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HUMAN SALMONELLOSIS – IMPACT OF TRAVEL AND TRADE FROM A SWEDISH PERSPECTIVE

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To Nosse and Mille

ABSTRACT

Background: Salmonellosis is one of the most important gastrointestinal infections in humans. The vast majority of Swedish *Salmonella* cases have acquired the disease abroad, reflecting both a large number of Swedish travellers and a very favourable domestic *Salmonella* situation compared to other countries, mainly due to various control programs implemented in the animal food production and import regulations (e.g. on reptiles). Some of these programs and regulations were changed when in 1995 Sweden joined the European Union (EU). The aim of this thesis is to describe and analyse the impact of travel and trade on the epidemiological situation of human salmonellosis in Sweden.

Methods: The basis for the five studies in the thesis is the national database on notified *Salmonella* infections, from which we extracted case-based information on age, sex, area of residence, country of infection, *Salmonella* serotype, and reptile contacts. For comparison in Papers II and III, we used a comprehensive database on the patterns of over-night travels abroad among Swedish residents as travel denominator to calculate risks per 100,000 travellers, but also to estimate the incidence of this infection in the various EU countries, using Norway as reference. In Paper IV, *Salmonella* isolates isolated from humans and sewage sludge from the same residential areas were compared using genetic typing method and antibiotic susceptibility testing. Paper V was a case-control study, comparing knowledge and behaviours and risk of salmonellosis in reported travel-associated cases and randomly selected travellers from destinations outside EU.

Results: In Paper I, we could show rapidly increasing incidences of reptile-associated salmonellosis, after EU harmonisations of import rules, but also how this trend could be broken by active information. In Paper II, we showed the risk of travel-associated salmonellosis to be highest in East Africa and the Indian subcontinent. Children aged 0-6 years were at higher risk than travellers of other ages. There are also marked geographical differences in serotype distribution between various regions of the world, in Europe *S*. Enteritidis was especially dominating. In Paper III, we estimated the "true" European salmonella incidence to be highest in Bulgaria, Turkey and Malta, and that a severe under-reporting in the official figures from some countries make official data useless for comparisons. As the egg-related *S*. Enteritidis was the dominating serotype, limiting *Salmonella* in European poultry could have a major public health impact. In Paper IV, we could demonstrate that *Salmonella* isolates from sewage treatment plants probably originated from infected humans and survived the treatment at the plants. It also highlighted the risk of spreading antibiotic resistant *Salmonella* from sewage sludge to the environment. In Paper V, we could show that knowledge and advice are not enough to decrease the risk of travel-associated salmonellosis, while actual behaviour of strictly avoiding high risk food-items reduces the risk by approximately 70%. Still the weekly risk for salmonellosis in these travellers would be more than 100 times higher compared to staying at home in Sweden.

SAMMANFATTNING

Bakgrund: Majoriteten av de salmonellafall som rapporteras har smittats utomlands, vilket visar att svenskarna är ett resande folk men också att den svenska salmonellasituationen är mycket god jämfört med andra länder. Denna goda situation beror på att olika kontrollprogram inom den animala livsmedelsproduktionen har funnits i landet länge, även importrestriktioner har medverkat till det goda läget. Vissa av dessa regler ändrades när Sverige gick med i EU. Syftet med denna avhandling var att beskriva och analysera vilken påverkan resande och handel har på det epidemiologiska läget när det gäller human salmonellainfektion i Sverige. Följande frågor ställdes:

1. Vilken påverkan fick EU-harmonisering av importregler för reptiler på den svenska humana salmonellasituationen
2. Reseassocierad salmonellos hos svenska turister - finns det länder/regioner där svenskar har en högre risk att få salmonellainfektion
3. Kan hemvändande svenska turister ge en jämförbar uppskattning av salmonellatrycket i EU:s olika medlemsstater, associerade och kandidatländer
4. Finns det ett samband mellan de salmonellastammar som isolerats från avloppsslam och humana salmonellafall
5. I vilken grad påverkar reseråd, kunskapsnivå när det gäller riskabla livsmedel och drycker samt det aktuella beteendet under resan risken att insjukna i salmonellos.

Metoder: Grunden för dessa fem olika projekt var Smittskyddsinstitutets nationella databas över anmälda fall av salmonellainfektion. Från denna databas har vi hämtat information rörande ålder, kön, bostadsort, smittland, serotyp och eventuell reptilkontakt. I studie 1 jämfördes åren innan EU-harmoniseringen med åren efter det att harmoniseringen trätt i kraft med avseende på antalet rapporterade fall av reptil-associerad salmonellos (RAS). För studierna 2 och 3 användes en databas innehållande svenskars utlandsresande, vilken användes som nämnare när risken att insjukna vid resa till olika länder/regioner beräknades. Dessutom användes i studie 3 antalet officellt rapporterade salmonellafall från respektive land, med Norge som referensland vid skattningen av salmonellaincidensen i de olika länderna. I studie 4 jämfördes salmonellastammar isolerade från avloppsslam med humana salmonellastammar med molekylärbiologisk metod och antibiotikaresistenstest. Studie 5 var en fall-kontrollstudie omfattande 400 fall och 1 600 kontrollpersoner. Där fallen valdes från salmonellapositiva resenärer som vistats utanför EU och kontrollpersonerna bland de personer som bokat en resa utanför EU via en stor researrangör.

Resultat: Studie 1 visade att reptiler inte är en ovanlig smittkälla för salmonellos, speciellt bland barn. Importrestriktioner var ett effektivt skydd mot RAS. En dramatisk ökning av antalet fall av RAS noterades 1996, men med information lyckades antalet RAS-fall minskas. Studie 2 visade att risken var störst bland resenärer från Östafrika och Indien med grannländer, och att barn löpte störst risk att insjukna. Geografiska skillnader i distributionen av serotyper över världen kunde konstateras, med *S.* Enteritidis helt dominerande i Europa. I studie 3 visade skattningen de högsta incidenserna av salmonellos i Bulgarien, Turkiet och Malta samt att en betydande underrapportering gör de officiella siffrorna oanvändbara för jämförelser mellan länderna. Studie 4 visade att salmonellastammar isolerade från avloppsslam troligen har

humant ursprung samt att dessa stammar kan vara multiresistenta vilket bör beaktas vid spridning av slam på åkermark. Studie 5 visade att kunskapen om vad som borde undviks eller hur informationen erhållits inte påverkade risken att insjukna. Däremot var det aktuella beteende under resan den faktor som påverkade risken att insjukna mest då ett strikt undvikande av alla riskabla maträtter reducerade risken med cirka 70 %. Trots detta var risken att insjukna i salmonellos som utlandsresenär 100 till 400 gånger högre per vecka, beroende på resmål, jämfört med att stanna hemma.

LIST OF PUBLICATIONS

This thesis is based on the following papers:

I. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. *Emerg Infect Dis 2005; 11: 398-403*

II. Ekdahl K, de Jong B, Wollin R, Andersson Y. Travel-associated non-typhoidal salmonellosis: geographical and seasonal differences and serotype distribution. *Clin Microbiol Infect* 2005; 11: 138-144

III. de Jong B, Ekdahl K. The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. *BMC Publ Health 2006; 6:4.*

IV. Sahlström L, de Jong B, Aspan A. Salmonella isolated in Sewage Sludge traced back to human cases of salmonellosis *Lett App Microbiol 2006;43:46-52.*

V. de Jong B, Ekwall E, Rombo L, Ekdahl K. Food and drink, should the traveller bother? - A case-control study on behaviour and risk for travel-associated salmonellosis. *Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

CDC	The Centers for Disease Control and Prevention
CI	Confidence interval
CMO	County Medical Officer of Communicable Disease Control
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EHB	Environmental Health Board
EU	European Union
HACCP	Hazard analysis and critical control point
MIC	Minimum inhibitory concentration
OR	Odds ratio
PFGE	Pulsed-field gel electrophoresis
PT	Phage type
RAS	Reptile associated salmonellosis
S.	*Salmonella*
SBL	National Bacteriological Laboratory
SMI	Swedish Institute for Infectious Disease Control
STP	Sewage treatment plant
SVA	National Veterinary Institute
TD	Travellers' diarrhoea
TDB	Swedish Travel and Tourist Database
WHO	World Health Organization

INTRODUCTION

Salmonellosis

Infections caused by *Salmonella* bacteria are common worldwide. Salmonellosis is a zoonotic disease, which means that both humans and animals can contract the infection. Domestic as well as wild animals, including cattle, poultry, swine, rodents, cats, dogs and reptiles, can serve as reservoir [1].

Salmonellosis is an important public health problem, causing substantial morbidity, and thus also having a significant economic impact. Although most infections cause a mild to moderate self-limiting illness, serious disease leading to death does occur. In the United States, it is estimated that 1.4 million non-typhoidal *Salmonella* infections with 400 deaths occur annually [2]. Calculations from England and Wales for the year 1995, resulted in an estimation of 102,227 indigenous cases with 3,412 hospital admissions and 268 deaths [3].

The infective dose is usually high (> 100,000 bacteria), but *Salmonella* grow well in most foodstuff. In food with a high fat content, e.g. chocolate and cheese the infective dose is very low, and just a few bacteria may be sufficient to cause infection [4,5]. The susceptibility to infection varies; in infants, elderly, or compromised hosts, the critical infective dose is lower [6].

The onset of disease is often sudden with diarrhoea, stomach pain, nausea and vomiting [7]. The incubation period is 1–3 (range <1–10) days. The carrier state is normally 4–6 weeks, but a few percent may be asymptomatic carriers for months or even years [4,8]. Complications from the joints may occur. Antibiotic treatment is normally not given in uncomplicated disease, but sometimes rehydration is needed. No vaccine is available against non-typhoidal salmonellosis.

Culturing of faecal samples verifies the diagnosis in most cases, but the bacteria could also be found in blood and urine. A *Salmonella* infection is often considered to be a rather mild infection. However, a study on persons from an outbreak of salmonellosis showed that 33% of the affected people still had symptoms of fatigue, diarrhoea and menstrual disturbance 2 months after the onset of disease and 8 % still had symptoms after 5 months [9].

The bacteria and typing

The *Salmonella* bacteria were first described in 1886, by Daniel Elmer Salmon and his co-worker Theobald Smith. The bacterium is a Gram-negative, non-spore forming, facultatively anaerobic, motile rod of about 0.5 x 1-3 µm size. Non-typhoidal salmonellae express two antigens: “H” or flagellar antigen and “O” or somatic antigen. H antigen may occur in either or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. In 1934, the first Kauffmann-White scheme was published and it described 44 different serovars of *Salmonella* using antisera reaction to different the O and H antigens. From 1949 to 1965, Fritz Kauffmann in Copenhagen described a number of new serovars [10,11]. Using the Kauffmann-White scheme, today more than 2,500 *Salmonella* serovars have been identified [12]. The *Salmonella* genus consists of two species; *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies; *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salmae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae, S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica.* These six subspecies are often given symbols: I = enterica, II = salmae, IIIa = arizonae, IIIb = diarizonae, IV = houtenae, VI = indica. The symbol V is due to habits used for serovars of *S. bongori*. This taxonomy may be confusing, but most of the *Salmonella* strains that affect humans belong to *S. enterica* subsp. *enterica* and have serovars that are given names, i.e. the correct name of *S.* Enteritidis is *S. enterica* subsp. *enterica* serovar Enteritidis. In most literature regarding salmonellosis the word “serotype” is used instead of “serovar”, these two words should be regarded as interchangeable and both words are used in these thesis.

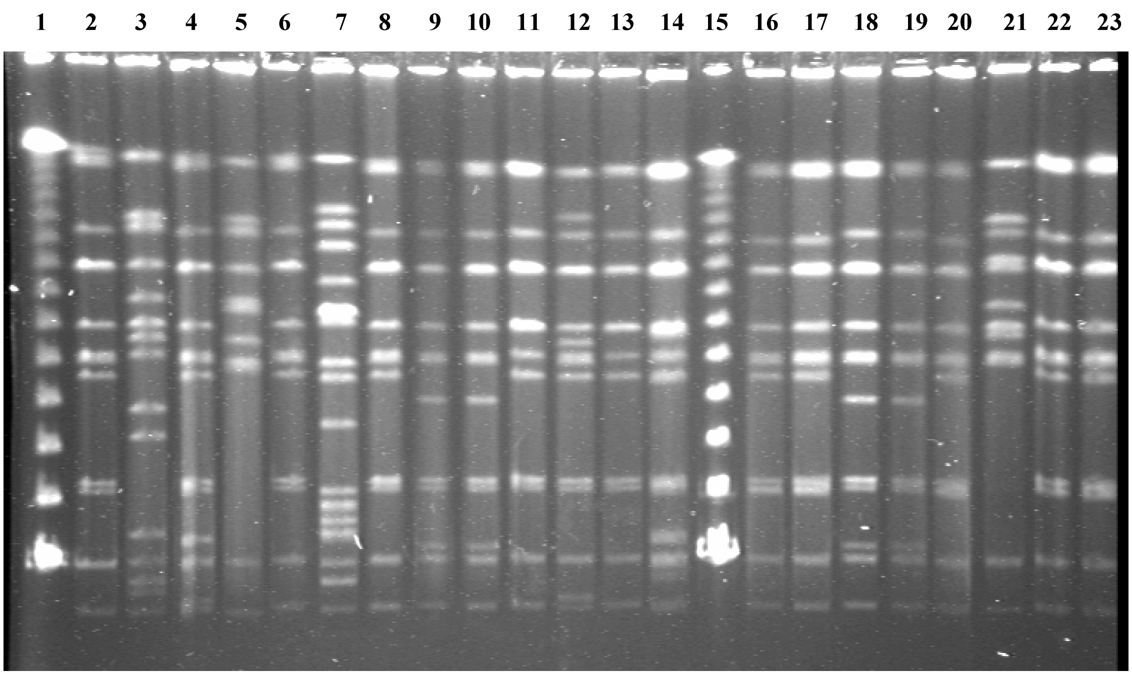

Photo: S. Löfdahl/M. Ramberg, SMI

1, 15	**Markers**
2-5, 7, 12, 21	**Unrelated isolates**
6, 8, 16, 17, 20, 22, 23	**Outbreak isolates, Stockholm-Uppsala**
9, 10, 18, 19	**Cluster Gothenburg**
11, 13, 14	**Returning travellers from Brasil**

Figure 1. PFGE gel used for subtyping of *S.* Saintpaul isolates in an outbreak situation in Sweden 2002.

Subtyping with different strains of bacteriophages (phage typing) is also a well-established method and could be performed on several different serovars [13,14]. In Sweden isolates of *S.* Enteritidis and *S.* Typhimurium are phage typed. During the last decades much progress has been made in the field of molecular typing and several new methods have been developed. The most used method, pulsed-field gel electrophoresis (PFGE), is now used world wide in different international and national networks [15,16] (Figure 1). Antibiotic resistance pattern could also provide useful information about an isolate.

The Swedish situation

Salmonellosis used to be one of the infectious diseases with greatest impact on public health in Sweden in the late 19th century. But measures taken to provide a better sanitary situation, in particular the building of sewage treatment plants in the beginning of 20th century, improved the situation and fewer cases were reported (Figure 2).

In the summer of 1953, a large outbreak of salmonellosis occurred, affecting almost 9,000 cases including 90 fatal cases [17,18]. The causative agent in the outbreak was *Salmonella* Typhimurium and the source was a slaughterhouse in the town of Alvesta, from which infected meat and meat products were distributed to many different places in Sweden. This outbreak was an alarm clock to the Swedish Government and the Department of Epidemiology at the National Bacteriological Laboratory (SBL, nowadays SMI) was established in order to carry out surveillance of human salmonellosis in Sweden.

In the 1960s, Swedes started to go on package tours to countries with a warmer and sunnier climate but also with a higher burden of salmonellosis, which is clearly visible in the number of reported cases of salmonellosis (Figure 2).

Another milestone in the Swedish history of salmonellosis was a large outbreak in 1977. In Tensta, a suburb to Stockholm, all school children and teachers were served a fish dish with cold mayonnaise sauce. The sauce was contaminated with *Salmonella* Enteritidis, and caused infections in about 3,000 pupils and teachers attending 28 different schools that day [19].

In the middle of the 1980s, a pandemic of egg-associated *S.* Enteritidis commenced in Europe and the United States. Thanks to very strict rules in the breading of egg-laying hens, Sweden was not affected of this pandemic. However, Swedes returning from travel outside Sweden were affected in high numbers.

Reporting system

Since 1968, any physician diagnosing a patient with salmonellosis has to notify the patient to the Department of Epidemiology at the Swedish Institute for Infectious Disease Control (SMI) – before 1993, the National Bacteriological Laboratory (SBL). The cases are notifiable both by the doctor having seen the patient (clinical notification) and the laboratory having diagnosed the bacteria (laboratory notification).

Notifications are submitted in parallel to the SMI and to the County Medical Officer of Communicable Disease Control (CMO). Clinical notification should contain relevant epidemiological information, including suspected source of infection and country of infection. The information in the clinical notifications facilitates the surveillance of salmonellosis in Sweden. Among the cases reported as being infected in Sweden (indigenous infection) many have consumed imported food items or have been secondarily infected by another person having contracted the infection abroad. About 3,500 to 5,000 cases are notified each year. Most of the cases are imported cases, often in tourists returning from the Mediterranean area or Thailand [20].

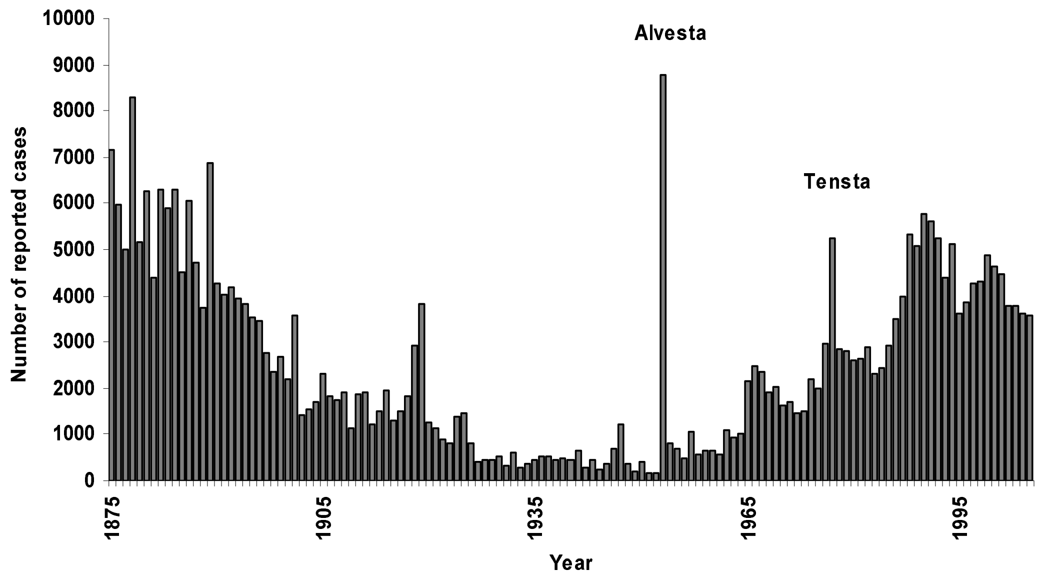

Figure 2. Number of reported cases of salmonellosis in Sweden 1875-2005. Source: SMI

Reported cases

As seen in Figure 3, the Swedish *Salmonella* statistics is greatly influenced by the annual number of journeys abroad by Swedish residents. But the statistics is also influenced by public health measures introduced in countries with popular tourist destinations. One example was the legislations introduced in 1995, in Denmark, Germany and Spain, prohibiting the use of raw shell eggs. This had a great impact also on the number of imported cases of salmonellosis from these countries.

The number of Swedes travelling abroad was continuously increasing in the 1990s, and in 2000 about 8.2 millions Swedish residents went on leisure tours outside Sweden [21]. This increase was abruptly halted in 2001/2002 after the terrorist attacks in the USA (September 11, 2001) and Bali (2002); in 2003 only 1.8 millions Swedes went on a package tour. Also the tsunami in 2004 had a negative effect on people’s wish to travel.

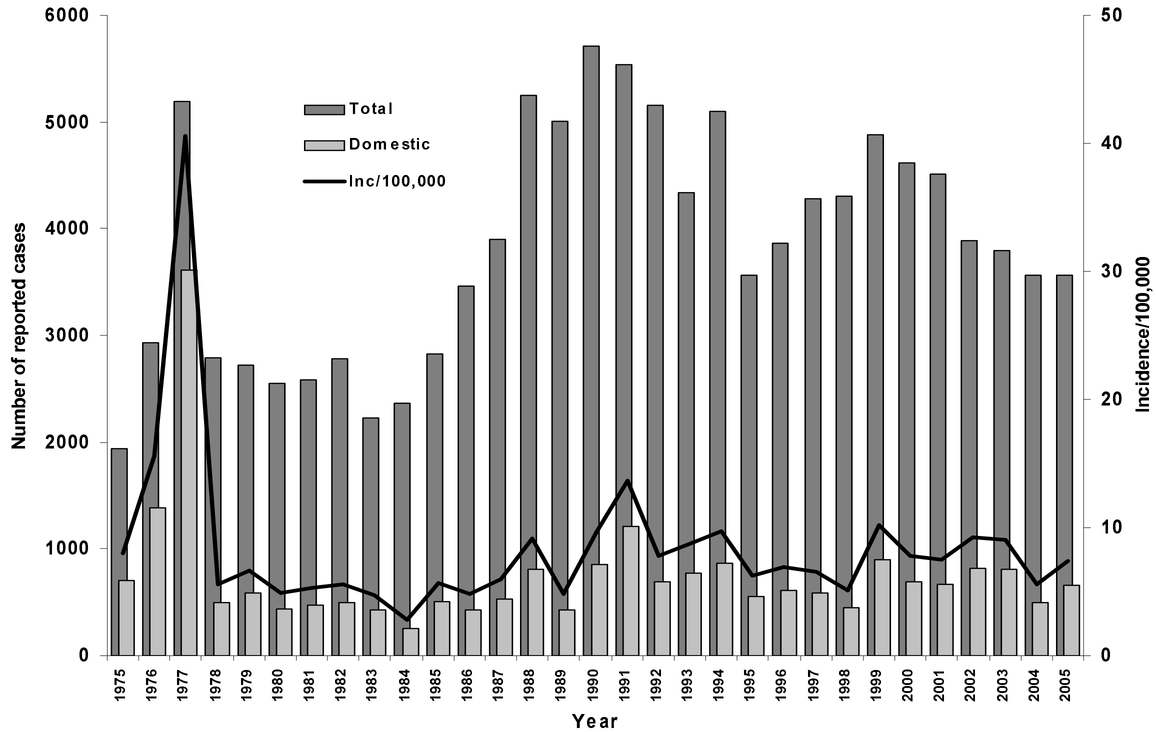

Figure 3. Number of reported cases of salmonellosis in Sweden 1975-2005 and incidence of domestically acquired cases per 100,000 inhabitants. Dark grey bars represent total number of reported cases, light grey bars number of domestic acquired and curve the domestic incidence per 100,000 inhabitants.

Age and gender

Among the reported cases the 1-year olds dominate when the infection is acquired in Sweden (Figure 4). The attractiveness of celebrating the 50th and to a lesser extent the 40th and 60th anniversaries abroad is also clearly shown in the statistics of reported cases of salmonellosis in Sweden (Figure 5). No difference in gender is observed.

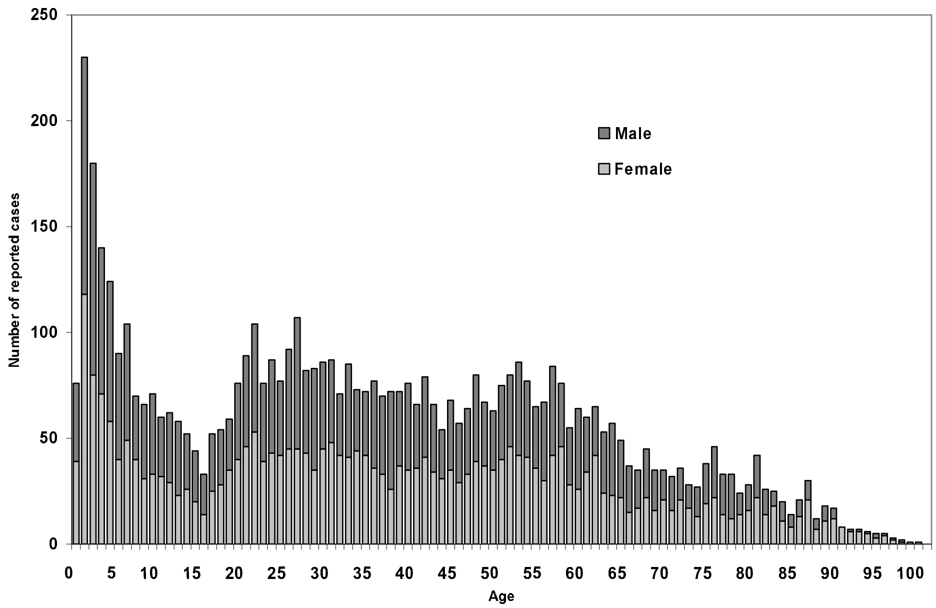

Figure 4. Age and gender of reported cases with a domestic acquired *Salmonella* infection in Sweden 1997-2005. Source: SMI

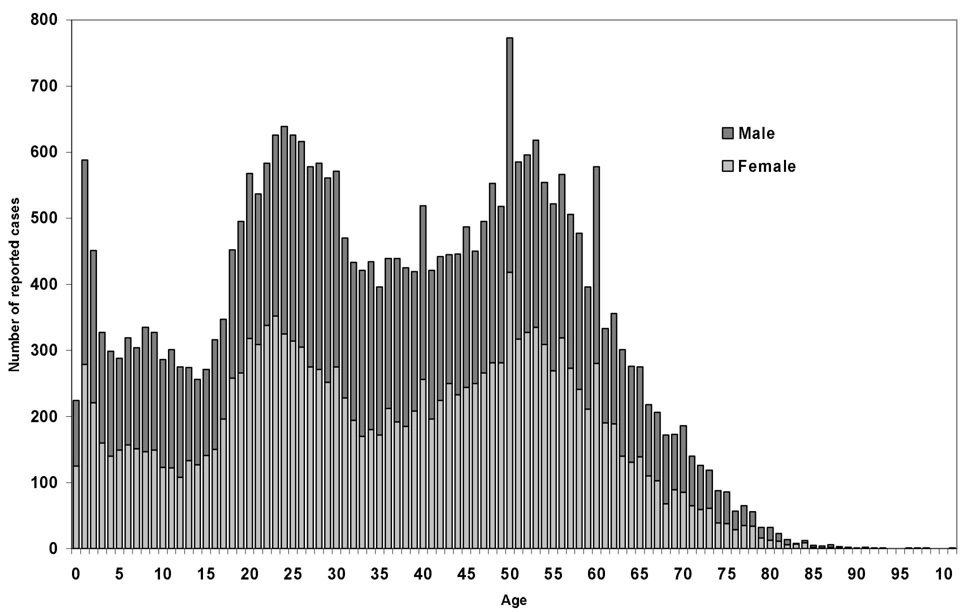

Figure 5. Age and gender of reported cases with a *Salmonella* infection acquired outside Sweden, 1997-2005. Source: SMI

Sources of infection

Sweden has a very favourable situation regarding salmonellosis, shared only with our Nordic neighbours, Norway, Finland and Iceland. This situation is due to the various control programs that were introduced in the agricultural area during the 1960s (see p. 11). Thanks to the control program for poultry, Sweden was one of very few countries that managed to be unaffected of the egg-associated pandemic that started to spread in the 1980s. However, in 1991 one single farm with egg-laying hens caused a large number of solitary cases and eight different outbreaks of salmonellosis (Figure 3). More than 1,200 people were reported with indigenously acquired infection that year.

To identify the source of infection in solitary cases is often very difficult. Nonetheless, in a study of patients with indigenously acquired salmonellosis in 1990 to 1994, it was shown that food was the most common source of infection. However, it was also shown that 8 % of the cases had acquired the infection from another person; secondary person-to-person transmission [22].

As part of the demands to achieve harmonization with the European Union (EU) legislation, reptile import regulations were changed in 1996, so that Sweden no longer required certificates stating that imported reptiles were free from *Salmonella*. This resulted in a large increase of imported reptiles in Swedish homes, and a subsequent increase in the number of reptile-associated *Salmonella* infections (RAS).

Serovars

S. Typhimurium and *S*. Enteritidis are the most commonly reported serovars causing disease in humans, in Sweden and worldwide. As shown in Tables 1 and 2, *S*. Typhimurium is the most common serovar in persons with an indigenously acquired infection. *S*. Typhimurium is also the dominating serovar in outbreaks of salmonellosis in Sweden, causing 64 reported outbreaks since 1980, followed by *S*. Enteritidis with 34 outbreaks during the same period [20].

During the increase of reptile-associated cases in 1996 and 1997, *S*. Typhimurium and *S*. Enteritidis were the dominant serovars, causing 33% of the reptile-associated cases.

In Europe, *S*. Enteritidis has been the most reported serovar since the middle of the 1980s [23]. In the developed world salmonellosis due to *S*. Enteritidis is most often associated with consumption of poultry and eggs [2,24].

Serovar	Number of cases
Typhimurium	1,452
Enteritidis	1,118
Hadar	220
Saintpaul	152
Agona	102
Stanley	74
Newport	68
Oranienburg	56
Virchow	52
Thompson	44

Table 1. The most common serovars among persons infected in Sweden and number of reported cases 1997-2003. Source: SMI

Serovar	Number of cases
Enteritidis	12,525
Typhimurium	1,815
Virchow	1,028
Hadar	947
Stanley	667
Agona	425
Newport	412
Panama	327
Braenderup	296
Blockley	244

Table 2. The most common serovars among persons infected abroad and number of reported cases, 1997-2003. Source: SMI

Outbreaks

The ability to grow well in different food items make the *Salmonella* bacteria belong to the most recognized agents in foodborne outbreaks. This is also why a timely surveillance is needed to detect these outbreaks before they affect a large number of persons.

The World Health Organization (WHO) defines a foodborne outbreak as an incident in which two or more persons experience a similar illness after ingestion of the same food, or after ingestion of water from the same source, and where the epidemiological evidence implicates the food or water as the source of illness [25].

In average, four to six such outbreaks have occurred per year in Sweden, but during the later years there has been a slight increase in the number of outbreaks. There have been no changes in the surveillance system that could explain this increase. A clear trend has been noticed in the last 15 years with more outbreaks being caused by vegetables and fruits in Sweden and in other industrialised countries [26, 27]. Bean sprouts have caused numerous outbreaks worldwide, and is now the vegetable causing most of the vegetable-associated outbreaks both in Sweden and elsewhere [28-31]. In Sweden bean sprouts have caused at least 10 outbreaks in the last 20 years. In Europe, also iceberg lettuce and various vegetables (tomatoes, cucumber and paprika) have caused several outbreaks [26,32]. In the United States, outbreaks caused by pre-sliced melons and raw tomatoes have been described [33-36]. In 2001, tahini and helva caused two separate outbreaks and several analyses have shown that *Salmonella* bacteria have been present in these products [37]. Tahini and helva are produced from sesame seeds. With the high fat content of these seeds, the infective dose is probably very low, especially since these products are normally not kept in a refrigerator.

Different meat products have also caused a number of *Salmonella* outbreaks. During recent years, kebab has been identified as the source in several outbreaks [20]. One example is an outbreak occurring in the summer of 2003, when at least 150 persons fell ill after consuming kebab made from imported Danish pork. The meat was delivered to several restaurants in southern Sweden, and cases occurred from restaurants with inadequate heat treatment.

In the autumn of 2003, several persons in Sweden fell ill with *S.* Hadar in at least 3 different outbreaks due to imported chicken, served in different salads and ready-made sandwiches. The chicken had undergone inadequate heat treatment after importation to Sweden.

In 2004, *S.* Thompson was found in a routine sample of a pasta salad taken by the environmental health board (EHB) in a Swedish municipality. When the first sample was found to be *Salmonella* positive a second sampling was done on the various ingredients in the salad, and rocket salad was found to be contaminated with *S.* Thompson. This finding was communicated to relevant authorities in Europe, and subsequently an increase of cases with rocket salad-associated *S.* Thompson was reported from Norway, England and Wales. These salads were all imported from the same Italian producer [38,39]. This international outbreak was thus detected by a single routine sampling.

Salami sausages imported from Italy was in the end of December 2005 found to be the source of another outbreak. The sausages were contaminated with both *S.* Typhimurium and *S.* Infantis, but only *S.* Typhimurium was found in human Swedish cases [40].

Year	Serovar	Source	Number of cases
1996	Bredeney	Pea soup	21
	Oranienburg	Salad	11
	Enteritidis	Bean sprouts	14
1997	Hadar	Unknown	60
	Oritamerin	Unknown	16
1999	Typhimurium	Roast beef	31
	Typhimurium	Turkey	33
	Typhimurium	Salmon	11
	Blockley	Bean sprouts	19
	Enteritidis	Béarnaise sauce	87
2000	Typhimurium	Unknown	18
	Typhimurium	Unknown	26
	Typhimurium	Parsley	22
	Typhimurium	Unknown	11
	Enteritidis	Mixed vegetables (imported)	11
2001	Livingstone	Fish gratin	16
	Typhimurium	Tahini (imported)	55
	Typhimurium	Helva (imported)	27
	Typhimurium	Meat	33
2002	Oranienburg	Chocolate (imported)	12
	Saintpaul	Bean sprouts	87
	Enteritidis+Hadar	Chicken (polish ferry)	353
2003	Enteritidis	Sun flower/bean sprouts	18
	Agona	Unknown	17
	Typhimurium	Buffet	16
	Typhimurium	Kebab	148
	Hadar	Chicken products	53
	Typhimurium	Christmas buffet	74
2004	Thompson	Rocket salad (imported)	13
2005	Typhimurium	Salami (imported)	15

Table 3. Outbreaks of salmonellosis in Sweden affecting >10 persons/outbreak, 1996-2005. Source: SMI

Enter-net, the international surveillance network

Enter-net is an EU-funded surveillance network for human gastrointestinal infections in Europe. The aim of the Enter-net is to maintain and develop international laboratory-based surveillance of two major enteric bacterial pathogens (*Salmonella* and *E. coli* O157) through a co-ordinated network in which the microbiologists responsible for national reference services and the epidemiologists responsible for national surveillance, of these bacteria are actively involved. Enter-net, and its predecessor

Salm-Net, has since 1994 on numerous occasions demonstrated that the timely exchange of information between experts in different EU countries could lead to effective public health action in Europe [41-47].

Antimicrobial resistance

Unlike other Nordic countries no routine general surveillance of antimicrobial resistance in human *Salmonella* isolates is done in Sweden, although *Salmonella* isolates were introduced in the Swedish annual resistance surveillance and quality survey in 2002 [48]. This survey, based on 100 consecutive isolates from each of the 30 bacteriological laboratories in the country, does not discriminate between indigenous and imported isolates, making it impossible to assess the resistance situation in isolates circulating within Sweden. Furthermore, there are too few isolates included in the survey to obtain conclusive results, although fluoroquinolone resistance has been high (20-25 % of analysed isolates) possibly due to imported isolates [49].

Economic consequences of *Salmonella* infections

A few estimates have been made to assess the economic consequences of *Salmonella* infections in Sweden. A milk-borne outbreak in 1985, with 108 reported cases generated an estimated cost of 16 millions SEK (1.7 millions €) [50]. A study performed after the outbreak of salmonellosis in 1999, estimated the cost of one human case of salmonellosis to be at least 26,000 SEK (2,800 €) [9]. This is an underestimation since the costs borne by the insurance companies could not be assessed due to personal secrecy policies.

Nevertheless, if this minimum cost had been applied to all domestic cases during 1999, the total cost for domestic cases of salmonellosis would had been at least 2.5 million €. In the Netherlands a study on costs of gastroenteritis estimated the cost of salmonellosis to be 4 millions € in 1999 [51].

The Swedish *Salmonella* control programme

The Swedish *Salmonella* control programme started in 1961. The general aims of the programme are to prevent *Salmonella* in any part of the animal food production chain ("from stable to table"), from feed to food of animal origin, to monitor the whole chain, and to eradicate *Salmonella* whenever found [52]. In 1995, certain parts of the programme, covering cattle, pigs, poultry and eggs, were approved by the EU (95/50/EC) and an extended surveillance programme was initiated [53].

Governmental authorities, as well as consumer organisations and the industry support the programme, which is of major importance for the successful implementation. All serotypes of *Salmonella* are regarded as unacceptable and the legislation and programs include all serotypes.

Salmonella in feedstuffs

Domestic feed materials of animal origin are controlled, and each batch produced is sampled and kept under quarantine until the *Salmonella* investigation has ended. Major domestic producers of feed materials of vegetable origin are required to analyse their products for the presence of *Salmonella*. Imported feed materials of vegetable origin are investigated for *Salmonella* when the delivery has reached its Swedish destination or at the point of exportation.

Since 1972, all broiler feed must be heat-treated. Hazard analysis and critical control point (HACCP) control programmes are in place for *Salmonella* control in all feed plants

Salmonella in poultry and eggs

Sampling strategies are outlined in the Swedish *Salmonella* control programme and approved by the EU. Microbiological sampling of breeding flocks is carried out according to Council Directive 92/117/EEC. Breeding animals are imported as grandparents and isolated and frequently tested for *Salmonella* before being allowed to enter the production chain.

Breeding animals are sampled every month throughout their lives and every batch of eggs is sampled in the hatchery. Sampling of laying flocks with more than 200 layers from establishments not placing eggs on the market and of all laying flocks from establishments placing their eggs on the market is carried out three times during egg production.

All meat production flocks of broilers, turkeys, ducks, ratites and geese are investigated by faecal sampling 1-2 weeks before slaughter. Within to the control programme, neck skin samples are taken from poultry at slaughterhouses.

Salmonella in cattle and pigs

Sampling strategies are outlined in the Swedish *Salmonella* control programme and approved by the EU. Random sampling of slaughtered animals is carried out in abattoirs. Samples consist of intestinal lymph nodes and swabs taken from parts of the carcass where the chances of finding *Salmonella* are considered to be optimal. Faecal samples are collected annually in elite breeding herds, gilt-producing herds and twice annually in so-called sow pools.

In addition to the *Salmonella* control programme, all weaner pig producing/integrated herds affiliated to a health control programme run by the industry, are tested by faecal samples collected annually. Samples are also taken at autopsies.

Measures taken in case of *Salmonella* isolation

Regarding poultry, all premises where *Salmonella* is found are put under restrictions, and after destruction of the flock (“stamping out”), the premises are cleaned and disinfected. An investigation of the feed supplier involved is also initiated. Feedstuffs are destroyed or decontaminated. Grandparent and parent flocks are immediately destroyed if found infected, as are broilers and other meat producing poultry and layers, irrespective of *Salmonella* serotype isolated. Isolation of *Salmonella* in neck skins collected at slaughter is considered to be a contamination at slaughter and will lead to hygiene measures being taken at the slaughterhouse.

If *Salmonella* is isolated from cattle, pigs and other food producing animals it is indicating an infection in the herd of origin and action is always taken. This involves restrictions put on the herd. Animals are not allowed to enter or leave the herd. The herd is sampled and a sanitation plan is established, involving the elimination of chronically infected animals, cleaning and disinfection, manure and sludge treatment, disinfection or treatment of feedstuffs. An investigation of the feed supplier involved is also initiated. Restrictions are lifted when cleanup procedures are completed and faecal samples from all animals in the herd are negative.

If swabs samples from the carcasses of slaughtered animals are positive for *Salmonella*, the carcass is considered contaminated and hygiene measures are taken at the slaughterhouse.

Carcasses that are found to be contaminated with *Salmonella* are deemed unfit for human consumption.

If *Salmonella* is found in food of animal origin, investigations are undertaken on the farm of origin. Food contaminated with *Salmonella* bacteria is destroyed or returned to the country of origin.

Any finding of *Salmonella enterica*, irrespective of subspecies, in animals, feed and food of animal origin is compulsory notifiable. Action, including an investigation to clarify the source of infection, is always taken. Feed contaminated with *Salmonella* bacteria is destroyed or treated to eliminate the contamination.

Due to the control programme, both meats from cattle, pigs and poultry as well as table eggs produced in Sweden are almost free from *Salmonella*. The Swedish *Salmonella* control programme shows that the overall prevalence is below 0.1%, which is such a low number that many persons outside Sweden has hard to believe it.

Since many changes have been implemented in agriculture and food production and science has made improvements, the Swedish *Salmonella* control programme will be revised during 2006.

AIMS

The general aim of this thesis is to describe and analyse the impact of travel and trade on the epidemiological situation of human salmonellosis in Sweden.

The specific aims are:

- ❖ to study the impact of strict import regulations on the epidemiology of reptile-associated salmonellosis in humans;
- ❖ to assess whether awareness campaigns can decrease the number of reptile-associated salmonellosis in human cases;
- ❖ to estimate the risk of contracting non-typhoidal salmonellosis in various regions of the world;
- ❖ to investigate the serotype epidemiology in returning travellers from various countries;
- ❖ to give an estimate of the comparative burden of non-typhoidal salmonellosis in different European countries;
- ❖ to investigate whether *Salmonella* detected in sewage sludge was identical with isolates isolated from human cases of *Salmonella* infection; and
- ❖ to investigate to what extent pre-travel advice, level of pre-travel knowledge about dietary risk factors and actual behaviours during travelling affected the risk of acquiring non-typhoidal salmonellosis during travelling.

MATERIAL AND METHODS

Epidemiological data

The basis for Papers I-V in the thesis was the Swedish national database on notified *Salmonella* infections at the Swedish Institute for Infectious Disease Control. From this database we extracted case-based information on age, sex, area of residence, country of infection, *Salmonella* serotype, and reptile contacts.

In Paper I, all 8,208 reported cases from January 1990 to December 2000, with reported domestically acquired infection were examined for contact with reptiles.

In Papers II and III, notification data for the period January 1997 to December 2003 were used. Cases with stated domestically acquired infection or cases for which information on the likely country of infection was either missing or 'unknown' as well as newly entered immigrants and refugees were excluded. In Paper II the number of cases included in the study was 24,803. For comparison in Papers II and III, we used a comprehensive database, the Swedish Travel and Tourist Database (TDB) [21], with data on over-night travels abroad among Swedish residents as travel denominator to calculate risks per 100,000 travellers.

In Paper III we also included the number of reported cases of salmonellosis from each country to estimate the incidence of this infection in the European Union Member States, associated and candidate countries, and EEA/EFTA countries, using Norway as reference. During the study period a total of 15,864 cases were notified with a *Salmonella* infection after a journey in Europe.

In Paper IV, *Salmonella* isolates isolated from humans and sewage sludge from the same residential areas were compared using genetic typing method and antibiotic susceptibility testing. Notification data on human cases from January 1997 to December 2002, a total of 27,269 cases were used. The sewage treatments plants (STP) were sampled every second month during a period of 1 year, starting in July 2000, and two additional samplings were performed from each STP, 6 and 12 months after the last sampling, ending in June 2002.

Paper V describes a case-control study, comparing knowledge and risk behaviours in reported travel-associated *Salmonella* cases and randomly selected travellers from destinations outside EU. Cases were extracted from the national database on notified cases from May 2002 to April 2003, a total of 400 cases. The first ten *Salmonella* cases notified each week with infection acquired outside EU were selected as cases. 1600 control persons were randomly selected among persons travelling outside Europe with a major tour operator.

Microbiological methods

Human strains were isolated by routine culturing, using selective agar media and including a pre-enrichment step, at different local microbiological laboratories in Sweden [54]. These isolates were then sent for typing to the national reference laboratory at SMI. In the sludge from STP's analyses were performed according to the method issued by the Nordic Committee on Food Analysis; Nordisk Metodikkommitté för Livsmedel (NMKL) 71:5:1999 [55].

Molecular typing was performed with macro restriction enzyme analysis and pulsed-field gel electrophoresis (PFGE) [56]. DNA from each isolate was cut with three different restriction enzymes, Xba 1, Spe 1 and Bln 1 and isolates were considered indistinguishable if the restriction patterns were indistinguishable from all three enzymes used.

Antimicrobial susceptibility was analysed with VetMIC™ at SVA, Uppsala, Sweden. The following antimicrobial agents were tested: ampicillin, ceftiofur, chloramphenicol, enrofloxacin, florfenicol, gentamicin, nalidixic acid, neomycin, streptomycin, oxitetracycline and trimethoprim.

Statistical methods

In Paper II the risk of salmonellosis per 100,000 travellers was calculated using the number of notified cases as the numerator and the estimated total numbers of travellers from the TDB as the denominator. The actual number of individuals interviewed was used to calculate 95% confidence intervals (CI) for the estimates. Odds ratios (OR) with corresponding 95% CI were calculated to assess the risk factors for being notified with salmonellosis. The respondents in the TDB were used as controls (with the lowest incidence in each category used as the reference). To adjust for confounding and test for interaction, a logistic regression model was used, which included as variables the country/area of destination, age, gender and month. For each region, the OR for disease per month was analysed, adjusted for age, gender, and number of cases per travellers.

In Paper III, the risk of disease per 100,000 travellers was calculated as described for Paper II. Data for each country on the reported number of salmonellosis cases were retrieved from WHO Surveillance Program for Control of Foodborne Infections and Intoxications in Europe, 8th report, year 2000. An under-detection index was calculated by dividing the incidence per 100,000 inhabitants in the country with the risk per 100,000 Swedish travellers who visited the country and this quotient was then dived with the quotient from the reference country (Norway). This index denotes estimated number of salmonellosis cases not notified for every notified case [57]. To measure the burden of salmonellosis in each country this index was multiplied with the reported incidence from the actual country. A higher risk among returning travellers and a higher "under-detection index", the higher the burden of salmonellosis will be in a specific country/region.

In Paper V, OR with corresponding 95% CI were calculated as risk measures to assess the association between pre-travel advice, knowledge of recommendations, and actual behaviour and being notified with disease. To adjust for confounding and test for interaction, we used a logistic regression model, including a number of potential confounders. The variables age group, number of travels abroad in the last 5 years, main purpose of travel, main type of destination, length of travel and region of travel were included in the logistic regression model.

All analyses were done using the Stata 6.0 software (Stata Corporation, College Station, Tx, USA)

Ethical considerations

In all Papers (I-V), data used on human cases of salmonellosis were compiled as part of routine national surveillance of communicable diseases, as regulated in the Swedish Communicable Disease Act. The subset of the notification database extracted for this project did not contain any information that could be linked to a specific person.

The TDB contains anonymous data only.

The Ethical Committee of the Karolinska Institute, Stockholm, Sweden, approved the studies for Papers I-III and V, while the Research Ethics Committee at Uppsala University, Uppsala, Sweden, approved the study for Paper IV.

RESULTS

Paper I

A total of 339 RAS cases were reported during the study period. From a very low proportion of RAS (1.2%; 5–16 cases) in 1990 to 1994 when reptile import restrictions were in force, the proportion increased to 4.5% (25 cases) in 1995, as "*Salmonella* certificates" were no longer required. The proportion of RAS increased even more (to 11.6%; 68–71 cases) in the two following years, when all import regulations had ceased.

Starting in late 1997 when the RAS problem was recognised, the authorities informed the public, mainly through the media. In 1998, a small decrease was noticed, and in the following years (1999–2000) the proportion of RAS decreased further to 4.8 % (43 and 34 cases), but did not reach the low levels seen before 1995.

Salmonella Enteritidis was the most frequent serotype identified among a total of 51 different serotypes from the RAS-infected individuals, followed by *S.* Typhimurium.

Children and young adults were the most affected age group among RAS cases (Figure 6). RAS cases with infections acquired from turtles were younger than snake/lizard-associated cases; median age 8 years vs. 17 years, reflecting preferences for different pets in different age groups.

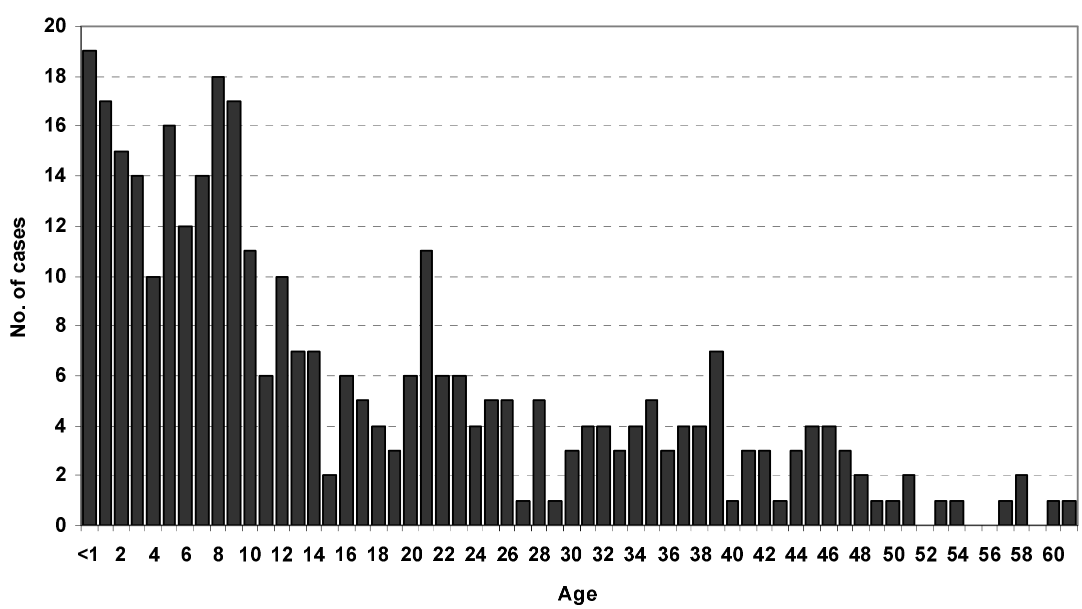

Figure 6. Age at time when contracting salmonellosis from a reptile among RAS-cases 1990-2000, Sweden.

Paper II

During the study period, 31,679 patients were notified with salmonellosis. Most of these infections (24,803; 78 %) were travel-associated, with cases infected in 151 different countries. The most frequently reported countries were Spain, Thailand and Greece.

The 16,255 respondents in the TDB with overnight travel to different regions during the study period formed the basis for the estimates of travel risks to different regions. The overall risk of being notified with salmonellosis was 36.5 per 100,000 travellers. The lowest risk was seen in the Nordic countries (1.7 per 100,000 travellers). The highest individual risk was seen in developing countries, specifically India and neighbouring countries (474 per 100,000 traveller; 95% CI 330-681), East Africa (471 per 100,000 traveller; 95% CI 294-755), West Africa (279 per 100,000 traveller; 95% CI 180-432) and East Asia (270 per 100,000 traveller; 95% CI 247-295), (Figure 7). The risk decreased with increasing age.

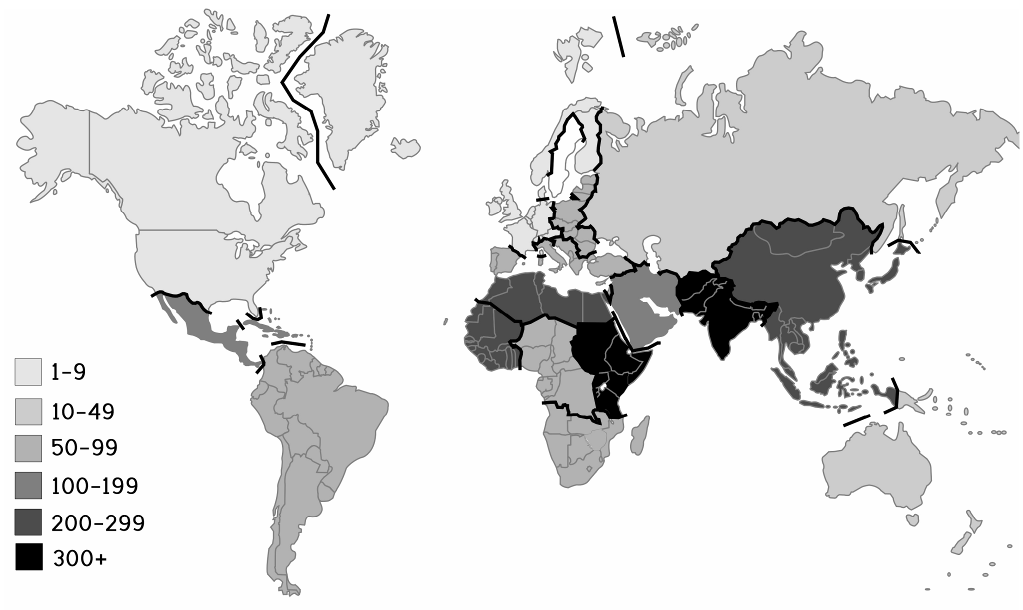

Figure 7. The risk of being notified with a non-typhoidal salmonellosis after travel in different regions of the world per 100,000 travellers.

A total of 202 different serotypes were recorded during the study period (serotype was not available for 1.8 % of cases). Regional differences in the distribution of serotypes were noted. *S.* Enteritidis was the most common serotype worldwide and in Europe more than two thirds of all human cases were due to this serotype. Serotypes were much more heterogeneous in tropical countries than in temperate regions.

Some distinct seasonal patterns could also be distinguished, with the highest risks existing during June–September in Europe, and in November–December in East Asia.

Paper III

A total of 15,864 cases were notified with a *Salmonella* infection after over-night travel in Europe and of them 10,607 (66.9 %) had an infection caused by *S*. Enteritidis. No cases of salmonellosis were reported among travellers from Luxembourg or Liechtenstein.

Risk

The total risk of being notified with salmonellosis after a European journey was 26.2/100,000 travellers. Travel to Norway and Finland was associated with a very low risk, 0.2 and 0.4 per 100,000 travellers respectively. The highest risks were observed in travellers returning from Bulgaria (129/100,000 travellers), Turkey (110/100,000 travellers and Malta (101/100,000 travellers).

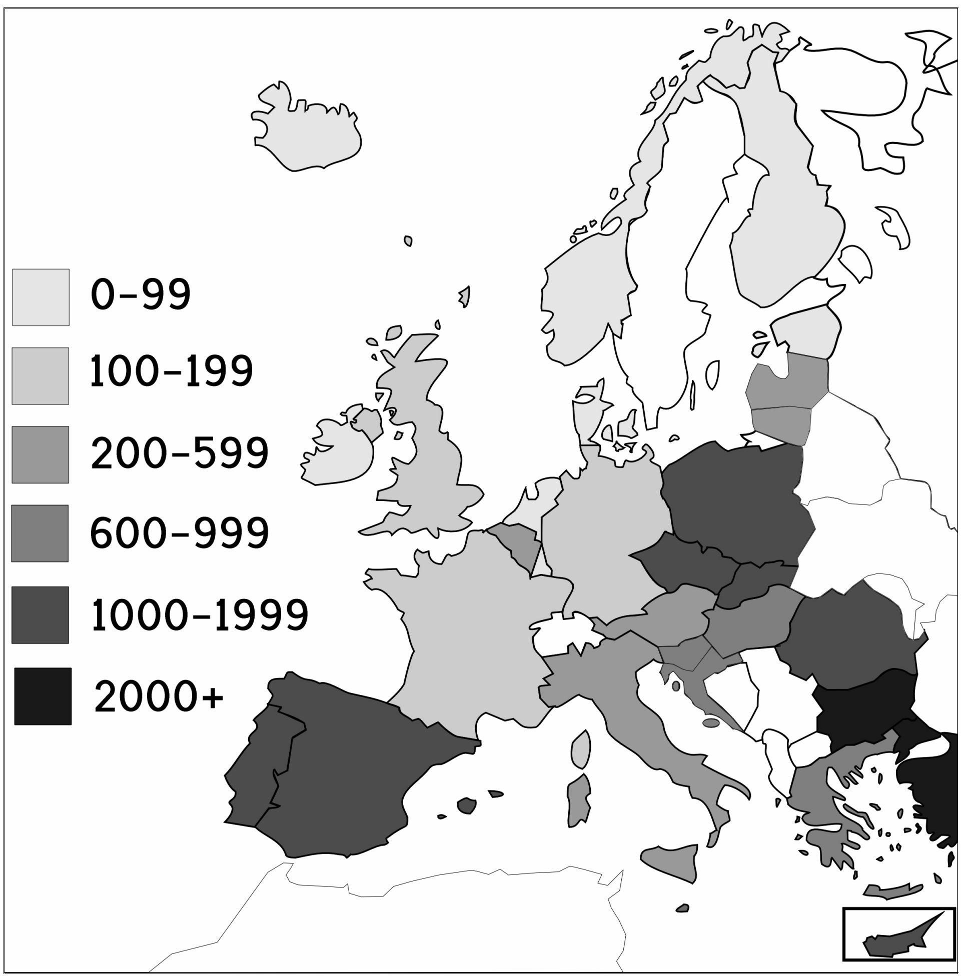

Figure 8. The burden of non-typhoidal salmonellosis in different European countries, estimated number of cases per 100,000 inhabitants.

The total risk of being notified with a *Salmonella* infection caused by *S*. Enteritidis, all phage types, was 17.5/100,000 travellers, the risks per country is shown in table 4.

Country/region	Risk per 100,000 travellers	Country/region	Risk per 100,000 travellers
Norway	0.05	Baltic countries	17.7
Finland	0.11	Cyprus	23.8
Iceland	0.56	Romania	27.1
Ireland	1.08	Greece	27.3
Switzerland	1.49	Hungary	29.3
Denmark	2.55	former Yugoslavia + Albania	33.7
Netherlands	3.29	Czech Republic + Slovakia	46.8
Great Britain	4.66	Turkey	53.5
France	4.97	Spain	57.5
Germany	6.59	Poland	59.3
Italy	6.87	Malta	60.0
Austria	10.0	Portugal	66.4
Belgium	11.5	Bulgaria	87.5

Table 4. The risk per 100,000 travellers of contracting *S.* Enteritidis, all phage types, in European countries.

Overall incidence of non-typhoidal salmonellosis

Norway had the lowest risk per 100,000 travellers and was for this reason used as reference in the analysis estimating the overall incidence of disease in the countries under study. The calculated incidence of disease per country was an estimate of the *Salmonella* situation if the country had the same reporting performance as Norway. Bulgaria was the European country with the highest estimated incidence; 2,741 cases per 100,000 inhabitants.

The countries with the lowest estimated incidence were situated in the northern parts of Europe, while countries with a higher incidence were situated in the southern parts of Europe. Poland was the only country in the northern part of Europe with a comparatively high estimated incidence. Countries in the eastern parts of Europe also tended to have a higher incidence than the countries situated in the western parts of Europe (Figure 8).

The proportion of *S.* Enteritidis was 67 % in returning travellers, which clearly shows the high burden of this serotype. However, the proportion of *S.* Enteritidis cases from the different countries varied from 25 % in Iceland and up to 98 % in Latvia. The second most frequent serotype was *S.* Typhimurium accounting for 9 % of all cases.

Of the 10,607 cases of *S.* Enteritidis, data on phage type (PT) was available for 10,479 (99 %) cases. Forty-eight different phage types were represented among the notified cases throughout the study period. *S.* Enteritidis PT 4 was the most dominating phage type, accounting for 35% of all *S.* Enteritidis cases, while PT 1 accounted for 22 %. On the Iberian peninsula and in some eastern parts of Europe PT 1 was the dominating phage type, while PT 14b dominated in Greece and PT 8 in Czech Republic, Denmark and Slovakia.

Paper IV

The comparison of the PFGE patterns between *Salmonella* serotypes isolated from humans and from sewage sludge revealed eight indistinguishable matches according to all three enzymes used, Xba 1, Spe 1 and Bln 1. Four different serotypes were represented among the matches: *S.* Emek, *S.* Bredeney, *S.* Enteritidis PT 1 (3 human isolates) and *S.* Hadar also with three isolates from humans.

Of these eight PFGE matches, six human strains were also indistinguishable from the isolates from sewage sludge when comparing the antibiotic resistance pattern. The two human isolates that did not have identical antibiograms with the corresponding isolate from sludge, despite indistinguishable patterns according to PFGE, belonged to *S.* Enteritidis PT 1.

One additional pair of *S.* Enteritidis differed simply by one enzyme in the PFGE and was identical according to the antimicrobial susceptibility analysis.

In four of the identical matches, the human *Salmonella* serotype was isolated or the human case had an onset of disease within one month before the actual serotype was isolated from the sludge (*S.* Hadar (two matches), *S.* Bredeney and *S.* Emek). In one pair of *S.* Enteritidis PT 1, the strain was isolated in the sludge two months after the onset of disease in the human case. A further strain of *S.* Hadar was isolated in the sludge four months after the human case was diagnosed. Additionally, in one match of *S.* Enteritidis PT 1, the strain in sewage sludge was isolated more than 2 years after onset of disease in the human case, with indistinguishable PFGE pattern, but in this pair the strains did not have the same antibiogram.

There were five diverse serotypes isolated from STPs on more than one occasion that are not listed among the 36 most common serotypes in humans. These include (with number of times isolated in brackets): *S.* Bardo (5), *S.* Otmarschen (5), *S.* Berta (4), *S.* Waral (2), and *S.* subspecies II [9,46:g,m,t] (2).

Antimicrobial susceptibility testing revealed that 12 (12 %) out of 101 tested strains from sewage sludge were resistant to at least one antimicrobial agent. In addition, there were 7/101 (7 %) multiresistant *Salmonella* strains isolated from the sewage sludge, i.e. resistant to three or more antimicrobials. Among the 12 strains isolated from humans analysed, 9 (75 %) were resistant to at least one antimicrobial agent and 5 (42 %) were multiresistant.

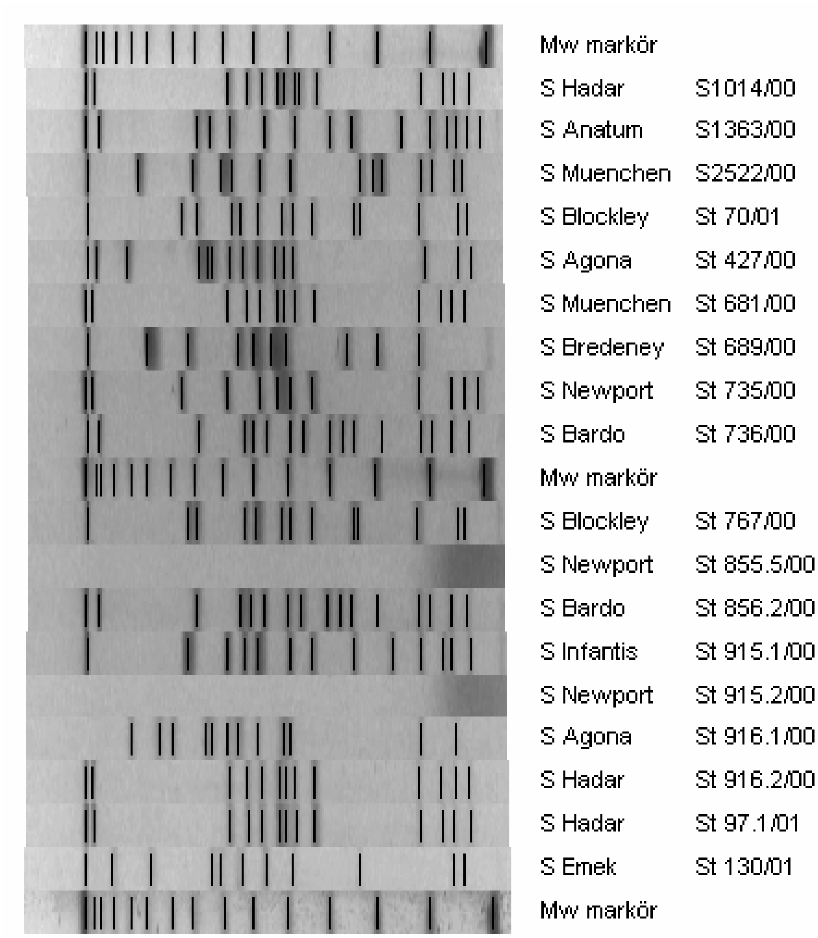

Figure 9. A PFGE gel with different isolates and the Xba enzyme, isolates with a number beginning with S are of human origin and with St are from sewage sludge. Mw markör is a standardised marker. Source: A. Aspan, SVA.

Paper V

The overall response rate for the case control study was 56 %; 271 cases (68 % response rate) and 840 controls (53 %). The response rate for different age groups and gender among cases is shown in Figure 10, where it is obvious that males have a much lower response rate than females which could create an undesired bias if not controlled for in the analysis. This difference in gender has also been observed in by other researchers [58]. Since the questionnaire was anonymous no such data are available for the controls. The youngest age group, 18-29 years, was overrepresented among the cases. Other main differences between the two groups were a larger proportion of more primitive travel such as backpackers and "travel and learn" among the cases. There was also a higher proportion of travel to beach resorts among the controls, and a higher proportion of trips exceeding 2 weeks among the cases. Furthermore, there were important differences in the travel destinations, with a majority of the controls having returned from Eastern Mediterranean, while East Asia was the most common destination among the cases.

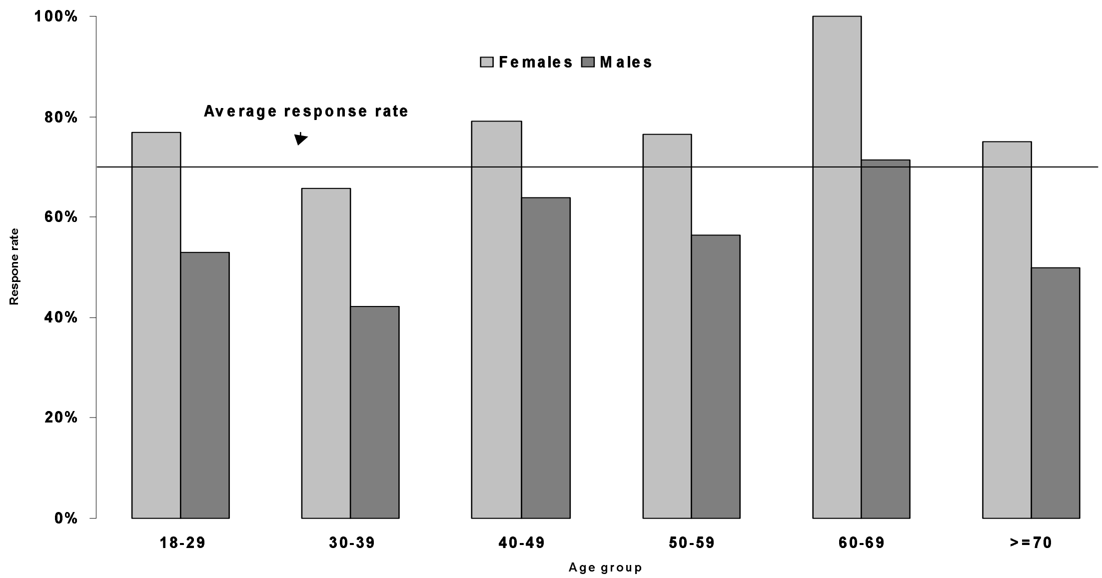

Figure 10. Response rate in different age groups and gender among cases.

The pre-travel knowledge of risk factors was high: only 17 % of cases and 20 % of controls stated that they had no previous knowledge nor had they received advice on what food and drinks to avoid in order to prevent travel-related diarrhoea. The risk of salmonellosis did not significantly differ between those having received pre-travel advice and those not having. The source of information had no significant effect on the risk, although those travellers having received their information on the Internet had a (non-significant) tendency of lower risk, as was also seen in those travellers with a previous knowledge of risk factors.

Knowledge of the importance of avoiding raw vegetables was associated with a lower risk of salmonellosis (OR 0.51; 95% CI 0.31-0.82), while knowledge of other risk behaviours on food and drink consumption were not associated with lower risk.

The actual behaviour during the travel period did however to a large degree affect the risk of salmonellosis. Completely avoiding a number of risky food items such as raw vegetables, green salad, raw seafood, hamburgers, kebab, grilled chicken, non-packaged ice cream, pastries, mayonnaise and cold sauces and not eating food from street vendors were significantly associated with a lower risk. Avoiding bloody meat, non-bottled water and ice cubs did not reduce the risk of salmonellosis. By completely avoiding all risky food items as well as avoid eating from street vendors the traveller could reduce the risk by more than two thirds (multivariate OR 0.30; 95% CI 0.11-0.81).

Those persons stating that they had used antibiotics as prophylaxis against diarrhoea during the trip had a borderline significant elevated risk of salmonellosis.

DISCUSSION

Notification system

This thesis is based on the routine surveillance data from the national register of notified communicable diseases at the Department of Epidemiology at the Swedish Institute for Infectious Disease Control. The objective of a communicable disease surveillance system is to detect changes in overall incidences, or incidences of specific serotypes/clones of important microbiological agents in order to take appropriate preventive measures in a timely manner. The data obtained in a surveillance system could in addition be used in monitoring different trends or used for evaluation of an intervention [59-61].

According to the Swedish Communicable Diseases Act the doctor, who was responsible for sending a sample for microbiological analysis, must notify all cases of salmonellosis within 24 hours to the local CMO and SMI. At the laboratory the doctor in charge is also responsible for notifying the newly diagnosed case of salmonellosis. No active searching for cases is performed.

However, there is a long chain of events before a person with salmonellosis could be notified to the authorities. Not all infected persons experience symptoms. If a person has symptoms, they must be severe enough for the person to seek health care. The health care system may advice the person to wait a couple of days during which period the symptoms may subside. If the person is actually seeing a doctor, a decision to take a sample must be taken, and even then just one faecal sample will detect only 93-97 % of *Salmonella* positive persons, while two faecal specimens will detect 99 % of all positive cases [62-64]. Furthermore, the sample must be handled correctly in the laboratory, and finally the doctor and/or the laboratory must notify the patient.

The performance of Swedish surveillance system is comparatively good in an international perspective and about 99 % of all diagnosed cases are notified from the laboratories to the authorities [65,66]. In comparison an English study showed that only 32 % of all cases of salmonellosis in the community were reported to the national surveillance authority [67]. In Germany it was estimated that 20 % of persons experiencing diarrhoea will seek health care and out of them 5% will deliver a sample for analyse of *Salmonella* [68]. An illustration of all these events are shown in Figure 11, but to reflect the Swedish *Salmonella* situation the top of the pyramid should be more quadratic.

When discussing number of reported cases the discussion often tends to talk about under-reported cases but it should concentrate more on discussions on under-diagnosed cases. In Sweden it appears that the persons experiencing symptom often are not asked to deliver a relevant sample if they have not been travelling abroad. This will create an under-diagnosing and thereby underestimation of domestically acquired salmonellosis cases. This “bad habit” could be due to a general notion that most cases of salmonellosis have been infected abroad, and specimens from persons with a resent history of travelling outside the country thus will have a greater chance of being positive compared to specimens from persons without a travel history. From an epidemiological point of view this is unsatisfactory since it is even more important to detect indigenous cases and outbreaks for which active public health measures could be instigated. However, tourists can be used as sentinel persons for different purposes as described in Papers II and III. Furthermore, they can provide useful information on what *Salmonella* strains are circulating at the moment, which could be valuable when tracing imported food items related to an outbreak situation.

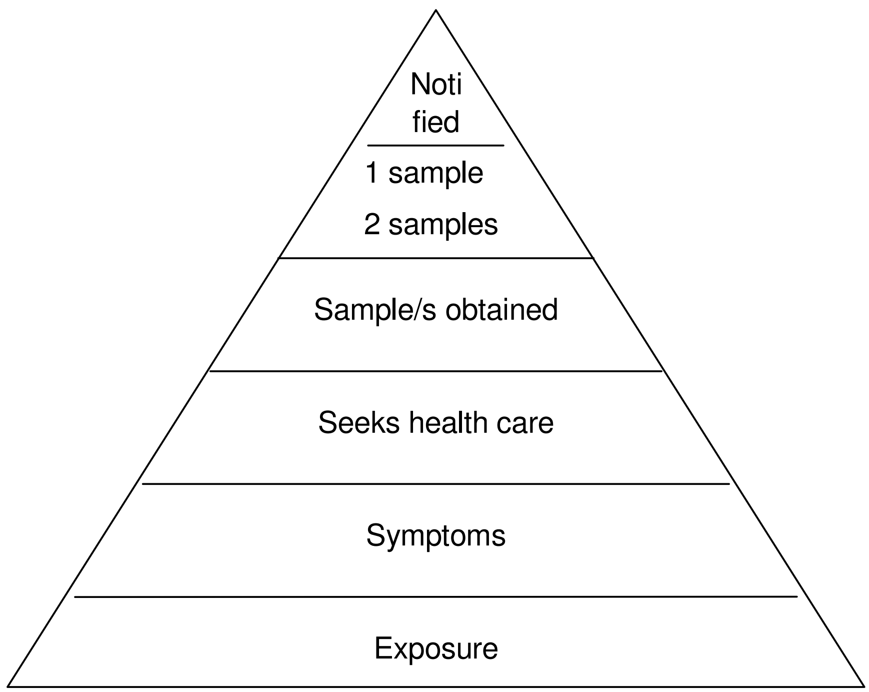

Figure 11. Notification pyramid; chain of events that must happen before a case is notified to authorities.

In conclusion, the notification system will only show the tip of an iceberg consisting of probably more severe cases than the average person experiencing an episode of salmonellosis. This said, the Swedish surveillance data hold a good quality, and reflect what is happening in the society, even though each notified case will correspond to several cases in the general public.

The Swedish *Salmonella* situation

Sweden together with its neighbouring countries, Norway, Finland and Iceland, has a unique situation compared with the rest of the world. Nowhere, there is such a low number of food producing animals that are found to be *Salmonella* infected [69]. In Sweden this is due to the control programs that were initiated already in 1961. The different control programs have undergone changes during the time and are now approved by the EU [70,71]. The number of reported human cases confirms that the control programs are working. In 2003, there was a huge, feed borne, outbreak among pigs in Sweden but no persons were affected in that outbreak which indicates that the barriers in the program are effective [72].

This favourable situation can easily be changed since implementations of new rules could have a negative influence on the situation. Seemingly small changes in legislation could have a major effect on the *Salmonella* situation. This is what happened when the importation rules for reptiles were harmonised with the EU rules. A sharp increase of domestically acquired cases was the result as described in Paper I.

The general Swedish *Salmonella* situation is to a high degree reflecting the situation in countries with major tourist resorts visited by Swedes. If more virulent *Salmonella* strains start to circulate in these places, this will show in the Swedish surveillance system as it did during the 1980s when the egg associated *S.* Enteritidis started to spread over the world (Figures 2-3). The fact that the Swedes are a travelling people is reflected in the notification system (Figure 3). The returning traveller may carry *Salmonella* strains that could affect the circulating Swedish environmental bacterial flora and spread onwards to humans. Therefore, it is important to be aware of that sewage sludge, as presented in Paper IV, could pose a risk that could alter the present favourable situation.

Pet reptiles

The knowledge that reptiles harbour *Salmonella* bacteria that could affect humans has been communicated for more than 60 years [73-75]. Today figures shows that 50-90% of reptiles are carriers of *Salmonella* [76-78]. Paper I demonstrates well what could happen in a country when restrictions regarding reptile trade are altered resulting in a sharp increase of reptile associated salmonellosis (RAS) cases, mostly children. In a case-control study in fives states in USA, the population attributable fraction for reptile or amphibian contact was 6% for all sporadic salmonellosis cases and 11% among persons <21 years old [79]. Earlier studies in USA have estimated the proportion of RAS to be 7% [80]. In Denmark it is estimated that 3-5% of human *Salmonella* cases are due to exotic pets, particularly reptiles [81]. In Norway no figures are available but the import and holding of reptiles are forbidden. However, illegal importation from Sweden is substantial [82]. In Sweden the proportion of RAS cases went down to 6% after information about the risk of contracting salmonellosis from a reptile had been communicated to the public. Since two different studies from different countries shows the same level of RAS and other estimates are in the same level, this indicates that of all sporadic cases of salmonellosis 6% could be reptile associated. In Sweden this will result in about 50 notified cases per year, corresponding to some 1,000 cases in the community. Having a pet reptile has become very popular during recent years in USA and the same thing has probably happened in Sweden, although no Swedish figures are available [83,84]. However, the alteration in importation rules has made reptiles much easier to buy in Sweden than before.

Since pet reptile keeping in private homes is entirely non-essential, good information to prospective buyers is an important public health measure. It has been suggested that their obstetricians shall inform pregnant women since it could be too late to be informed at the first visit to a paediatrician [85]. The following guidelines released by the Association of Reptilian and Amphibian Veterinarians in USA are a good example of information on how to prevent RAS [86].

- Always wash your hands with hot, soapy water after handling reptiles, reptile cages and equipment, and the stool of reptiles.
- Do not allow reptiles to have access to the kitchen, dining room, or any other area in which food is prepared. Also, do not allow reptiles to have access to bathroom sinks and tubs or to any area where infants are bathed. Consider keeping your reptiles caged or limiting the parts of the house where reptiles are allowed to roam free. Always wash your hands after coming into contact with any area where reptiles are allowed to roam free.
- Do not eat, drink, or smoke while handling reptiles, reptiles cages, or reptile equipment. Do not kiss reptiles or share food or drink with them.
- Do not use the kitchen sink, kitchen counters, bathroom sinks or bathtubs to bathe reptiles or to wash reptile cages, dishes or aquariums. Reptile owners may wish to purchase a plastic basin or tub in which to bathe or swim their reptiles. Waste water and faecal material should be disposed of in the toilet instead of the bathtub or household sink.
- The Centers for Disease Control and Prevention (CDC) recommends that children less than five years of age avoid contact with reptiles and that household with children less than one year of age not own reptiles. The Association of Reptilian and Amphibian Veterinarians encourages reptile owners with young children to discuss steps to minimize risks associated with owning reptiles with their reptiles' veterinarian and their physician. Children should be supervised when they are handling reptiles to ensure that they do not place their hands or objects that a reptile has contacted in their mouths. Reptiles should not be kept in child care centers.
- Immunocompromised persons should avoid contact with reptiles.
- Follow instructions from your reptile's veterinarian concerning proper diet and environment for your reptile. Healthy reptiles living in proper environments are less likely to shed *Salmonella* bacteria.

There is sometimes a false belief that a reptile is salmonella-free if it is born in Sweden. However, the animal could have been infected before birth through trans-ovarial transmission or be infected as newborn. It is shown that colonization of the pet snake offspring during pregnancy and birth results in 65 % of the newborn to be positive for *Salmonella* [87]. Treating the reptiles with antibiotics will not result in salmonella-freeness and could instead promote the selection of resistant strains.

The international trade in reptiles may also bring other diseases from remote parts of the world since salmonellosis is not the only disease that could be transmitted from reptiles to humans [88]. This situation emphasizes the importance of hygiene and education; people should be informed that there is always a risk when handling these animals, especially when eating afterwards without washing their hands.

Non-typhoidal salmonellosis and travellers

Ever since the Vikings, the Swedes have a sustained and proud tradition of travelling abroad, as traders, soldiers and more lately as sun-seeking tourists. As seen in Figure 3 the increase of reported cases of salmonellosis started around 1960. An increase in the number of different serotypes was also seen at this time. In 1954, 30 different serotypes were isolated in Sweden compared to 82 different types in 1964 [89] and in 118 different serotypes in 2004 [20]. This increase in number of reported cases and different serotypes is closely related to the travel habits of the Swedes. In 1959, 448,000 Swedes visited non-Scandinavian countries and in 1964 the corresponding number was 875,000 [89]. During the study period in papers II and III, 1997-2003, it is estimated that about 68 millions overnight journeys outside Sweden were done, corresponding to almost 10 millions journeys per year.

In Papers II and III the risk of being notified with a *Salmonella* infection after overnight travel outside Sweden is described and thereby how travellers could be used as sentinels for disease in a certain area [57,90,91]. The *Salmonella* figures presented in these papers could be compared with the figures available in an old Swedish study [89], concerning salmonellosis acquired abroad during 1963, Table 5. The number of travellers to each country in the 1963 year study was obtained from the tourists agencies of the country concerned.

	Number of positive persons[1] 1963	**Number of travellers[1] 1963**	**Risk per 100.000 travellers 1963**	**95% CI 1963**	**Risk per 100.000 travellers 1997-2003**	**95% CI 1997-2003**
USA	0	13991	0.0	-	2.5[2]	1.9-3.3[2]
Hungary	0	1500	0.0	-	41.8	33.7-51.8
France	4	110000	3.6	0.1-7.2	8.4	7.2-9.7
UK	5	57000	8.8	1.1-16.5	5.6	4.8-6.5
Netherlands	10	46000	21.7	8.3-35.2	4.6	3.2-6.6
Yugoslavia	12	27640	43.4	18.9-68.0	40.1[3]	32.7-49.1[3]
Portugal	4	6927	57.7	1.2-114	79.9	68.0-93.9
Italy	256	371200	69.0	60.5-77.4	12.8	11.2-14.6
Israel	4	4558	87.8	1.8-174	54.1[4]	51.2–57.1[4]
Spain	112	121503	92.2	75.1-109	72.0	68.5-75.7
Greece	125	24748	505.1	417-593	39.2	36.4-42.2
Romania	59	3000	1966.7	1470-2464	68.6	39.5-119

[1] From Ringertz and Mentzing [89], [2] Combined travel data from USA and Canada, [3] Combined travel data from Croatia and Slovenia, [4] Travel data from Eastern Mediterranean

Table 5. Number of persons with salmonellosis and country of infection reported in Sweden 1963 with corresponding risk of *Salmonella* infection per 100,000 travellers and the risk for travellers measured 1997-2003.

When comparing the figures from these studies, there are remarkable similarities despite the time span passed between the two studies. In the 1963 year study, some numbers are rather small which gives a greater uncertainty in the results. However, there has been a remarkable decrease in the risk for travellers to Romania even if Romania still has the "top" position in this comparison. Greece and Italy has also a significant decreased risk, while in some other countries no risk reduction is noticed.

In June 2006, the European Food Safety Authority (EFSA) released a preliminary report on the analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus* [92]. This report shows a large variation of the prevalence in the different European countries. In Paper III the risk of being notified with salmonellosis is measured as incidence per 100,000 travellers, see Figure 8, and when comparing our incidence figures with the prevalence among laying hens in the different countries a very high correlation (Pearson correlation coefficient 0.96) was noticed, Table 6 and Figure 12.

	All *Salmonella* serotypes		***S.* Enteritidis and *S.* Typhimurium**	
Country	**In laying hen flocks [1]**	**Per 100,000 travellers [2]**	**In laying hen flocks [1]**	**Per 100,000 travellers [2]**
Austria	52/334 (15.6 %)	12.1	36/334 (10.8 %)	10.2
Baltic Republics [3]	7/25 (28.0 %)	19.4	5/25 (20.0 %)	17.9
Belgium	46/130 (35.4 %)	13.8	34/130 (26.2 %)	12.9
Czech Republic	42/64 (65.6 %)	54.8 [4]	40/64 (62.5 %)	43.9 [4]
Denmark	2/85 (2.4 %)	3.8	1/85 (1.2 %)	3.1
Finland	1/249 (0.4 %)	0.4	1/249 (0.4 %)	0.3
France	88/511 (17.2 %)	8.5	41/511 (8.0 %)	6.0
Germany	150/522 (28.7 %)	8.7	127/522 (24.3 %)	7.7
Greece	40/107 (37.4 %)	39.3	24/107 (22.4 %)	30.6
Hungary	117/267 (43.8 %)	42.1	90/267 (33.7 %)	30.7
Ireland	2/146 (1.4 %)	3.2	0/146 (0 %)	1.9
Italy	89/295 (30.2 %)	12.8	24/295 (8.1 %)	9.1
Netherlands	62/392 (15.8 %)	5.0	31/392 (7.9 %)	4.6
Norway	0/236 (0 %)	0.2	0/236 (0 %)	0.1
Poland	224/290 (77.2 %)	77.3	162/290 (55.9 %)	61.4
Portugal	35/44 (79.5 %)	80.9	21/44 (47.7 %)	72.0
Spain	352/481 (73.2 %)	72.5	248/481 (51.6 %)	62.8
Sweden	0/97 (0 %)	--	0/97 (0 %)	--
United Kingdom	49/413 (11.9 %)	5.7	33/413 (8 %)	5.1

[1] Data from the European Food Safety Authority [92]; [2] Data from Paper III; [3] Combined data for Estonia, Latvia and Lithuania; [4] Combined travel data from Czech Republic and Slovakia.

Table 6. *Salmonella* in laying hen flocks of *Gallus gallus* and in returning Swedish travellers (all *Salmonella* serotypes and *Salmonella* Enteritidis and *Salmonella* Typhimurium separately) Source: de Jong and Ekdahl [93]

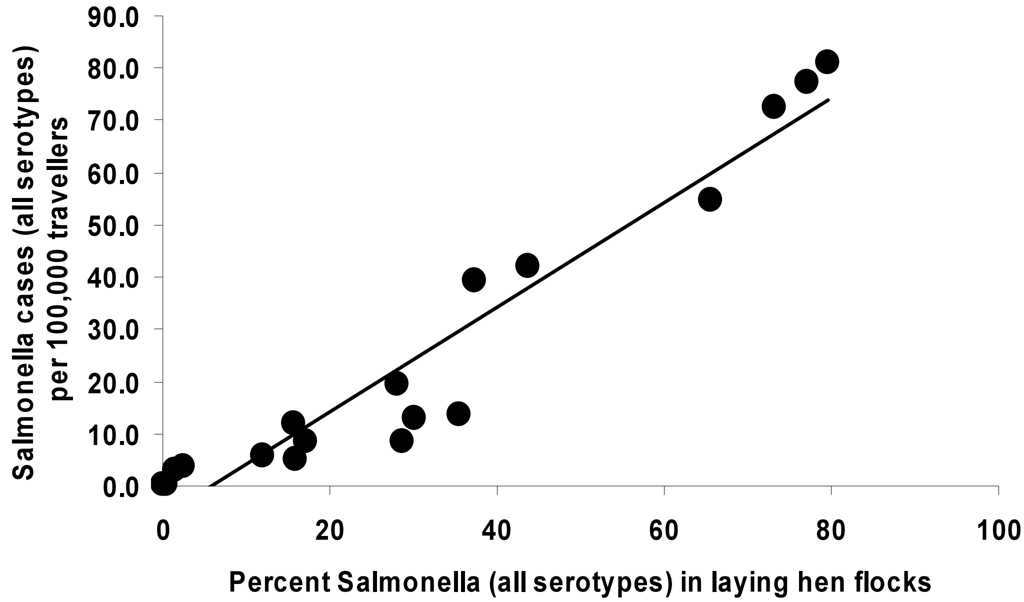

Figure 12. Travel-related salmonellosis (all serotypes) as a function of prevalence of Salmonella in laying hen flocks of *Gallus gallus*. Each dot represents a country. Source: de Jong and Ekdahl [93]

This very high correlation confirms that data on *Salmonella* from returning travellers, captured within a single surveillance system, can be used for sentinel purpose to describe the burden of salmonellosis in a single country. Furthermore our data suggest a clear, causal relationship between the prevalence of *Salmonella* in the egg production and human disease.

The average domestic incidence in Sweden is about 7 per 100,000 inhabitants and year, giving a weekly incidence of about 0.13 per 100,000, Figure 3 [20]. In Paper II it is shown that the overall risk for salmonellosis in returning Swedish travellers is 36.5 per 100,000 travellers, the risk for travellers from the EU is 20 per 100,000 travellers while from outside the EU 140 per 100,000 travellers. Assuming that the Swedish travellers spends 2 weeks abroad each year this will correspond to a 140 times increased overall weekly risk when travelling, while travelling inside the EU has a 100 times increased weekly risk and an almost 400 times increased weekly risk when travelling outside Europe. These figures clearly show how favourable the *Salmonella* situation is in Sweden.

The number of trips outside Sweden is today the single factor having the greatest impact on the incidence of salmonellosis in Sweden. About 3,000 cases are notified each year with travel-associated salmonellosis and the true number of cases is considerably higher. One way to counteract this is to give pre-travel health advice. A previous study on Swedish tourist has shown a high level of knowledge that diarrhoeal diseases are transmitted through food and beverages and that Swedes often seeks pre-travel advice [94]. But to be able to get through with the message one must understand how to communicate it, and what to communicate. The study presented in Paper V shows that it is very important not only to give information about what kind of food should be avoided in order not to fall ill with salmonellosis but also to stress that the actual behaviour is the most important factor. This could be hard and most tourists do make dietary mistakes [95,96]. Our study supports the old advice "boil it, cook it, peel it, or forget it" in contrast to most other studies on prevention of travellers' diarrhoea (TD) [97-103]. This could depend on that the focus of our study was on one important cause of TD, non-typhoidal *Salmonella*, and not including other causes of TD and cases without a specified microbiological finding. The study also indicated that traveller using antibiotics, as a prophylaxis against diarrhoea could be more at risk to contract salmonellosis. This could be due to the fact that a more risky behaviour is applied since the traveller may feel protected and that the *Salmonella* strain has reduced susceptibility against the antibiotics taken, but might also be a selection bias in that the most susceptible persons are those that are given antibiotic prophylaxis. Several studies have shown an increased risk of salmonellosis when exposed to antibiotics [104-106]. Preliminary results from a study by Weir et al. indicate that there may be a genetic linkage between virulence and drug resistance [107].

Focusing prevention of salmonellosis on egg and poultry associated *S.* Enteritidis infection will have a major impact from a public health perspective in most European countries and this will also reduce the number of travel-associated cases reported in Sweden. However, the most effective preventive measures regarding tourist on package tours will probably be if the tour operators only use hotels and resorts, which could show that they had implemented programs for good food hygiene, preferably using HACCP methods. Since the trend today goes towards more and more "all-inclusive hotels" this will have a large impact on preventing diarrhoeal illness among tourists. In 1998, British tour operators decided to use only hotels in the Dominican Republic that contracted a commercial food hygiene training and auditing company [108]. This resulted in a decrease of TD in British package holiday tourist from 57% to 29% experiencing TD in 2000. At Jamaica it is now are required to submit an illness surveillance report, on a weekly basis, to the Public Health Department and all hotels require annual food safety and environmental health standard audits before they can be health certified [109]. Significant reductions in the incidence of TD, about 80%, were recorded from the different tourist regions. These are two examples showing that improvement of food hygiene resulted in a dramatic decrease in the incidence of TD among tourists to these destinations.

The trend of booking travel over the Internet including hotel booking may also raise hotel owner's awareness that healthy guests will post favourable remarks to the hotel on the Internet sites where these hotel bookings are done and this could result in upgraded food hygiene.

Sewage sludge and human salmonellosis

"The traveller can be seen as an interactive biological unit who picks up, processes, carries and drop off microbiological genetic material" is a quotation from ME Wilson and these few words describe exactly what happens when a human becomes infected with *Salmonella* [110]. As mentioned, the majority of Swedish cases have acquired the infection outside Sweden and thereby the *Salmonella* strategies in other countries could have an impact on the Swedish *Salmonella* situation.

In 1977, several studies were published showing the presence of *Salmonella* in sewage sludge from Swedish sewage treatment plants (STP) [111]. In paper IV we describe how *Salmonella* bacteria can also be isolated today, and a comparison with human isolates is done. The techniques for sampling and isolation are described elsewhere [112]. Pulsed-field gel electrophoresis (PFGE) was chosen as the method to carry out the molecular typing as it has been widely recognised as a sensitive method for molecular fingerprinting in several *Salmonella* serotypes [113, 114]. To use three different enzymes together with antibiotic susceptibility is a rather strict method to test similarity between strains and it resulted in only a few pairs of indistinguishable strains. However, knowing that there is a wide variety of different PFGE patterns within each serotype, strict criteria was applied to have a high degree of confidence that the strains were similar and thereby considered to be of the same clonal origin [115].

The serotypes isolated from sewage sludge were not those serotypes that are dominating among humans. In travellers reported in Sweden *S.* Enteritidis, especially phage type 4, is dominating (Paper III). This has also been noticed in a Spanish study where *S.* Hadar dominated among the strain isolated from wastewater, 38 %, while it only accounts for 5 % of all human cases in Spain [116]. In our study no isolates of *S.* Typhimurium were found to be indistinguishable even if this serotype is the only one that could be considered endemic in Sweden.

The antibiotic susceptibility testing showed that the isolates originating from humans with an infection acquired abroad had a high level of antibiotic resistance. Since most human *Salmonella* infections are imported, the number of *Salmonella* bacteria with a reduced susceptibility to several different antibiotics, which are flushed into Swedish sewage systems must be substantial. A recent Finnish study showed an increase over the years in reduced fluoroquinolone susceptibility among human domestic *Salmonella* isolates, with an even higher increase in isolates from travellers. The overall conclusion was that reduced fluoroquinolone susceptibility continues to grow rapidly in many parts of the world, including EU countries [117]. Since there are no big differences in which places the Finnish and Swedish travellers visit, the antibiotic susceptibly situation among the *Salmonella* strains brought into Sweden by travellers can be considered to be the same as in Finland. These findings indicate that there are risks with using sludge from STP's as fertiliser in Swedish agriculture since bacteria with reduced antibiotic susceptibility or even multiresistance could be spread to the Swedish ecosystems. A sensitive surveillance program for detecting changes in the serotypes isolated from domestic human cases and domestic animals is essential to measure if a spread to domestic sources will occur and more research is needed to elucidate the risk with sewage sludge in Swedish agriculture.

CONCLUSIONS

- Import restriction requiring certificates that reptiles are not carrying *Salmonella* is an effective public health measure for protecting the general population from reptile-associated salmonellosis.

- Reptile-associated salmonellosis poses a threat to human health that cannot be ignored and children are the most affected age group. However, it is possible to decrease the number of reptile-associated salmonellosis cases through active information to the public.

- Returning tourists as a sentinel population can provide a useful base for comparison of disease burdens in different countries/regions. The highest risk of disease was seen in travellers returning from East Africa and the Indian subcontinent. Children aged 0–6 years were at higher risk than travellers of other ages. Marked geographical differences in serotype distribution were noted. *Salmonella* Enteritidis was especially dominant in Europe and the highest burden of salmonellosis in Europe was estimated for Bulgaria followed by Turkey and Malta.

- Focusing prevention of salmonellosis on egg- and poultry-associated *S.* Enteritidis infection will have a major public health impact and will significantly lower the burden of disease in most European countries.

- *Salmonella* bacteria originating from infected humans can survive the treatment in sewage treatment plants, and thereby the risk of *Salmonella* being spread with sewage sludge to the environment and then to people and animals is enhanced. The threat to society is even worse if the bacteria are resistant to antimicrobial agents.

- Knowledge and advice are not enough to decrease the risk of travel-associated salmonellosis, while actual behaviour of strictly avoiding high risk food-items reduces the risk. Pre-travel advice should therefore focus even more on changing behaviours rather than informing on risks.

- Sweden has a unique *Salmonella* situation with a very low domestic incidence of human salmonellosis. However, this can without doubt be changed if not considering alteration in legislations and regulations with care.

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