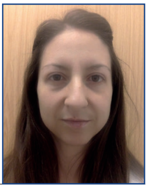
ORIGINAL SCIENTIFIC ARTICLE / IZVORNI ZNANSTVENI ČLANAK

Diversity and prevalence of *Salmonella* spp. in gulls caught at a landfill, Zagreb, Croatia



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Abstract

Gulls are a group of seabirds distributed worldwide that are an important reservoir of *Salmonella* spp. Salmonellosis is the second most commonly reported gastrointestinal infection in humans, and understanding the role wild birds have in spreading *Salmonella* can help to improve the health of humans and domestic animals. The mobility and migration capacity of gulls makes them an interesting group for research given their potential role in spreading pathogens. This paper presents the diversity and prevalence of *Salmonella* spp. in different gull species caught at a landfill in Zagreb in the winter months over a nine-year period from 2014-2022. In total, 1083 cloacal swabs were sampled from six gull species: Black-headed Gull (*Larus ridibundus*), Yellow-legged Gull (*L. michahellis*), Caspian Gull (*L. cachinnans*), Common Gull (*L. canus*), Lesser Black-back Gull (*L. fuscus*) and Herring Gull (*L. argentatus*). The prevalence of *Salmonella* was 5.82%, and 16 *Salmonella* serotypes were identified; *S.* Typhimurium had the highest prevalence (47.62%) followed by *S.* Enteritidis (12.69%) and *S.* Infantis (9.52%). To date, 82 *Salmonella* serotypes have been isolated in research on gulls in Europe, with *S.* Typhimurium as the most common, followed by *S.* Agona and *S.* Enteritidis. In this study, we found three serotypes not previously reported in gulls, *S.* Yalding, *S.* Reading and one with the antigenic formula O:17; H:z_{10}; H:e,n,x,z_{15} (IIIb).

Key words: *gull; Salmonella; wild birds; prevalence; serotype*

Introduction

There is strong interest in researching the epidemiology of pathogenic bacteria in wildlife, especially birds. Many species are natural carriers of bacteria and other microorganisms in their intestinal tract (Refsum et al., 2002; Reed et al., 2003; Pennycott et al., 2006). They can shed bacteria into the environment via faeces, thus

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spreading them to other animals and humans (Hudson et al., 2000). According to EFSA and ECDC (2021), salmonellosis is the second most commonly reported gastrointestinal infection in humans. A major source of infection for humans comes from poultry, pork and eggs (EFSA and ECDC, 2021). Understanding the role that wild birds play in spreading these bacteria is important for human and domestic animal health. Research to date has primarily been performed on birds already suspected in the spread pathogens, either directly to humans or through production farms, particularly those that are opportunistic feeders visiting various potential sites of infection (beaches, landfills, farmyards, fishponds, fish markets, farmland, cities, and water reservoirs) in close vicinity to human settlements (Reed et al., 2003; Antilles et al., 2021; Hubalek, 2021).

Gulls are a widespread group of seabirds of the family *Laridae*, known to be an important reservoir of *Salmonella* spp. and other pathogens like *Campylobacter* spp. (Quessy and Messier, 1992; Wahlström et al., 2003; Kinzelman et al., 2008; Rodríguez et al., 2012; Antilles et al., 2015; Dolejska et al., 2016; Toro et al., 2016; Moré et al., 2017). They are a gregarious species, breeding in colonies of varying size, roosting and feeding together, especially in winter (Harrison et al., 2021). Their mobility and migration capacity makes them interesting for the potential role in the spread of pathogens. Depending on the species, age, area, food availability and breeding season, they can remain near their colonies (less than 50 km), while during the non-breeding season they can travel hundreds of kilometres from their colonies (Arizaga et al., 2010; Juvaste et al., 2017; Enners et al., 2018; Fijn et al., 2022).

This paper presents the diversity and the prevalence of *Salmonella* spp. in different gull species caught at a landfill in Zagreb over a nine-year period from 2014–2022 and compares the results with those of other gull studies in Europe.

Materials and methods

In the winter period, from December 2014 to March 2022, gulls were captured using a cannon net at the Zagreb city landfill (45.765 N, 16.025 E). Each caught bird was ringed with steel and plastic rings, aged and identified. During every session, between 20 and 83 individual cloacal swabs were collected for *Salmonella* analysis.

Detection and isolation of *Salmonella* strains was performed according to the standard EN ISO 6579-1 method. Briefly, within 24 hours of sampling, swabs were placed in buffered peptone water (bioMérieux, France), and upon completion of 18 ± 2 h incubation at 37ºC, 0.1 mL of the sample was inoculated on three spots of one Modified Semi-solid Rappaport Vassiliadis (Biokar Diagnostics, France) agar plate. This was incubated at 41.5 ± 1ºC for 24 ± 3 h and for an additional 24 ± 3 hours in case of a negative result. If bacterial growth was observed, one loop from the migration zone was inoculated onto Xylose Lysine Deoxycholate agar (Oxoid Ltd, United Kingdom) and Rambach Chromogen agar (Merck, Germany). After the appropriate incubation time, the selective plating media were checked for the presence of colonies considered to be presumptive *Salmonella*. The selected, presumptive colonies were sub-cultured onto Columbia agar (bioMérieux, France) as a non-selective medium for the purpose of further biochemical identification and serotyping as the combination of these test results indicates whether an isolate belongs to the genus *Salmonella*. Pure colonies showing typical reactions for *Salmonella* on media for biochemical confirmation (Triple Sugar Iron agar: alkaline – red, slants and acid – yellow, butts, with gas formation and formation of hydrogen sulphide – blackening of the agar; Urea agar – remains unchanged; Lysine Decarboxylase agar – purple colour of the agar) were also tested for the presence of *Salmonella* O- and H- antigens by slide agglutination using polyvalent and monovalent antisera. Before the detection of the specific O and H antigens, pure colonies cultured on a non-selective agar medium were checked for auto-agglutination using saline solution. Only non-auto-agglutinating strains proceeded to the determination of the whole antigen formula. Serotyping was performed according to the CEN ISO/TR 6579-3 using polyvalent and monovalent O and H antisera (Bio Rad, France; Statens Serum Institut, Denmark).

Results

In total, 1083 cloacal swabs were collected from six gull species: Black-headed Gull (*Larus ridibundus*) (*n*=753), Yellow-legged Gull (*L. michahellis*) (*n*=296), Caspian Gull (*L. cachinnans*) (*n*=20), Common Gull (*L. canus*) (*n*=11), Lesser Black-back Gull (*L. fuscus*) (*n*=2) and Herring Gull (*L. argentatus*) (*n*=1). The overall *Salmonella* prevalence in the period 2014–2022 was 5.82%, with 16 different *Salmonella* serotypes identified (Tab. 1). *S.* Typhimurium had the highest prevalence (47.62%) followed by *S.* Enteritidis (12.69%) and *S.* Infantis (9.52%). The species with the highest *Salmonella* prevalence was the Caspian Gull (15.00%) followed by Black-headed Gull (5.71%) and Yellow-legged Gull

Table 1. Number of Salmonella serotypes found in six gull species caught at a landfill near Zagreb, Croatia from 2014–2022

Year	Species	No. of samples	No. of positive samples	No. of *Salmonella enterica* serotypes	% positive samples
2014	*L. michahellis*	23	0	/	0.00
	L. ridibundus	10	0	/	0.00
	L. fuscus	1	0	/	0.00
2015	*L. ridibundus*	105	4	Typhimurium (*n*=1) Infantis (*n*=1) Enteritidis (*n*=2)	3.81
2016	*L. michahellis*	59	6	Typhimurium (*n*=1) Kottbus (*n*=1) Enteritidis (*n*=1) Infantis (*n*=2) Derby (*n*=1)	10.17
	L. ridibundus	101	4	Newport (*n*=1) Typhimurium (*n*=3)	3.96

2017	*L. michahellis*	72	5	Goldcoast (*n*=1) Infantis (*n*=1) Typhimurium (*n*=3)	6.94
	L. ridibundus	102	12	Typhimurium (*n*=7) 4,5,12:i:- (*n*=2) Paratyphi B (*n*=1) Coeln (*n*=1) II_7:k:- (*n*=1)	11.76
	L. canus	3	0	/	0.00
	L. cachinnans	16	2	Infantis (*n*=1) Abony (*n*=1)	12.50
2018	*L. ridibundus*	56	6	Enteritidis (*n*=1) Typhimurium (*n*=4) IIIb_17:z_{10}: e,n,x,z_{15} (*n*=1)	10.71
	L. michahellis	81	4	Derby (*n*=2) Typhimurium (*n*=2)	4.94
	L. fuscus	1	0	/	
	L. canus	1	0	/	
	L. cachinnans	4	1	Enteritidis (*n*=1)	25.00
2019	*L. ridibundus*	133	7	Reading (*n*=1) Typhimurium (*n*=5) Derby (*n*=1)	5.26
	L. michahellis	2	0	/	0.00
	L. canus	5	0	/	0.00
2020	*L. argentatus*	1	0	/	0.00
	L. ridibundus	87	6	Enteritidis (*n*=2) Typhimurium (*n*=2) Coeln (*n*=1) Yadling (*n*=1)	6.89
	L. michahellis	59	1	Infantis (*n*=1)	1.69
2021	*L. ridibundus*	80	4	Hadar (*n*=1) Agona (*n*=1) Typhimurium (*n*=2)	5.00
2022	*L. ridibundus*	80	1	Enteritidis (*n*=1)	1.25
	L. canus	2	0	/	0.00

(5.41%) while the other three species were not positive for *Salmonella*, though this is likely due to the small sample size. Black-headed Gulls had the highest number of *S.* Typhimurium isolates (*n*=26) including two with its mono-phase variant (4, 5,12:i:-).

Discussion

Despite the general interest in the epidemiology of pathogens in wild birds, there is still a great need to better understand how birds are involved in pathogen epidemiology. So far, most research has been conducted on Black-headed and Yellow-legged Gulls, with data collected in breeding colonies or rescue centres. Various prevalence rates of *Salmonella* positive samples have been recorded for these two species, ranging from 6.28% to 31.08% and 1.33% to 26.26%, respectively (Literák et al., 1992; Ferns and Mudge, 2000; Wahlström et al., 2003; Palmgren et al., 2006; Ramos et al., 2010; Masarikova et al., 2016; Migura-Garcia et al., 2017; Antilles et al., 2021; Ebani et al., 2021; Russo et al., 2021). The literature to date reports the isolation of 82 *Salmonella* serotypes and by far the most common was *S.* Typhimurium, followed by *S.* Agona and *S.* Enteritidis. According to EFSA and ECDC (2021), *S.* Typhimurium is the second most common serotype (after *S.* Enteritidis) causing infections in humans and it is mostly related to broilers and pig products while *S.* Enteritidis is primarily related to broilers. *S.* Agona is among the top 20 serotypes causing human infections and it is associated with various foods such as sushi, ready-to-eat savoury snacks and cereal (Killalea et al., 1996; Russo et al., 2013; Thompson et al., 2017; EFSA and ECDC, 2021). In the present study, the *S.* Typhimurium serotype had the highest prevalence, followed by *S.* Enteritidis and *S.* Infantis. *S.* Infantis is the third most common serotype causing salmonellosis in humans and it is strictly related to broiler sources (EFSA and ECDC, 2021). According to the research in Europe to date, the present study adds three *Salmonella* serotypes that were not previously reported in gulls: *S.* Yalding, *S.* Reading and *S. enterica* subsp. *diarizonae* (IIIb_17:z_{10}:e,n,x,z_{15}).

Considering the large number of *Salmonella* serotypes found in gulls, it is likely that they become carriers after being infected somewhere in the environment, e.g., at feeding places close to productions farm harbouring *Salmonella,* or by scavenging at landfills or on sewage (Wahlström et al., 2003; Pennycott et al., 2006; Skov et al., 2008; Masarikova et al., 2016; Antilles et al., 2021). The presence and prevalence of *Salmonella* serotypes in wild birds generally varies substantially and most studies are not comparable (Skov et al., 2008) and have not examined *Salmonella* prevalence in relation to other factors such as season, age, feeding behaviour, movement or environment. As such, systematic studies are required to determine the role these birds have in spreading pathogens. Also, additional molecular methods such as whole genome sequencing could help to unravel the similarities among *Salmonella* isolates from different sources to pinpoint the origin of infections.

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Raznolikost i prevalencija Salmonella spp. u galebovima ulovljenih na odlagalištu otpada, Zagreb, Hrvatska

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Galebovi su skupina morskih ptica raširenih diljem svijeta koje su važan rezervoar *Salmonella* spp. Salmoneloza je druga najčešće prijavljena gastrointestinalna infekcija u ljudi i razumijevanje uloge koju divlje ptice imaju u širenju *Salmonella* spp. može pomoći u poboljšanju zdravlja ljudi i domaćih životinja. Mobilnost i migracijska sposobnost čini galebove vrlo zanimljivom skupinom za istraživanje zbog njihove potencijalne uloge u širenju patogena. Kroz ovaj rad prikazujemo raznolikost i prevalenciju *Salmonella* spp. kod nekoliko vrsta galebova ulovljenih na odlagalištu otpada tijekom zime u Zagrebu kroz devetogodišnje razdoblje, 2014.-2022. Ukupno je uzorkovano 1083 obrisaka kloake od šest vrsta galebova: riječni galeb (*Larus ridibundus*), galeb klaukavac (*L. michahellis*), pontski galeb (*L. cachinnans*), burni galeb (*L. canus*), tamnoleđi galeb (*L. fuscus*) i srebrnasti galeb (*L. argentatus*). Ukupna prevalencija *Salmonella* spp. je 5,82 % sa 16 identificiranih serotipova. *S.* Typhimurium ima najveću zastupljenost (47,62 %), zatim *S.* Enteritidis (12,69 %) i *S.* Infantis (9,52 %). Prema istraživanjima prisutnosti serotipova *Salmonella* spp. u galebova u Europi izolirana su njih 82, a najčešće dokazani je *S.* Typhimurium, zatim *S.* Agona i *S.* Enteritidis. Tijekom ovog istraživanja identificirana su tri serotipa koja ranije nisu izdvojena iz galebova *S.* Yalding i *S.* Reading te jedan iz podvrste *S. enterica* subsp. *diarizonae* (IIIb_O:17; H:z10; H:e,n,x,z15).

Ključne riječi: *galeb, Salmonella, divlje ptice, prevalencija, serotip*