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SHORT AND LONG TERM EFFECTS OF BACTERIAL GASTROINTESTINAL INFECTION

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Stockholm 2008

Published by Karolinska Institutet, Stockholm, Sweden.
Grafisk Form: Charlotte Hansson Design.

ISBN 978-91-7409-025-3

ABSTRACT

The objectives of this thesis were to increase our understanding of the mortality and complications associated with bacterial gastrointestinal infections, with focus on *Salmonella* and *Campylobacter* enteritis. The effect of antibiotics vs. placebo on duration of diarrhea in *Campylobacter* enteritis was also examined.

Persons reported with culture verified bacterial enteritis to the Swedish Institute of Infectious Disease Control 1997–2004 formed the base for three of the studies in this thesis. From the national database, case-based information on age, sex, type of bacteria, date of debut of illness, and country of infection were extracted. Each person in this retrospective cohort was followed until death, an event took place or until study termination. We used the National Tax Board registers to identify deaths in the cohort, and the national Hospital Discharge Register from the National Board of Health and Welfare to observe complications associated with bacterial gastrointestinal infection. Standardized mortality/incidence ratios (SMR/SIR) were used to estimate the relative risk of death or short and long term complications.

For persons infected with *Campylobacter*, the SMR among those infected within Sweden was 2.9 with 95% confidence interval (CI) 1.9–4.0 during the first month after infection, and for those who had acquired *Salmonella* at home 5.6 (95% CI: 3.4–8.2). No increased SMR within the first 30 days after infection could be found among those infected with *Campylobacter* or *Salmonella* abroad: 0.3 (95% CI: 0.04–0.8) and 0.6 (95% CI: 0.2–1.2). We are probably observing a 'healthy traveler effect', i.e. persons who travel are healthier than the general population.

The effect of antibiotics on *Campylobacter* infection has only been studied in quite small studies. This led us to conduct a quantitative summary analysis of all published randomized controlled trials (RCTs). Eleven RCTs which included a total of 479 study participants were included in a meta-analysis. The summary effect in our random effect model showed a reduction of 1.32 days (95% CI: 0.64–1.99) with symptoms in favor of antibiotics compared to placebo.

If one had been infected at home or abroad did not have any interaction effect on the complications observed among patients in our cohorts. We could confirm the associations between EHEC and hemolytic uraemic syndrome, *Campylobacter* infection and Guillain-Barré syndrome, and *Yersinia* enteritis and reactive arthritis. We found evidence of an association between *Salmonella* enteritis and aortic aneurysm, SIR 6.4 (95% CI: 3.1–11.8). Transient bacteremia with non-typhoid *Salmonella* can probably cause a localized endothelial infection that result in an aneurysm or the enlargement of a pre-existing aneurysm. *Salmonella* infection was associated with an increased risk for ulcerative colitis, SIR 3.2 (95% CI: 2.2–4.6) and this was also found among those with campylobacterosis, SIR 2.8 (95% CI: 2.0–3.8). No significant increased risk for Crohns disease was shown in the *Salmonella* cohort, SIR 1.4 (95% CI: 0.8–2.3) or among those reported with *Campylobacter* enteritis, SIR 1.6 (95% CI: 1.0–2.3). Although similar results have been found elsewhere, more work is needed to refute or confirm our findings.

Key words: Gastroenteritis, acute infectious diarrhea, *Salmonella*, *Campylobacter*, mortality, complications, morbidity, late effects, epidemiology, hospitalization, cohort study.

LIST OF PUBLICATIONS

The thesis is based upon the following papers:

I. Ternhag A, Törner A, Svensson Å, Giesecke J, Ekdahl K.
Mortality following Campylobacter infection: a registry-based linkage study.
BMC Infect Dis. 2005; 5: 70–5.

II. Ternhag A, Törner A, Ekdahl K, Giesecke J.
Salmonella-associated deaths, Sweden, 1997–2003.
Emerg Infect Dis. 2006; 12: 337–9.

III. Ternhag A, Asikainen T, Giesecke J, Ekdahl K.
A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with Campylobacter species.
Clin Infect Dis. 2007; 44: 696–700.

IV. Ternhag A, Törner A, Svensson Å, Ekdahl K, Giesecke J.
Short- and long-term effects of bacterial gastrointestinal infections.
Emerg Infect Dis. 2008; 14: 143–8.

CONTENTS

LIST OF ABBREVIATIONS

CI	Confidence interval
e.g.	For example (*exempli gratia*)
EHEC	Enterohemorrhagic *E. coli*
GBS	Guillain-Barré syndrome
HUS	Hemolytic uraemic syndrome
ICD	International Classification of Diseases
i.e.	That is (*id est*)
NTS	Non-typhoid *Salmonella*
RCT	Randomized controlled trial
SIR	Standardized incidence ratio
SMI	Swedish Institute for Infectious Disease Control
SMR	Standardized mortality ratio
UC	Ulcerative colitis
vs.	Versus
WHO	World Health Organization

1 INTRODUCTION

Acute diarrheal illness is a major public health problem in most parts of the world. Contaminated food and drinking water in low-income countries causes diarrhea that results in malnutrition and mortality in infants and young children (Baqui and Ahmed, 2006; Bryce et al., 2005; Lopez et al., 2006). In high-income countries have acute gastrointestinal illnesses been estimated to affect up to 30% of the population during one year. Major foodborne diseases from bacteria include salmonellosis and campylobacterosis, the two major causes of acute gastroenteritis reported in Sweden and Europe (Fisher and Meakins, 2006). In these countries has gastroenteritis due to *Salmonella* and *Campylobacter* often been regarded as – at least from the medical community – an unpleasant but generally harmless and self-limiting infection (O'Brien, 2005).

A Danish group published 2003 a study on mortality due to foodborne bacterial gastrointestinal infections (Helms et al., 2003). In this study, 2.2% of persons with gastrointestinal infections were dead within one year after infection. The relative mortality was 3.1 times higher in patients than in controls. This study became the inspiration and starting shot for this thesis. There was a need to confirm or reject the findings, and the conditions in Sweden are very favorable for these kinds of epidemiological studies with our large, high-quality patient registers.

Not only mortality, but also other complications have been attributed to bacterial gastrointestinal illness. These complications can come early or late after the infection. Because bacterial gastroenteritis is such a common disease, even a small increase in relative risk for a complication can have a significant public health impact. The burden of disease in terms of complications and sequaele after an episode of acute bacterial gastroenteritis is therefore important to estimate.

Even if many bacterial gastrointestinal infections are self-limited, antibiotic treatment in addition to rehydration are in many situations practiced. Setting aside mortality and complications for a while, one has to ask if antibiotic treatment could shorten the duration of diarrhea in persons with gastroenteritis. For *Salmonella*, it has been shown in a Cochrane report (Sirinavin and Garner, 2000) that antibiotic therapy does not have any significant effect on the acute disease, and it could even prolong the excretion of *Salmonella* from faeces. No large randomized controlled trial, or meta-analysis, exists that answers the same clinical relevant question for campylobacterosis. There is in this respect a need for a study that can serve the same purpose.

2 BACKGROUND

2.1 *ACUTE DIARRHEA AND MICROBIOLOGICAL DIAGNOSTICS*

There are many conditions – apart from infections – that cause acute diarrhea: food poisoning, inflammatory bowel disease, colon cancer, fecal impaction, proctitis, irritable bowel syndrome, medicines and side-effects to mention a few (Sabol and Carlson, 2007).

Acute diarrhea defined as ≥3 loose stools per day with a duration of ≥24 h and a negative effect on daily life is very common. A population based American study (Jones et al., 2007) reported a prevalence of 5.1% among study subjects corresponding to 0.6 episodes of acute diarrhea per person and year.

Many types of acute infections cause diarrhea (Musher and Musher, 2004). Viral gastroenteritis is the most common cause of acute diarrhea in community cases (de Wit et al., 2001a). Several viruses can cause acute diarrhea: rotaviruses, noroviruses, enteric adenoviruses etc. Diagnosis is in most of these cases based on clinical signs and symptoms.

There are parasites that causes diarrhea. Two examples are amoebic dysentery (*Entamoeba histolytica*) and giardiasis (*Giardia lamblia*). These protozoa's are endemic in many parts of the world, and cause disease among travelers (Freedman et al., 2006) and are also more common in specific groups, e.g. men who have sex with men (Stark et al., 2007) and institutionalized individuals (Gatti et al., 2000). Cryptosporidium are another important protozoal intestinal pathogen, not at least among children in low-income countries (Huang et al., 2004).

Examples of bacteria's that are intestinal pathogens include *Vibrio cholerae*, diarrheogenic *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, cytotoxic *Clostridium difficile* (Bartlett, 2006; DuPont, 2005; Guerrant, 2006). The patient history and clinical signs, symptoms and laboratory findings can be used to assess the type of diarrheal illness (inflammatory or non-inflammatory) and give some clues to the causative agent (Thielman and Guerrant, 2004). To have a definitive diagnose of bacterial gastroenteritis, a stool culture must be done. However, even if one strongly suspect infectious diarrhea and active search for enteropathogens is done one will not for certain find any causative agent. In a 1-year Swedish prospective study on adults with diarrhea admitted to hospital, a potential pathogen (either bacteria, virus, or protozoa) was found in only 56% of patients (Svenungsson et al., 2000).

A freshly obtained stool sample is preferable for stool culture, but a rectal swab is also acceptable. Because the gastrointestinal tract is far from a sterile environment, the main microbiological problem is to sort out any pathogens from the normal intestinal flora. One way to solve this problem is to use selective growth medias (includes antibiotics) and they may also contain indicator substances that aid in initial identification. Repeated fecal specimens for culture are increasing the probability of

a positive stool culture (Petersen et al., 1996) (Ethelberg et al., 2007).

Once a suspected enteric pathogen has been identified on a growth plate, further microbiological testing must in most cases be performed (Hallander et al., 2002. [In Swedish].). One exception is *Campylobacter*, where many laboratories do not distinguish between *Campylobacter jejuni/coli*. A typical morphology on CCDA-agar, and oxidase/catalase positive colonies can be enough for bacteriological typing. Suspected *Enterobacteriaceae* is routinely fermented and each species has a typical profile that allow determination of specie. Another technique used is agglutination where the bacteria clump in the presence of antibodies targeted against specific bacterial antigens. For other bacteria e.g. enterohemorrhagic *E. coli* (EHEC) PCR-technique are used in the routine diagnostics.

2.2 *SWEDISH SURVEILLANCE SYSTEM*

The Swedish Communicable Diseases Act from 2004 and its ordinance regulate how the surveillance system works. The communicable diseases have been listed in four different categories, based on their threat to public health, and different measures can be undertaken accordingly to prevent further spread. The more serious a disease is, the more extensive are the measures that are forced upon the individual.

Approximately 60 infectious diseases are governed by the Swedish Communicable Diseases Act and are called 'notifiable diseases'. A physician diagnosing a patient with a notifiable disease has to report it without delay to the County Medical Officer and to the Swedish Institute for Infectious Disease Control (SMI). Laboratories that find a pathogen which is on the list of notifiable diseases are also obliged to report the findings to the same authorities. This means that Sweden has a dual reporting system, and studies have shown that this increases the rate of reporting into the surveillance system (Jansson et al., 2005).

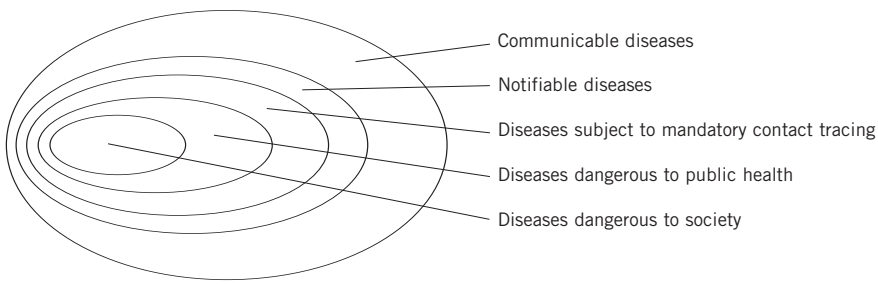

***Figure* 1.** Categories according to the Swedish Communicable Disease Act. Source: National Board of Health and Welfare

Infection with *Campylobacter*, EHEC, *Salmonella* and *Shigella* is classified as 'diseases dangerous to public health'. Patients infected with these organisms gets personal guidelines in order to stop the spread of the disease. If a patient does not follow these rules, there are legal possibilities to isolate a person by force. Contact tracing is also performed to found further cases.

Compared to the gastrointestinal pathogens already mentioned, *Yersinia* infection is perceived as a smaller threat to public health, and is categorized as a 'diseases subject to mandatory contact tracing'. This means that a patient must leave information regarding how he or she got infected and who else may have been exposed to infection. These latter persons are contacted and asked to see a physician.

2.3 *DESCRIPTION OF BACTERIA: NOMENCLATURE, MICROBIOLOGY AND CLINICAL ILLNESS*

2.3.1 *Salmonella*

The genus *Salmonella* belongs to the family *Enterobacteriaceae* (Murray Patrick R, 2003). *Salmonella* are gram-negative, facultatively anaerobic rods. *Salmonella* nomenclature is difficult and complex, partly due to historical reasons. Kauffmann initially proposed that each serotype identified by their O (somatic) and H (flagellar) antigen was a separate species (Brenner et al., 2000). Using the serological scheme by Kauffmann-White, there are currently over 2500 *Salmonella* serotypes (Popoff et al., 2004), but with this initial concept we would have over 2500 species of *Salmonella*.

The genus is divided into two species: *Salmonella enterica* and *Salmonella bongori* (formerly subspecies V) (Uzzau et al., 2000). This became clear 1973 when Costa used DNA-DNA hybridization technique to demonstrate that former serotypes and subgenera (I, II, IIIa, IV) belonged in a single species (Brenner et al., 2000). The only exception was *Salmonella bongori* (V) which a few years later was described as a distinct species. *Salmonella enterica* has been further subdivided into six subspecies: subsp. *enterica* (I), subsp. *salamae* (II), subsp. *arizonae* (IIIa), subsp. *diarizonae* (IIIb), subsp. *houtenae* (IV) and subsp. *indica* (VI).

The vast majority of human pathogenic serotypes belong to *Salmonella enterica* subsp. *enterica* (Pegues D, 2007). The other subspecies (including *Salmonella bongori*) are rarely isolated from humans, but usually from cold-blooded animals and the environment. In most situations including this thesis are a more convenient short version used in the description of serotypes, e.g. *Salmonella* Typhimurium instead of *Salmonella enterica* subsp. *enterica* serotype Tyhpimurium. *Salmonella* Typhimurium and *Salmonella* Enteritidis are two of the most commonly isolated serotypes (Galanis et al., 2006).

Salmonella Typhi and *Salmonella* Paratyphi are human pathogens with no animal reservoir (Heymann, 2004). Many of the other non-typhoid *Salmonella* (NTS) serotypes are pathogenic to both human and animals and have a large reservoir among animals.

NTS infection is usually manifested as a self-limited gastroenteritis with diarrhea, nausea, and fever (Kim Arthur Y, 2006) (Saphra and Winter, 1957). Other more rare manifestations include bacteraemia and localized infections such as septic arthritis, cholecystitis, osteomyelitis, septic aneurysms etc. Those with bacteraemia have often prolonged and sustained fever and chills, and the diagnosis is confirmed by blood culture.

Salmonellosis is caused by ingestion of contaminated food or water. Undercooked eggs have in some countries been an important source of infection (Heymann, 2004). Infants are a particular risk group for sporadic NTS infection and a recent case-control study revealed that infants with *Salmonella* infection were less likely to have been breastfed and more likely to have had exposure to reptiles (Jones et al., 2006). NTS can also be transmitted by contact with infected animal carriers such as pet reptiles. Faecal-oral transmission directly between persons rarely occurs, but may be the cause in small children. Predisposing factors for NTS infection include achlorhydria (secondary to medication or gastroduodenal surgery) and immunosuppression.

2.3.2 *Campylobacter*

The family *Campylobacteraceae* includes 18 species in the genus *Campylobacter* (the other genuses belonging to the family are *Arcobacter* and *Sulfurospirillum*). *Campylobacter jejuni* and, to a much lesser extent, *C. coli* are the most common *C.* species associated with gastroenteritis in humans and most laboratories are not distinguishing between these organisms.

Campylobacter jejuni is best cultured on selective media in an atmosphere of reduced oxygen tension at a temperature of 42° C (Blaser Martin J). *Campylobacter* are motile, gram-negative rods with a characteristic comma-shaped form that can be seen in microscopy.

Infection with *Campylobacter* typically results in diarrhea, abdominal pain, malaise and fever after an incubation period of 2–5 days (Opal Steven M, 2007). *Campylobacter* may occasionally be spread in the bloodstream and in rare cases result in disseminated infection. Infection occurs after consumption of contaminated foodstuff such as meat (often poultry), nonchlorinated water, or unpasteurized milk. Infection from pets of various kind and farm animals has also been reported (Heymann, 2004).

2.3.3 *Nomenclature of Yersinia, Shigella and Enterohemorrhagic Escherichia coli*

The genus *Yersinia* belongs to the family *Enterobacteriaceae* and includes 11 species among which *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* are human pathogens. *Y. pestis* and *Y. pseudotuberculosis* are highly related genetically, but cause different disease (*Y. pestis* are the cause of plague which is not further discussed in this thesis).

Y. enterocolitica is the most common *Y.* specie isolated from humans with diarrhea (*Y. pseudotuberculosis* is primarily an animal pathogen) and can be divided into 6 biotypes

based on specific chemical reactions, and can also be divided into one of 78 serogroups based on the characterization of their O-antigen. Certain serogroups such as O3 and O9 within biogroup 2, 3 and 4 are over represented as human pathogens among *Y. enterocolitica* isolates in Europe.

Shigella are gram-negative rods and members of *Enterobacteriaceae* family. There are four subgroups of the bacteria: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* (Lan and Reeves, 2002). The subgroups have historically been treated as species, but from a genetic standpoint would the four *Shigella* species be regarded as a biotype of *E. coli* because of their nucleotide similarity. *Sh. Dysenteriae* serotype 1 produce an exotoxin called shigatoxin (Stx1) which is identical to a toxin produced by EHEC.

E.coli is one of five *Escherichia* species, and belongs to *Enterobacteriaceae*, and is part of the normal gut-flora. Certain *E. coli* have virulence factors such as toxin producing capabilities, adhesive functions or invasiveness. There are four categories of *E. coli* that cause diarrhea, one of which is enterohemorrhagic *E. coli* (EHEC) (Robins-Browne and Hartland, 2002). This bacterium is also labeled Shiga-toxin (or Verocytotoxin) producing E. coli (STEC or VTEC) (Clarke, 2001). There are several serotypes of EHEC capable of producing toxin (Stx1 or Stx2), but the one most frequently isolated is O157:H7. The serologic classification is based on the O antigen (somatic) and H antigen (flagellar). In Sweden is infection with EHEC O157 mandatory to report (not other serotypes producing Stx).

2.4 *DESCRIPTIVE EPIDEMIOLOGY*

It is difficult to estimate the number of annual cases of bacterial gastroenteritis in any country. Figures derived from surveillance systems will never capture every single case, and comparisons between countries are complicated due to highly variable surveillance systems.

The incidence of *Campylobacter* in Sweden is about 70 per 100,000 per year and has declined during the 2000s. The incidence usually peak during summer time. About 60% of reported cases have been infected abroad.

Salmonella incidence rate is about 40 per 100,000 per year in Sweden and 70–80% have been infected abroad. NTS also have a cyclic pattern during a year, with an accumulation of cases during summer. This cyclic pattern has been explained by an increase in travel during summer (Ekdahl et al., 2005) and barbecuing (Olsen et al., 2001). It seems that this seasonal variation is highest among mild cases of salmonellosis, and then diminishes with increased severity (Gradel et al., 2007).

The largest outbreak of salmonellosis in Sweden occurred during the summer of 1953 in Alvesta, a small town in the southern part of the country. Approximately

9000 cases of *S.* Typhimurium were diagnosed and 90 persons died (Olin, 1955). The epidemiological investigation could trace the point source of infection to a local slaughter house from which contaminated meat was distributed to other parts of Sweden. Several unlucky circumstances coincide which ultimately resulted in the large number of cases: a temporary high production volume at the slaughter house, a simultaneous shortage of refrigerator space, and a heat-wave in Sweden the same period.

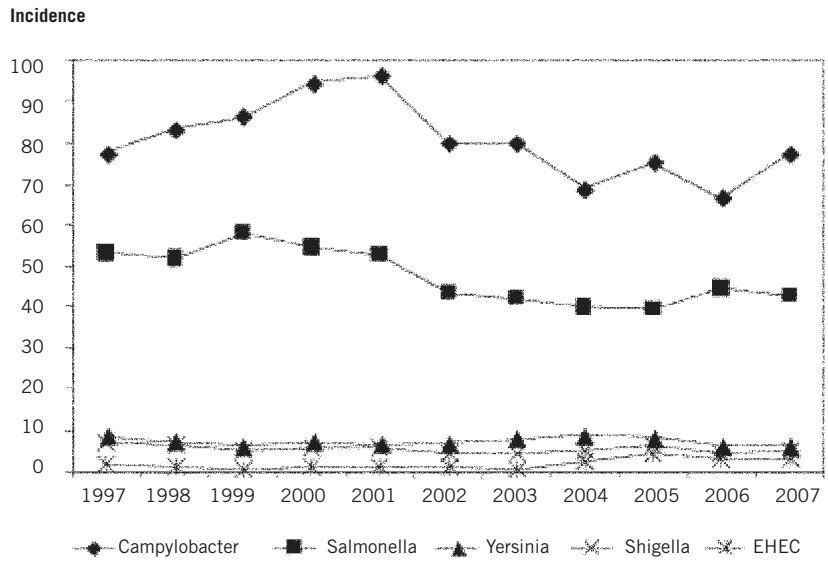

Figure 2. Incidence rate of Campylobacter, Salmonella, Yersinia and EHEC per 100,000 per year in Sweden, 1997–2007. Source: National surveillance data.

The Swedish situation, with a steady decline of *Salmonella* during 2000s, is true for most Europeans countries. During 2004, over 135,000 cases of *Salmonella* were reported to Enter-net (Fisher, 2006), which is a European disease surveillance network (of which 6500 was reported from Sweden). In United States, the mean annual incidence of *Salmonella* is 13.4 per 100,000 (Voetsch et al., 2004). Although incidence figures between countries are difficult to compare, a strong correlation has been shown between the prevalence of *Salmonella* in laying hen flocks in a single country and human disease (de Jong and Ekdahl, 2006).

Campylobacter is in most industrialized countries the dominating pathogen among reported cases of bacterial gastrointestinal infection (Allos, 2001) (Takkinen and Ammon, 2003). The average annual incidence of culture confirmed *Campylobacter* cases in United States exceed that of *Salmonella* and is estimated to be 21.9 per 100,000 (Samuel et al., 2004).

Yersinia infection affects children much more than *Salmonella* and *Campylobacter*. The highest prevalence of *Yersinia* is seen in the age groups 0–4 years of age. The overall incidence rate was 6.2 per 100,000 per year 2007. Three fourth of the cases were domestically infected. During 2007, *Shigella* incidence rate was 5.1 per 100,000 per year in Sweden and 84% were infected abroad. The incidence rate for EHEC infection was 2.9 per 100,000 per year 2007 and most of the cases are found among children 0–4 years.

Because a large proportion of the reported cases of gastrointestinal infection are infected outside Sweden, factors that influence travel pattern will have great impact on the number of annual cases. For example, a recession with lower disposable income in the households would probably reduce charter tours, and therefore reported cases. People's will to travel is also negatively affected by events such as terrorist attacks or large natural catastrophes. And if the risk of contracting disease is greatly reduced by public health measures in a specific country, then will the number of cases among returning travelers diminish.

2.5 *ANTIMICROBIAL DRUG RESISTANCE IN SALMONELLA AND CAMPYLOBACTER*

One cannot write an introduction on bacterial gastroenteritis without saying a few words on antibiotic resistance. This has been an issue since the first cases of multidrug-resistant *Salmonella* Typhimurium DT 29 were reported in England during 1960's. The situation has unfortunately not improved since.

Strains of *Salmonella* resistant to fluoroquinolones (often tested by using nalidixic acid, a first generation quinolone) have risen in the United States, from 0.4% in 1996 to 2.3% in 2003 (Stevenson et al., 2007). In Finland, during 2004, had 10% of domestic *Salmonella* reduced susceptibility to ciprofloxacin, whereas the corresponding figures were much higher for isolates collected from returning travellers (e.g. Thailand 52%, Spain 73%) (Hakanen et al., 2006). The same story is true for *Campylobacter*: in United States were none of the tested human isolates resistant to ciprofloxacin in 1990, while in 1999 were 18% resistant and in 2001 41% (DuPont, 2007). The Finnish situation were quite the same with 46% of human isolates resistant to ciprofloxacin during 1995–2000 (Hakanen et al., 2003). We have no routine surveillance of antimicrobial resistance of *Salmonella* and *Campylobacter* in Sweden. This is instead monitored voluntarily by sentinel surveillance once a year by the Swedish microbiological laboratories. However, data are too sparse and do not contain information whether the isolates are imported or domestic and are therefore not shown here.

Why has a situation with increasing antimicrobial drug resistance described above evolved? One important factor is the use of antimicrobial agents in agriculture which by far exceeds the use in humans and produce resistance among enteric pathogens (Witte, 1998). Antibiotics are used in agriculture not only for disease treatment, but for prophylaxis or growth promoters. Mass-treatment, long-term administration and

sub-therapeutic concentrations contribute to resistance development (McEwen, 2006). There are at least two observations that support the view that extensive use of antibiotics in food animal production creates resistance among human isolates. First, human isolates of *Salmonella* and *Campylobacter* were not resistant to fluoroquinolones when they were introduced and it was not until the introduction of the agents in agriculture in 1990s that resistance showed up (Threlfall et al., 1999) (Angulo et al., 2004). Second, countries such as Australia that never allowed quinolones in agriculture do not have the same problems with quinolone resistant isolates of enteric pathogens collected from humans (Unicomb et al., 2006). However, fluoroquinolones have fortunately been banned in both United States and European Union for use in food animal production (DuPont, 2007). But there are many countries in the world, and other classes of antibiotics, so there is still a need to reduce unnecessary use of antibiotics in agriculture. We will in paper III argue for a very restrictive policy when it comes to antibiotic treatment of human campylobacterosis.

2.6 *BACTERIAL GASTROENTERITIS AND COMPLICATIONS*

2.6.1 *Mortality*

The cause of death is a problematic concept if one stops for a minute and think about it. In January 1988, Sweden adopted a new law that said that a person is dead when all the activity in the brain is forever lost. This means, by definition, that the cause of death ultimately is when the brain no longer performs any of its functions. When the brain dies, the person dies. Many deaths are related to cardiac arrests. And a person with cardiac arrest looks dead upon clinical investigation. But in a strict sense, the person is alive for a few more moments until the brain dies from loss of oxygenated blood. The cause of death concept is therefore more a way of formulating chains of events that ultimately leads to the irreplaceable loss of all the brain functions.

What the cause of death was for a person can be determined in two principal ways. Many of us dies within hospital and the clinical doctor can either determine the cause of death based on clinical history or perform an autopsy. An autopsy is obviously a more precise way to determine the cause of death. The number of autopsies have declined in Sweden from about 50 percent at the beginning of the seventies to about 14 percent in 2004. The largest proportion of autopsies is done in the youngest age groups and decline with the increase of age. The second way to determine cause of death is by clinical investigation. This is of course subject to great variation in quality. In certain cases is the patient very well characterized and has undergone exhaustive investigations so the cause of death can be very precise. In other situations are the patient almost unknown to the doctor who is establishing the time and cause of death, and the accuracy of these comments are naturally much lower.

There are studies that have tried to measure the accuracy of death certificates (which can be based on clinical/forensic autopsies or clinical examination). The cause of death determined by clinical examination and by autopsy differed in ICD-category

in about 12–29% of cases (Armstrong et al., 1999). Other studies have used record linkage to see if last main diagnose in the national Hospital Discharge Register agree with the underlying cause of death. Results show that the final main condition differed from the underlying cause of death in 54% of cases (Johansson and Westerling, 2000). The diagnoses correlates quite good between the two registers for patients who dies at hospital or nearly after discharge, and for certain diagnosis (cancer), but in other cases were the correlations not that good.

The Cause of Death Register also overestimates diseases that are acute and dramatic, and probably underestimate non-acute, non-dramatic illnesses. For patients infected with *Salmonella* was the diagnosis present as an underlying cause of death in only half of death certificates (Kennedy et al., 2004), which suggests an underreporting of deaths due to *Salmonella* infection.

For diseases with high mortality, or case fatality rate, the problem of competing risks is negligible. For example, Ebola hemorrhagic fever has a case fatality rate between 50–90% among clinically ill cases (Cohen, 2004; Lamunu et al., 2004). If one follows clinical diagnosed cases there should be no problems to calculate the proportion that dies during follow-up. On the other hand, diseases that generally have a mild clinical course are more difficult to calculate the exact proportion of deaths among infected cases. The problem is two-fold: first, there can be hard to see any deaths in a study sample if this is an uncommon event. Second, persons die from many different causes. One would therefore like to have a 'basal death rate' in a study sample so any elevated mortality due to a specific agent is observed.

Influenza is an example of a disease that kills many of the oldest during epidemics. This is confirmed by studies were persons vaccinated have much lower mortality compared to those who are not (Christenson et al., 2001). There is also evidence of higher mortality among the oldest in years with high occurrence of influenza compared to seasons with low influenza prevalence (Thompson et al., 2003). But these findings would be hard to see if one, for example, followed a small sample of persons hospitalized with influenza during a few months.

This line of reasoning is applicable to mortality associated with bacterial gastroenteritis. To capture mortality among persons with bacterial gastroenteritis one need a sample large enough and some measure of what rate of all cause mortality one can expect.

2.6.2 *Short and long term effects – biological plausibility*

Short term effects are complications that take place within days-weeks after an acute infection and long term effects within months-year. Short term effects could be a result of immunological changes with loss of tolerance to self antigens, whereas long term effects may be the result of chronic inflammation.

Several immunological mechanisms have been described where an infectious agent induces mechanisms that ultimately lead to tissue damage. Molecular mimicry is a process where cross-reactivity between a microbial product and a self-antigen leads to autoreactive lymphocytes (Wucherpfennig, 2001) (Rose, 1998). An example is rheumatic fever, a disease associated with pharyngitis and caused by molecular mimicry between antibodies to group A streptococcal protein found in the cell wall, and certain matrix proteins (myosin, laminin) in the endocardium.

Superantigen stimulation is a process in which microbial substances stimulates a wide range of lymphocytes. Various bacterial components, such as staphylococcal enterotoxins can act as superantigen and via cascade reactions result in toxic and septic chock.

Viruses such as hepatitis C that infects lymphocytes can increase the production of immunoglobulins that form complexes which circulate in the blood and deposit in various organs, such as blood vessels, skin, joints, peripheral nerves, and kidneys (mixed cryoglobulinemia). This immune complex mediated disease can develop many years after infection with hepatitis C virus.

Some infectious agents, e.g. HIV, hepatitis B- and C viruses, *Helicobacter pylori*, and human papilloma virus, have the ability to escape immun clearence and become a chronic infection. The long time period of viral replication, or the chronic inflammation, can initiate the transformation to cancer. This phenomena has been shown for at least seven microbiological agents: hepatitis B, hepatitis C, human papilloma virus, Epstein-Barr virus, HIV and HTLV-1 (Mueller, 2003).

Gram negative bacteria express lipopolysaccharides (LPS) on their surface which is a potent stimulator of cytokine release from monocytes and macrophages, and can also trigger production of protease and oxygen radicals from neutrophil granulocytes (Cohen, 2002). Perhaps this immunologic response can have negative effect not only in the short time period, such as the harmful pathophysiologic effects in a gram negative sepsis (Taveira da Silva et al., 1993), but also trigger late term effects? The role of host genetics in the susceptibility to complications of *Salmonella* and *Campylobacter* infection has been studied, and single nucleotide polymorphisms (SNPs) in interferon-gamma gene was associated with reactive arthritis and recurrent diarrhea (Doorduyn et al., 2007). Another study found that polymorphism in genes coding for CD1 molecules, which presents glycolipids to antigen-specific T-cells, were correlated to GBS in *Campylobacter* patients (Caporale et al., 2006). We will probably see more

studies like this in the future and other host genetics (besides these two and HLA-B27) may be recognized as important in development of complications and chronic sequelae.

Bacterial gastroenteritis is, in a minority of cases, associated with complications and late effects. Some of these are extraintestinal focal infections and *Salmonella* is probably the enteric pathogen that is guilty of most of these manifestations. Examples of focal infection associated with septic salmonellosis include endocarditis, mycotic aneurysms, abscesses, osteomyelitis and arthritis (Banky et al., 2002; Karim and Islam, 2002; Santos and Sapico, 1998; Shimoni et al., 1999; Soravia-Dunand et al., 1999). But there are other, not focal infections, but focal or systemic disease caused by inflammation that has originated from bacterial enteritis. The most feared complications are probably Guillain-Barré syndrome (GBS) associated with *Campylobacter* infection (Nachamkin, 2001), and Hemolytic uraemic syndrome (HUS) caused by EHEC infection (Tarr et al., 2005). For more examples, see table 1.

***Table* 1**. Some examples of immunmediated bacterial gastrointestinal complications. Source: (Crushell et al., 2004; Dworkin et al., 2001; Leirisalo-Repo et al., 2003; Rees et al., 2004)

Complications	
Irritable bowel syndrome (IBS)	Hemolytic uraemic syndrome (HUS)
Reactive arthritis	Erythema nodosum
Guillain-Barré syndrome (GBS)	Reiter´s syndrome (uveities, urethritis, arthritis)

3 AIMS OF THE STUDIES

The aims of the studies included in this thesis were:

- to estimate the mortality associated with *Salmonella* and *Campylobacter* infection.
- to explore if country of infection could be an effect modifier on mortality in epidemiological studies that uses routine surveillance data on *Salmonella* and *Campylobacter* infections.
- to evaluate if antibiotic therapy have any effect on duration of diarrhea compared to placebo in *Campylobacter* infection.
- to analyze if length of the time period between debut of campylobacterosis symptoms and antibiotic treatment influence the efficacy of therapy.
- to estimate hospital treated complications after bacterial gastrointestinal infection.

4 MATERIALS AND METHODS

4.1 *STUDIES* I *AND* II

4.1.1 *The cohort and the follow-up*

These two studies are population-based retrospective cohort studies. The study participants were all reported to SMI in accordance to the Swedish Communicable Disease Act during the years 1997–2004. The reason why we did not elongated our cohort further back in time is because 1997 was the year SMI started to store all data in a modern data base. For every study subject we have information on type of bacterial pathogen, social security number (which include date of birth), sex, date of disease onset, date of diagnosis, reporting date, and country of infection. If date of disease onset or date of diagnosis was missing, we used the median time from patients who had all three dates to back-calculate a date for disease onset.

These cases were then via the social security number linked to a database that contains information from the National Tax Board on every deceased person in Sweden and their date of death.

Follow-up times were then calculated in each five-year age group, from the date of disease onset until either an event took place (death) or until November 1, 2004. To be clear, age group 10–14 means 'from the date of the 10th birthday to the day before the 15th birthday'. The underlying assumption is that mortality rates do not differ much within each age group. Note that a study participant can start in one age group and pass to another during the study period.

The following time strata were used: 0–1 month, 1–3 months, 4–12 months and >12 months after the onset of disease. We constructed the first time strata to be long enough so that an acute effect of even prolonged illness was not missed. The other cut-offs were chosen so that effects between strata could be evident (e.g. a declining risk with time) and that each time strata contained a sufficient amount of person-time for robust estimations.

From Statistics Sweden we obtained sex- and age-specific death rates, which were used to obtain the expected number of deaths. Expected number of deaths was calculated by multiplying the observed number of person-years in the cohort by age- sex- and calendar-year-specific death rates from the general population (table 2).

Table 2. Observed (Obs) and expected (Exp) number of deaths and Standardized mortality ratios (SMR).

	Study population			General population			Exp
Age group	Obs	Person-time	Rate	Obs	Population		Rate
0–4	I_1	PT_1	I_1/PT_1	B_1	N_1	B_1/N_1	$E_1=PT_1*(B_1/N_1)$
5–9	I_2	PT_2	I_2/PT_2	B_2	N_2	B_2/N_2	$E_2=PT_2*(B_2/N_2)$
10–14	I_3	PT_3	I_3/PT_3	B_3	N_3	B_3/N_3	$E_3=PT_3*(B_3/N_3)$
etc.							
Total	I_t						$E_1+E_2+E_3=\sum E_j$

With the assumption that those who had acquired their infection abroad and those who had contracted it at home were two fundamentally different groups – the former probably healthier than the latter ('healthy traveler effect') – we divided our *Campylobacter/Salmonella* cohort into two groups, imported and domestic cases.

4.1.2 *Statistical methods*

Standardized mortality ratios (SMRs) were calculated by dividing the observed number of deaths in the cohorts with the expected number of deaths. If the result was >1.0, then the mortality in the *Campylobacter/Salmonella* cohort was higher than in the general population, and conversely, if the ratio was <1.0 then the mortality among our cases was lower than in the general population.

$$SMR = \frac{I_t}{\sum E_j} = \frac{Obs}{Exp}$$

The SMR is a weighted average of all age groups in the *Campylobacter/Salmonella* cohort. This indirect standardization was done with 5-year age strata (e.g. 0–4, 5–9, etc.) but is presented in the two articles either as SMR, or grouped into broader categories e.g. 0–14 years, 15–64 years and >65 years.

Exact confidence intervals and tests were calculated assuming that the number of deaths in each stratum was Poisson distributed. Within each time stratum the observed SMRs for each age group were compared using an exact test. The observed number of cases was assumed to be multinomially distributed over the age classes with probabilities proportional to the expected values.

4.2 *STUDY* III

4.2.1 *Design*

Study III is a meta-analysis. This is a method used to combine results from several studies in a systematic and quantitative way. Sampling variability means that estimates of a treatment effect will vary even between identical studies conducted in the same source population (Egger et al., 2001). And the smaller the sample, the larger is the variability. A meta-analysis is a way to, if well conducted, provide more precise estimates of a treatment effect than a single study. In a meta-analysis, the researcher follows exactly the same steps as in any other scientific study (specify research question, design, data extraction, analysis, interpretation etc.) and others can replicate the results if they like to challenge the findings.

We had in advanced stated criteria's for the studies to be selected and used in our meta-analysis. We search PubMed, Web of Science, Embase, Cochrane Central Register of Controlled Trials (issue 2, 2006) and ClinicalTrials.gov for studies to include. Both free text word searches and searches were we used MeSH-terms were performed. All citations in the studies retained for detailed evaluation were also checked. Publications published up to June 2006 were included. 446 potentially relevant studies were found in the searches, and 11 studies were included in the meta-analysis.

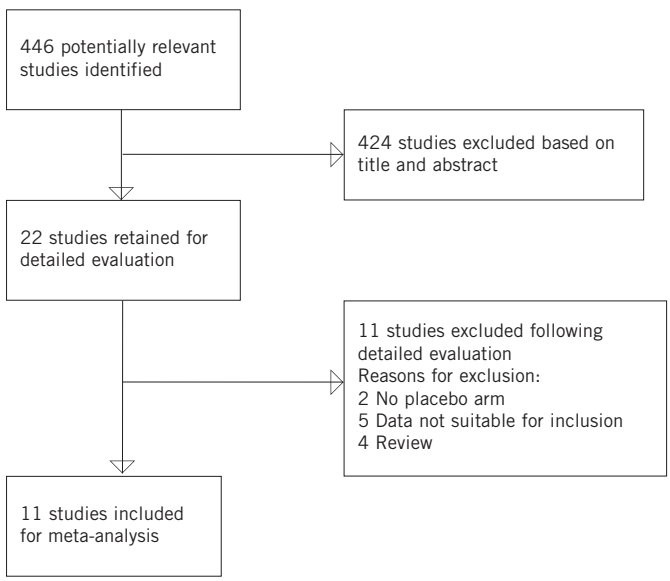

***Figure* 3**. Flow chart of the inclusion of eligible RCTs in the meta-analysis.

4.2.2 *Statistical methods*

Results from different studies are pooled together in order to obtain an average effect across all studies. The underlying assumption is that there is a common effect that can be measured. Each study will receive weight proportional to the amount of information that it contains, i.e. a big study will have a big weight and a small study a small weight. More formally (w = weight, var = variance):

$$w_i = \frac{1}{var_i}$$

The general principle for pooling looks like:

$$Effect_{pooled} = \frac{\sum Effect_i \cdot w_i}{\sum w_i}$$

If there is a large similarity between the outcomes from different studies, then there are no problems to combine results. This can be the result in a situation where different studies have used the same source population, and used identical protocol and procedures. The observed differences in outcome are a result of sampling variation alone.

In many cases though are the differences between treatment effects in individual studies not that homogeneous, due to differences in the underlying population, different study protocols and methods used etc (Dickersin and Berlin, 1992). There are statistical methods that assess heterogeneity between studies (Higgins et al., 2003), but one can many times from a forrest-plot have visual information on any lack of homogeneity between studies.

If no heterogeneity between studies exists, then one can use a fixed-effect model to combine all results. But if there is evidence of heterogeneity between studies, a random-effect model is preferred (Egger et al., 1997). This model takes into account variability between studies and also sampling variation within a study. A random-effect model will give wider confidence intervals around the combined estimate of treatment effect (R. Brian Haynes, 2006).

Even if we only included double-blinded, placebo-controlled RCT, the population varied between studies in respect to age and geographical location. Different antibiotics (macrolides or quinolones) were also used between studies. We therefore used a random-effect model.

4.3 *STUDY* IV

4.3.1 *The cohort and the follow-up*

Study IV is a retrospective, population-based cohort study. Persons reported to SMI in accordance to the Swedish Communicable Disease Act either with *Campylobacter* spp, non-typhoid *Salmonella* spp, *Yersinia enterocolitica*, *Shigella* spp or EHEC infections during 1997–2004 were used in this study. The cohort consists of 101,855 persons. All infections have been microbiologically confirmed by culture.

Each patient were followed either until the time of diagnosis of a complication, death, or date of study termination. We were interested both in short term complications (within 3 months) and long term effects (within 1 year) after infection. The type of events had been pre-determined before the study started, in order to avoid the problem of signicant results by chance (massignificans). The events were identified using record linkage to the Hospital Discharge Register and Cause of Death Register.

We looked for interaction by dividing the cohort in two groups: those infected abroad and at home, but the result was negative. We therefore used the whole cohort regardless of country of infection in the further analysis.

4.3.2 *Statistical methods*

Standardized incidence ratios (SIR) were calculated by dividing the observed number of cases with the expected. The expected number of cases was calculated by multiplying the observed number of person-years in the cohort by age- and sex-specific incidence rates from the year 2000 for the Swedish general population.

We used 95% confidence intervals to analyze if any SIR was different from 1 and thus statistically significant. The number of observed complications was assumed to be Poisson distributed in these calculations.

5 RESULTS AND COMMENTS

5.1 *STUDY* I

Mortality following *Campylobacter* infection: a registry-based linkage study

Our study on campylobacterosis and mortality included 48,025 study subjects, of whom 28,930 were infected abroad and 16,710 within Sweden. The personal identification number was wrong or the country of infection was missing for 2,385 persons and they were excluded from the analysis.

For those infected at home, SMR was 2.9 (95% CI: 1.9–4.0) within the first month after infection and equaled 1 after one year (table 3). For those infected abroad, the relative mortality risk was lower than for the general population throughout the whole time period. This indicates that those who travels are healthier compared to the general population. Infection with *Campylobacter* in this group of persons is an unpleasant, but far from life-threatening experience.

***Table* 3**. Standardized mortality ratio (SMR) and 95% CI for patients diagnosed with campylobacterosis, Sweden, by time since infection.

		Latency interval after acute campylobacterosis (months)			
		<1	1–3	4–12	>12
Infected in Sweden	No. of observed deaths	30	31	123	379
	No. of expected deaths	10.4	20.7	93.9	388.6
	SMR	2.9	1.5	1.3	1.0
	95% CI	1.9–4.0	1.0–2.1	1.1–1.6	0.9–1.1
Infected abroad	No. of observed deaths	2	10	28	182
	No. of expected deaths	6.8	13.7	63.3	330.6
	SMR	0.3	0.7	0.4	0.6
	95% CI	0.04–0.8	0.4–1.3	0.3–0.6	0.5–0.6

5.2 *STUDY* II

Salmonella-associated deaths, Sweden, 1997–2003

In this study, we found a very clear difference in SMR between those infected abroad and those infected at home, and therefore handled these groups separately. After we had excluded 239 persons due to lack of information on country of infection, a total of 30,438 were included in the analysis. Out of these, 25,060 had been infected

abroad and 5,139 at home. The median age for those infected abroad was 40 years (interquartile range: 25–56) and for those infected in Sweden was 36 years (interquartile range: 20–56 years).

For the group of persons infected within Sweden we found an increased SMR for every time period during the first year after infection (figure 4). After a period of one year, there is still a higher relative mortality risk in this group compared to the Swedish general population. This may be interpreted as a frailty phenomena: this group of persons are probably more vulnerable then the Swedish general population. In a situation when they are exposed to acute salmonellosis, they will therefore have a high relative mortality risk initially.

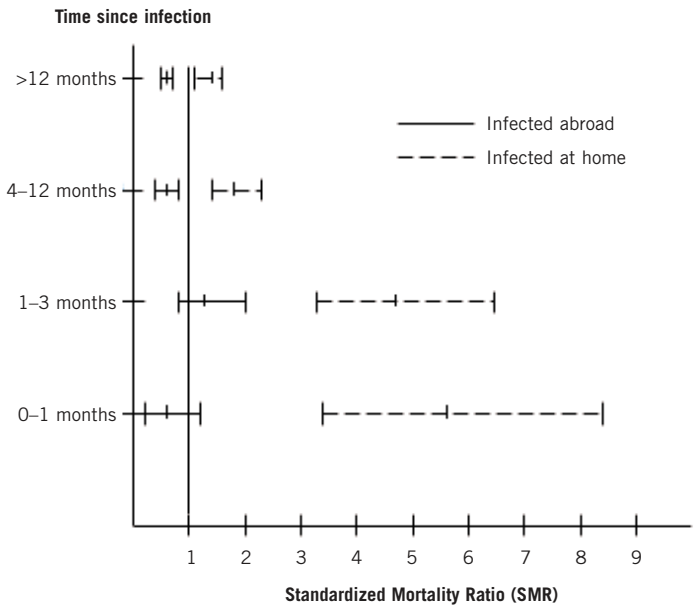

***Figure* 4**. Standardized mortality ratio (SMR) among persons infected abroad and at home with non-typhoid Salmonella.

We investigated homogeneity in the results by calculating relative mortality risk for the age-groups groups 0–14 years, 15–64 years and >65 years separately (table 4). If the high SMR in the group of persons infected at home would be an effect of mortality in a specific age-group (such as the oldest), we would expect different SMRs for the three age-groups within the same time-period after infection. We did not found any large differences between age groups, and the assumption that the oldest age-group would be responsible for the SMR is highly unlikely.

Table 4. Standardized mortality ratio (SMR) and 95% CI for patients diagnosed with domestic salmonellosis, Sweden, by age-group and time since infection.

		Latency interval after acute salmonellosis (months)			
		<1	1–3	4–12	>12
0–14 years	No. of observed deaths	0	0	0	1
	No. of expected deaths	0.1	0.1	0.4	1.0
	SMR	0	0	0	1.0
	95% CI	0–59.6	0–30.3	0–7.3	0.03–3.8
15–64 years	No. of observed deaths	6	6	11	27
	No. of expected deaths	0.5	1.1	4.7	16.9
	SMR	11.2	5.6	2.4	1.6
	95% CI	4.1–21.8	2.1–10.9	1.2–3.9	1.1–2.2
>65 years	No. of observed deaths	15	28	44	118
	No. of expected deaths	3.2	6.0	25.3	89.4
	SMR	4.7	4.7	1.7	1.3
	95% CI	2.6–7.4	3.1–6.5	1.3–2.3	1.1–1.6

We found no increased SMR for the group of persons who had acquired their Salmonella infection abroad (figure 4). This is probably a 'healthy traveller effect' and in line with our findings from study I.

This was not a study designed to analyse mortality associated with specific subtypes of *Salmonella*. All isolates were not subtyped, but among the isolates that were, *S.* Enteritidis and *S.* Typhimurium dominated both among persons infected at home and abroad. *S.* Dublin and *S.* Wirchow, which sometimes are believed to be more pathogenic than others (O'Brien and Feldman, 2003), constituted together around 1% of the isolates among persons infected within Sweden and 2.6% among persons infected abroad.

5.3 *STUDY* III

A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with *Campylobacter* species

Our quantitative review of RCT on antibiotics vs. placebo in campylobacterosis showed a small, but statistically significant effect of antibiotic treatment (figure 5). In a random-effect model, antibiotic treatment shortened the duration with diarrhea with 1.32 days (95% CI: 0.64–1.99) Antibiotics also shortened the time period of bacterial shedding from faeces, but we were unable to quantify this effect due to lack of data.

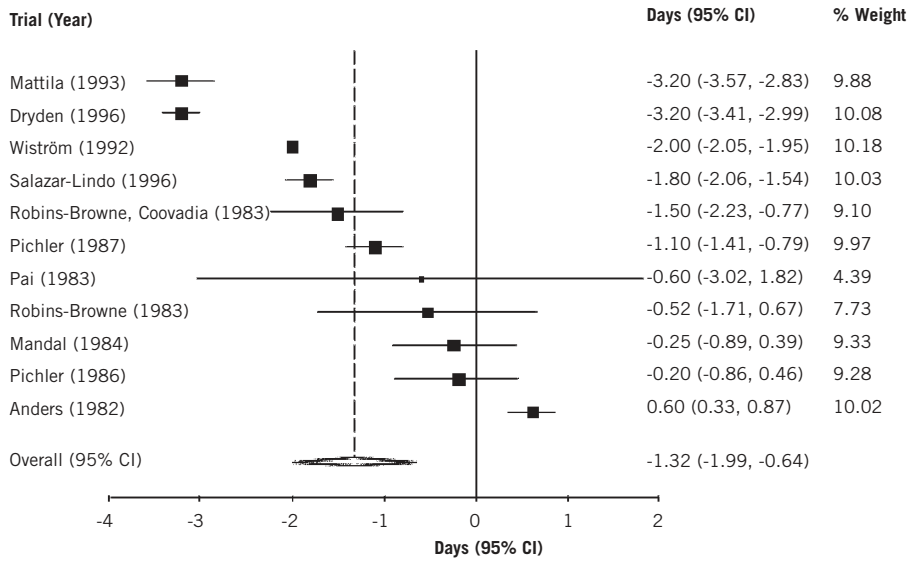

***Figure* 5**. Forest plot of RCTs of antibiotics vs. placebo in campylobacterosis.

The mean number of days with symptoms before antibiotics or placebo was administered differed between trials. The range was 0.8–5.6 for antibiotics, and 0.8–6.5 in the placebo group. We tried to estimate the effect of duration of illness before therapy on the duration of diarrhea after therapy in a linear regression model. The results were non-significant (p=0.30) due to too few observations and showed a decrease of 0.35 days per day earlier treatment. By dividing the data into two groups: those treated within three days of illness, and those who received treatment after three days or more had passed, we saw that the duration of diarrhea after therapy was 2.38 days in the first group and 4.11 days in the second group.

Publication bias can be a problem in meta-analysis. The articles that are most likely to be unpublished are those with small sample sizes and no or limited treatment effects. Figure 6 visualize this and we cannot see any gap, i.e. a lack of articles 'in the lower left corner' in the funnel plot. We therefore do not believe that publication bias was any major problem in our study.

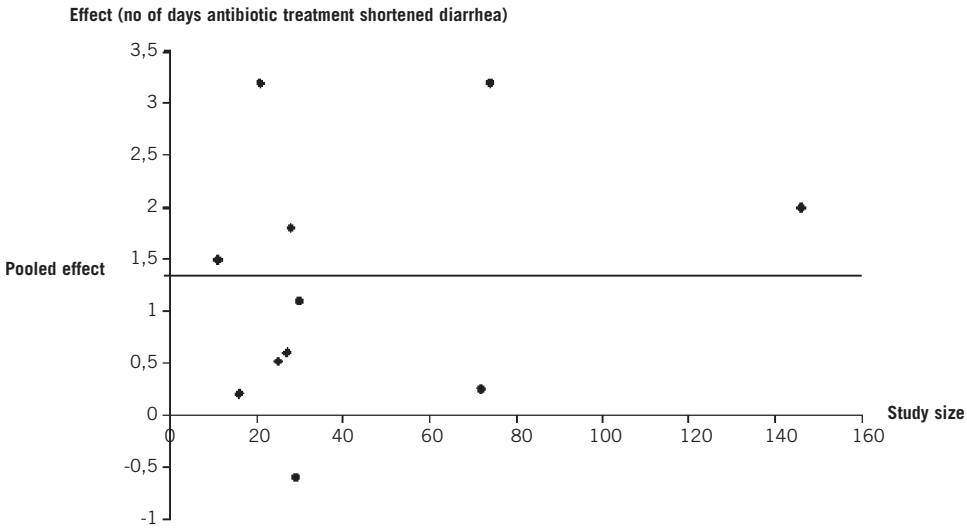

***Figure* 6**. Funnel plot of RCTs of antibiotics vs. placebo in campylobacterosis.

5.4 *STUDY* IV

Short- and long-term effects of bacterial gastrointestinal infections

In this large, population-based, retrospective cohort study we found an elevated absolute risk for HUS after EHEC infection (1.6%), GBS after campylobacterosis (0.02%) and reactive arthritis after infection with *Yersinia* spp (0.2%).

We further examined the relative risk for complications from several organ systems, both in a short (<3 months) and a long (<12 months) time period. The short term complications were either extraintestinal infections, acute surgical complications, or immunmediated diseases with a short latency period. The late effects, or chronic sequaele, were autoimmune diseases with a presumed long latency period.

Within the short time period, the most intriguing finding was that of an increased relative risk for aortic aneurysm after *Salmonella* infection (SIR 6.4; 95% CI: 3.1–11.8). No statistically significant increased SIR for aneurysm was found among those with *Campylobacter* infection. Study participants in the *Salmonella* cohort also had an increased risk during one-year follow-up for ulcerative colitis (SIR 3.2; 95% CI: 2.2–4.6). This increased risk was also evident in the *Campylobacter* cohort (SIR 2.8; 95% CI: 2.0–3.8). We could not find any statistically significant increased risk for Crohns disease either among cases with salmonellosis (SIR 1.4; 95% CI: 0.8–2.3) or

campylobacterosis (SIR 1.6; 95% CI: 1.0–2.3), but the risk for other types of noninfective colitis (e.g. eosinophilic gastroenteritis) was increased. The risk for reactive arthropathies was increased among all groups of patients, and most notably for Yersinia (SIR 47.0; 95% CI: 21.5–89.2) and *Salmonella* infection (SIR 18.2; 95% CI: 12.0–26.5). For all the results, see table 5 and 6 below.

***Table* 5**. Complications associated with gastroenteritis, 3-months after infection among 101,855 patients with bacterial gastrointestinal infection.

Disease	Infecting organism	Obs	Exp	SIR	95% CI
Respiratory system					
Bacterial pneumonia, Pneumonitis due to food and vomit	NTS	24	13.5	1.8	1.1–2.6
	Campylobacter	17	21.4	0.8	0.5–1.3
	EHEC	1	0.3	3.1	0.1–17.2
	Shigella spp	1	1.1	0.9	0.02–5.2
	Yersinia spp	4	2.3	1.8	0.5–4.5
Blood					
Hemolytic-uremic syndrome	NTS	1	<0.05	55.5	1.4–309.1
	Campylobacter	2	<0.05	81.0	9.8–292.7
	EHEC	13	<0.05	18333.4	9761.8–31350.6
Circulatory system					
Aortic aneurysm	NTS	10	1.6	6.4	3.1–11.8
	Campylobacter	5	2.4	2.1	0.7–4.8
	Yersinia spp	1	0.2	5.2	0.1–28.9
Endocarditis	NTS	2	0.4	5.7	0.7–20.5
Digestive system					
Peritonitis	NTS	1	0.6	1.9	0.05–10.1
	Campylobacter	2	0.9	2.3	0.4–8.4
Perforation of intestine (non traumatic)	NTS	1	0.1	9.7	0.3–54.0
	Campylobacter	2	0.2	12.4	1.5–44.7
	EHEC	1	<0.05	655.3	16.6–3651.0
Idiopathic acute pancreatitis	NTS	6	2.6	2.3	0.9–5.1
	Campylobacter	7	4.1	1.7	0.68–3.5
Hepatic failure	NTS	1	0.3	4.0	0.1–22.2
Infectious diseases					
Septicemia	NTS	10	2.6	3.9	1.8–7.1
	Campylobacter	14	4.1	3.4	1.9–5.7
	Shigella spp	1	0.2	5.1	0.1–28.2
Nervous system					
Guillain-Barré syndrome	Campylobacter	13	0.2	66.6	35.5–114.0
Musculoskeletal system					
Pyogenic arthritis	NTS	4	0.8	5.2	1.4–13.4
	Yersinia spp	1	0.1	10.1	0.3–56.2
Osteomyelitis	NTS	3	0.6	5.4	1.1–15.7

Non-typhoid Salmonella (NTS), observed number of cases (Obs), expected number of cases (Exp), standardized incidence ratio (SIR), confidence interval (CI), Enterohemorrhagic Escherichia coli (EHEC).

***Table* 6**. Complications associated with gastroenteritis, 1-year after infection among 101,855 patients with bacterial gastrointestinal infection.

Disease	Infecting organism	Obs	Exp	SIR	95% CI
Digestive system					
Crohn's disease	Campylobacter	27	17.1	1.6	1.0–2.3
	NTS	14	10.3	1.4	0.8–2.3
	Shigella spp	1	1.1	0.9	0.02–5.2
	Yersinia spp	2	1.1	1.8	0.2–6.4
Ulcerative colitis	Campylobacter	42	14.8	2.8	2.0–3.8
	EHEC	1	0.1	6.8	0.2–37.7
	NTS	29	9	3.2	2.2–4.6
	Yersinia spp	3	1	2.9	0.6–8.5
Other specified/unspecified noninfective gastroenteritis and colitis	Campylobacter	37	14.9	2.5	1.8–3.4
	NTS	30	9.2	3.3	2.2–4.6
	Yersinia spp	10	1.3	7.6	3.7–14.0
Irritable bowel syndrome	Campylobacter	15	5	3.0	1.7–5.0
	NTS	5	3	1.7	0.5–3.9
	Yersinia spp	3	0.4	7.8	1.6–22.9
Intestinal malabsorption	NTS	1	0.6	1.7	0.04–9.3
	Yersinia spp	1	0.1	7.9	0.2–43.7
Musculoskeletal system					
Postdysenteric arthropathy, Reiter's disease, Other reactive arthropathies	Campylobacter	15	2.4	6.3	3.5–10.4
	NTS	27	1.5	18.2	12.0–26.5
	Shigella spp	2	0.1	13.4	1.6–48.4
	Yersinia spp	9	0.2	47.0	21.5–89.2
Rheumatoid arthritis	Campylobacter	22	22.5	1.0	0.6–1.5
	EHEC	1	0.2	5.8	0.2–32.1
	NTS	9	14.7	0.6	0.3–1.2
	Shigella spp	1	1.2	0.8	0.02–4.7
	Yersinia spp	3	1.5	2.0	0.4–5.7
Other arthritis	Campylobacter	8	3.8	2.1	0.9–4.2
	NTS	4	2.5	1.6	0.4–4.1
	Shigella spp	1	0.2	4.3	0.1–24.1
	Yersinia spp	1	0.4	2.4	0.06–13.4
Other necrotizing vasculopathies (Goodpasture's syndrome, TTP, Wegener's granulomatosis, Giant cell arteritis)	Campylobacter	10	3.3	3.1	1.5–5.6
	EHEC	0	<0.05	32.8	0.8–183.0
	NTS	1	2.1	0.5	0.01–2.7
Systemic lupus erythematosus	Campylobacter	5	3.4	1.5	0.5–3.4
	NTS	2	2.1	1.0	0.1–3.5
Systemic sclerosis	Campylobacter	2	1.7	1.2	0.2–4.4
	NTS	3	1.1	2.8	0.6–8.1

Other systemic involvement of connective tissue (Sjögren's syndrome, Mixed connective tissue disease, Polymyalgia rheumatica)	Campylobacter	12	5	2.4	1.2–4.2
	NTS	4	3.1	1.3	0.4–3.3
	Shigella spp	1	0.2	4.2	0.1–23.3
Ankylosing spondylitis	Campylobacter	2	1.1	1.8	0.2–6.4
	NTS	1	0.7	1.5	0.04–8.1

Non-typhoid Salmonella (NTS), observed number of cases (Obs), expected number of cases (Exp), standardized incidence ratio (SIR), confidence interval (CI), Enterohemorrhagic Escherichia coli (EHEC).

6 GENERAL DISCUSSION

6.1 *METHODOLIOGICAL CONSIDERATIONS*

6.1.1 *Study design*

We have in papers I, II and IV done retrospective cohort studies in order to estimate associated mortality and disease risks after bacterial gastroenteritis. Our study base consists of the whole country over an 8-year period and because the frequency of our exposure (bacterial gastroenteritis) was quite common, we ended up with a large cohort.

But one must also take into account the incidence of the outcome of interest, and if this is a rare disease, the case-control design can be more efficient compared to a cohort study. However, we concluded that our cohort was large enough and the diseases we were interested in not too rare, so a cohort design would fulfill our needs. Generally speaking, a cohort study is efficient if one has a single exposure and is looking for multiple outcomes (as in paper IV). Whereas a case-control study probably are a better choice if one has a single outcome and is interested to find out which one, out of several exposures, is a cause of the disease.

In paper III we present a meta-analysis on antibiotics vs. placebo in *Campylobacter* enteritis. Another way to answer the same question would have been to conduct a RCT, often regarded the gold standard in evidence based medicine. However, almost a dozen published RCT already exists and if we would have done yet another one it should have included more study participants than any of the previous ones, and naturally be very expensive.

6.1.2 *Selection bias*

Papers I, II and IV have a population-based design which in theory means that results from the study population are valid for the entire population. In the Swedish health care system must all hospitals without exceptions report data on discharges to the Hospital Discharge Register. The patient register on reported infectious diseases at SMI is also nationwide and covers not only hospitals, but all clinics and microbiological laboratories within the health care sector. This means that all patients with acute infectious diarrhea who sought health care, and were diagnosed with reportable bacteria are included in the SMI-register. Both these registers are of good quality and continuously updated (Jansson et al., 2005).

If there is a biased sampling of subjects from the theoretical cohort of all eligible participants to the study population, or if subjects are lost to follow-up in a differential way, there exists a problem of selection bias (Rothman, 2002). This is of concern in papers I, II and IV and perhaps to some extent also in the meta-analysis in paper III.

The cohorts used in papers I, II and IV are not a representative sample of the whole population. On the contrary, there exist selection mechanisms in every single step from the moment a person becomes ill with acute diarrhea, to the moment he/she is reported to SMI. A Dutch study showed that a stool sample was collected only in 12% of cases with gastroenteritis presented at general practitioners (van den Brandhof et al., 2006). The corresponding figure is probably higher in Sweden, were most of the health care are governmental funded and the costs for microbiological samples do not affect individual care providers.

One way to illustrate this selection process is to consider the reported cases 'the top of the iceberg' (figure 7). The ratio between NTS infection recorded by passive surveillance and actual NTS infection in the population has been estimated in United Kingdom (ratio 3.2) (Wheeler et al., 1999) and United States (ratio 38) (Voetsch et al., 2004). In general, the more severe the infections are, the less will the underdetection ratio be (de Wit et al., 2001b).

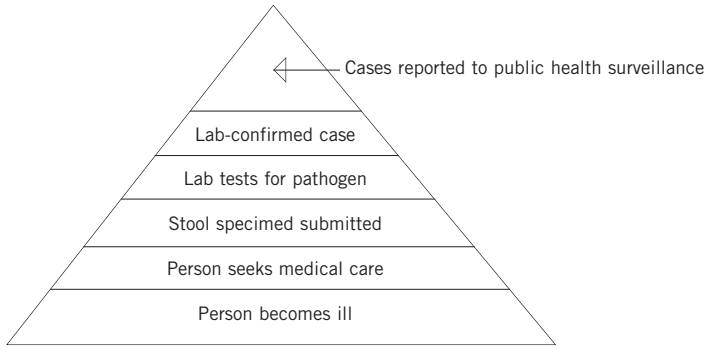

Figure 7. Steps where selection occurs when patients with acute diarrhea are reported to national surveillance. Source: Adopted from (Scallan, 2007).

In the meta-analysis in paper III we looked for unpublished data in data bases of RCTs, but we did not found any, so the meta-analysis is based on published data. We tried to estimate publication bias (which may be considered a variant of selection bias) in a funnel plot but we did not see any obvious signs of that. Publication bias is an unquestionable problem in medical research in general. However, in the specific case of RCTs that have studied antibiotics vs. placebo in *Campylobacter* infection, we do not believe that there are any, or at least not many, RCTs with the same aim and design who did not published their (negative) results. This is because many of the RCTs included in our meta-analysis did not show any effect of antibiotics.

6.1.3 *Diagnostic bias*

The term diagnostic (detection) bias (a form of selection bias) refers to situations where the exposure of interest affects the probability of being diagnosed with the disease (John Wiley & Sons, 2007). In our example, paper IV, if people have had bacterial gastroenteritis they may be more likely to be diagnosed with reactive arthritis if they have symptoms of joint inflammation compared to other patient groups. This would lead to an over-estimation of the risk for reactive arthritis following gastrointestinal infection. Because we only have disease data from Hospital Discharge Register we miss all of those who are diagnosed with reactive arthritis at general practitioners and such. This means that we will not detect some of the cases of reactive arthritis.

The net effect of our over-estimation due to diagnostic bias, and our under-estimation due to missing cases is hard to predict. The net effect becomes even harder to estimate accurately if we take into consideration the problem of 'top of the iceberg' reporting of cases with acute infectious diarrhea. If we compare our findings with

others, the best guess is that we underestimate the risk for reactive arthritis because we only have data from hospitals, not outpatient clinics.

Severe complications such as GBS and HUS are most likely diagnosed in the same numbers regardless of a known exposure to bacterial intestinal pathogen or not.

The problem with diagnostic bias in register based epidemiological studies is perhaps most pronounced in situations with common exposures and rare diseases with long latency periods. This is discussed in a very nice study on gastric or duodenal ulcer and the risk for stomach cancer (Hansson et al., 1996). In this study the author's showed that patients with symptoms from the stomach underwent gastroscopy more than those without symptoms and therefore had a higher probability than the population to be misdiagnosed with benign ulcers, when it later became clear that the correct diagnose was stomach cancer. If one would use those identified with ulcers at their first endoscopy in the Hospital Discharge Register and link this to the Cancer Register to look for stomach cancer during follow-up, one would consequently overestimate the risk initially.

We believe that we do not have the same problem in our studies on acute *Campylobacter* and *Salmonella* infection and mortality risk and the risk for other diseases for a number of reasons. First, our exposure variable is culture confirmed gastrointestinal infections reported to SMI. The misclassification of diagnoses (i.e. duodenal ulcer and not stomach cancer) is therefore not the same problem as in epidemiological studies based on ICD-9 codes from the Hospital Discharge Register. Second, we have studied relative acute effects of an infectious episode on mortality and to construct an exposure window (e.g. 6 months) prior to the event would be inappropriate. Third, the findings of an increased relative mortality risk close to infection that gradually diminishes and equals 1 after one year supports a causal relationship. Fourth, the findings of different mortality risks among travelers and non-travelers could be a selection effect, but the findings of no increased mortality among travelers at any time-period after the infection makes diagnostic bias as the only explanation to our mortality figures highly unlikely. Fifth, to order a stool culture do not cost the individual doctor or patient anything in Sweden. This means that there are no economic incitements to withhold a microbiological test for those with mild disease. This category of patients may be also tested for epidemiological reasons only. And last, the plausibility of our mortality figures may be further supported by common knowledge about *Salmonella* and *Campylobacter* severity, i.e. NTS infection was associated with a higher mortality than *Campylobacter* infection in our studies.

6.1.4 *Information on exposure and outcome*

All patients in studies I, II and IV where reported with bacterial gastroenteritis that had been microbiologically confirmed. For patients infected with *Salmonella*, *Shigella*, *Yersinia* and EHEC had the local laboratory in most cases sent the isolate to SMI for confirmation and further subtyping. Some cases of bacterial gastroenteritis may be missed because the sensitivity of a stool culture is not perfect, but the specificity is high. Anyway, the problem of misclassification of exposure is most likely non-differential i.e. we probably miss the same proportion of cases regardless of the disease status of interest (any complication or death after the acute infection).

In papers I and II we linked our cohort of cases to the Swedish National Tax Board to determine date for any possible deaths during follow-up. This is the most accurate and updated Swedish register on deaths and since death is a binary outcome and easy to diagnose, it should be no misclassification on outcome in these studies.

In paper IV we used the Hospital Discharge Register to determine sickness date and type of disease during follow-up in our gastroenteritis cohort. We only looked at a restricted number of diseases, which had been decided in advance, based on a hypothesis of an increased risk for the same list of diseases among gastroenteritis patients. The Hospital Discharge Register has been validated, using the diagnose of acute myocardial infarction, and the under reporting was <1%, main diagnose was missing in <1% and correct diagnose was present in 86% of cases .

6.1.5 *Confounding and effect modification*

It is easy to mistake the role of an extraneous factor in any observational study. The result would be that the effect of the exposure of interest is disturbed. This may be a problem in the studies we have conducted. There are probably some, known or unknown, risk factors for GBS, reactive arthritis, HUS, aortic aneurysm, and ulcerative colitis that it would have been nice to have information on (in order to adjust our data in a multivariate analysis). Particularly other co-existing illnesses, smoking and type of occupation are such potential risk factors that could have altered our sex- and age-adjusted risk estimates. In a Danish retrospective cohort study on food-borne infections and complications (Helms et al., 2006) and deaths (Helms et al., 2003), they had information on co-existing illnesses, but that did not change the risk estimates in any substantial way. Note that a strong association with an estimated relative risk of >3.0 is less likely to be explained by a confounding factor compared to a weak association (Axelson, 1980).

In papers I and II we found evidence of effect modification: i.e. the effect of exposure on disease risk varies between groups (John Wiley & Sons, 2007). We found that the mortality among persons with *Salmonella* and *Campylobacter* differed between those who had been infected abroad and at home. We solved this problem by stratifying

the analysis by these two groups. The selection bias of cases to the SMI-register, and consequently our cohort, are one possible explanation to these findings. People who have recently traveled and become ill with gastroenteritis are more likely to seek health care and be diagnosed with bacterial gastroenteritis, compared to those who have not been abroad (Tam et al., 2003). People who do not travel are probably as a group less healthy, compared to those who travel. Our findings of a lower mortality among gastroenteritis patients infected abroad compared to that of the population as a whole is most likely a 'healthy-traveler effect' (Kelman et al., 2003) (An equivalent to the 'healthy-worker effect', i.e. people in the workforce have lower mortality rates than members of the general population.).

6.1.6 *Chance*

We estimated 95% confidence intervals around our point estimates and calculated p-values were appropriate, in order to estimate the precision in our results. The large sample sizes in papers I, II and IV were good enough to prove moderately strong associations. Also, the predetermined hypotheses in paper IV reduced the problem of massignificans and individual chance findings.

6.2 *INTERPRETATIONS AND IMPLICATIONS*

6.2.1 *Bacterial gastrointestinal infection and mortality (Studies* I *and* II*)*

Our aims in papers I and II were to estimate the mortality associated with an acute episode of campylobacterosis and salmonellosis. We used a large cohort of patients reported with culture-confirmed infection in accordance with the Swedish Communicable Disease Act. We also had information on geographical place of infection, which served as a surrogate marker for general health status. We observed an increased relative mortality risk among persons infected at home, and the mortality risk was highest in the acute phase. The mortality was higher after NTS infection than after *Campylobacter* infection. These findings could probably be explained by *Salmonella's* invasive capabilities and extraintestinal manifestations. We have in these studies estimated mortality risk while adjusting for competing mortality risks, something that have been lacking in other studies. We identified our diseased persons in the cohort using register data from the Swedish National Tax Board. This makes our estimate more robust in respect to misclassifications and underreporting. The mortality observed is probably an effect of acute complications such as severe dehydration, bacteremia with our without an extraintestinal focus, or uncommon complications such as GBS after campylobacterosis.

Our findings are in coherence of others, such as an English study (Adak et al., 2002) where more *Campylobacter* (56.6%) than NTS (6.6%) were found among isolates (n=634,568) of bacterial, viral, protozoal enteric pathogens, but NTS were considered as a cause of death in 29.2% of cases (total no of deaths=407) and *Campylobacter* in 21.1%. These results are in line with an American study (Kennedy et al., 2004)

on bacterial gastroenteritis were *Campylobacter* constituted 44% of cases (n=34,296) and NTS 33%. Among those who died (n=153) in this study, 12 had campylobacterosis (case-fatality rate 0.1%) and 58 salmonellosis (case-fatality rate 0.6%). The case-fatality rates (mortality within 30 days of infection) associated with *Campylobacter* infection was in our studies 0.07% and for infection with *Salmonella* 0.08%, i.e. lower than the American and Danish (Helms et al., 2003) figures.

The findings of a different mortality risk among those who acquired their infection abroad and at home, suggests that the latter are a more vulnerable population than the former and probably have more underlying illnesses. This could contribute to the different mortality risks. In a population-based study on NTS bacteremia in 111 patients, a multivariate analysis showed that old age and co-morbid diseases were independently related to the elevated 30-days and 180-days mortality they had found (Gradel et al., 2006). Old age and co-morbid illnesses also affected the length of hospital stay for NTS in an American study (Trevejo et al., 2003). However, even if we would have had information on co-morbid diseases and had adjusted our estimates for that, the main effects would probably still be evident (Helms et al., 2003).

Deaths due to infectious diseases in general have declined during the last one hundred years in Western countries, and this is true also for bacterial gastrointestinal diseases (Armstrong et al., 1999) (Mokdad et al., 2004). Because bacterial gastrointestinal infections still affect a large number of individuals, even rare complications can, taken together, result in some mortality.

6.2.2 *Antibiotic therapy to minimize short term effects (Study* III)

This study was designed to answer the question whether antibiotic therapy has any effect on the duration of diarrhea compared to placebo in patients with campylobacterosis. Our findings support that antibiotic therapy shortens the duration of diarrhea with 1.32 days and also shortens the time period of excretion of bacteria from faeces. Furthermore, it seems that the effect of treatment is better the earlier it is administered. For every day won of treatment, the duration of diarrhea shortens with 0.35 days (p=0.30). However, our material was to small to yield any statistical significant results in this linear regression model.

Uncomplicated campylobacterosis seems to be easier to treat with antibiotics compared to infection with NTS. A meta-analysis of antibiotic therapy in uncomplicated NTS infection included 12 trials and 778 patients in all ages and the results showed that antibiotic therapy not only lacked effect, but could prolong detection of NTS in stool culture (Sirinavin and Garner, 2000). Others have found some support for effects of antibiotic treatment in situations with travelers' diarrhea were the causative agent is unknown (De Bruyn et al., 2000).

Even if antibiotic therapy has a proven effect in several RCTs, the clinical usefulness

of therapy is limited in most cases. There are two reasons for this. First, *Campylobacter* infection is in the majority of cases an unpleasant but self-limited and harmless disease. Second, antibiotic therapy produces resistance among *Campylobacter* isolates (Engberg et al., 2001; van Hees et al., 2007). It is therefore important to avoid unnecessary use of quinolones for campylobacterosis in human as well as veterinary medicine (Gallay et al., 2007; Nelson et al., 2007). Our study is by no means an argument to treat *Campylobacter* enteritis with antibiotics, on the contrary, we support the present opinion that uncomplicated *Campylobacter* enteritis should not be given any antibiotics.

It would have been interesting to see if antimicrobial treatment also could have an effect on complications, e.g. GBS, but this was not an aim in the present study.

6.2.3 *Bacterial gastrointestinal infection and complications (Study* IV*)*

In this study, we aimed to estimate the hospital treated complications after bacterial gastrointestinal infection. We could confirm well-known complications such as GBS after *Campylobacter* infection, reactive arthritis after *Yersinia* infection and HUS after an episode of EHEC.

Our risk estimate for HUS in patients infected with EHEC is lower than previously reported (Karch et al., 2005; Welinder-Olsson and Kaijser, 2005). An explanation to this could be that we only used ICD-codes specific for HUS. Several of these cases may in fact be classified under nonspecific ICD-codes that also include a large proportion of cases unrelated to HUS. But if we had included them in the analysis, any association with the infections would have been diluted. The risk for GBS among patients with campylobacterosis is in line with previous studies (McCarthy and Giesecke, 2001; Tam et al., 2006).

We found that acute salmonellosis was associated with increased risk of aortic aneurysm. This has been described in previous studies (Chen et al., 2007; Nielsen et al., 2006; Soravia-Dunand et al., 1999), and may not be that uncommon that is generally perceived among clinical doctors. In patients with atherosclerotic disease, or in those with pre-existing aneurysms, transient bacteremia with NTS can result in vascular infection (Chen et al., 2007; Fernandez Guerrero et al., 2004; Nielsen et al., 2006). Bacteria can in these cases invade the arterial intima and cause a localized endothelial infection that results in an aneurysm or the enlargement of a previously existing aneurysm. This may be the explanation to the association between *Salmonella* infection and aortic aneurysm in this study

Perhaps more unexpected was the association between *Salmonella* infection and an increased risk for ulcerative colitis (UC). The increased risk was also evident in the cohort of *Campylobacter* patients, but to a minor degree. Others have found the same association (Garcia Rodriguez et al., 2006) (Helms et al., 2006), and the seasonal

variation in the onset of UC, and reports that excessive childhood infections are associated with higher risk for UC, may support that infections could be triggers of disease (Farrell and Peppercorn, 2002). Note that 13 of 29 (44%) persons with UC in the *Salmonella* cohort, and 18 of 42 (43%) in the *Campylobacter* cohort had been diagnosed with UC in the 10-years period prior to infection. To be honest, we can not say whether we are observing a causal relationship between *Salmonella/Campylobacter* and the initiation of UC in predisposed individuals or relapse of UC among those with known disease. The findings could merely be a result of extensive numbers of stool cultures among patients with diarrhea and diagnosed or undiagnosed UC.

Because our study on short and long term effects after bacterial gastroenteritis is based on an in-patient hospital material, we will underestimate diseases that do not need hospitalization. This is obvious in the case of IBS (IBS is in this text used synonymously with FBD, functional bowel disorder: PI-FBD, postinfectious functional bowel disorder: PI-IBS, postinfectious irritable bowel syndrome). A recent meta-analysis has estimated the occurrence of IBS after gastroenteritis to 9.8% compared to 1.2% in the control group (OR 7.3; 95%: CI 4.7–11.1) (Halvorson et al., 2006). These findings seem to be valid also for Swedish conditions (Tornblom et al., 2007), and what have been suggested elsewhere (Rhodes and Wallace, 2006). Reactive arthritis is also a disease that does not need hospital treatment in many patients (Leirisalo-Repo et al., 2003) and although our risk estimates are in line with others (Rees et al., 2004), we probably underestimate the true risk.

6.2.4 *Public health impact*

Primary prevention of food-borne diseases has probably the best effect on mortality and complications due to bacterial gastroenteritis. There are some modifiable risk factors that should be communicated to the public. Raw poultry and meat must be stored in a cool place and should be properly cooked. It is also wise to separate raw food from cooked food to avoid contamination. Untreated water, unpasteurized diary products and raw eggs are high-risks food products that can lead to infection. Proper handwashing when handling raw meat in the kitchen as well as good kitchen hygiene is essential in all food preparation. Furthermore, handwashing with soap has been shown to minimize not only diarrheal illnesses, but also respiratory infections and impetigo among children in developing countries (Luby et al., 2005).

Travelers to areas where bacterial gastrointestinal infections are common need to be aware of the routes of transmission so that they can take precautions to avoid infection. Many buffet items are for example served at too low temperature and could harbor pathogenic bacteria.

In agriculture could unclean water supply infect animals that ultimately could infect humans. The strong association between the prevalence of *Salmonella* among laying hen flocks and risk for NTS among travelers (de Jong and Ekdahl, 2006), indicate

that control measures which reduce *Salmonella* prevalence in agriculture will reduce *Salmonella* among humans. Lack of hygiene procedures at slaughterhouses can contaminate carcasses with faeces and is another potential source of infection. Pet reptiles at home can be a source for NTS infection (de Jong et al., 2005; Swanson et al., 2007).

Improvement of drinking water quality will probably have the greatest impact in reducing food-borne infections (Clasen et al., 2007) and lower mortality and complications of bacterial gastroenteritis on a global scale.

6.2.5 *Future research*

The result of these investigations give rise to questions that needs to be addressed in future research. In epidemiological cohort studies are a sample of individuals at risk for the disease of interest followed over time, their exposure status is determined, and the goal is to determine the occurrence of some outcome. However, different forms of bias can disturb the incidence rate estimates.

The individuals in our cohorts have been enrolled based on the occurrence of a reported bacterial gastrointestinal infection. We have already mentioned problems of selection bias due to sampling (our cohort may differ in certain aspects from a theoretical cohort of all eligible participants). Another variant of selection bias we also have discussed previously is that of diagnostic bias: our patients with bacterial gastroenteritis may be more likely than the general population to be diagnosed with certain complications because the physicians suspect a relationship between for example *Campylobacter* infection and GBS. One way of solving this problem is to use a latency period (or left truncate data) between the time of exposure and the disease. But this may be problematic if the natural cause of events between exposure and disease is short. We therefore plan a future study were we investigate this relationship in detail and see what effect various latency periods and modeling approaches have on incidence rates.

Another study that we plan to do is to explore risk factors for NTS-bacteremia. Of all persons that are infected with NTS only a small fraction develop septicemia, or at least are findings of NTS sparse in blood cultures at most hospitals. We have designed a study were we plan to collect all positive samples of NTS in faeces and in blood during 2000–2007 at Karolinska University Hospital, Huddinge (a Swedish tertiary hospital). We will then look into medical journals for co-morbidities and medications and use a multiple logistic regression model to see if these factors have any effect on the risk to develop NTS-bacteremia.

7 CONCLUSIONS

- NTS infection and, to a lesser extent, *Campylobacter* infection is associated with an increased mortality risk among persons infected within Sweden.
- No increased mortality risk is evident for those infected with NTS or *Campylobacter* abroad.
- Antibiotic therapy shortens the duration of diarrhea compared to placebo in patients with *Campylobacter* infection.
- The effect of antibiotic therapy seems to be better the earlier in the course of disease it is administered.
- Due to the self-limited nature of *Campylobacter* infection, and the risk of pro ducing resistance, antibiotic therapy is not advisable in the normal case.
- Infection with NTS is associated with an increased risk for aortic aneurysms.
- NTS infection and *Campylobacter* infection may contribute to the initiation or relaps of UC, but these findings needs to be further explored.
- The risk for GBS, HUS and reactive arthritis, are increased in infection with *Campylobacter*, EHEC and *Yersinia* respectively.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING (SWEDISH SUMMARY)

Syftet med den här doktorsavhandlingen är att uppskatta vilken risk patienter med tarminfektion orsakad av *Salmonella* eller *Campylobacter* har att avlida och att utveckla vissa komplikationer i efterförloppet till den akuta infektionen. Ett annat mål har varit att undersöka om antibiotikabehandling förkortar sjukdomstiden vid tarminfektion orsakad av *Campylobacter*.

Vi har haft tillgång till Smittskyddsinstitutets nationella databas över patienter som är anmälda enligt Smittskyddslagen med tarminfektioner orsakade av *Salmonella* och *Campylobacter*, men även mindre vanligt förekommande bakterier såsom *Yersinia, Shigella* och enterohemorragisk *Escherichia coli* (EHEC). Totalt omfattar studiematerialet 101,855 deltagare som blivit anmälda med någon av dessa tarminfektioner under perioden 1997–2004. Förutom uppgifter om personernas ålder, kön och vilken bakterie som orsakade infektionen, har vi också uppgifter om när man blev sjuk och om man insjuknande utomlands eller i Sverige. Just den sista uppgiften är viktig eftersom en stor andel blir smittade på utlandsresa. Via personnummer har vi länkat patientuppgifterna i Smittskyddsinstitutets databas till Riksskatteverkets register över avlidna i Sverige. Vi har också gjort länkningar till Socialstyrelsens slutenvårds- och dödsorsaksregister. Från Statistiska Centralbyrån har vi fått uppgifter om dödligheten under olika år uppdelat på kön och åldersgrupper i Sverige.

Med hjälp av dessa register kunde vi identifiera personer som avlidit och tidpunkten för dödsfallen och vi kunde också bestämma vilka av studiedeltagarna som vårdats på sjukhus för komplikationer i anslutning till tarminfektion. Dessa observationer jämförde vi sedan med vad man kan förvänta sig att finna bland studiedeltagarna om dödligheten och sjukligheten vore densamma som hos motsvarande kvinnor och män i samma åldersgrupper i Sverige. Kvoten mellan vad man observerar i sin studiepopulation av antal döda eller insjuknade och vad man kan förvänta sig finna kallas standardized mortality/incidence ratio (SMR/SIR). Om SMR 1 så är dödligheten i patientgruppen lika med den i riket, om den är >1 är den förhöjd och om den är <1 så är den mindre än för Sveriges befolkning. Den slumpmässiga variationen i resultaten har beräknats genom att konstruera 95% konfidensintervall (CI). Ett sätt att tolka dessa är att säga att vi är 95% säkra på att det sanna resultatet ligger inom det uppgivna intervallet.

Resultaten visar att man blir tvungen att studera risken att avlida separat för gruppen som smittats utomlands och gruppen som smittats av *Salmonella/Campylobacter* i Sverige. För de som smittats av *Salmonella* i Sverige är SMR 5.6 (95% CI: 3.4–8.2) under de närmaste 30 dagarna efter insjuknande och för de med *Campylobacter* är risken att avlida 2.9 gånger större (95% CI: 1.9–4.0) jämfört med dödligheten i befolkningen under de första 30 dagarna efter insjuknande. För personer som smittats utomlands kunde vi inte påvisa någon ökad risk att avlida, tvärtom var den lägre

jämfört med förväntat: SMR för Salmonella var 0.6 (95% CI: 0.2–1.2) och för *Campylobacter* 0.3 (95% CI: 0.04–0.8).

Huruvida antibiotikabehandling är bättre än sockerpiller vid *Campylobacter*-infektion har enbart studerats i relativt små kliniska prövningar. Vi gjorde därför en analys av alla publicerade kliniska prövningar och slog ihop resultaten och räknade om dem på nytt i en så kallad meta-analys. Elva randomiserade kliniska prövningar som omfattade totalt 479 personer som antingen hade fått antibiotika eller placebo kunde identifieras. Resultatet visar att antibiotika minskar antalet dagar med diarré med i genomsnitt 1.32 dagar (95% CI: 0.64–1.99). Det verkar dessutom som att effekten av behandling är bättre ju tidigare man sätter in den. Det är viktigt att understryka att även om antibiotika har effekt vid *Campylobacter*-infektion så ska man undvika att använda detta av två skäl: För det första är infektionen i de allra flesta fall självläkande. För det andra bidrar användning av antibiotika till spridning av resistens hos bakterier.

I det avslutande arbetet i avhandlingen har vi undersökt förekomsten av komplikationer i efterförloppet till tarminfektion och om infektionen kan utgöra en riskfaktor för andra sjukdomar. Vi kunde bekräfta kända komplikationer såsom hemolytiskt uremiskt syndrom (sönderfall av röda blodkroppar och njursvikt) i anslutning till infektion med EHEC, Guillan-Barré syndrom (en förlamningssjukdom) och *Campylobacter*-infektion och reaktiv artrit (ledinflammation) i efterförloppet till infektion med *Yersinia*. Vi såg också att risken var ökad för aorta aneurysm (pulsåderbråck) hos gruppen med *Salmonella*-infektion, SIR 6.4 (95% CI: 3.1–11.8). Antagligen kan *Salmonella* förekomma kortvarigt i blod under en infektion och fastna i kärlväggen och orsaka en lokal infektion som kan leda till ett aneurysm eller öka storleken på ett aneurysm som redan finns där. Risken för ulcerös colit (inflammatorisk tarmsjukdom) var ökad i både gruppen som haft *Salmonella*, SIR 3.2 (95% CI: 2.2–4.6) och *Campylobacter*, SIR 2.8 (95% CI: 2.0–3.8). Vi kunde däremot inte se någon signifikant ökning av risken för Crohns sjukdom (en annan inflammatorisk tarmsjukdom) vare sig för patientgruppen med *Salmonella*, SIR 1.4 (95% CI: 0.8–2.3) eller *Campylobacter*, SIR 1.6 (95% CI: 1.0–2.3). Även om andra forskargrupper gjort liknande fynd så kan vi inte dra några säkra slutsatser om huruvida bakteriella tarminfektioner kan initiera ulcerös kolit hos mottagliga individer.

9 ACKNOWLEDGEMENTS

This research has involved many people who have contributed directly or indirectly to the progress of this work. I wish to thank you all, and especially:

Professor *Johan Giesecke*, my principal supervisor, for introducing me to the field of science, for never losing the overall perspective, but mostly for always supporting my work. I first met Johan 1996 when he involved me as a medical student to write a letter on EHEC to Lancet (it was refused) during my summer job at his department. He has taken me onboard several projects since then (including work during SARS epidemic), and is the person who has influenced my professional career the most so far.

Professor *Karl Ekdahl*, my co-supervisor, for enthusiasm, help, input and not at least rapid communication despite a great workload. I have in many ways learnt the handicraft of scientific writing during my employment at SMI where I had my room next to his.

Statistician *Anna Törner*, for carrying out the statistical analysis and for intelligent explanations, patience and stimulating collaboration during the analysis and writing of these papers. I also enjoy her advice on child care and opinions on popular TV-shows such as Melodifestivalen.

Statistician *Tommi Asikainen*, for the statistical analysis in the meta-analysis study and for stimulating discussions.

Åke Svensson, professor of mathematical statistics, for sound advice and help throughout my studies.

Fredrik Husander, programmer at SMI, for help with database management, and for squasch games during my time at SMI.

Dr *Pasi Pentinnen*, my former boss at SMI, for supporting my work initially.

Dr *David Lindström*, surgeon at Södersjukhuset, fellow doctoral student and friend, for comments on the cover story and whose competitiveness has spurred me during my studies.

All my *friends and former and present colleagues* – Department of Epidemiology SMI, Department of Infectious Diseases and Department of Acute Internal Medicine, both at Karolinska University Hospital – for encouragement and interest. You know who you are!

My parents, *Ingela* and *Gunnar* and my sister *Anna* for support at all times.

Finally, I acknowledge my partner, *Susanne*, and my children *Björn* and *Svante* for being around and making my time joyful.

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