Importance of wild birds in the spread of Salmonella
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Helena Palmgren

SUMMARY

Salmonella is one of the most important enteropathogenic bacteria. It is responsible for about 5000 reported cases of human gastroenteritis each year in Sweden. Salmonellosis is a zoonotic disease, and the bacterium has the ability to infect a variety of both domestic and wild animal species.

In studies of Swedish wild bird populations, we found that Black-headed gull may be the main reservoir for Salmonella in birds, and that Salmonella infection is expressed as carriage with no obvious disease manifestations. Black-headed gull is a migratory bird and can transport strains of Salmonella with virulence traits like antibiotic resistance, from sources outside Sweden. Genetic molecular methods, PFGE and IS200, also demonstrate that Black-headed gull play a role in the transmission chain of Salmonella in Sweden.

In a study of the Swedish Peregrine Falcon population, Salmonella amager and Campylobacter jejuni were found. There were indications, based on serotyping of Salmonella and genetical typing by PFGE of Campylobacter that these isolates were transmitted to the falcons from a human or domestic animal source. This bird of prey has sparse contact with humans but may be infected by Salmonella of human origin by feeding on other birds, like gull.

Salmonella was found in penguins, albatrosses and mainly in seals in a study in Antarctica. Several features of the Salmonella serotypes found indicate a human source for Salmonella infection in these animals, and also a spread of Salmonella within and between animal species in Antarctica.

Key words: Salmonella, wild birds, Black-headed gull, Peregrine falcon, Antarctica, penguins, seals, PFGE, IS200, Salmonella carriage.

Umeå 2002
Importance of wild birds in the spread of *Salmonella*

Helena Palmgren

Umeå 2002
"Det kunde ha varit värre"

Görgen Göstas
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SUMMARY

*Salmonella* is one of the most important enteropathogenic bacteria. It is responsible for about 5000 reported cases of human gastroenteritis each year in Sweden. Salmonellosis is a zoonotic disease, and the bacterium has the ability to infect a variety of both domestic and wild animal species.

In studies of Swedish wild bird populations, we found that Black-headed gull may be the main reservoir for *Salmonella* in birds, and that *Salmonella* infection is expressed as carriage with no obvious disease manifestations. Black-headed gull is a migratory bird and can transport strains of *Salmonella* with virulence traits like antibiotic resistance, from sources outside Sweden. Genetic molecular methods, PFGE and IS200, also demonstrate that Black-headed gull play a role in the transmission chain of *Salmonella* in Sweden.

In a study of the Swedish Peregrine Falcon population, *Salmonella amager* and *Campylobacter jejuni* were found. There were indications, based on serotyping of *Salmonella* and genetical typing by PFGE of *Campylobacter* that these isolates were transmitted to the falcons from a human or domestic animal source. This bird of prey has sparse contact with humans but may be infected by *Salmonella* of human origin by feeding on other birds, like gull.

*Salmonella* was found in penguins, albatrosses and mainly in seals in a study in Antarctica. Several features of the *Salmonella* serotypes found indicate a human source for *Salmonella* infection in these animals, and also a spread of *Salmonella* within and between animal species in Antarctica.

**Key words:** Salmonella, wild birds, Black-headed gull, Peregrine falcon, Antarctica, penguins, seals, PFGE, IS200, Salmonella carriage.
SAMMANFATTNING

(Summary of the thesis in Swedish)

Salmonella är en av de viktigaste enteropathogena bakterierna. I Sverige rapporteras varje år ca. 5000 fall av salmonellos hos människa. Salmonellos är en zoonos och bakterien kan infektera de flesta arter av både husdjur och vilda djur.


I en studie av förekomsten av Salmonella och Campylobacter i hos svenska pilgrimsfalkar, fann vi båda dessa bakterier hos boungar. Epidemiologiska markörer visade att de funna bakterierna kan ha spridits till falkarna från människa eller husdjur. Pilgrimsfalkar har få kontakter med människa, men kan smittas av bakterie stammar med humant ursprung via sin föda som består av andra fåglar, t.ex. skrattmås.

I Antarktis fann vi Salmonella hos både sälar, pingviner och albatrosser. Serotypning och fag typning av dessa Salmonella isolat visar att de kan ha spridits till dessa djur från människa. Molekylärbiologiska metoder indikerade att Salmonella kan ha spridit sig mellan olika sälpopulationer och mellan sälar, pingviner och albatrosser.
ORIGINAL PAPERS

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>phage type of <em>Salmonella enteritidis</em></td>
</tr>
<tr>
<td>DT</td>
<td>definitive type, phage type of <em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>mr-DT104</td>
<td>multi-resistant <em>S. typhimutium</em> definitive type 104</td>
</tr>
<tr>
<td>EHEC</td>
<td>enterohemorrhagtic Escherichia coli</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>O-antigen</td>
<td>outer membrane antigen of <em>Salmonella</em></td>
</tr>
<tr>
<td>H-antigen</td>
<td>flagellar antigen of <em>Salmonella</em></td>
</tr>
<tr>
<td>Vi-antigen</td>
<td>capsular antigen of <em>Salmonella</em></td>
</tr>
<tr>
<td>DVC</td>
<td>direct viable count</td>
</tr>
<tr>
<td>SP</td>
<td><em>Salmonella</em> pathogenicity island</td>
</tr>
<tr>
<td>BPW</td>
<td>buffered peptone water</td>
</tr>
<tr>
<td>XLD</td>
<td>xylose-lysine-desoxycholate agar</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>PFGE</td>
<td>pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>XbaI, SpeI, NotI, BlnI</td>
<td>restriction enzymes</td>
</tr>
<tr>
<td>Mb</td>
<td>megabase</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>IS200</td>
<td>DNA insertion sequence 200</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>AT</td>
<td>Antarctic Treaty</td>
</tr>
<tr>
<td>UV-light</td>
<td>ultraviolet light</td>
</tr>
</tbody>
</table>
INTRODUCTION

Salmonella induced disease

*Salmonella* is an enteric bacterium with the ability to cause disease in humans and in most animal species, from mammals to molluscs (Okoh 1980, Minette 1984, Brand 1988, Wray 1990, Wernery 1992, Malmqvist 1995, Battisti 1998). It comprises a genus of facultative aerobe, gram-negative rods, belonging to the family *Enterobacteriaceae*.

The most frequent manifestation of *Salmonella* disease in humans is gastroenteritis and the most severe conditions are typhoid or paratyphoid fever caused by the *Salmonella* serotypes *S. typhi* and *S. paratyphi*.


The disease spectrum in animals is much the same as in humans, but *Salmonella* also cause abortion in several animal species like horses, sheep and cows (Leondidis 1984, Foley 1994, Uzzau 2000).

All *Salmonella* serovars, including *S. typhi* and *S. paratyphi*, are transmitted through the oral-faecal transmission route and are considered both food-borne and
water-borne (D’Aoust 1997). *Salmonella* also has a propensity to induce carriage, both in humans and animals, and healthy carriers are one of the main reservoirs of *Salmonella* (Hohmann 2001, Kaufmann 2001).

**Salmonella classification**

**Salmonella species and subspecies**

Based on multilocus enzyme electrophoresis, *Salmonella* can be divided into two main species: *S. enterica* and *S. bongori* (Reeves 1989, Popoff 1997). Whereas both species contain the pathogenicity island SP1, that encodes a number of *Salmonella* virulence traits, only *S. enterica* has acquired a second pathogenicity island designated SP2 (Bäumler 1998). *S. enterica* is further divided into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae* and *indica*, also called subspecies I-VI.

**Salmonella serotyping**

Differences in O- (outer membrane lipopolysaccharides, LPS), H- (flagella) and Vi - (capsular) antigens, are used to further divide *Salmonella* subspecies into over 2300 serotypes or serovars (D’Aoust 1997, Uzzau 2000) according to the serotyping scheme of Kauffmann-White (1957). I have chosen to use the word serotype in the following text, for denunciation of the antigen subdivision of *Salmonellae*. *S. enterica*, subspecies *enterica*, also called *S. enterica* subspecies I, comprises 1405 of these serotypes (D’Aoust 1997), including *S. typhi* and *S. paratyphi*.

**Salmonella phage typing**

The serotypes *S. typhi*, *S. paratyphi* A and B, *S. virchow*, *S. enteritidis* and *S. typhimurium*, can be further subdivided into different phage types, by their ability to take up and be lysed by different *Salmonella* bacteriophages (Ward 1987).

In *S. enteritidis*, a phage type is denoted PT (phage type), while in *S. typhimurium* it is called DT (definitive type) (Anderson 1977, Ward 1987). The best known of the *S. enteritidis* phage types is PT 4. It is the main phage type responsible for the *S.*
**Introduction**

*enteritidis* pandemic that has swept over the world during the last decade (Rodrigue 1990, Threlfall 1993a, Boyce 1996). *S. typhimurium* DT 104 has been much debated in recent years because of its ability to become multi-resistant to antibiotics (Low 1997, Hancock 2000, Cloeckert 2001).

Alteration of phage types has been described, resulting in the conversion of one phage type to another (Chart 1989, Threlfall 1993b, Rankin 1995, Baggesen 1997). This has to be considered when using phage typing as a tool in epidemiologic surveys of *Salmonella*.

**Salmonella in the environment**

*Salmonella* is well adapted to survival in different environments. The optimum growth temperature for *Salmonella* is 30-45° C, but the bacterium grows actively in a temperature range from +2° C to +47° C, depending on growth media, pH and *Salmonella* serotype (D’Aoust 1991). The pH optimum is 6.5-7.5 but the pH range for proliferation is 4.5-9.5 (D’Aoust 1991). High salt concentration is a common preservative method for foods due to its bactericidal effect, and *Salmonella* growth is normally inhibited in the presence of 3-4% NaCl, but its salt tolerance increases with increasing temperature (D’Aoust 1991). Drying is another method used for preservation of mainly spices and fruits. *Salmonella* has repeatedly proved its high resistance to drying by causing outbreaks due to dried spices and fruits, and even dried cocoa beans (D’Aoust 1977).

**Water**

Through faecal pollution and unsatisfactory treated sewage, Salmonella and other enteric pathogens can spread in water. Due to the physiological adaptability of Salmonella, the bacteria can survive up to several months in aquatic environments (Pokorny 1988, Smith 1994). The survival time of Salmonella in cold water is up to two times longer than in temperate water (20°C) (Smith 1994).)

The effect of nutrients in the water on survival time is unclear. In relatively low
Introduction

nutrient cold seawater, e.g. polar marine environments, the direct viable count (DVC) increases with increased nutrient content in the water (Smith 1994). In surface water from rivers, Salmonella viability decreases with increasing organic pollution, so the bacterium has decreased survival time in sewage with high organic content when compared to unpolluted drinking water (Pokorny 1988). This may be due to antagonistic effects from competition with other bacteria (Wray 1975). Other studies have shown that DNA content, adhesion and therefore also the virulence of the bacterium decreases over time in low nutrient water (Galdiero 1994).

Soil and other solids

*Salmonella* is able to survive for long periods under starvation and desiccation conditions on solid media (Oliver 1993, Lesne 2000). *Salmonella* is repeatedly found in environmental samples of the habitats of domestic animals, in slaughterhouses and in animal feed (Reilly 1981, Lahellec 1986, Wray 1990, Murray 1991, Davies 2001). Several environmental factors influence the survival time of *Salmonella* in soil or on other solid matter. For example, survival time is increased in wet or moist surroundings, and in shade when compared to areas with sun exposure (Murray 1991). Reported survival times of *Salmonella* have been 120 days in pasture soil, 280 days in garden soil and up to 30 months in dried bovine manure (Morse 1974, Murray 1991).

Domestic animals

A considerable number of control measures are used to prevent humans from eating *Salmonella* infected meat, eggs and other animal products (Wray 1990, Eld 1991, Murray 1991, Wierup 1995, National Veterinary Institute 2001). The greatest problems in the last decades have been in the poultry industry, in particular with *S. enteritidis* (Rodrique 1990, Pohl 1991, van de Giessen 1992, Altekruse 1993)..
Wild animals other than birds
A number of studies have reported both *Salmonella* carriage and salmonellosis in wild animals. Almost all animal species have been implicated, including amphibians, insects, fish, reptiles, birds and mammals (McCoy 1974, Morse 1974, Kourany 1976, Okoh 1980, Minette 1984, Minette 1986, Rolland 1985, Venkateswaran 1989, Devi 1991, Baker 1995, Gopee 2000, Heinitz 2000, Olsen 2000, Warwick 2001). The best known *Salmonella* carriers in the wild are reptiles like snakes, turtles and lizards (Gopee 2000) *Salmonella* is considered to be part of the normal enteric bacterial flora of reptiles (Warwick 2001). Reptiles are also the group of wild animals in which *Salmonella* disease is most often found (Gopee 2000). Turtles, snakes and lizards have for several decades been kept as pet animals, and in USA about 280 000 reptile-associated cases of human salmonellosis are seen per year (Warwick 2001).

The serotype that seems to be most pathogenic to wild animals is *S. typhimurium*. Although not so dominating in carriers, it is the serotype most commonly encountered in *Salmonella* disease in wild animals (Gopee 2000).

In a study on captive zoo animals in Trinidad (Gopee 2000), a high percentage of *Salmonella* isolates were antibiotic resistant, which indicates that the animals were infected from human or domestic animal sources. Likely sources for infection were infected meat and feed.

**Host adaptation in Salmonella serotypes**

*S. enterica* subspecies I is isolated from a wide variety of mammalian and avian hosts. The other six subspecies of *S. enterica* and *S. bongori* are associated with cold-blooded vertebrates like reptiles (Uzzau 2000).

The majority of *Salmonella* serotypes or serovars have the ability to infect several different animal species. These serotypes are referred to as unrestricted (Uzzau 2000). A small number of *Salmonella* serotypes are so called host-restricted
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(D'Aoust 1997, Bäumler 1998, Uzzau 2000) indicating association with one single animal species. Although the transmission route is oral-faecal, host-restricted serotypes tend to cause primary septicaemia in their host rather than enteritis. The host-restricted serotypes *S. typhi* and *S. paratyphi A* are strictly human pathogens (Kelterborn 1979, Uzzau 2000), whereas *S. abortus-ovis* and *S. gallinarum* cause septic disease and abortion in sheep and fowl, respectively. (Kelterborn 1979, Uzzau 2000).

There is also a group of serotypes, called host-adapted (Uzzau 2000), which have a predominant animal host but may occasionally infect other animals including humans. Examples of host adapted *Salmonella* serotypes are *S. dublin* and *S. cholerasuis*, for which cattle and pigs are the predominant hosts (Uzzau 2000).

About 50 *Salmonella* serotypes are more frequent causative agents of human enteric salmonellosis. Since the introduction of the Kauffman-White serotyping scheme in 1957, *S. enteritidis* and *S. typhimurium* have been the enteric *Salmonella* most frequently isolated in human salmonellosis all around the world (Kelterborn 1979, Rodrigue 1990). *S. saintpaul, S agona, S. virchow, S. stanley, S. badar, S. newport*, and *S. dublin* (Kelterborn 1979, Swedish Institute for Infectious Disease Control 2002) are other common human enteric pathogens.

**Salmonella and antibiotic resistance**

Until the beginning of the 1990s, enteric *Salmonellae* have been fairly antibiotic sensitive. Oral treatment with ampicillin, trimethoprim, sulfa, tetracycline and, in the last two decades, quinolones, have been used routinely in human salmonellosis.

Already in the late 1960s there was criticism of the use of antibiotics in animal husbandry and veterinary medicine, due to the effect it could have on antibiotic susceptibility of certain pathogens (Swann Committee 1969). This led to restrictions on the use of antibiotics in animal husbandry in Europe and North America, especially those used for growth promotion. In Sweden, all antibacterial
growth promoters were banned in 1986 (Humphrey 2001, Spring 1999). Reports of antibiotic resistance in *Salmonella* from humans and livestock have slowly increased during the last decades (Swann Committee 1969, Riley 1984, Wray 1993, Threlfall 2000), especially in *S. typhi*, the *Salmonella* most commonly treated with antibiotics in humans (Hancock 2000).

The late 1980s saw the emergence of multi-resistant *S. typhimurium* DT 104 (mr-DT104). This strain now accounts for 8-9 % of all *Salmonella* isolates in the USA (Hohmann 2001), and is the most common phage type of *S. typhimurium* in several countries in Europe (Hancock 2000). The emergence of mr-DT104 has not influenced the overall percentage of *S. typhimurium* compared to other *Salmonella* serotypes (Hancock 2000, Humphrey 2001), indicating that this *S. typhimurium* clone has expanded in competition with other *S. typhimurium* clones (Hancock 2000).

The first reported case of *Salmonella typhimurium* DT 104 in nondomestic birds came in 2000, when isolates of DT 104 were found in two parrots from the same pet shop (Hudson 2000).

It is rare that *Salmonella* isolates found in wild animals are antibiotic resistant, and a *Salmonella* isolate from wild animals with antibiotic resistance strongly suggests contamination from a human or domestic animal source (Rolland 1985).

**Salmonella serotypes described in the present work**

**Salmonella typhimurium**

*Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S. typhimurium*) is one of the most common enteric *Salmonella* serotypes. It is also one of the most unrestricted *Salmonella* serotypes, with an ability to infect almost all animals (Uzzau 2000). *S. typhimurium* is, together with *S. enteritidis*, the most common enteric *Salmonella* serotype in man, (Kelterborn 1979). Besides being a common cause of enteritis, it is also by far the most experimentally studied of the *Salmonellae*. It has many features in common with *Escherichia coli*, and more is known about the genetic
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and metabolic proprieties of these two bacteria than about any other cellular form of life (Schaechter 1987).

Salmonella enteritidis

*Salmonella enterica* subspecies *enterica* serovar Enteritidis (*S. enteritidis*) is another important enteric *Salmonella* serotype. It shows a high grade of heterogeneity and can be divided into 27 phage types. Since the 1970s, human salmonellosis caused by *S. enteritidis* has increased dramatically (Rodrique 1990). In 1997, *S. enteritidis* accounted for 85% of all human cases of salmonellosis in Europe (Guard-Petter 2001). A four to six fold increase of salmonellosis caused by this serotype has also been seen in North America (Guard-Petter 2001). At the same time, other serovars causing human salmonellosis have remained stable (Bäumler 2000, Guard-Petter 2001).

The *S. enteritidis* pandemic did not reach Sweden, and *S. enteritidis* phage type 4 has only rarely been found within Swedish poultry (Wierup1995). Despite this, phage type 4 has clearly been the dominating phage type during the ongoing pandemic, especially in Europe (Rodrique 1990, Boyce 1996, Guard-Petter 2001).

Eggs are virtually the only source of outbreaks of *S. enteritidis* in humans (Coyle 1988, St Louis 1988, Guard-Petter 2001). Hens infected with *S. enteritidis* show no sign of disease and their egg production can even increase when they are infected (Guard-Petter 2001).

Salmonella havana

*Salmonella enterica* subspecies *enterica* serovar Havana (*S. havana*) has been implicated in a number of outbreaks of human salmonellosis (Schiff 1939, Makarem 1982, Menon 1994, Backer 2000), with a tendency to cause more extra-intestinal infections than is usual for enteric *Salmonellae* (Backer 2000). In domestic animals, poultry and pigs have been implicated in *S. havana* infection (Soerjadi-Liem 1984, Chandler 1981) and Australian meat and bone meal have been highly contaminated with *S. havana* (Bensink 1979).
In Sweden, S. havana is only rarely found in human enteritis (Katouli 1992), but is quite common as a fodder contaminant (Anna Aspán, personal communication).

**Salmonella newport**

*Salmonella enterica* subspecies *enterica* serovar Newport (*S. newport*) is one of the most common *Salmonella* serotypes. It is also considered to be one of the six most common sources of enteric salmonellosis in man (Kelterborn 1979).

In the Nordic countries, *S. newport* has been relatively uncommon in both animals and humans in recent years. In Finland it accounted for only 1.3 % of all human cases in 1996 (Lyytikäinen 2000). *S. newport* is also found in wild birds and mammals (Adesiyun 1998), and is the most frequently reported serotype from seals (Gilmartin 1979, Baker 1995, Thornton 1998)

**Salmonella in Sweden**

The incidence of human salmonellosis in Sweden is low compared to other European countries, with about 55 cases/100,000 inhabitants per year in 1999 and 2000 (Swedish Institute for Infectious Disease Control 2002). The vast majority of human cases of *Salmonella* infection in Sweden are associated with international tourism. Spain, Thailand and Greece are among the most common destinations for Swedish tourists, and are also the most common sources for non-domestic cases of salmonellosis (Swedish Institute for Infectious Disease Control 2002).

The domestic cases represent only 14-18 % of the total number of human salmonellosis in Sweden. The dominating serotypes are *S. typhimurium* and *S. enteritidis*. In the year 2000, the most common *S. typhimurium* phage types in reported human domestic *Salmonella* cases were DT 104 and DT 40, while *S. enteritidis* was dominated by PT 4.
Table. Human cases of *Salmonella* infection in Sweden 1999 and 2000. (Swedish Institute for Infectious Disease Control 2002)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of Salmonella cases</th>
<th>Non-domestic cases of Salmonella infection</th>
<th>Domestic cases of Salmonella infection</th>
<th>S.typhimurium</th>
<th>S.enteritidis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Salmonella</em> cases per 100 000 inhab</td>
<td>% of total number</td>
<td>% of total number</td>
<td>% of domestic cases</td>
<td>% of domestic cases</td>
</tr>
<tr>
<td>1999</td>
<td>8.861 milj</td>
<td>5141</td>
<td>4238</td>
<td>903</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.0/100 000</td>
<td>82%</td>
<td>18%</td>
<td>34%</td>
</tr>
<tr>
<td>2000</td>
<td>8.883 milj</td>
<td>4845</td>
<td>4154</td>
<td>691</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.5/100 000</td>
<td>86%</td>
<td>14%</td>
<td>37%</td>
</tr>
</tbody>
</table>

The Swedish Salmonella Control Program was initiated in 1961, and in 1995 the EU approved certain parts of the programme, covering pigs, cattle, poultry and eggs (National Veterinary Institute 2000). Any *Salmonella* isolated from animals, humans, feed and food is required by law to be reported, and extensive inspections are regularly made of feed mills, feed material, pet food, eggs and food producing animals (National Veterinary Institute 2000). Results of these inspections show that Swedish meat and eggs are virtually *Salmonella* free. The overall prevalence of *Salmonella* in meat and eggs produced in Sweden is less than 0.1 % (National Veterinary Institute 2000). The vast majority of *Salmonella* infected animal feed, animals and meat products in Sweden are imported.

*Salmonella* isolated according to the Swedish Salmonella Control Programme are serotyped and *S. typhimurium* and *S. enteritidis* are phage typed. A variety of serotypes are found in feed, live animals and meat. The most common *Salmonella* phage types found in feed mills and feed material are uncommon as human pathogens, e.g. *S. seftenberg*, *S. livingstone* and *S. mbandaka* (National Veterinary Institute 2000, National Veterinary Institute 2001). *S. dublin* dominates in cattle herds, but in all other animals, including poultry, *S. typhimurium* is the most common serotype (National Veterinary Institute 2001).
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In humans, the most common phage type of *S. enteritidis* was PT 4, while only one isolate of *S. enteritidis* was found in domestic animals, also PT 4, in the year 2000. *S. typhimurium* phage types NST, DT 41, DT 120 and DT 193 were found in feed mills and NST, DT 40, DT 41, DT 120 and DT15a in food producing animals the same year (National Veterinary Institute 2000).

**Wild birds and Salmonella**

Wild birds are suspected of being a threat to humans through faecal contamination of crops or water. They may also contaminate meat or eggs through faecal contamination of animal fodder (Hatch 1996). There are, to my knowledge no reports of human outbreaks of salmonellosis where wild birds have been implicated as the direct source of infection. Transmission from wild birds has more frequently been indirect, via infected milk-producing animals and milk products (Johnston 1979, Reilly 1981, Kapperud 1989, Kirk 2002) or via infected water (Johnston 1981, Koplan 1978, Kapperud 1998). Wild birds are more likely to be dispersal agents for *Salmonella* than serve as a primary source (Hatch 1996).

When the studies of the present dissertation were planned, a wide variety of bird species had been found to be *Salmonella* carriers. The most frequent carriers were reported to be wild bird species living in close contact with humans, or domestic birds like pigeons (Faddoul 1966, Casanovas 1995), geese (Dieter 2001) and Corvids (Nielsen 1981). Top predators, such as hawks, owls, eagles and falcons had also been a focus of interest, as they feed on other birds and can mirror the *Salmonella* prevalence in the whole bird population. Birds of prey, both captive and free-living have been found to carry *Salmonella* (Keymer 1972, Lüthgen 1978, Kirkpatrick 1986a, Kirkpatrick 1986b), and have also been the victims of *Salmonella* disease (Lüthgen 1978, Sykes 1981, Wernery 1998).

At the time when my studies started, the majority of previous reports had found *S. typhimurium* to be the most common serotype carried by wild birds.
Introduction


Salmonella in gulls


In a study of the prevalence of Salmonella in different bird species in the Czech Republic, 2890 birds of 61 species were examined with cloacal swabs. The results showed that Salmonella carriage was restricted to Black-headed gulls (Larus ridibundus). The only other bird species that carried Salmonella were house sparrows and serins feeding in the vicinity of a farm where there was an ongoing outbreak of salmonellosis among the farm animals (Cizek 1994).

In another Czech study, where 756 birds of 57 species were examined for the incidence of Salmonella carriage, 38 out of 40 (95%) of the Salmonella positive samples found were from Black-headed gulls (Húbalek 1995). In these two Czech studies, Salmonella carriage was most common among juveniel Black-headed gulls (Cizek 1994, Húbalek 1995), with 19.2 % of juveniel Black-headed gulls infected, compared to 4.2 % for adult gulls (Cizek 1994). This difference was attributed to differences in feeding habits of juveniel compared to adult birds, and to young birds being more susceptible to infections.
Most gulls are omnivorous, with a wide range of food, including small mammals, dead animals, fish, insects, worms, seed, fruit and waste (Cramp 1990). They rarely feed on other birds (Kenneth Bengtsson, personal communication). The gull populations, especially Herring gulls and Black-headed gulls, have increased dramatically in numbers during the last decades (Cramp 1990, Girdwood 1985, Monaghan 1985, Hatch 1996). This increase may be due to cessation of previous persecution, with laws now preventing both shooting and egging (Hatch 1996). Population growth has forced the gulls to change both their feeding and breeding habits. Because of their ability to adapt their feeding habits to environmental changes, some gull species have solved the population problem by “urbanisation”. They are now found in close proximity to human activities where food is abundant, such as at refuse dumps and sewage outlets, behind fishing boats and in newly sown fields (Cramp 1990, Girdwood 1985, Monaghan 1985, Hatch 1996). A few studies have shown a direct relationship between the availability of food of human origin and demographic parameters of gull populations, e.g. breeding success Pons 1992).

There are few reports of disease outbreaks caused by Salmonella in gulls (Brand 1988). The gulls seem to be carriers, with the ability to shed Salmonella in their faeces but with no obvious disease in the birds (Butterfield 1983, Girdwood 1985, Monaghan 1985, Húbalek 1995, Hatch 1996).

Salmonella excretion in gull faeces is low, 150-200 bacteria per gram of faeces (Fenlon 1981, Girdwood 1985). This makes direct transmission to humans via gull faeces unlikely, as an infectious dose of approximately $10^7$ bacteria is required to cause human Salmonella enteritis in a immunocompetent host (Blaser 1982). Salmonella infected gulls are more likely to pose a threat to humans by infecting water, at bathing resorts, fish processing plants and open water reservoirs, where many birds gather and the water becomes polluted with bird faeces (Berg 1972, Fennell 1974, Benton 1983, Lévesque 1993, Hatch 1996).
Gulls and other wild birds are presumably more often infected through human activities than vice versa (Hatch 1996), for example when gulls gather in great numbers at city dumps and sewage outlets. In a study of gull faeces collected at a sewage disposal works in Hamburg, Germany, 78% of the samples were positive for *Salmonella* and the range of serotypes from gulls matched exactly the serotypes found in the Hamburg rivers (Muller 1965). Similar studies of gulls feeding at sewage treatment works in Scotland demonstrated a close association between the *Salmonella* serotypes found in the gull faeces and those in the sewage sludge (Fenlon 1983, Fricker 1984).

Very little is known about the duration of *Salmonella* carriage in wild birds. In the report by Girdwood and colleges (1985), 17 *Salmonella* positive Herring gulls were kept in captivity with precautions taken to prevent reinfection. They excreted *Salmonella* in their faeces for a mean of two days and a maximum of four days (Girdwood 1985).

Seasonal differences in the prevalence of *Salmonella* carriage in gulls have previously been reported (Girdwood 1985, Húbalek 1995). In a study by Girdwood and colleges, the incidence of carriage in Herring gulls was highest in autumn, August to November, and coincided with the period when human salmonellosis was known to reach its peak. In the study by Húbalek and colleges (1995), the highest *Salmonella* prevalence occurred from May to July, and was attributed to a higher frequency of juvenile birds, and a greater diversity of food.

Girdwood and colleges also found that *S. virchow* and *S. typhimurium* were the two most common *Salmonella* serotypes in Scottish gulls, and also two of the three most common *Salmonella* serotypes found in both humans and cattle in Scotland during the same period (Girdwood 1985). This was considered to indicate a close relationship between *Salmonella carriage* in gulls and human salmonellosis.
Gull migration

Gulls have a worldwide distribution but are most common in temperate regions in the Northern Hemisphere (Hatch 1996). It has been estimated that 46 species of birds belong to the gull family (*Laridae*) (Hatch 1996). In Sweden, eight species are regularly encountered (Cramp 1990). Five of these species, the Black-headed gull (*Larus ridibundus*), Common gull (*Larus canus*), Herring gull (*Larus argentatus*), lesser Black-backed gull (*Larus fuscus*) and great Black-backed gull (*Larus marinus*) are common breeding birds in Sweden and in the rest of northern Europe (Cramp 1990, Girdwood 1985, Monaghan 1985).

The Black-headed gull is the smallest of the five gull species most commonly found in Sweden. In contrast to the four other common gull species in Sweden, it is a medium range migrator. The majority of Black-headed gulls spend their winters in Denmark, Germany, Holland, Belgium, Great Britain and France and move as far as the North African coast (Bengtsson 1996, Bengtsson 2001). Their winter quarters depend to some degree on where in Sweden the birds breed. Black-headed gulls from Skåne and other provinces of southern Sweden, migrate to more southern destinations like France and North Africa, whereas birds from northern Sweden spend their winters mostly in Denmark and northern Germany (Bengtsson 1996, Bengtsson 2001).

Wildlife of Antarctica

Only 2% of the Antarctic continent is accessible to terrestrial plants and animals, the rest is covered with ice. Consequently, the wildlife of Antarctica consists mainly of birds that obtain their food from the sea. The only mammals in the region are seals, whales and dolphins, except for reindeers that were introduced by humans on South Georgia and some rodent species on a few subantarctic islands.

Of the 17 known penguin species, seven are found in Antarctica: Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarctica*), Emperor (*Aptenodytes forsteri*),...
Gentoo (*Pygoscelis papua*), Macaroni (*Eudyptes chrysolophus*), Rockhopper (*Eudyptes chrysocome*) and King (*Aptenodytes patagonicus*) penguins. Antarctic penguins spend most of their life in colonies on land or fishing at sea. Their main feed is krill and fish. (About Antarctica 2002)

Apart from penguins, a number of bird species breed on the Antarctic continent, e.g. species of albatrosses, skuas, terns, sheathbills, gulls and petrels. The majority of these are long-distant migrators, spending their winters in South America, Africa, Australia and New Zealand. Their main food is fish, but some species are also scavengers, feeding on dead animals and refuse.

Of 35 known species of seals, only five live in Antarctica: Antarctic fur seals (*Arctocephalus gazella*), Weddell seals (*Leptonychotes weddellii*), Leopard seals (*Hydrurga leptonix*), Ross seals (*Omnatophoca rossi*) and Crabeater seals (*Lobodon carcinophagus*). In the beginning of the twentieth century some of the Antarctic seal species, notably Antarctic fur seals, were almost exterminated by extensive hunting. In the last fifty years, however, Antarctic seals have been protected and now comprise the majority of the world’s total seal population.

Seals spend most of their life in water, spending only the breeding season on land, when they gather in large colonies along the Antarctic coasts. The main food of seals is fish, krill and occasionally penguins. Some of the larger seal species also feed on smaller seals. (About Antarctica 2002)

The Antarctic Treaty

The waters around the Antarctic continent have been exploited by humans, mainly fishermen, seal hunters and whale hunters, for hundreds of years. The land exploration of Antarctica is more recent, most of it occurring during the twentieth century. The improved technology of the century also allowed colonisation of Antarctica, and by the 1950s nine countries had territorial claims on the continent. To avoid political and military conflict and to facilitate further scientific co-
operation, the nations active in Antarctica decided on a treaty to avoid future territorial conflicts and guarantee continued freedom to conduct scientific research.

In 1961 the Antarctic Treaty (AT) entered into force. In 1964, the Agreed Measures for the Conservation of Antarctic Fauna and Flora, to protect native wildlife and plants, were additionally adopted by the AT member states. To improve regulations on fishing for krill, which is one of the key species in the Antarctic food web, and to protect the Antarctic environment from the effects of increasing human activities on the continent, additional acts were included in the Treaty in 1980 and 1991. Today 44 nations have signed the treaty, among them Sweden. (Antarctic treaty 2002)

The wildlife of Antarctica is considered particularly sensitive to environmental hazards caused by human activities in the Antarctic region. This has been recognised for the past few decades and the AT has focused on measures to minimise the risks for introduction of infectious diseases. No such diseases are known to have been introduced to Antarctic wildlife through human activities, but few studies have been made on this subject.

Four recorded incidents of mass mortality among seals and birds are suspected to be the result of infectious diseases. Avian cholera caused by Pasteurella multocida was suspected in an incident with 90% mortality in a population of skuas in 1981 (Trivelpiece 1981), but this was not proved. In 1998, sea lions on a New Zealand sub-Antarctic island died from unknown causes, but a new Campylobacter-like bacterial species was suspected (Baker 1999)

Salmonella (Oelke 1973), Pasteurella multocida (Parmelee 1978, Lisle 1990), Mycobacterium tuberculosis (Bastida 1999), Campylobacter (Broman 2000), and avian paramyxovirus, causing Newcastle disease (Morgan 1981) are potential pathogens that have been isolated in Antarctic animals. Reports of Salmonella in Antarctic wildlife have been sparse. In 1970-71, Oelke and Steiniger (Oelke 1973) found several different Salmonella species in faecal samples from Adélie penguins and
South polar skuas on Ross Island.

There are few human settlements in Antarctica and the majority of humans on the continent stay at research stations only for short periods. The Antarctic Treaty regulates the number of residents at each research station. Numerous regulations have been made to safeguard the environment from harmful effects of human presence.

The population of scientists in Antarctica has remained fairly constant in the last decade. The growing concern is Antarctic tourism, which is rapidly increasing. There was more than a five-fold increase between 1980 and 1990, from 855 to 4842. In 1997-1998 over 10,000 tourists, mostly shipborne, were expected (Provic 1998).

The introduction of humans into Antarctica greatly increases the risk of negative influence on the environment and directly or indirectly on the wildlife. The introduction of live non-indigenous animal and plant species on the continent is prohibited. The most likely risk factors for the introduction of infectious diseases are contaminated food and untreated sewage emanating from research stations and tourist or commercial ships. An incident with accidental pollution by sewage in 1990-1991 at the Scott base on Ross Island had devastating effects on the sea fauna in the affected area, with a high mortality of marine animal species (Meyer-Rochow 1992).

**Risk factors for introduction of infectious diseases in Antarctica**

Factors that could influence the introduction and spread of infectious diseases among Antarctic wildlife include the properties of the infecting microbe, behaviour of the affected animals and human activities.

The introduced disease agent must be pathogenic to the animal. It has to be able to survive in the harsh Antarctic environment with its extreme temperatures. Bacteria, parasites and viruses that are vector transmitted cannot survive in the
fauna if their vector is not present. The only potential disease-transmitting vectors found in Antarctica are ticks, *Ixodes*, (Bergström 1999a, Bergström 1999b, Murray 1990) and lice (Murray 1990, Harder 1991).

Animal behaviour is another important factor. Scavengers are more likely to have contact with pathogens through feeding on human waste. Some seal and bird species congregate in dense colonies during the breeding season and infectious agents can spread rapidly through these colonies. Some animal species form colonies together with other species, like Antarctic fur seals and gentoo penguins on Bird Island, which enhance the threat of interspecific spread of pathogens.

The migration pattern of the birds and seals is also of great importance. Migrating birds and seals have the ability to spread pathogens over long distances, both within the Antarctic continent and from neighbouring continents outside Antarctica where they spend the winter season.

Epizootics in the 27 countries active in Antarctica could spread to the Antarctic continent through equipment, clothing or personnel from those countries.

Treatment of waste and sewage are regulated in the Antarctic region, but treatment of sewage is only required for populations of more than thirty persons. A number of research stations restrict their personnel to less than thirty. Kitchen waste must either be incinerated or removed from the continent, alternatively stored in a safe way to prevent contact with scavengers.

The scientists may in the course of research be in direct contact with Antarctic animals. They may themselves unconsciously transport pathogens from an infected animal population to uninfected populations through their equipment, clothes or hands. Faecal pathogens could, for example, be transferred on footwear that has been in contact with faecal material from an infected animal population.

*Salmonella* is a bacterium that fills a number of the criteria for being a potential threat to Antarctic wildlife. It has been reported as a pathogen in seals from other
parts of the world (Schroeder 1973, Stroud 1980) and in several bird species (see above). *Salmonella* has the ability to survive in the cold waters of Antarctica (Smith 1994). It is distributed worldwide and causes epidemics and epizootics in all of the Antarctic Treaty member states. It is a faecal pathogen that is spread through wastewater, food products and sewage. Because asymptomatic carriage is a common feature of *Salmonella* in humans, persons with no obvious signs of disease can bring it to the Antarctic continent.
METHODS

Collection of samples

_Salmonella_ is most frequently isolated from faeces. In extraintestinal disease, the bacterium may also be isolated from blood, urine, cerebrospinal fluid and tissue specimens. In humans, faecal samples are taken preferably from faecal deposits, but can also be obtained by rectal swabs. Cotton tipped swabs are used and the samples are placed in transport medium to maintain viability during transport to the laboratory.

In birds, several methods for faecal sampling have been described. In dead animals, the most reliable is total gut culturing. The gut is divided into pieces and faecal material from each piece cultured (Girdwood 1985).

In live birds there are several applicable methods. Faecal samples can be taken from bird droppings, either on the ground, or from a cage. With droppings on the ground, the risk of false negative results increases rapidly through desiccation and UV-light radiation (Girdwood 1985). The value of droppings sampled from the ground is limited, as it can be difficult to know from which species of birds, or which individual, the sample came. Moreover, one infected bird may leave several droppings, with implications for statistics. A more reliable method is to catch the birds and cage them until they deliver a fresh dropping. This diminishes the risk for contamination of the sample and also allows for the result to be connected both to bird species and to the individual bird.

The equivalent to rectal swabs in humans are called cloacal swabs, whereas cloacal lavage means to insert fluid, like buffered peptone water (BPW), into the cloaca and then take out the fluid with its faecal content (Fricker 1983, Girdwood 1985). A comparison of cultures obtained by these two methods showed that
Cloacal swabs missed 23% of *Salmonella* detected with cloacal lavage, while only 1% of *Salmonella* isolates were missed with cloacal lavage compared to cloacal swabs (Girdwood 1985). Cloacal swabs yielded only 62% of *Salmonella* isolates when compared to whole gut culturing (Girdwood 1985). Cloacal lavage is, however, more time-consuming and potentially more dangerous to the birds than cloacal swabbing. We have been using cloacal swabs in study I, III, IV and V, while in study II and on the passerines in study I samples were taken from droppings of caged birds.

**Culturing**

We used charcoal transport medium, selenit selective broth for enrichment, and XLD solid media for primary culture. In study II, Rappaport selective broth was used instead of selenit broth. This is in accordance with the routine methods recommended for use in Swedish accredited bacteriological routine laboratories for detection of human *Salmonella* isolates (Hallander 2002). These culture methods have been established for several years in the Clinical Bacteriological laboratory in Umeå, where our samples were analysed. The method allows simple detection of *Salmonella enterica* subspecies I, because they form black colonies on the XLD medium. The vast majority of *Salmonella* found in warm-blooded mammals, avian species and humans (Uzzau 2000) belongs to subspecies I. However, there are clones of *Salmonella* serotypes other than those belonging to subspecies I that do not form black colonies on XLD agar. In our studies II-IV, the method was modified so that all isolates that yielded a change in colour of the XLD medium from orange-red to red-violet were further investigated.

**Molecular typing methods**

**PFGE**

Pulsed field gel electrophoresis, PFGE, is a molecular genetic method, based on
electrophoretic separation of large DNA fragments, generated by digestion of
genomic DNA with restriction endonucleases, and analysing restriction fragment
length polymorphism, RFLP. The method was introduced in 1983 (Schwartz 1983),
and has since become an important tool in molecular epidemiology.

A standard agarose electrophoresis permits only DNA molecules up to a size
of 20-50 kb (kilobase) to separate (Maule 1998, Bustamante 1993). Using the
PFGE method, DNA fragments up to 12 Mb (megabase) can be separated (Maule
1998).

In a standard agarose electrophoresis, the matrix of the agarose gel traps DNA
molecules larger than 20 kb (kilobase). The changing electric fields in PFGE, allow
larger DNA molecules to “zigzag” through the agarose gel (Maule 1998) with the
motion of crawling snakes (reptation) (Bustamante 1993).

DNA preparation for PFGE is dependent on the type of DNA used. Free
DNA cannot be used in solution, but has to be embedded in an agarose “plug” to
avoid shearing leading to breakage of DNA strands (Maule 1998). DNA embedded
in an agarose “plug” is then cut by a suitable restriction enzyme. Depending on the
size of the DNA and the restriction enzyme used, the pulse time and running time
of the PFGE are selected.

By using molecular weight markers and standardised methods, different gel
runs can be compared in a specially designed computer program, and relatedness
between strains can be demonstrated by clonality dendrograms (Garaizar 2000,
Rementeria 2001).

To interpret the PFGE-pattern, the most frequently used criteria are those
described by Tenover and colleges (Tenover 1995). According to these criteria, the
epidemiological analysis should be based on at least 10 distinct fragments visualised
by PFGE.
Analysis of IS200

IS200 is a short insertion sequence of about 708 base pairs, first described in the DNA of *Salmonella* (Millemann 2000). Although highly conserved, it is subject to variations in copy number and location of copies in the genome (Millemann 1995). It is not restricted to *Salmonella* spp. but also found in some other bacteria, notably *Escherichia coli* (Millemann 1995). All *Salmonella* spp. do not harbour IS200 insertion sequences (Millemann 1995).

IS200 profiles of Salmonella DNA can be analysed by digesting the DNA with restriction enzymes that do not cut within the IS200 sequence, followed by Southern Blot analysis (Stanley 1994). They could also be analysed by IS200-PCR (Millemann 2000). The latter method was used in our study IV.

PFGE, IS200 and Salmonella


Comparative evaluation of PFGE, IS200 and other molecular typing methods does not conclusively show which method is the most efficient in analysing *Salmonella* (Baquar 1994, Thong 1995, Olsen 1997). The discriminatory properties of the different methods are probably dependent on the *Salmonella* serotype analysed. Today, subdividing of *Salmonella* by the use of PFGE and IS200 analysis are both considered helpful tools when analysing *Salmonella* isolates with a known or suspected epidemiological interrelationship.
AIMS

• To investigate if wild birds are important reservoirs for enteropathogens like *Salmonella*, *Campylobacter* and EHEC in Sweden.

• To determine which wild bird species may serve as frequent carriers of *Salmonella*.

• To investigate the presence of antibiotic resistance in *Salmonella* found in wild birds.

• To determine the duration of *Salmonella* carriage in gulls.

• To investigate how gulls are affected by *Salmonella* infection.

• To investigate the prevalence of *Salmonella* in Swedish Peregrine falcons, a bird of prey at the top of the food web.

• To compare *Salmonella* isolates in birds with those from humans and domestic animals by the use of molecular typing methods.

• To investigate *Salmonella* occurrence in Antarctic wildlife.
RESULTS

Original paper I:


In 1997, 151 wild birds in Southern Sweden were investigated for prevalence of the enteropathogens Salmonella, Campylobacter and enterohaemorrhagic Escherichia coli, EHEC O157:H7. 50 faecal samples were from gull species; Black-headed gull (Larus ridibundus) and Common gull (Larus canus). 101 samples were from passerines; starling (Sturnus vulgaris), blackbird (Turdus merula), redwing (Turdus iliacus), song thrush (Turdus philomelos), fieldfare (Turdus pilaris) and ring ouzel (Turdus torquatus). The birds were sampled in spring, in the south of Sweden, when returning from their winter quarters in the southern parts of Europe.

Salmonella was found in 4.9 % of Black-headed gulls and in no other birds. Campylobacter jejuni was found in 3 % of passerines. EHEC was not found in any bird species.

The two Salmonella isolates belonged to serotype typhimurium. They were phage typed as NST and DT 22. Both isolates showed multiple antibiotic resistance. The three Campylobacter jejuni isolates were resistant to one or two antibiotics, respectively.

In this study we demonstrated that migrating birds coming into Sweden might carry Campylobacter and Salmonella. The Black-headed gull was the only Salmonella positive bird species and S. typhimurium was the serotype isolated. The Salmonella isolates found were antibiotic resistant, suggesting that they were derived from a human or domestic animal source.
Results

Original paper II:
Bonnerdahl J., Palmgren H., Hernandez J, Waldenström J., Olsen B. *Salmonella* in migrating birds – myth or reality?

This study was designed to determine whether any *Salmonella* reservoir might be found in migrating wild birds. 1087 migrating birds of 90 species were randomly sampled when passing Ottenby bird observatory, Öland, Sweden in the autumn of 2001. Every tenth bird ringed was also sampled for *Salmonella*, irrespective of bird species. A majority of migrating bird species breeding in Sweden was represented. 97.7 % of the samples were positive in Rappaport broth, but no *Salmonella spp* were found. *Citrobacter youngae, Citrobacter braakii, Citrobacter freundii, Escherichia vulneris, Escherichia coli, Hafnia alvei, Klebsiella pneumoniae ozaenae, Yersinia kristen senii* were isolated. These bacterial species may belong to the commensal enteric flora of birds.

The results of this study gave strength to our previous finding that gulls, notably Black-headed gulls, are the main *Salmonella* carrier among wild birds in Sweden.

Original paper III:

In this study, we wanted to further investigate *Salmonella* carriage in Black-headed gulls and the role of this bird species in *Salmonella* epidemiology in Sweden. 1047 faecal samples were collected from Black-headed gulls in Malmö, in the province of Skåne, Sweden during 1998, 1999 and 2000. In 1998 we sampled adult gulls coming to Sweden under the same conditions as in study I.

In 1999 and 2000, each faecal sample was associated with the ring number of
Results

the bird. Thereby we were able to get further information on individual *Salmonella* positive birds through resampling the bird, and also by ornithologist observations sent to the Swedish Bird Ringing Centre, Swedish Museum of Natural History.

This method allowed us to study the duration of *Salmonella* carriage in Black-headed gulls and look for possible signs of disease in *Salmonella* positive birds.

25 % of *Salmonella* positive gulls were sampled at least twice. No gull was *Salmonella* positive on more than one occasion. The results of inspection of gulls on the sampling occasions, and the reports on dead and sick gulls in the Malmö area, led us to conclude that there were no signs of morbidity or mortality in *Salmonella* positive gulls. Three of the gulls were reported to have accomplished their migration to Denmark and Holland after being found *Salmonella* positive.

Sampling was also done throughout the year, and the gulls were divided into five categories: spring migrating (March-April), nestlings (June), juveniel birds (July-August), autumn migrating (October-November) and stationary (January-February). *Salmonella* was found in 2.7% of all Black-headed gulls. The only difference in *Salmonella* carriage among the different categories of gulls was a significantly higher prevalence (7.8 %) in juveniel birds (p<0.001).

The predominant serotype, *S. typhimurium*, was found in 82 % of *Salmonella* positive samples. DT 41 was the predominating phage type. Other phage types were DT 195, DT 120 and NST.

PFGE and IS200-PCR of *S. typhimurium* DT 41, DT 195 and NST from gulls, humans and domestic animals, revealed a high genetic homogeneity among all DT 41 isolates, and a high genetic heterogeneity among NST isolates. Among DT 195 isolates, a genetic identity was found between isolates from gulls and domestic animals. There was also a genetic identity between DT 195 isolates from gulls and human isolates of domestic origin, but not from human isolates with non-domestic origin.

A new strategy was used in this study, based on ringing and bird observations
by ornithologists. The results suggested that *Salmonella* does not cause morbidity or mortality in Black-headed gulls, and that *Salmonella* carriage does not affect their ability to migrate. By taking advantage of ringing, we also received data indicating that *Salmonella* carriage is of short duration in gulls.

The majority of *S. typhimurium* phage types found in gulls were also the predominant phage types in *S. typhimurium* isolates reported in 1999 and 2000 from Swedish domestic animals. PFGE and IS200-PCR demonstrated a genetic relatedness between gull isolates of *S. typhimurium* DT 195 and human and domestic animal isolates from Sweden, but not with human isolates brought into Sweden from other countries. These results indicate that gulls play a role in the transmission of *S. typhimurium* in Sweden.

**Original paper IV:**


In this study we wanted to examine whether the feeding habits of wild birds may influence their carriage of human-associated enteropathogens. A carnivorous bird species, the Peregrine falcon (*Falco peregrinus*), was selected for the purpose, because it was in less direct contact with humans or human garbage than other birds. As top predators, Peregrine falcons feed on birds further down the food web.

As part of the Swedish recovery program for Peregrine falcons, 157 Peregrine falcon nestlings were ringed in the year 2000. Of these nestlings, 69 (44%) were sampled for *Salmonella* and *Campylobacter*.

*Salmonella amager*, a serotype known to be a human pathogen, was isolated from two nestlings in one brood. Seven *Campylobacter* isolates were found, including three
isolates of *Campylobacter jejuni*. The source of *S. amager* could not be established. There is no known reservoir of this serotype in Sweden. An identical PFGE pattern to a previously recovered human isolate could be demonstrated in the *Campylobacter jejuni* isolates. Remains of Black-headed gulls were found in all broods where nestlings were infected. To our knowledge, our reports of *Salmonella* in free-living Peregrine falcons were the first ever made for this cosmopolitan species.

**Original paper V:**


We studied the occurrence of *Salmonella* among birds and seals in the Antarctic area. The study was performed on Bird Island, South Georgia in 1996 and 1998. In 1996, 220 and in 1998, 446 penguin and albatross chicks and seal pups were sampled for *Salmonella*. The species were Gentoo penguin (*Pygoscelis papua*), Macaroni penguin (*Eudyptes chrysolophus*), Black-browed albatross (*Diomedea melanophrys*), Grey-headed albatross (*Diomedea chrysostoma*) and Antarctic fur seal (*Arctocephalus gazella*).

In 1996, *Salmonella* was found in 5% of Antarctic fur seals and in 7% of Gentoo penguins. Three serotypes, *S. typhimurium*, *S. enteritidis* and *S. havana*, were found. In 1998, 22% of Antarctic fur seals and 2% of Black-browed albatrosses were *Salmonella* positive. *S. enteritidis*, *S. havana* and *S. newport* were the serotypes found this year. Phage typing of *S. enteritidis* yielded PT4 or a PT4-like phage type, and in *S. typhimurium* DT 150.

Three serotypes, *S. havana*, *S. enteritidis* and *S. newport* were found in two animal species each, Antarctic fur seal and Gentoo penguin (*S. havana*, *S. enteritidis*) and seal and Black-browed albatross (*S. newport*). Two serotypes, *S. havana* and *S. enteritidis* were found in both 1996 and 1998.
PFGE revealed a total genetic homogeneity within each serotype of *S. havana*, *S. newport* and *S. enteritidis*, with the exception of one *S. newport* isolate that had one band difference from the other isolates of the same serotype. This genetic homogeneity occurred irrespective of sampling year or animal species.

The antibiotic susceptibility test showed no resistance to seven antibiotics with the exception of one isolate of *S. newport* that had reduced susceptibility to streptomycin.

In 1998, the populations of Gentoo penguins and Antarctic fur seals were reduced compared to 1996. This was due to reduced breeding success and increased pup mortality in the seal population and an almost nil breeding success in the Gentoo penguin population.

Our study showed that *Salmonella* has been introduced in Antarctic wildlife, both in seals and birds. There are indications of spread from human sources, both by the fact that the *Salmonella* serotypes and phage types found are all known human pathogens, and by the genetic homogeneity established in isolates of the same serotype by PFGE. This genetic homogeneity indicates relatively recent introduction of these *Salmonella* serotypes in the region.

Interspecific spread between seals and birds was indicated by the recovery of genetically identical isolates of the same serotype from seals and penguins, and from seals and albatrosses. The congregation of seals and penguins in dense colonies during the breeding season is a significant risk factor for rapid spread of introduced infectious agents like *Salmonella*.

The reduced breeding and the augmented pup mortality found in this study could be due to increased *Salmonella* infection of the seals and penguins. More likely, deteriorating nutritional status of the seals and penguins, due to natural fluctuations in krill, increase the secretion of *Salmonella* from the gut.
DISCUSSION

We wished to study the interaction between wild birds and humans in Salmonella epidemiology. As a hypothesis, we assumed that Salmonella infected wild birds might serve as a reservoir for this pathogen and a source for salmonellosis in humans, either directly or via transmission to domestic animals. We also wanted to study the impact human presence might have on infectious disease prevalence of wild animal populations in remote areas such as Antarctica. According to our results, the Black-headed gull is a predominant carrier of Salmonella among wild birds in Sweden, and S. typhimurium is the predominant serotype. Salmonella can be found in wild birds, both in Sweden and in Antarctica.

Salmonella in wild bird species

In our first study, we found Salmonella in Black-headed gulls but in none of the other eight bird species investigated (I). Since this study was too small to identify all bird species that are hosts for Salmonella, we did an extended study (II). In 1087 birds of 90 species that were examined, all birds were Salmonella negative (II), but positive to other enteric bacteria such as Escherichia coli, Hafnia spp., Klebsiella spp., Yersinia spp. and Citrobacter. These bacterial species possibly belong to the commensal enteric flora of wild birds and were a control on the validity of sampling and culturing methods used in the study. The prevalence of Salmonella in Black-headed gull was 4.9 % and 2.7 % in studies I and III, respectively, while we found no Salmonella in other migrating bird species. This strengthens the presumption that gulls are the main wild bird reservoir for this bacterium.

This is probably due to their migratory and feeding habits. They are omnivorous birds with a diverse diet. The rapid growth of gull populations in many European countries during the last decades has forced Black-headed gulls into urban areas (Girdwood 1985, Monaghan 1985, Hatch 1996). In this way, they have
come to live in close contact with humans and are now an abundant bird species in
cities and farmlands. They take a high proportion of their food in areas where they
are exposed to human sewage, city dumps and other garbage. Through their
feeding sites and food, they are easily infected with pathogens of human origin. The
Black-headed gulls examined in studies I and III were highly urbanised birds,
cought in Pildammsparken, a central park in Malmö, the city with the third highest
human population in Sweden.

Salmonella serotypes in wild birds
From the results of studies I and III, we concluded that *S. typhimurium* was the
predominant *Salmonella* serotype found in Black-headed gulls. It was the only
serotype found in study I, and in study III it represented 82% of the isolated
*Salmonella* serotypes.

*S. typhimurium* was the predominant serotype found in the majority of studies of
This serotype is also the exclusive cause of *Salmonella* disease outbreaks reported in

The predominance of *S. typhimurium* in Black-headed gulls is difficult to explain.
In 1998, 1999 and 2000, when the gull study was made, *S. typhimurium* and *S.
enteritidis* were the two predominant serotypes of domestic cases of human
salmonellosis in Sweden, and the prevalence of the two serotypes was almost the
same (Table 1). Still, we found that only 3.6% of *Salmonella* isolates in gulls were *S.
enteritidis*, while 82% were *S. typhimurium*. This was surprising, as *S. enteritidis* is the
most common *Salmonella* serotype in poultry in most European countries, although
rarely found in Swedish domestic birds.

The repeated finding of *S. typhimurium* as the most common *Salmonella* serotype
in wild birds might be due to host adaptation. One approach in studies to further
investigate this would be to screen for binding affinities of various serotypes to gut
mucosa of gulls and other wild birds.

The role of Black-headed gulls in Salmonella epidemiology in Sweden

The incidence of domestic *Salmonella* is low in Sweden, compared to European and North African countries where Black-headed gulls spend their winters (Cramp 1990, Bengtsson 1996, Bengtsson 2001). This fact and the results of study I, led us to advance the hypothesis that spring migrating gulls may be the predominant group of *Salmonella* carriers among the Swedish gull population. Investigating *Salmonella* prevalence in Black-headed gulls during all seasons (III) tested this hypothesis.

Contrary to our hypothesis, we found that *Salmonella* carriage was not higher in spring migrating Black-headed gulls than in gulls sampled in other seasons. Instead, *Salmonella* carriage was most prevalent in juvenile birds, the population of Swedish Black-headed gulls that had never been outside Sweden (III). This indicated that *Salmonella* sources within Sweden played an important role for *Salmonella* carriage in Black-headed gulls. The prevalence of *Salmonella* carriage in juvenile birds was still low compared to the results in previous studies in Scotland and in the Czech republic (Monaghan 1985, Hübalek 1995, Sixl 1997) where 15 %, 25 % and 51% of juvenile birds were found to be *Salmonella* positive, respectively.

To further investigate possible Swedish sources for *Salmonella* infection of Black-headed gulls, we compared the *S. typhimurium* phage types of the gull isolates with phage types of *S. typhimurium* found in humans and domestic animal in Sweden in the study years 1999 and 2000. The majority of *S. typhimurium* phage types carried by gulls were also the dominating phage types reported from cases of *S. typhimurium* in domestic animals and animal feed (III).

One of the *S. typhimurium* phage types found in gulls, humans and domestic animals was DT 195. PFGE and IS200 on *S. typhimurium* DT195 showed genetic identity between Swedish isolates from gulls and domestic animals, and also with
one case of human salmonellosis from a person living in Lund, a town close to Malmö where the *S. typhimurium* DT 195 gulls were caught. The conclusion from these results is that a transmission route for *Salmonella* involving gulls is present in Sweden. The exact role of gulls as a link in this transmission chain is not clear, but there are several possible transmission routes between gulls, humans and domestic animals. Previous studies have reported on wild birds, including gulls, contaminating the environment of domestic animal habitats with *Salmonella* and thereby contributing to *Salmonella* infection in domestic animals (Johnston 1979, Reilly 1981, Butterfield 1983, Cizek 1994, Hatch 1996, Craven 2000, Davies 2001). Gulls often roost and feed at sewage outlets where they may get infected by pathogens from both humans and domestic animals found in insufficiently treated sewage (Fenlon 1983, Fricker 1984), and also infect water supplies with *Salmonella* (Fennel 1974, Benton 1982). There are also reports of possible *Salmonella* transmission by gulls to humans through faecal contamination of bathing water (Lévesque 1993).

Antibiotic resistance in *Salmonella* isolates from gulls

Antibiotic resistance in *Salmonella* and other human pathogens, has been a growing concern for the last three decades (Swann Committee 1969, Riley 1984, Wray 1993, Threlfall 2000). The multi-resistant strain *S. typhimurium* DT 104, has rapidly become one of the major problems in human salmonellosis in a growing number of European countries. Sweden has managed to control the problem of antibiotic resistance, through extensive legislation on imported feed, food and animals for feed production, and a strict use of antibiotics in humans, domestic animals and in animal husbandry. To investigate if wild birds could be vectors of enteropathogens carrying antibiotic resistance, all *Salmonella* isolates found in Swedish wild birds in studies I, III and IV were tested for sensitivity to antibiotics, commonly used in treatment of salmonellosis.

We did not find any mr-DT104, but we did find a total of four multiple
antibiotic resistant isolates of *S. typhimurium* of other phage types (I, III). These multiple antibiotic resistant isolates were all found in spring migrating birds, just entering Sweden from their winter quarters in other European countries (I, III), where antibiotic resistant *Salmonella* is common in humans and domestic animals. We also found vancomycin-resistant *Enterococci* in the spring migrating Black-headed gull population described in article I. We have previously reported this discovery (Sellin 2000). Migratory birds may carry virulent isolates of *Salmonella* and other enteropathogens from *Salmonella* sources abroad and thus have an impact on the epidemiology of these pathogens in Sweden.

Impact of *Salmonella* on health and survival of gulls and duration of carriercship

In study III, we introduced a strategy that may become more generally applied to study different features of infection in birds. The strategy was based on cooperation with bird ringers and other ornithologists. Bird ringing makes it possible to identify individual birds, recapture infected birds and follow the movements of individual birds through observations registered at the Swedish Bird Ringing Centre.

Ringing and ornithologist reports enabled us to investigate if there were any disease manifestations of *Salmonella* infected gulls. General appearance of the gulls was registered at the time of sampling, and no signs of disease were noted in the *Salmonella* positive birds. There were no reports of morbidity or mortality in *Salmonella* positive birds during the study period that could be attributed to salmonellosis. The condition of the ringed birds was followed by ornithologists working in the Malmö area, where the study was done, and by checking reports of sick and dead birds sent to the Bird Ringing Centre. Through the Bird Ringing Centre, we could even get observation reports on three *Salmonella* positive birds that had made their autumn migration to Denmark and Holland, shortly after being demonstrated to be *Salmonella* positive. The results indicate that Black-headed gulls are not affected by *Salmonella* carriage. This is in accordance with the fact that
Salmonella disease has rarely been reported in gulls (Brand 1988). The method of ringing and resampling individual birds also allowed us to address the question of duration of Salmonella carriage in Black-headed gulls (III). To our knowledge, no previous reports have been made on the duration of Salmonella carriage in wild living gulls and only one previous report has been made on the duration of Salmonella carriage in captured gulls (Girdwood 1985). Girdwood and colleges reported a mean duration of two and a maximum duration of four days (Girdwood 1985). Our results suggest that carriage is of short duration also in the wild. Six of 24 Salmonella positive Black-headed gulls were sampled at least twice. All of them were Salmonella positive only at one occasion. Two were Salmonella negative before and four after being Salmonella positive. The shortest time between the positive and the negative sampling was thirteen days.

Several features of Salmonella carriage in Black-headed gulls make us believe that Salmonella, as opposed to Campylobacter, is not part of the commensal intestinal flora of this bird species. The low carriage rate (I-III), the almost exclusive presence in gulls (I-II), a bird species that lives in close contact with humans and easily picks up human pathogens, and the short duration of carriage (III) all point in that direction. Thus, the role of Black-headed gulls, in Salmonella epidemiology seems to be rather as vectors than as a reservoir. As vectors they may, however, play an important role as they may rapidly transport Salmonella over long distances.

Occurrence of Salmonella in Peregrine falcons, a predator of other birds. Black-headed gulls are omnivorous birds that eat a wide variety of foods and often live in urban environments. They easily come in contact with both humans and human enteropathogens like Salmonella. To further investigate the role of feeding habits on the carriage of enteropathogens in wild birds, faecal samples from Swedish Peregrine falcons (Falco peregrinus), a raptor that preys on other birds, were analysed for Salmonella and Campylobacter (IV). Salmonella has previously only been

Peregrine falcons have only sparse contact with humans in the wild. Thus, any findings of *Salmonella* in Peregrines would most likely come from the prey birds they eat.

Both *Salmonella* and *Campylobacter jejuni* were also found in the Peregrine falcon population. Spread of these enteropathogens from human sources to the falcon population was indicated by the fact that *S. amager*, the *Salmonella* serotype found, has mostly been reported from cases of human salmonellosis (Beliakov 1969, Kelterborn 1979, Oak 1984).

The three *Campylobacter jejuni* isolates found showed genetic identity to a previously described human *Campylobacter jejuni* isolate, a fact which also suggests spread from a human source (IV). A possible explanation of how the falcons became infected with enteropathogens from human or domestic animal sources was suggested by the finding of gull remains in the broods of infected falcons (IV).

The possible effect of *Salmonella* carriage on Peregrine falcons needs further investigation. Falcon mortality from salmonellosis has only been reported when the birds were co-infected with other pathogens like chlamydia and poxviruses (Wernery 1998).

The Swedish Peregrine falcon population was once on the verge of extinction through human spread of chemical compounds (Gärdefors 2000, Lindberg 1983). It is possible that various entero-pathogens from human sources could have devastating effects on this sensitive animal species, although it must be emphasised that there is no evidence of such effects occurring now.

**Salmonella in birds and mammals in Antarctica**

In our studies on gulls, falcons and other wild bird species (I-IV), transmission of *Salmonella* between wild birds and humans was implicated. We therefore wanted to study the occurrence of human-associated *Salmonella* infection in Antarctic wildlife.
Antarctica is the last continent on earth to be explored by man, the wildlife there being undisturbed by humans until the end of the 19th century. To our knowledge, there existed only one previous report on *Salmonella* in Antarctic animals (Oelke 1973).

We found *Salmonella* in Antarctic seals, Gentoo penguins and albatrosses (V), and identified from these animals the following four *Salmonella* serotypes: *S. typhimurium, S. enteritidis, S. newport* and *S. havana*. All four serotypes are among the 50 of the now more than 2300 *Salmonella* serotypes that are considered human pathogens (Kelterborn 1979, Uzzau 2000). *S. enteritidis, S. typhimurium* and *S. newport* are three of the six most common human *Salmonella* serotypes reported by Kelterborn (1979). The phage type of the *S. enteritidis* we found in Antarctic birds and seals was a variant of PT4, the most common *S. enteritidis* phage type in Europe for the last twenty years (Olsen 1996). This suggested a human source for *Salmonella* in the Antarctic animals we studied.

The precise means by which *Salmonella* was introduced to Antarctic wildlife is not clear and needs further investigation. One possible source of transmission is insufficiently treated sewage from research stations that can contaminate the waters where seals and birds feed. Accidental contamination of seawater from sewage outlets has been reported in the Antarctic area (Meyer-Rochow 1992), and research stations with less than 30 personnel are not required by law to treat their sewage and clean up their latrines (Antarctic Treaty 2002). Seals and penguins may also pick up *Salmonella* when foraging for food where fishing boats and other commercial ships dump their sewage into the sea.

Just as *Salmonella* can be spread to Sweden from foreign countries via a medium-distance migrant, the Black-headed gull, long-distant migrants like albatrosses could conceivably bring *Salmonella* from human sources to Antarctica from other continents. During the winter season, migrating Antarctic bird species, e.g. albatrosses and skuas, often feed around sewage outlets in the waters outside
the major cities of South America, Australia and Africa (Peter Prince personal comm.).

Genetic identity with PFGE among isolates of one and the same serotype was shown for *S. enteritidis*, *S. havana* and *S. newport*. One isolate of *S. newport* differed in one band from the other 21 isolates of this serotype. PFGE was not done on *S. typhimurium* because only one isolate was encountered. This genetic identity could be seen in isolates from both study years, from different seal colonies at Bird Island and with different animal species. This fact suggests that *Salmonella* may quickly spread between seal populations and between different animal species. The extremely crowded breeding colonies of both seals and penguins would seem to constitute an excellent ground for the rapid spread of *Salmonella*. Interspecific transmission between seals and penguins can be explained by the fact that these two species often breed on the same shores in large, mixed colonies.

In Antarctic seals and penguins, there was a reduced number in seal pups and penguin chicks in 1998 compared to 1996. At the same time, *Salmonella* incidence in seal pups had increased from 5% to 22%. Gentoo penguins were found to be *Salmonella* infected in 1996. In 1998 the breeding success in the Gentoo penguin population was almost nil, which was the reason why Gentoo penguins were not examined for *Salmonella* that year. The amount of krill, the most important food for both seals and penguins, was sparse in the waters of Bird Island in 1998, which might have resulted in reduced nutritional status of both seals and penguins.

To our knowledge, *Salmonella* disease has not been reported in penguins. In a number of seal species, there are reports on *Salmonella* disease and seal mortality from salmonellosis (Gillmartin 1979, Stroud 1980, Baker 1995, Thornton 1998).

It should be emphasised, however that we have no evidence indicating a causal relation between the increased prevalence of *Salmonella* in Antarctic fur seals or the finding of *Salmonella* in Gentoo penguins in 1996, and the decimation of these animal populations on Bird Island in 1998.
The effect of nutritional depletion on *Salmonella* disease expression has previously been described in wild birds. *Salmonella* epizootics in the populations of small birds like passerines occur mostly during the winter season when food is sparse (Macdonald 1969, Locke 1973, Hurvell 1974, Nesbitt 1974, Fichtel 1978, Pennycott 1998, Tauni 2000). If *Salmonella* is introduced in a small bird population, it usually causes no harm to the birds under good living-conditions. In winter, the stress of cold weather and nutritional depletion can cause salmonellosis in birds infected with *Salmonella*.

The reduced nutritional status of seals may also have augmented the faecal shedding of *Salmonella* observed as an increased prevalence of the bacteria in seals in 1998, compared to 1996. This explanation is supported by a previous observation that hens show an increased faecal shedding of *Salmonella* if they are exhibited to reduction in feed and water (Holt 1992, Holt 1994).
CONCLUSIONS

• Black-headed gull is the main wild bird reservoir for *Salmonella* in Sweden and *S. typhimurium* is the dominating serotype.

• The duration of *Salmonella* carriage is relatively short and the morbidity, mortality and migratory capacity in Black-headed gulls are not influenced by *Salmonella*.

• Epidemiological investigations of *S. typhimurium* from gulls, humans and domestic animals, indicate a possible role for gulls in the transmission chain of this *Salmonella* serotype in Sweden.

• Migrating birds can be vectors for antibiotic resistant *Salmonella* and other virulent enteropathogens from sources outside Sweden.

• Birds of prey can be infected by *Salmonella* and *Campylobacter* of suspected human origin, through feeding on gulls and other “urbanised” bird species.

• The Salmonella serotypes and phage types in Antarctica indicate a human source for transmission to wildlife.

• Genetic homogeneity in *Salmonella* found in Antarctic wildlife indicates a spread between different reservoirs as birds and seals.
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