Salmonella in Black-headed gulls (Larus ridibundus); prevalence, genotypes and influence on Salmonella epidemiology

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SUMMARY

During a period of 3 years, 1998–2000, 1047 faecal swabs from Black-headed gulls were sampled at one location in Southern Sweden. *Salmonella* spp. was found in 28 individuals (2·7%) and the dominating serotype found was *S*. Typhimurium (83%). Twenty-five per cent of the *Salmonella*-infected gulls were later recaptured and re-sampled. We found that *Salmonella* infection in Black-headed gulls was of short duration, and that infection in this bird species was predominantly expressed as carriage without disease manifestations. All *S*. Typhimurium isolates were subjected to antibiotic resistance profiling and molecular characterization by pulsed-field gel electrophoresis and IS*200* polymerase chain reaction. The *S*. Typhimurium gull isolates were compared to human and domestic animal isolates of the same serotype and phage type. We found genetic relatedness of *S*. Typhimurium DT195 isolates from gulls, domestic animals and humans, indicating that Black-headed gulls might play a role in the spread of *S*. Typhimurium in Sweden.

INTRODUCTION

Salmonella is a zoonosis with a number of wild and domestic animal hosts [1–6]. There are more than 2500 serovars or serotypes of this bacterium known today. Some serotypes have developed host adaptation and are, therefore, found in only a few animal reservoirs [7–11]. However, most serotypes are ubiquitous or unadapted and can be found in a number of animal hosts.

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As a result of legislation and strict control measures applied to the domestic animal food production chain, Sweden has less *Salmonella*-infected domestic animals than the majority of European countries [6]. Human salmonellosis in Sweden is dominated by cases associated with travelling abroad (~85% of reported clinical cases). Domestic and non-domestic cases are dominated by two *Salmonella* serotypes *S.* Typhimurium and *S.* Enteritidis. *S.* Typhimurium is found in a number of animal reservoirs, while *S.* Enteritidis is often associated with poultry and other fowl [12–16].

The role of wild animals in *Salmonella* epidemiology is debated. A survey of wildlife was performed

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Year	No. of samples	Salmonella positive	S. Typhimurium	Other Salmonella serovars	S. Typhimurium phage types			
					DT41	DT120	DT195	NST
1998	198	4	2	2 S. Kedougou (1), S. Newport (1)	0	0	0	2
1999	440	11	9	2 S. Enteritidis (1), S. Newport (1)	7	0	1	1
2000	409	13	12	1 S. Infantis (1)	7	1	4	0
Total	1047	28	23	5	14	1	5	3

Table 1. Total number, serovars and S. Typhimurium phage types (DT) in Black-headed gulls sampled in Malmö, Sweden, 1998–2000

NST, Non-serotypable.

in Sweden in 1999, including deer, hares, moose, wild boars, Canada geese and gulls. Of these only gulls yielded positive Salmonella samples [17]. Studies focusing on wild birds as carriers of Salmonella have shown that bird species which live close to humans and feed on human garbage or sewage are more likely to carry Salmonella and that carriage in the wild bird population is low [18-21]. Wild birds spreading enteropathogenic bacteria to the food and shelters of domestic animals, or food-processing plants like dairies are suggested as a risk factor for human and domestic animal salmonellosis [22-27]. Previous studies have pointed to gulls, especially the Blackheaded gull (Larus ridibundus), as the most important wild bird Salmonella reservoir in Europe [21, 23, 28–30], and to S. Typhimurium as the most common serotype found in wild birds [28, 29].

The aim of this study was to further investigate the role of Black-headed gulls as carriers of *Salmonella* and their impact on *Salmonella* epidemiology. We report on the prevalence of *Salmonella* in Blackheaded gulls of different ages and populations, captured at one site in Sweden. Using re-capture and re-sampling, we had a unique opportunity to study *Salmonella* carriage longitudinally in the Blackheaded gull population.

MATERIAL AND METHODS

Sampling strategy

During a period of 3 years, 1998–2000, a total of 1047 faecal samples were collected from Black-headed gulls. All gulls, excluding nestlings, were caught in a central park, in Malmö, southern Sweden (Table 1).

The birds were captured in cage-like traps, identified as to species, age-determined and ringed. Faecal samples were collected by inserting a cotton-coated swab in the cloak, and transported to the laboratory in charcoal transport media (Transwab, BioDisc, Solna, Sweden). Each faecal sample was marked with the corresponding ring number. When *Salmonella*-positive gulls were re-captured a new sample was collected. Information on the movements of individual gulls was obtained from the Swedish Bird Ringing Centre, Swedish Museum of Natural History. The Swedish Bird Ringing Centre collects reports of ornithologists and the public on ringed birds, either by ringing reports, or by observation or capture of ringed birds.

To be able to look into seasonality of *Salmonella* carriage in Black-headed gulls, the samples were divided into five seasonal groups. In January–February samples were taken from stationary birds, in March–April from spring-migrating birds, in June from nestlings, in July–August from juvenile birds and in October–November from autumn-migrating birds (Table 2). In 1998, only spring-migrating birds were sampled. In 1999 all groups, excluding nestlings, were sampled, while in 2000 all groups were represented. In total, 198 birds were sampled in 1998, 440 in 1999 and 409 in 2000. Thirty nestlings were sampled in a colony in central Malmö in June 2000.

Isolation and identification of Salmonella

Sampling and re-sampling were done according to the same procedure. Our aim was to identify human associated *Salmonella*, therefore, the analysing

	1998		1999		2000	
Season	No.	Salmonella positive	No.	Salmonella positive	No.	Salmonella positive
January–February (stationary birds)	n.d.	n.d.	57	0	39	3
March–April (migrating birds)	198	4	176	0	145	0
June (nestlings)*	n.d.	n.d.	n.d.	n.d.	30	1
July-August (juvenile birds)†	n.d.	n.d.	90	7	89	7
October–November (migrating birds)	n.d.	n.d.	117	4	106	2
Total	198	4	440	11	409	13

Table 2. Number of sampled Black-headed gulls according to season and age

method used was the recommended procedure of a Swedish accredited laboratory for clinical human medicine. The bacteriological analyses were performed 1–3 days after sampling. Samples were sent to the laboratory by mail, or otherwise stored at 4–8 °C until analysed. Each sample was enriched in selenite broth (Oxoid AB, Stockholm, Sweden), incubated at 37 °C for 18-24 h, and subcultured on xyloselysine-desoxycholate agar. Black H₂S precipitating colonies were verified as Salmonella by their reaction in fermentation tests and with a Salmonella polyvalent phage FO-1 (Reagensia AB, Stockholm, Sweden). Isolates were then sent for serotyping and phage typing to the Swedish Institute of Infectious Disease Control [31]. Serotyping was carried out according to the Kaufmann-White serotyping scheme [32]. Phage typing of S. Typhimurium was done according to Anderson [33].

Characterization of isolated Salmonella strains

All Salmonella isolates collected from Black-headed gulls were characterized by pulsed-field gel electrophoresis (PFGE), IS200 polymerase chain reaction (PCR) and antibiotic resistance typing. In the two former investigations, isolates collected from domestic animals and human clinical cases were included for comparison. PFGE was performed according to Palmgren [34] using three restriction enzymes, SpeI, BlnI and XbaI (Boerhinger–Mannheim, GmbH, Germany). IS200 typing was done by PCR amplification of fragments located between the IS200 elements, as described by Millemann [35]. Antibiotic

resistance to sulfisoxazole, streptomycin, nalidixic acid, ciprofloxacin, gentamycin, ampicillin, trimethoprim, and chloramphenicol was determined by the disk diffusion method with paper disks on PDM agar (AB Biodisk, Solna, Sweden), according to instructions from the Swedish Reference Group for Antibiotic and Resistance Methods (RAF-M; http://ltkronoberg.se/ext/raf/raf.htm). The zone of growth inhibition was measured with calipers and classified as sensitive, intermediate or resistant using the break-points predetermined after standardization by RAF-M.

Strains used for comparative studies by PFGE and IS200 typing

Fifty-four S. Typhimurium DT (definitive type) 41 isolates originating from human clinical cases (n=31) and domestic animals (n=23), as well as 13 isolates of S. Typhimurium DT195, nine human and four from domestic animals, were included for comparison. All isolates used in the study were from the three study years 1998–2000.

Human isolates were recovered from the Swedish Institute of Infectious Disease Control where the reference laboratory for serotyping and phage typing of *Salmonella* is situated. The *Salmonella* isolates from domestic animals came from the National Veterinary Institute. All isolates in the study were serotyped and phage typed according to the same methods and all phage typing was done at the same laboratory at the Swedish Institute of Infectious Disease Control.

n.d., Not done.

^{*} Only nestlings were sampled and ringed in June.

[†] All captured birds in July-August were juvenile birds. The adult birds do not reside in the capture area at this time of the year.

S. Typhimurium DT41 isolates

Human

All 31 human DT41 isolates came from clinical cases of salmonellosis or *Salmonella* carriage. Twenty-four isolates came from presumed domestic cases, while four patients had a previous history of travel outside Sweden (Table 3). For three of the isolates no epidemiological information as to country of origin was stated.

Domestic animals

The 23 isolates from domestic animals all had Sweden as country of origin. They were divided according to animal species (Table 3). Isolates came from poultry (3), goose (4), pig (9), cattle (5) and horse (2).

S. Typhimurium DT195 isolates

Human

Nine isolates from human clinical cases or carriers were divided according to country of origin. Four isolates were considered domestic while five isolates came from patients with a history of travelling abroad prior to infection (Table 3).

Domestic animals

Isolates from a Swedish goose and three pigs were also included in the epidemiological study.

RESULTS

Of the 1047 Black-headed gulls sampled in 1998, 1999 and 2000, 28 (2.7%) individuals were Salmonella positive (Table 1). Twenty-four of the 28 Salmonella isolates were collected in 1999 and 2000, in Blackheaded gulls sampled at different seasons of the year (Table 2). Fourteen of these 24 isolates (58%) were found in juvenile gulls born in Sweden (Table 2). Salmonella carriage in juvenile birds was significantly higher (7.8%, P < 0.001, χ^2 test) than in adult birds. All the Salmonella-positive juvenile birds were captured in the summer period July-August when they constitute the vast majority of Black-headed gulls at the capture site in Malmö. During this part of summer, when the nests are empty, the adult birds are found at sea and are not captured in the city area. In 2000, one nestling (3.3%) was found to be Salmonella positive. The remaining 13

Salmonella-positive samples were found in birds considered as adult birds, aged ≥ 1 year. A total of 2% (4/198 samples) of the birds sampled in the spring migration period were Salmonella positive while $3\cdot1\%$ (3/96) of the stationary birds sampled in the winter of 2000 and $2\cdot7\%$ (6/223) of the autumn-migrating birds were Salmonella positive.

Out of the 28 isolates retrieved in the whole study, 23 were identified as *S*. Typhimurium. Fourteen of these belonged to DT41, five to DT195, one to DT120 and three isolates were non-serotypable (NST). Furthermore, two isolates were identified by serotyping as *S*. Newport and one each as *S*. Enteritidis, *S*. Infantis and *S*. Kedougou (Table 1).

All of the Salmonella-positive birds appeared to be in good health at the time of the Salmonella-positive sampling. Six (25%) of the 24 birds found positive for Salmonella in 1999 and 2000 were sampled at least twice. None of these birds was found to be positive on more than at one occasion. Two of them had been sampled previously, and are, therefore, known to have been Salmonella negative at 8 and 63 days respectively, before being sampled and found to be Salmonella positive. Four birds were re-sampled as Salmonella negative at days 13, 18, 71 and 119 respectively, after being Salmonella positive.

History of recaptured birds

A juvenile Black-headed gull born in Malmö in May 2000 was sampled in August, and was found to be positive for *Salmonella*. The bird was later re-captured and sampled on two occasions, once in October and once in November. On both these occasions it was found to be *Salmonella* negative and apparently healthy. Two weeks after the third sampling, the bird was found dead in a city park in Malmö. No apparent cause of death could be determined on inspection. The bird carcass was not available for sampling for cultivation.

Another individual was sampled as an adult, during the sampling session of stationary birds in February 2000, and found to be *Salmonella* positive. This bird was subsequently re-sampled and found to be negative on three occasions: 3 weeks, 2 months and 9 months after the positive sampling.

Molecular characterization of isolates

S. Typhimurium isolates from Black-headed gulls, of DT41 (n=14) and DT195 (n=5), were subjected

Table 3. PFGE type and IS200 type of isolates of S. Typhimurium DT41 and DT195 found in Sweden 1998–2000

	S. Typhimurium DT41					
Species	PFGE type DT41	IS200 type DT41	No. of isolates	Country of origin		
Human	1	1	1	Not stated		
Human	2	1	20	Sweden		
Human	2	1	2	Not stated		
Human	2	1	1	Italy		
Human	3	1	1	Spain		
Human	4	2	1	Tunisia		
Human	5	3	1	Sweden		
Human	6	1	1	Sweden		
Human	7	1	2	Sweden		
Human	7	1	1	Spain		
Total			31			
Poultry	2	1	3	Sweden		
Goose	2	1	4	Sweden		
Pig	2	1	9	Sweden		
Horse	2	1	2	Sweden		
Cattle	2	1	4	Sweden		
Cattle	8	1	1	Sweden		
Total			23			
Black-headed gull	2	1	9	Sweden		
Black-headed gull	7	1	2	Sweden		
Black-headed gull	9	1	1	Sweden		
Black-headed gull	10	1	2	Sweden		
Total			14			

S. Typhimurium DT195

Species	PFGE type DT195	IS200 type DT195	No. of isolates	Country of origin		
Human	1	1	1	Sweden		
Human	2	2	1	Italy		
Human	3	3	1	Netherlands		
Human	3	4	1	Greece		
Human	4	1	1	Sweden		
Human	5	1	1	Sweden		
Human	6	2	1	Sweden		
Human	7	5	1	Spain		
Human	8	2	1	Italy		
Total			9	•		
Goose	3	1	1	Sweden		
Pig	3	1	3	Sweden		
Total			4			
Black-headed gull	3	1	4	Sweden		
Black-headed gull	9	1	1	Sweden		
Total			5			

Human isolates with different country of origin via the Swedish Institute for Infectious Disease Control (SMI). Domestic animal isolates via the National Veterinary Institute (SVA) and the Black-headed gull isolates from this study. 43 S. Typhimurium DT41 and 18 S. Typhimurium DT195 were analysed with both typing methods.

to further characterization by PFGE and IS200 typing. The S. Typhimurium DT41 isolates were compared to isolates of the corresponding phage type collected from domestic animals (n=25) and human clinical cases (n=31) between 1997 and 2000.

By IS200 PCR, three different banding patterns were found (*IS200* types 1–3, Table 3). All *S.* Typhimurium DT41 isolates of gull and animal origin and the majority of isolates from humans were found to be identical and belonged to IS200 type 1. Only two human isolates differed in banding patterns (Table 3).

PFGE analysis, however, showed a number of minor differences between isolates, resulting in 10 PFGE subtypes, denoted PFGE type DT41 1–10 (Table 3). The vast majority of the isolates from gulls, domestic animals and human clinical cases, 57 out of 70 analysed, were of PFGE type 2. Some PFGE types, namely types 1 (n=1), 3 (n=1), 4 (n=1), 5 (n=1) and 6 (n=1), were unique to human isolates. PFGE types 9 (n=1) and 10 (n=2) were found in isolates from Black-headed gulls only and PFGE type 8 (n=1) was unique for a *Salmonella* isolate found in cattle, whereas PFGE type 7 was found both among human (n=3) and gull (n=2) isolates.

Furthermore, gull isolates of S. Typhimurium DT195 were compared to DT195 isolates collected between 1997 and 2000 from domestic animals (n=4) and human clinical cases (n=9). IS200 PCR typing resulted in five different IS200 types. Using PFGE typing, seven subtypes could be identified. All domestic animal and gull isolates were identical with both methods. With IS200 PCR typing, three of the human isolates were grouped with the isolates of animal origin, but by PFGE only one of these isolates showed complete identity with the animal isolates.

Antibiogram

All 28 Salmonella isolates were tested for antibiotic resistance by the disk diffusion method. Multi-resistance, defined as antibiotic resistance against three or more antibiotics, was found in two isolates of serotypes Newport and Typhimurium NST, both collected during the spring migration in 1998. One of these was resistant to sulfisoxazole, ampicillin and trimethoprim, the other to sulfisoxazole, ampicillin, streptomycin and cloramphenicol. No other antibiotic resistance was observed.

DISCUSSION

From a total of 1047 Black-headed gulls, caught at one location in Sweden between 1998 and 2000, 28 Salmonella spp. (2.7%) were isolated. The dominating serotype was S. Typhimurium (83%), the other serotypes found being S. Enteritidis, S. Infantis, S. Kedougou and S. Newport. Based on results of a study performed in 1997 [29], the sampling focused on Black-headed gulls in 1998, 1999 and 2000. In 1999, we introduced a strategy of sampling and re-sampling of Black-headed gulls, that allowed us to, under natural conditions, study the duration of Salmonella carriage in Black-headed gulls and determine if positive birds showed any signs of disease. By registering the faecal sample together with the ring number of each gull, we could get further information on individual Salmonella-positive birds, either by resampling the bird or by ornithologists' observations.

Twenty-five per cent of the *Salmonella*-positive gulls were sampled at least twice. There were no signs of morbidity or mortality in *Salmonella*-positive gulls, and we had reports on three occasions that previously *Salmonella*-positive gulls had completed their migrations to Denmark and Holland respectively.

Girdwood and colleagues found the average duration of Salmonella excretion in a related species, the Herring gull (Larus argentatus), to be 2 days with a maximum duration of 4 days, in a challenge experiment with caged birds [36]. Our study in Blackheaded gulls support their observations that Salmonella does not belong to the normal enteric bacterial flora of the gulls and the duration of Salmonella carriage is relatively short. This is also supported by the fact that none of the gulls were Salmonella positive on more than one sampling occasion, even if there was a short period between the time when the positive sample was taken and the re-sampling. Furthermore, the low incidence of Salmonella in adult Black-headed gulls is another observation pointing in the same direction.

Even if Black-headed gulls are present in the study area throughout the year, ringing recoveries have shown that several different populations can be found in the area [37]. Some individuals are stationary, spending the entire year in the region. Another subpopulation breeds in Malmö and spends the winter in Western Europe. Still other birds breed further to the northeast, mainly in southeast Sweden, Finland, the Baltic states, Belarus and in parts of Russia, and either remain in the Malmö area during winter, or just

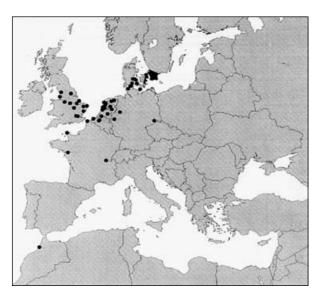


Fig. Recoveries of Black-headed gulls (*Larus ridibundus*) hatched and ringed in Malmö, Sweden.

stop over on their migration southwest in autumn and northeast in spring (Fig.). It is, therefore, important to note that the population of Black-headed gulls sampled by us is not uniform. Seasonal differences in *Salmonella* carriage were examined by sampling Black-headed gulls throughout the year, and dividing the gulls into four groups, spring-migrating birds, juveniles, autumn-migrating birds, and birds stationary in Sweden over the winter period.

We found that Black-headed gulls seem to be unaffected by *Salmonella* carriage. This is consistent with earlier studies on Herring gulls [36, 38]. The *Salmonella*-infected gulls in our study have shown no sign of disease and have been able to migrate to their winter quarters, flying long distances, after testing *Salmonella* positive.

Like many European countries, Sweden has had outbreaks of the multiresistant *S.* Typhimurium DT104. Other *Salmonella* strains isolated in Sweden show very little antibiotic resistance [6]. Wild birds flying over long distances in a short time can bring more resistant *Salmonella* strains into Sweden, as they can be carriers of strains from southern parts of Europe were antibiotic-resistant *Salmonella* in domestic animals is a larger problem. Black-headed gulls usually winter in Denmark, Germany, Holland, England, Belgium, France and have been found as far south as North Africa [39] (Fig.). During winter, they often congregate in large flocks in estuaries, wetlands and in farmlands. Two multiresistant *Salmonella* isolates were found in spring-migrating birds in 1998,

while the other 26 *Salmonella* isolates were either antibiotic sensitive or resistant to sulfisoxazole only. This is consistent with a previous study of migrating Black-headed gulls in the same area, where multi-resistant *Salmonella* isolates were found in the spring-migrating gull population [29].

However, our hypothesis that birds migrating to Sweden from their wintering areas, where *Salmonella* is more commonly found both in human and domestic animal populations should carry more *Salmonella* than birds born or stationary in Sweden was not confirmed. In 1999 and 2000, we found no *Salmonella* in spring-migrating birds, while there were *Salmonella*-positive birds in all the other groups (nestlings, juveniles, autumn-migrating and stationary) (Table 2). Different feeding habits and an immature immune defence are probable explanations to the higher level of *Salmonella* carriage in juvenile birds compared to adult birds. This pattern has been described previously in studies of Herring gulls and Black-headed gulls [28, 38].

Of the domestic human cases of salmonelloses reported in Sweden, S. Typhimurium DT40 and DT41 are generally considered to be contracted within Sweden, and to have domestic sources. S. Typhimurium DT40 is known to be the cause of the small bird epizootics that from time to time severely affect passerine birds in Sweden and several other countries [40–46]. Where phage typing has been performed, the causative agent has been S. Typhimurium DT40 [46], except for the small bird epizootic in Sweden in the winter of 1998–1999, where a mix of DT40 and the newly described phage type U277 was responsible [40]. This outbreak spread in the small bird population throughout Sweden, in domestic cats and resulted in a few human cases as well [40]. During the same time period, we found no S. Typhimurium DT40 in the gull population. Subtyping by PFGE of the three isolates of S. Typhimurium NST found among the Black-headed gulls, showed no similarity between these isolates and the S. Typhimurium U277 found in the epizootic of small birds and cats. The isolates from gulls also had diverse PFGE patterns among them. The fact that we observed no sign of the 1998–1999 passerine bird epizootic in the Salmonella found in this study is consistent with what we know of the feeding habits of Black-headed gulls [47]. Even if it is omnivorous it is considered a very sporadic event for Black-headed gulls to prey on other birds.

As for S. Typhimurium DT41, the endemic source for human salmonellosis has been more difficult to

pinpoint. As this was the most commonly found Salmonella in Black-headed gulls, it would probably have the highest impact on human and domestic animal Salmonella infection. In an attempt to study the possible relation between isolates of domestic animal (n=25) and human (n=31) origin, collected between 1997 and 2000, subtyping of the isolates was performed and compared to the gull isolates by the two methods described as the most discriminatory for S. Typhimurium in the literature, PFGE and IS200 typing [48, 49]. However, only minor differences could be detected between the isolates analysed (Table 3). This indicates a close relationship between isolates of S. Typhimurium DT41, which makes it difficult to draw conclusions as to the importance of Blackheaded gulls in the epidemiology of S. Typhimurium DT41.

Isolates of S. Typhimurium DT195 showed greater variability when subtyped by the same methods as the S. Typhimurium DT41 isolates (Table 3). Of five gull isolates, four domestic animal isolates and nine isolates from human clinical cases contracted both abroad and within Sweden, five different types were found by IS200 PCR typing and seven by PFGE typing using three restriction enzymes. All domestic animal and gull isolates were identical with both methods. With IS200 PCR typing, three of the human strains were grouped with the isolates of gull and domestic animal origin. These three strains (2190/98, 3040/99 and 3199/99) were all from human clinical cases, which stated that they had contracted the infection within Sweden. By PFGE, only one of these strains showed complete identity with the animal isolates, and this particular strain (3199/99) was isolated from a person living in Lund, a city close to Malmö, where the Black-headed gulls were sampled.

Salmonella carriage in Black-headed gulls mirrors to some extent the Salmonella burden in the environment but its role in the epidemiology of Salmonella infection in Sweden is not clear. An interesting point is that we only found one gull infected with S. Enteritidis, although this is the second most common serotype of Salmonella found in domestic human cases in Sweden. S. Enteritidis is almost as common in human cases as S. Typhimurium [31], while in Blackheaded gulls S. Typhimurium constituted 82% of the Salmonella found and S. Enteritidis only 3.6%. This was an even more surprising finding considering that S. Enteritidis is a serotype with its main reservoir in birds.

Even if Black-headed gulls are short-term carriers of *Salmonella*, they may be a possible link in *Salmonella* epidemiology, serving as components for direct or indirect spread to domestic animals and humans. The fact that they seem to be unaffected by their *Salmonella* carriage makes this even more possible as they seem to be able to migrate in spite of being infected.

In this study we could not distinguish a clear connection between human, domestic animal and Blackheaded gull isolates in Sweden although there are some indications of transmission between the species. The possibility still remains that Black-headed gulls have the potential to pick up and transmit *Salmonella* serotypes or *Salmonella* with antibiotic resistance that are otherwise uncommon in Sweden due to strict control measures on food, feed and imported domestic animals.

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DECLARATION OF INTEREST

None.

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