashi *et al.* (2007) isolated *Salmonella* spp. from 5.8% (19 of 328) cloacal swabs obtained from wild birds, in which all *Salmonella* isolates were *Salmonella* Typhimurium. Other researchers (Hughes *et al*., 2008) obtained 32 *Salmonella* isolates from wild birds in northern England, of which 29 belonged to the *Salmonella* Typhimurium serovar, two isolates were *Salmonella* Newport and *Salmonella* Senftenberg, and one isolate was not identified by standard serotyping.

There have been several studies on the prevalence of *Salmonella* infection among wild birds kept in parks, gardens, or in cages. Out of 1,047 fecal swabs sampled at one location in southern Sweden from black-headed gulls

Table 1: Antimicrobial susceptibility profile of 19 *Salmonella* isolates to 30 antimicrobial drugs

Drugs	Resistant (%)	Intermediate Susceptible (%)	Susceptible (%)
Ciprofloxacin	0	15.8	84.2
Danofloxacin	0	0	100
Enrofloxacin	5.3	36.8	57.9
Levofloxacin	0	0	100
Norfloxacin	0	0	100
Ofloxacin	0	10.5	89.5
Nalidixic acid	47.4	21	31.6
Flumequine	36.8	0	63.1
Cephalothin	0	10.5	89.5
Ceftazidime	15.8	0	84.2
Ceftriaxone	10.5	5.3	84.2
Cefixime	10.5	10.5	79
Ampicllin	0	15.8	84.2
Amoxi-Clav	0	10.5	89.5
Carbenicillin	57.9	42.1	0
Piperacillin	15.8	21	63.2
Kanamycin	5.3	21	73/7
Neomycin	0	31.6	68.4
Streptomycin	52.6	15.8	31.6
Amikacin	0	0	100
Gentamicin	0	0	100
Tobramycin	0	0	100
Fosbac®	5.3	0	94.7
Fosfomicin	5.3	0	94.7
Linco-spectin	31.6	36.8	31.6
Chloramphenicol	5.3	31.6	63.1
Florfenicol	21	5.3	73.7
Furazolidone	26.3	10.5	63.1
Tetracycline	47.4	36.8	15.8
Trimethoprim-Sulfamethoxazole	31.6	5.3	63.1

Table 2: Multi-drug resistance[a] among Salmonella isolates of this study.

No. of antimicrobial drugs used	No of resistant isolates (%)
At least 1	16 (84.2)
> 1	14 (73.7)
> 2	11 (57.9)
> 3	9 (47.4)
> 4	8 (42.1)
> 5	6 (31.6)
> 6	6 (31.6)
> 7	6 (31.6)
> 8	3 (15.8)
> 9	2 (10.5)
> 10	1 (5.3)
> 11	0 (0)

[a]Multi-drug resistance was defined as resistance to more than one drug.

during a 3-year period (1998–2000), *Salmonella* was found in 28 (2.7%) individuals and *Salmonella* Typhimurium (83%) was the predominant serotype (Palmgren *et al.*, 2006). Georgiades *et al.* (2002) isolated *Salmonella* from 53 out of 618 pigeons (8.6%), 33 out of 182 canaries (18.1%) and 2 out of 71 psittacines (2.8%) from the Greater Thessaloniki area in Greece. *Salmonella* Typhimurium was the most frequently isolated serotype in pigeons (75.5% of isolates), followed by *Salmonella* Enteritidis (11.3%). In canaries, *Salmonella* Typhimurium was also the most frequently isolated serotype (90.9%) followed by *Salmonella* Enteritidis (6.1%). According to the study by Georgiades *et al.*, the prevalence of *Salmonella* infection in the examined birds was rather low, whereas *Salmonella* Typhimurium and *Salmonella* Enteritidis appeared to be the most frequent serotypes in sampled birds (Georgiades *et al.*, 2002). In a recent study in Iran, Madadgar *et al.* (2009) recovered 34 *Salmonella* Typhimurium isolates from samples obtained from 17 canary flocks located in different regions of Tehran. In our study, the highest prevalence of *Salmonella* infection was also found in canaries. In another study in Iran, Mirzaie *et al.* (2010) found 18 (3.8%) *Salmonella* isolates among 470 samples from house sparrows that were subjected to culture. Nine *Salmonella* Typhimurium serovars, eight *Salmonella* Enteritidis serovars, and one *Salmonella* M*ontevideo* serovar were identified among the 18 *Salmonella* isolates that were serotyped. Our results also showed that the group B *Salmonella* (possibly *Salmonella* Typhimurium) was the most prevalent serogroup.

Pigeons have close contact with human in parks, temples, shrines and public gardens and can be potential reservoirs for several pathogenic microorganisms including *Salmonella* (Tanaka *et al.*, 2005). In some studies, a low prevalence (3%–4%) of *Salmonella* infections has been reported in pigeons (Pasmans *et al.*, 2004; Tanaka *et al.*, 2005). In our study, five out of 138 (3.5%) sampled pigeons carried *Salmonella*, among which three isolates were *Salmonella* Enteritidis, one isolate belonged to serogroup B and one isolate to serogroup C. Despite the low prevalence of *Salmonella* among pigeons, several studies have shown the role of pigeons and sparrows in the maintenance of *Salmonella* at feedlots and dairies (Connolly *et al.*, 2006; Pedersen *et al.*, 2006). The aviary may include different species or focus only on a single species. Keeping multiple birds in close contact with each other in mixed aviaries provides the ideal environment for infectious disease to spread easily.

The present study showed that the resistance to antimicrobial agents among *Salmonella* serovars isolated from garden or cage birds was much lower than those from commercial poultry (Morshed and Peighambari, 2010). However, higher rate of resistance were found in our *Salmonella* isolates to certain antimicrobial agents, including nalidixic acid, carbenicillin, streptomycin, lincospectin, florfenicol, tetracycline, and trimethoprim+sulfamethoxazole compared with those from studies in commercial poultry (Morshed and Peighambari, 2010). A recent study in Iran on 18 *Salmonella* isolates from captured house sparrows found all isolates to be sensitive to norfloxacin, flumequine, ampicillin, and sultrim, and 35% were resistant to lincospectin (Mirzaie *et al.*, 2010).

The presence of a MDR pattern has been previously reported among avian *Salmonella* isolates from Iran (Madadgar *et al.*, 2008; Mirzaie *et al.*, 2010; Morshed and Peighambari, 2010). In the present study, 73.7% of *Salmonella* isolates demonstrated the MDR pattern and the number of antibacterial agents varied between two to 11 among MDR types. MDR bacterial isolates of animal origin may spread into human population by direct contacts and through animal-origin foods (Soulsby, 2008). These resistant bacteria may colonize the human intestinal tract and the genes that encode for antibiotic resistance can consequently be transferred to the bacteria of natural microflora or pathogenic bacteria. The resistant bacteria that are shed in the environment may infect animals, and then travel back through the food chain to humans. The development of resistance to antimicrobial agents among bacterial strains should be carefully monitored throughout the world.

In conclusion, the results of this study showed the presence of *Salmonella* infection among birds in the parks, gardens, and pet shops of Tehran. This study highlights that, as a result of the close physical contact that is possible between these birds and humans, caged birds pose a risk to public health.

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