Bacteriological and Epidemiological Studies of *Campylobacter* spp. in Swedish Broilers

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To whom it may concern

Abstract

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Campylobacter jejuni is the most frequently reported cause of bacterial gastrointestinal illness in humans in Sweden. Chicken products are considered an important risk factor for human infection. This thesis analyses and identifies sources and risk factors for colonisation of *Campylobacter* spp. in broilers at both farm level and slaughter. Slaughter groups with a low within-group prevalence were identified, split slaughter was confirmed as a risk factor and contamination of carcasses was shown to occur both during transport and during the slaughter process. Environmental campylobacter load was comparable on high and low incidence farms, indicating that hygiene regime is of greater importance than environmental load. Slaughter groups with a high within-flock prevalence had significantly higher campylobacter load in carcasses at slaughter than slaughter groups with a low degree of colonisation

Campylobacter jejuni is the *Campylobacter* sp. predominantly found in broilers. Strain characterisation below species level, so-called subtyping, is a helpful tool in epidemiological studies, *e.g.* in determination of transmission routes. Certain subtypes were shown to be common in Sweden and may have a higher ability to survive in the environment around broiler houses. The results from phylogenetic analysis indicated that typing based on 16S rRNA sequencing is not always sufficient for differentiating between *C. jejuni* and *C. coli*. However, nine different 16S rRNA types were identified among 47 Swedish campylobacter isolates from broilers.

About one-third of Swedish broiler producers seldom deliver any campylobacter-positive broilers to slaughter, demonstrating that it is possible to produce campylobacter-free chickens in Sweden. The factors identified as carrying the highest risk of producing campylobacter-positive broilers in Sweden were (i) insufficient general tidiness on the farm, (ii) split slaughter, (iii) an in-line position of the doors between the outside and access into broiler houses instead of an angled position. Furthermore, (iv) high risk farms often had other livestock such as cattle and pigs, and (v) high risk farms were more frequently situated in groves than in forest. Reducing the proportion of campylobacter-infected broiler flocks and the numbers of campylobacter on broiler carcasses would considerably lower the risk for consumers.

Keywords: *Campylobacter*, broilers, epidemiology, Sweden, surveillance, environment, transport, slaughter, 16S rRNA sequencing

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Sammanfattning

Campylobacter kan förekomma hos såväl djur som människor. Infektion med campylobacter är en zoonos, d.v.s. en sjukdom som kan smitta mellan djur och människa. I Sverige är campylobacter den bakterie som rapporteras orsaka de flesta bakteriella mag-tarminfektionerna hos människa. Kyckling och produkter av kyckling anses utgöra en viktig smittkälla, och smittöverföring sker oftast via otillräckligt värmebehandlat kött, eller via korskontamination i köket. Ett övervakningsprogram av campylobacter hos svensk slaktkyckling bedrivs i Svensk Fågels regi sedan 1991. Studierna i denna avhandling har gjorts i anslutning till övervakningsprogrammet.

Syftet med doktorandarbetet var att analysera och identifiera orsaker och riskfaktorer för campylobacter-infektion hos slaktkyckling i såväl uppfödar- som slakteriled. Resultaten kan senare ligga till grund för åtgärder för att minska andelen campylobacter-positiva kycklingflockar i primärproduktionen, vilket innebär att antalet campylobacter-positiva kycklingar kan minskas i konsumentledet. Förekomsten av campylobacter-positiva kycklingflockar varierar mellan olika regioner och uppfödare i Sverige. Cirka en tredjedel av uppfödarna levererar nästan aldrig campylobacter-positiva kycklingar till slakt. Studier har utförts för att klargöra skillnader mellan de uppfödare som ofta respektive sällan levererar campylobacter-positiva kycklingar. Analyser har utförts på prov tagna från stallar, förrum, ventilation, omgivning, insekter samt foder och vatten.

För att kunna skilja mellan olika stammar av campylobacter har s.k. subtypning utförts med molekylärgenetiska metoder. Subtypningen av campylobacter-stammarna tyder på att det är olika stammar vid olika uppfödningsomgångar, vilket kan tolkas som att campylobacter inte överlever i stallarna mellan uppfödningsomgångarna. Resultat från de kvantitativa undersökningarna visar att det finns ett samband mellan kycklingar med en hög andel campylobacter i kloaken och hög halt campylobacter i den konsumtionsfärdiga kycklingen. De kycklingar som blivit kontaminerade i samband med transport och slakt hade en lägre halt jämfört med de som blivit smittade under uppfödningsperioden, dvs. på gårdsnivå.

De faktorer som utgör de största riskerna för att en kycklingflock ska smittas av campylobacter är (i) bristande allmän ordning på gårdsnivå, (ii) förekomst av andra animalieproducerande djur på gården och (iii) delad slakt. Kycklingstallarnas utformning och läge har också betydelse, kycklingar uppfödda (iv) i stallar belägna i område med begränsad trädvegetation har ofta en högre campylobacterförekomst jämfört med de gårdar som finns i skogsområden. Stallar där (v) ytterdörren är i rak linje med dörren in till kycklingarna är oftare representerade bland de med stor andel campylobacter-positiva kycklingflockar till slakt.

Under 2000-talet har andelen campylobacter-positiva kycklingflockar visat en neråtgående trend. Studierna tyder dock på att andelen campylobacter-positiva kycklingflockar kan reduceras ytterligare.

Contents

Appendix

Papers I-VI

This thesis is based on the following studies, which are referred to in the text by their Roman numerals.

I Hansson, I., Engvall, E.O., Lindblad, J., Gunnarson, A. & Vågsholm, I. 2004. The *Campylobacter* surveillance program for broilers in Sweden, July 2001-June 2002. *Veterinary Record 155,* 193-196.

II Hansson, I., Ederoth, M., Andersson, L., Vågsholm, I. & Olsson Engvall, E. 2005. Transmission of *Campylobacter* spp. to chickens during transport to slaughter. *Journal of Applied Microbiology 99,* 1149-1157.

III Lindblad, M., Hansson, I., Vågsholm, I. & Lindqvist, R. 2006. Post-chill *Campylobacter* prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. *Journal of Food Protection 69*, 495-499.

IV Hansson, I., Vågsholm, I., Andersson, L. & Olsson Engvall, E. 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *Journal of Applied Microbiology*. Article published online: 7-Feb-2007 doi: 10.1111/j.1365-2672.2007.03291.x

V Hansson, I., Olsson Engvall E. & Vågsholm I. Risk factors for *Campylobacter* spp. in Swedish broiler flocks. Submitted.

VI Hansson, I., Persson, M., Svensson, L., Olsson Engvall, E. & Johansson, K-E. Sequence diversity of the 16S rRNA genes of *Campylobacter* spp. from broilers. Submitted.

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
CAT	Cefoperazone-Amphotericin-Teicoplanin agar
cfu	Colony forming units
DNA	Deoxyribonucleic acid
EC	European Commission
GBS	Guillain-Barré Syndrome
EFSA	European Food Safety Authority
ISO	International Organisation for Standardisation
mCCDA	modified Charcoal-Cefazolin-sodium Deoxycholate-amphotericin agar
MLST	Multilocus Sequence Typing
MS	Member States in EU
NMKL	Nordic Committee on Food Analysis
PFGE	Pulsed Field Gel Electrophoresis
PA	Preston agar
PCR	Polymerase chain reaction
PEB	Preston enrichment broth
REA	Restriction enzyme analysis
RFLP	Restriction fragment length polymorphism
rRNA	ribosomal Ribonucleic Acid
SMI	Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet)
SCP	Swedish Campylobacter programme
sp.	species (plural spp.)
subsp.	subspecies
SPMA	Swedish Poultry Meat Association

Background

The Genus *Campylobacter*

Historical aspects

The *Campylobacter* species were isolated and described for the first time in 1913 by McFadyean and Stockman as an important cause of bovine and ovine infertility and abortion. The bacterium involved was named *Vibrio* (now *Campylobacter*) *fetus*. The first time *Campylobacter* spp. were linked with diarrhoea was by Jones *et al*. (1931), who isolated a "Vibrio" in the intestine of cattle and calves with enteritis and named the organism *Vibrio* (now *Campylobacter*) *jejuni. Campylobacter* spp. were isolated from humans for the first time in conjunction with a milk-borne outbreak in the United States in 1938 (Levy, 1946). In the late 1950s, *Campylobacter* spp. were isolated from blood samples of children with diarrhoea and described by King (1957). She made three important observations, that the optimal temperature for the bacteria was 42°C, that the bacteria were isolated from humans with acute diarrhoea, and that the human strains were indistinguishable from the strains recently isolated from chickens. The crucial step was taken in Belgium in 1972 of isolating *Campylobacter* spp. from the blood and faeces of a previously healthy young woman with acute febrile haemorrhagic enteritis (Dekeyser *et al*., 1972). These observations elicited no response until Skirrow (1977) isolated the organism from a baby with febrile diarrhoea.

Taxonomy and classification

The genus *Campylobacter* belongs to the family *Campylobacteraceae* together with the genera *Arcobacter*, *Sulfurospirillum* and the generically misclassified species *Bacteroides ureolyticus,* which has been shown to belong to the genus *Campylobacter,* but its name has not yet been changed (Vandamme, 2000; http://www.bacterio.cict.fr/classifgenerafamilies.html#Campylobacteraceae).
Members of the genus *Dehalospirillum* that had been included as a genus within family *Campylobacteraceae* have recently been transferred to the genus *Sulfurospirillum* (Luijten *et al*., 2003). Species of the genus *Arcobacter* are the closest phylogenetic neighbours to the genus *Campylobacter* (Fig. 1) and were formerly included in the genus *Campylobacter*, as they are morphologically similar and share several genotypic and phenotypic features.

Scientific classification
Domain: *Bacteria*
Phylum: *Proteobacteria*
Class: *Epsilonproteobacteria*
Order: *Campylobacterales*
Family: *Campylobacteraceae*
Genus: *Campylobacter*

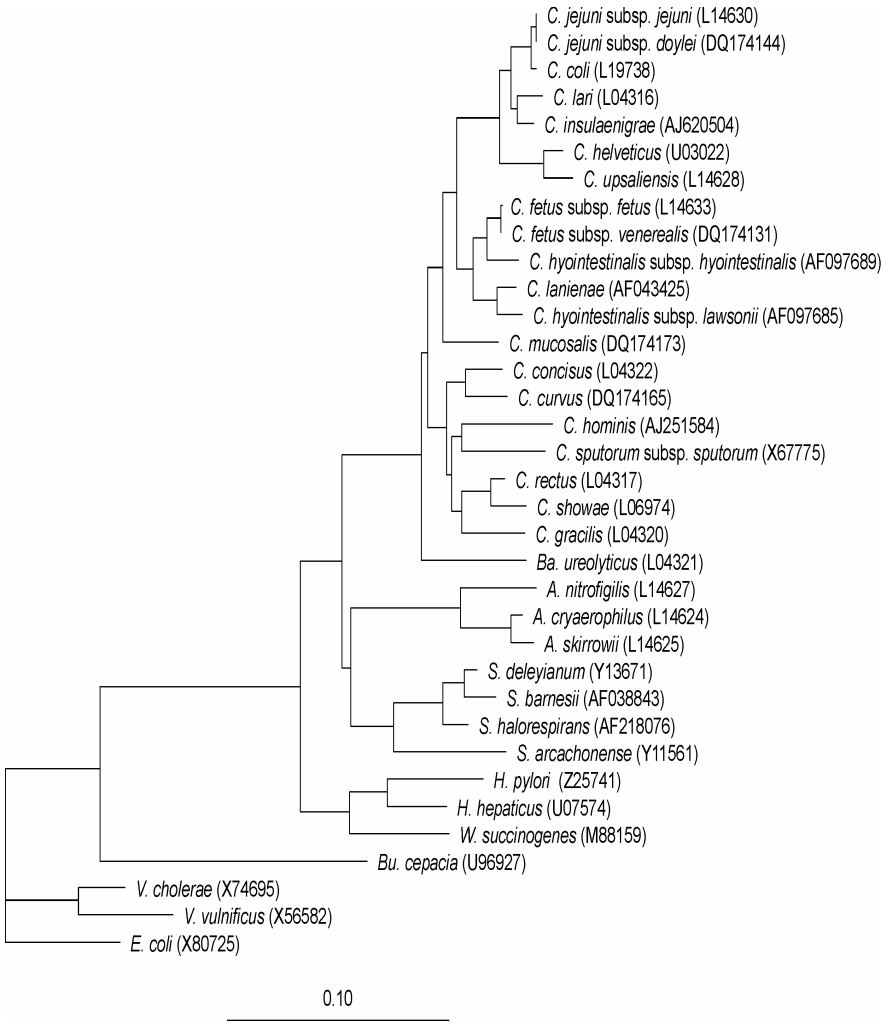


Figure 1. Phylogenetic tree based on the 16S rRNA genes of all members of the genus *Campylobacter* with officially approved species names. Some of the phylogenetically close neighbours, from the genera *Arcobacter* (*A*), *Sulfurospirillum* (*S*) and the generically misclassified species *Bacteroides (Ba) ureolyticus*, are included in the tree, as are some of the type strains from the genera *Helicobacter*, (*H*), *Burkholderia* (*Bu*), *Wolinella* (*W*) and *Vibrio* (*V*). The phylogenetic tree was constructed by neighbour-joining (Saitou & Nei, 1987) from a distance matrix comprising 1 450 nucleotide positions that was corrected for multiple substitutions at single locations by the two-parameter method (Kimura, 1980). The scale bar represents 10 nucleotide substitutions per 100 positions.

The genus *Campylobacter* was established in 1963 (Sebald & Véron, 1963) and its taxonomic structure has been revised over the years (Goodwin *et al*., 1989; Vandamme & De Ley, 1991; Vandamme & On, 2001). Earlier taxonomy was

mainly based on phenotypic data, such as serological and biochemical characteristics. However, the use of molecular methods in bacteriology has resulted in increased knowledge about biodiversity in bacteriology and within the genus *Campylobacter*. Sequence analysis of the 16S rRNA gene has proved useful for evolutionary studies in bacteriology (Woese, 1987) and is playing a major role in the rearrangement of the taxonomy for bacteria (Ludwig & Klenk, 2001). Subsequent developments in molecular biology have allowed revision of the genus. At present, the genus *Campylobacter* contains 17 species, four of which have been further divided into eight subspecies (http://www.bacterio.cict.fr/ 14-April-2007) (Fig. 1). The former suggested species *Campylobacter hyolei* has been transferred to *Campylobacter coli* (Vandamme & On, 2001).

The species *C. concisus*, *C. curvus, C. hominis, C. sputorum, C. rectus*, *C. showae* and *C. gracilis* are phylogenetically closely related (Fig. 1). Most of them have been found in the human oral cavity, but *C. hominis* has only been isolated in the human intestine and *C. sputorum*, which comprises two subspecies, has been found in the enteric and reproductive tract of animals such as sheep and cattle (On *et al*., 1998). *Campylobacter hyointestinalis* subsp. *lawsonii*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. laninae, C. fetus* subsp. *veneralis* and *C. fetus* subsp. *fetus* are also closely related as determined by phylogenetic analysis. *Campylobacter fetus* subsp. *veneralis* is mainly found in the bovine reproductive tract and is associated with bovine genital campylobacteriosis (epizootic bovine infertility). The main hosts of *C. fetus* subsp. *fetus* are sheep and cattle and this subspecies is most often connected with spontaneous abortion in cattle and sheep.

All *Campylobacter* spp. grow well at 37˚C The four species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* are often referred to as thermophilic *Campylobacter*, as most strains of these species exhibit optimal growth at a temperature of 42-43˚C. The species *C. jejuni* comprises two subspecies, *C. jejuni* subsp. *jejuni*, which is the most frequent cause of acute enteritis, and *C. jejuni* subsp. *doylei*, which may be more associated with paediatric diarrhoea in developing countries (Fernandez *et al*., 1997). *Campylobacter doylei* differs from *C. jejuni* in that it does not reduce nitrate or grow at 42˚C. In this thesis, *Campylobacter jejuni* subsp. *jejuni* is referred to as *C. jejuni* and *Campylobacter jejuni* subsp. *doylei* as *C. doylei.*

Morphology

Members of the genus *Campylobacter* are gram-negative, oxidase-positive and catalase-positive. Cells are curved, S-shaped or spiral rods 0.2-0.8 µm wide and 0.5-5 µm long, and sometimes when daughter cells remain joined, long spiral forms may be seen. The bacteria do not form spores and they do not ferment or oxidize carbohydrates. The bacteria are motile and move by a characteristic rotating rapid corkscrew-like motion, by unipolar or bipolar flagella. *Campylobacter* spp. are microaerophilic, but some species can also grow aerobically or anaerobically. Furthermore, *Campylobacter* spp. can undergo coccal transformation under stress, such as in old cultures and when exposed to oxygen. In the adaptation to coccoid morphology, *Campylobacter* spp. may lose their ability to grow on media, as they

can enter a viable but non-culturable stage (Rollinson & Colwell, 1986). However, the coccoid form is not necessarily associated with non-culturability. *Campylobacter* spp. are relatively slow-growing, fastidious bacteria that require specialised culture conditions. They grow best under reduced oxygen tension on nutritional basal media supplemented with 5-10% blood.

Laboratory diagnosis

There is no simple 'gold standard' for the routine isolation of all *Campylobacter* species. *Campylobacter* spp. are sensitive to environmental conditions such as dehydration and oxygen and both freezing and high temperatures, which can reduce their viability. Therefore transport to the laboratory should be as rapid as possible and preferably in suitable transport media, in order to protect the cells from drying out and from the toxic effect of oxygen (Cary & Blair, 1964; Luechtefeld *et al*., 1981).

Culture

Analysis of *Campylobacter* spp. is usually performed by direct plating on selective media or by enrichment followed by cultivation on solid selective media. Enrichment may be required if the bacteria are present in low numbers or have been damaged by environmental stresses such as temperature changes, nutrient deprivation, dehydration or exposure to atmospheric oxygen (Corry *et al*., 1995), whereas direct culture is recommended for samples in which a high level of campylobacter is suspected. A selective medium is necessary when a diverse flora is expected, such as in clinical samples from intestinal contents where large amounts of bacteria from the family *Enterobacteriaceae* are generally present. The selectivity of the medium is determined by the use of antibiotics. Cephalosporins are often used in combination with other antibiotics such as trimethoprim, vancomycin, amphotericin, rifampicin, since most *Campylobacter* spp. are resistant to these antibiotics. Several media are described in the literature for the bacteriological culture of thermophilic *Campylobacter* spp. (Corry *et al*., 1995). The main difference between these media is the amount and type of antibiotics used for inhibition of the contaminating flora. The media come from two main groups: blood-containing and charcoal-containing media. Blood components and charcoal remove toxic oxygen derivates such as peroxides, singlet oxygen and superoxide ions, which can be formed when media are exposed to light. These products are toxic to campylobacter due to the fact that they lack the enzymes superoxide dismutase and peroxidase (Corry *et al*., 1995). Pre-enrichment in particular commonly uses Exeter broth, Bolton broth, Preston broth, CEB (Campylobacter Enrichment Broth) and Park & Sanders broth. Commonly used selective media with blood components for campylobacter include Skirrow agar (Skirrow, 1977) and Preston agar (Bolton & Robertson, 1982), while selective media with charcoal include CAT agar (Aspinall *et al*., 1993), mCCDA (Hutchinson & Bolton, 1984) and Karmali agar (Karmali *et al*., 1986). In most protocols, a loopful of the enrichment is streaked onto a campylobacter-selective agar plate to obtain single colonies (ISO, 2006). Some media are favourable for some *Campylobacter* species,

for example some strains of *C. coli* are sensitive to polymyxin B and may therefore be inhibited in PEB and PA (Goossens *et al*., 1986) and some strains of *C. coli* and a few strains of *C. jejuni* may be missed due to their sensitivity to cephalotin (Brooks *et al*., 1986). For the moment, there is no single medium that allows growth of *C. jejuni* and inhibits *C. coli* or *vice versa*.

As thermophilic *Campylobacter* spp. in some samples can be difficult to culture, there are other methods available for isolation and identification, for example membrane filtration (Steele & McDermott, 1984), enzyme immunoassay (Endtz *et al*., 2000), and PCR-based methods (Lund *et al*., 2003, Lund *et al*., 2004).

Incubation of thermophilic *Campylobacter* spp. should preferably be performed at 42ºC to minimise the growth of contaminants. Most *Campylobacter* spp. are microaerophilic but a few species show a range of oxygen tolerance and some are almost anaerobic. Optimal growth is obtained in an atmosphere with 5-10% oxygen and 1-10% carbon dioxide (Bolton & Coates, 1983), and the growth of some species is enhanced by the presence of hydrogen (Goodman & Hoffman, 1983). Several methods are available to achieve the optimal gas mixture, by appropriate gas-generating envelopes such as Campygen (Oxoid Basingstoke UK) and Campypac (BBL Microbiology Systems,. Cockeysville,. Md.), or airtight jars with valves for evacuation and filling of gas, for example the Anoxomate system (Anoxomate, Mart-Netherlands). *Campylobacter* spp. grow in a spreading manner, characteristic campylobacter colonies are greyish and slightly pink with a metallic sheen on blood-containing agars. On charcoal-based media the campylobacter colonies are greyish to white with a metallic sheen (Fig. 2).

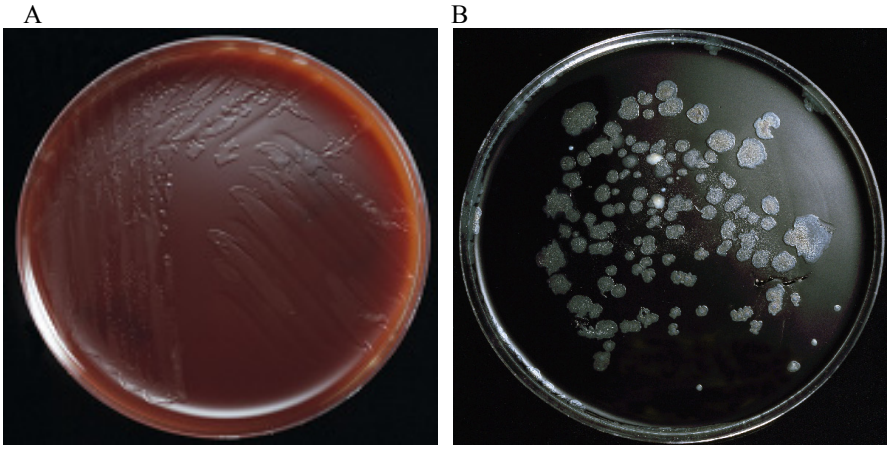


Figure 2. *Campylobacter jejuni* on (A) blood-based Preston agar and (B) charcoal-based mCCDA. (Photo: Bengt Ekberg).

Confirmation of *Campylobacter* spp. is mostly based on colony morphology, microscopic appearance and the following phenotypic characteristics: motility,

production of oxidase and catalase, and hippurate hydrolysis reaction (Nachamkin, 1995). *Campylobacter* spp. are typically spiral or curved rods with slender corkscrew motility, but bacteria from older cultures are less motile and coccoid forms occur. Bacteria from *C. jejuni* can be distinguished from other *Campylobacter* spp. on the basis of the hydrolysis of hippurate, as this is the only hippurate-positive species (Vandamme, 2000). However, hippurate hydrolysis-negative *C. jejuni* do exist (Totten *et al*., 1987). Biochemical speciation may be supplemented or replaced by molecular methods. A variety of polymerase chain reaction (PCR)-based methods for identifying thermophilic *Campylobacter* spp. have been developed (On, 1996; Linton *et al*., 1997; Fermer & Engvall, 1999; Vandamme, 2000).

Subtyping

Characterisation below species level, so-called subtyping, could be useful in epidemiological studies *e.g*. for determination of transmission routes and identification of sources of infection. A wide range of phenotyping and genotyping methods have been developed for subtyping *Campylobacter* spp. The chosen method should fulfil certain criteria. It should have the ability to subtype the majority of isolates, as a high discriminatory power is desirable to distinguish different subtypes, but should not be so sensitive that the association of each strain cannot be determined and the subtyping method should be reproducible. A number of genetic subtyping methods for *Campylobacter* spp. have been described, such as AFLP (amplified fragment length polymorphism) (Kokotovic & On, 1999), PFGE (pulsed field gel electrophoresis) (Gibson *et al*., 1995), ribotyping (Owen *et al*., 1990) and MLST (multilocus sequence typing) (Maiden *et al*., 1998; Dingle *et al*., 2001).

Campylobacter spp. have a small genome (Kim *et al*., 1993; Nuitjen *et al*., 1990; Taylor *et al*., 1992; Parkhill *et al*., 2000) and in previous studies it has been found that the *C. jejuni* and *C. coli* genomes are 1.7 to 1.8 Mb, which is only about 36% of the size of the *E. coli* chromosome (Smith *et al*., 1987). The DNA present is rich in A+T (adenine and thymine), with a G+C (guanine and cytosine) content of 29-36% (Véron & Chatelain, 1973; Lau *et al*., 1987; Taylor *et al*., 1992; Parkhill *et al*., 2000). Genomes of *Campylobacter* spp. only harbour three copies of the 16S rRNA gene, whereas *E. coli* has seven rRNA genomic loci (Nuitjen *et al*., 1990; Kim *et al*., 1992; Taylor *et al*., 1992). It is well known that *C. jejuni* comprises an extremely diverse population with a broad spectrum of subtypes (Wassenaar & Newell, 2000). The ribosome is present in all self-replicating cells and forms the heart of protein synthesis. In bacteria the ribosome is composed of two subunits, one small (referred to the 30S subunit) and one large (the 50S subunit). Both subunits contain ribosomal proteins and ribosomal RNA (rRNA). Of the bacterial rRNAs, 16S has received the most attention and has been used in a number of phylogenetic studies (Lau *et al*., 1987; Thompson *et al*., 1988). The evolutionary distance between two bacteria can be estimated from their sequence differences in the 16S RNA. Bacteria can also be identified by calculating the 16S rRNA sequence similarity with other bacteria using programmes such as BLAST, which is available at the website of the National Centre of Biotechnology Information

(http://www.ncbi,nlm.nih.gov) (BLAST option), and Sequence Match, which is available at the website of the Ribosomal Database Project, RDP-II (http://rdp.cme.msu.edu/).

Campylobacter in humans

During the 1970s, campylobacter enteritis was recognised as an emerging food-borne disease (Skirrow, 1977). One of the first documented outbreaks of campylobacteriosis, which was directly attributed to consumption of chicken, occurred in The Netherlands (Brouwer *et al*., 1979). Campylobacteriosis is a zoonosis and an important public health problem in most areas of the world, with considerable socio-economic implications. *Campylobacter* spp. can be transferred from animals to man directly after contact with animals or through consumption and handling of contaminated food products. In industrialised countries, people of all ages are affected by campylobacteriosis, but in developing countries infection by campylobacter is so frequent in children that they become immune, and therefore it rarely affects older children and adults (Skirrow, 1994).

The incubation period for campylobacteriosis is usually 2 to 5 days and the symptoms are mild to moderate, with diarrhoea (mild to severe, sometimes frequent, explosive and bloody). Most patients also have fever, abdominal pain, nausea and malaise. The clinical symptoms of campylobacter infection are often indistinguishable from those caused by other enteric pathogens such as salmonella and shigella. Campylobacter infections are usually self-limiting within a week, but in about 20% of patients the symptoms may persist for 1 to 3 weeks (Allos & Blaser, 1995). Post-infection complications include reactive arthritis and *C. jejuni* has been implicated as a trigger of Guillain-Barré syndrome (Altekruse *et al*., 1999; Nachamkin *et al*., 2000). The frequency of arthritis following infection with campylobacter is probably low. However no correlations have been found between the severity of gastrointestinal symptoms and the development of Guillain-Barré syndrome (Allos & Blazer, 1995).

Campylobacter spp. do not replicate in food, but replication is not necessary, as only a low dose is required to cause infection. A few reports are available on the infective dose of campylobacter. In two experimental infections in humans, *C. jejuni* has caused illness at an oral dose of 500 cfu (Robinson, 1981) and 800 cfu (Black *et al*., 1988). The molecular mechanisms involved in the pathogenesis of campylobacteriosis are still little understood. A number of *Campylobacter* species have been implicated in human disease, with *C. jejuni* and *C. coli* being the most common. Diagnosis of campylobacteriosis can only be established by detecting campylobacter in faeces. About 90% of the isolates from human campylobacteriosis are identified as *C. jejuni* and most of the remaining cases are identified as *C. coli* (EC, 2002), but other *Campylobacter* species, for example *C. lari, C. upsaliensis, C. fetus and C. consisus*, have also been associated with campylobacteriosis (Skirrow *et al*., 1993; Lindblom *et al*., 1995; Bourke *et al*., 1998).

Campylobacteriosis has been highlighted as the most frequently reported zoonotic disease in humans within the EU (EFSA, 2006). In most European countries, the number of reported cases of campylobacteriosis increased during the 1990s, with a total of 200,122 cases of campylobacteriosis in humans being reported in 22 Member States (MS) and two non-MS in 2005 (EFSA, 2006). The overall incidence of campylobacteriosis was 51.6 per 100,000 inhabitants, with a remarkably wide range of variation in the incidence among the reporting countries, as Poland reported 0.1 cases per 100,000 inhabitants, France 3.3 and Czech Republic 302.7. A higher similarity was apparent between the data from the Netherlands, the UK and the Nordic countries, as Iceland reported 43.6 confirmed cases per 100,000 inhabitants, the Netherlands 46.2, Norway 57.1, Sweden 66.2, Denmark 68.0, Finland 76.4 and the UK 88.5 (EFSA, 2006). Campylobacteriosis is also one of the most common intestinal disorders in non-European countries. In 2001, the incidence was 125 cases per 100,000 inhabitants in Australia and 14 in the United States (Vally *et al*., 2005). The highest national rate of reported campylobacteriosis in the developed world is in New Zealand, exceeding 400 cases per 100,000 inhabitants (Baker *et al*., 2006). However, these figures take no account of differences in healthcare systems or laboratory practices between the countries.

It has been established that campylobacteriosis is a multi-factorial problem. Case control studies (Tauxe, 1992; Kapperud *et al*., 1992; Kapperud *et al*., 2003) have suggested that a major source of human infection is handling and consumption of contaminated poultry meat. The majority of the campylobacter infections in humans are sporadic cases; major outbreaks are exceptional but happen occasionally, usually due to consumption of contaminated water (Melby *et al*., 1991; Andersson *et al*., 1997) or unpasteurised milk (Blaser *et al*., 1979; Hanninen *et al*., 2003).

Campylobacteriosis in Sweden

Campylobacteriosis is the most commonly reported bacterial gastroenteritis in Sweden and it is notifiable under the Communicable Diseases Act. A positive case is defined as a person from whom *Campylobacter* spp. has been isolated. The number of reported cases of campylobacteriosis in humans per year in Sweden has increased since the compulsory reporting system started in 1989 (Anonymous, 2004). The proportion of domestic cases has varied between 31-46% during the past 10 years (Fig. 3). The true number of campylobacteriosis cases is unknown, but it is surmised that only 10% of true cases are reported (Studahl, 1999). The number of cases of gastroenteritis is probably under-reported because many people with relatively mild symptoms do not seek medical care and physicians do not always send stool samples for analysis. As the majority of the cases are contracted abroad, travel patterns have a considerable impact on the total number of cases. Most cases are sporadic, which makes the investigation of the source and vehicles of a single case very difficult.

There is a seasonal variation in human campylobacteriosis in Sweden (http://www.smittskyddsinstitutet.se/statistik/campylobacterinfektion/) and in many other countries (Nylen *et al*., 2002; Kovats *et al*., 2005), with a peak during the summer. The peak coincides with seasonal habits of travelling abroad but domestically-acquired infections also rise during this period. This increase is distributed all over the country and throughout all age groups (http://www.smittskyddsinstitutet.se). The causes of the seasonal peak are not really known, but in Sweden, as in other northern countries, the amount of sunlight coupled with warmer temperatures during the summer months greatly affects human behaviour and may encourage outdoor activities, such as picnicking, camping, swimming and different eating habits, with more barbecues compared with the wintertime. There may also be an increased exposure to campylobacter in nature, which increases the risk of campylobacteriosis.

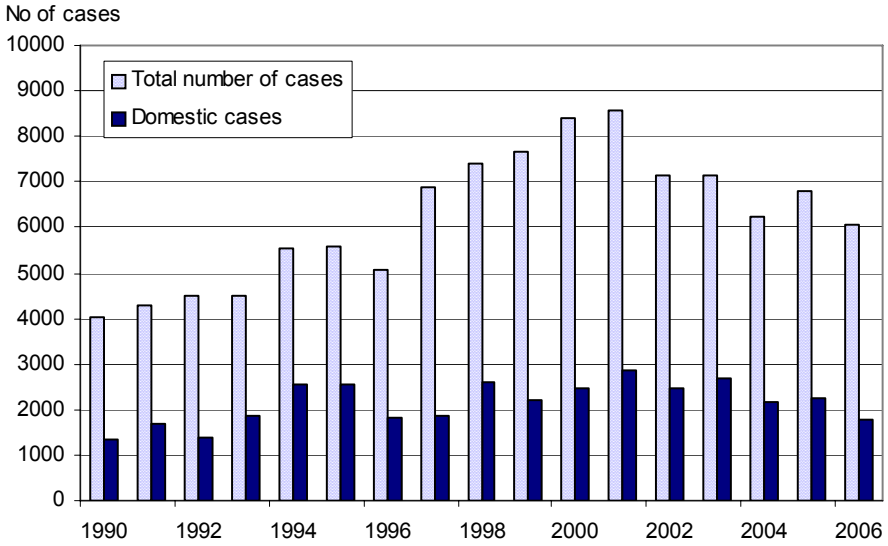


Figure 3. Reported number of cases of human campylobacteriosis in Sweden 1990-2006 (http://www.smittskyddsinstitutet.se).

In a recently published report from the Swedish Institute for Food and Agricultural Economics (http://www.sli.lu.se/pdf/Press_r20071.pdf), the costs of campylobacteriosis and salmonellosis are calculated. For the estimated number of 80,000 true cases of campylobacteriosis per year in Sweden, the total cost of both direct (medicine and hospital treatment and non-institutional care) and indirect decreases in productivity has been calculated to be 217 million SEK per year. The corresponding figure for the 17,000 estimated cases of salmonellosis is 80 million SEK per year.

Campylobacter in animals other than broilers

In many animal species, *Campylobacter* spp. occur as commensals in the gastro-intestinal tract. *Campylobacter jejuni* is predominantly found in poultry but has also been isolated from cattle, pigs and sheep (Manser & Dalziel, 1985; Quinn *et al*., 1994; Stanley *et al*., 1998; Stanley & Jones, 2003). *Campylobacter coli* is predominant in pigs but has also been isolated from poultry, cattle and sheep (Manser & Dalziel, 1985; Nielsen *et al*., 1997). Thermophilic *Campylobacter* spp. have not been associated with gastroenteritis in production animals, but *C. jejuni, C. helveticus, C. lari* and *C. upsaliensis* have been isolated from both diarrhoeic and healthy dogs and cats (Stanley *et al*., 1992; Moreno *et al*., 1993; Hald & Madsen, 1997; Sandberg *et al*., 2002; Olsson Engvall *et al*., 2003). *Campylobacter coli* has not been described as a cause of disease either in production or pet animals. Campylobacter infection in animals is not a notifiable disease in Sweden, except for bovine genital campylobacteriosis caused by *C. fetus* subsp. *veneralis*.

Campylobacter in broilers

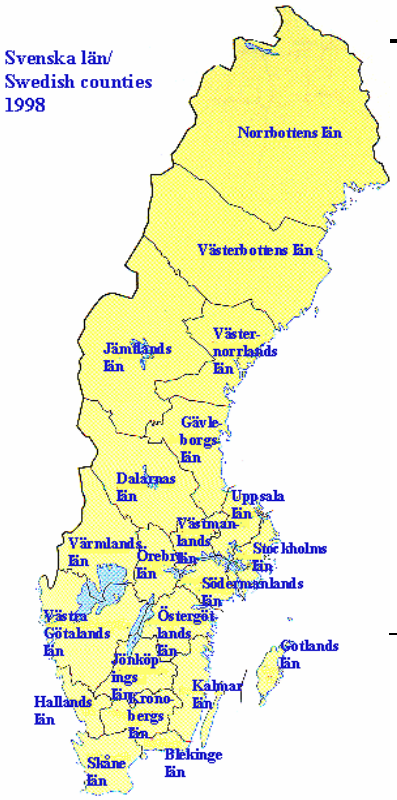
Birds appear to be the main reservoir for thermophilic *Campylobacter* spp. presumably because of their high body temperature. All kinds of birds can be colonised with *Campylobacter* spp. and even wild birds are frequently colonised (Waldenström *et al*., 2002). Broilers host campylobacter without showing any symptoms of disease. However, broilers colonised with campylobacter excrete large amounts of the bacteria, usually up to 10^8 cfu per gram faeces (Altmeyer *et al*., 1985; Stern *et al*., 1995). Within flocks, campylobacter can spread from chicken to chicken via coprophagy, but also by air and by contaminated feed and water lines in the house.

A decreasing trend in campylobacter incidence in broiler flocks was reported from 2000 to 2004 in Norway (Hofshagen & Kruse 2005), Denmark (Anonymous, 2006a) and Sweden (Hansson *et al*., 2007a). In the Community Summary Report on Trends and Sources of Zoonoses in the European Union in 2005 (EFSA, 2006), data on prevalence of campylobacter-positive poultry flocks are available from 12 of the EU member states. In the reported data from 2005, Sweden had a flock prevalence of 13.3%, Denmark 29.9%, Norway 3.4% and Finland 1.0%. Other countries in Europe reported a higher flock prevalence, *e.g*. France 85.2%, Germany 50.4% and Italy 45.3%. However, no consideration was given to method of detection or age and type of birds (conventional, free range or organic).

Swedish broiler production

The Swedish Poultry Meat Association includes and covers the entire production chain; feed manufacturers, producers of hatching eggs, hatcheries, broiler production farms and slaughter houses. Members of the Swedish Poultry Meat Association produce 98-99% of all domestic broilers. Members are obliged to participate in both mandatory and voluntary animal health programmes such as the salmonella control programme, the coccidiosis and clostridia control programme, and the welfare and classification programme, including foot health scoring as an indicator of welfare, and only to use feeds from approved feed factories.

Table 1. Regional distribution and differences in campylobacter-positive slaughter batches delivered from producers who are members of the Swedish Poultry Meat Association during a five-year period (2002-2006) within the Swedish Campylobacter Programme for broilers



	No of producers	Mean prevalence
County (Län)		
Skåne	35	21.9
Blekinge	25	20.0
Södermanland	11	16.1
Halland	20	15.5
Örebro-Västmanland	4	14.7
Kronoberg	5	11.4
Östergötland	5	10.0
Västra Götaland	21	9.0
Kalmar	14	8.5
Total number of producers	**140**	**16.2%**

There are two companies importing breeding stock for broiler production into Sweden. Swe-Chick import the breed Ross from Scotland and Blenta/North Chicken the breed Cobb from England. The breeding stock is brought in to Sweden as day-old grandparents. Three broiler hatcheries (four until 1 July 2002) supply the broiler producers with day-old commercial broiler chickens. In 2001, there were seven poultry slaughter companies within the Swedish Poultry Meat Association (SPMA), with nine slaughter houses, but the structure has changed so that by 2006 there were five slaughter companies with six slaughter houses. Swedish broiler production is concentrated to the southern part of Sweden, where the majority of Swedish grain is produced (Table 1). Most of the broiler producers also produce cereals, which are used as a feed complement as 'whole wheat'.

In Sweden, a broiler farm is a holding with one or several houses, each house consisting of one to four compartments. Between grow-outs there is a strict 'all in-all out' regime, which means that for a certain period of time, there are no broilers at all on the premises. On each holding, up to eight grow-outs per year are produced. One flock can be split into one or two (rarely three) slaughter batches, often referred to as split slaughter (or thinning). Broiler production is strictly scheduled, with preplanned date of hatch and date of slaughter. During 2001, 70.8 million broilers were delivered from 127 producers with 499 compartments. During 2005, 72.5 million broilers were delivered from 123 producers with 454 compartments, all members of SPMA. In 2006 there was a slight reduction in the total number of broilers produced to 71.5 million, but with an increase in broiler weight of 87 grams, resulting in an increased broiler meat volume of 4.5% In 2001, about 78% of the chicken consumed in Sweden originated from Swedish producers, but this figure had decreased to 60% in 2006, mainly as a result of chicken imports.

Swedish Campylobacter Programme

A surveillance programme for broilers operated by the Swedish Poultry Meat Association commenced in 1991 and involved sampling of all flocks at slaughter. An extended programme was initiated on 1 July 2001, based on the Swedish Board of Agriculture's regulation 1993:42 on organised health control and financed by the Swedish Board of Agriculture, the Swedish Poultry Meat Association and the European Commission (2001-2005). The aim was to reduce the occurrence of campylobacter in the food chain through preventive measures, starting with primary production, and in the long run to develop a campylobacter-free production system.

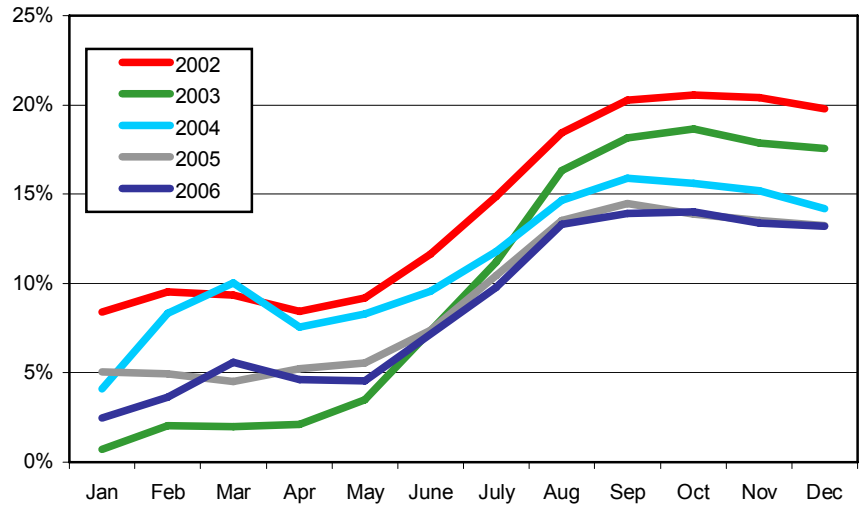


Figure 4. Cumulative incidence of campylobacter in broilers in Sweden. Based on cloacal samples in 2002-2005, caecal samples in 2006.

There are no compulsory sanctions or penalties for broiler producers in Sweden delivering campylobacter-positive broilers. Nevertheless, most of the slaughter companies pay a minor premium of 0.05 SEK per kg liveweight (1 EURO = 9.17 SEK, 12-April-2007) for campylobacter-free flocks. A decreasing trend has been identified during 2001-2006, as the prevalence of campylobacter-positive slaughter groups delivered by members of Swedish Poultry Meat Association has gradually been reduced from 20% to 13% (Fig. 4). Since broiler flocks once infected stay infected until slaughter, the cumulative incidence of campylobacter infection is equal to the flock prevalence at slaughter.

The aims of the thesis

The aims of this thesis were to analyse and identify sources and risk factors for colonisation of campylobacter in broilers both at farm level and at slaughter. Reducing the proportion of campylobacter-infected flocks and reducing the numbers of campylobacter on poultry carcasses may considerably lower the risk to consumers of campylobacteriosis. Although many studies have been performed on campylobacter in broilers, there is still lack of knowledge on the best way of preventing campylobacter colonisation in primary production.

The aims of the thesis were achieved by:

- Obtaining an overview of the presence of campylobacter in broilers both at farm level and at slaughter (Papers I, III and IV)
- Investigating the variation in prevalence of campylobacter in broilers at farm level and slaughter
  - during the season (Papers I, IV and VI)
  - within flocks (Papers I and II)
  - between producers (Papers I, IV and V)
- Studying the transmission of campylobacter to broilers
  - at farm level (Papers IV and V)
  - during transport to slaughter (Papers II and IV)
  - during the slaughter process (Papers I and III)
- Evaluating the relationship between the degree of intestinal colonisation and the prevalence and numbers of campylobacter on carcasses (Paper III)
- Investigating potential relationships between campylobacter presence in the environment and in broiler flocks and comparing the isolates by subtyping (Paper IV)
- Studying the relationship between campylobacter flock prevalence and the broiler house surroundings, design and impact of management (Papers IV and V).
- Evaluating the diversity of the gene for 16S rRNA from *Campylobacter* spp. and studying whether the sequence variation in the gene is sufficient to be used in subtyping of strains from broilers (Paper VI).

Considerations on Materials and Methods

A brief introduction and some additional information on materials and methods used in the analyses are presented here. Further details are given in Papers I-VI.

Sensitivity of pooled samples

In these studies, analyses were mostly performed on pooled samples consisting of ten individual samples. One of the questions raised during the studies concerned the sensitivity of pooled samples compared with individual samples. Therefore, a small study was performed in the laboratory to investigate this issue. Ten pooled samples each consisting of a positive caecal sample from each of ten individual caecae were analysed and campylobacter were found in all ten pooled samples. Furthermore, in ten pools consisting of one positive swab out of ten swabs, campylobacter were also found in all ten pooled samples. An evaluation of sampling and culturing methods has been performed within the Norwegian action plan against campylobacter in broilers (Sandberg *et al*., 2006). The results from this Norwegian study indicate that that three pooled cloacal samples are needed to identify 90% of the positive flocks. The results from the modelling of caecal data indicated that samples from seven individual carcasses were sufficient to identify 90% of the positive flocks. These results indicate that the sensitivity of the method used is high for the pooled samples, in that even if only one of the samples in a pool harbours campylobacter, the organism will mostly be detected in a pooled sample.

Direct culture versus enrichment

The presence of campylobacter was mainly detected by the NMKL method no. 119 with enrichment in Preston broth (PEB) followed by culture on selective Preston agar (PA) (Papers I, II, IV and VI). Several protocols are in current use for the bacteriological culture of *Campylobacter* spp. Until 2005, the bacteriological method used in the Swedish Campylobacter Programme (SCP) for broilers was NMKL method no. 119. In 2005, the cloacal samples analysed by PEB+PA were complemented with caecal samples analysed by direct culture on modified Charcoal-Cefazolin-sodium Deoxycholate agar (mCCDA). In the SCP during 2006 and 2007, the prevalence of campylobacter in broilers was determined by direct culture on mCCDA of caecal samples, as described in the OIE Terrestrial Manual (2004). In Paper III, chicken carcasses were analysed both by enrichment and by direct culture. The direct plating analysis used in Paper III resulted in a higher percentage of campylobacter-positive carcasses, mostly non-*C. jejuni*. This could be explained by the fact that certain strains of *C. coli* are known to be sensitive to polymyxin B (Goossens *et al*., 1986), one of the components of PEB, resulting in differences in the results between enrichment and direct plating (Paper III).

An evaluation of direct culture on mCCDA-agar and PEB + PA was performed in our laboratory. The following samples originating from the SCP were analysed: faecal droppings on the floor of the broiler house, cloacal samples of live broilers taken at farm level, and cloacal and neck skin samples from broilers at slaughter

(Table 2). All samples were cultured both in PEB followed by PA, and directly on mCCDA agar. In faeces and in cloacal swab samples, the results did not indicate any difference between the two methods. However, for neck skin samples, the use of enrichment in PEB followed by plating on PA resulted in 8% more positive neck skin samples compared with direct culture on mCCDA (Table 2).

Table 2. Comparison between mCCDA medium and Preston Enrichment Broth + Preston agar in 430 samples originating from the Swedish Campylobacter Programme for broilers

	mCCDA / PEB+PA			
Samples	Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg
Cloacal, farm level	5	0	1	90
Faecal, farm level	1	1	0	28
Cloacal, slaughter	36	4	2	154
Neck skin, slaughter	18	0	9	81
Total	60	5	12	353

Sampling routines

Sampling at farm level, faecal vs. cloacal and sock samples

Sampling was performed on live birds, on the environment around the broiler houses at farm level and on different body parts of broilers at slaughter (Papers I, II, III and IV). There is currently no universally accepted standard method for sampling and isolating *Campylobacter* spp. from farm samples. The similarities between samples from faecal droppings and cloacal swabs of live broilers were compared (Table 3). The results from the study indicate no significant difference between caecal and cloacal samples, as about 18% of the cloacal samples and 19% of the faeces samples tested positive for campylobacter and in 97% of the samples, the same result was found with both sampling methods (Table 3).

Table 3. Cloacal samples from live broilers compared with faecal droppings taken at the same time during 2003 from 664 slaughter groups sampled within the Swedish Campylobacter Programme for broilers, 2003

Faecal droppings	Cloacal	No. of slaughter groups
Negative	Negative	530 (80%)
Negative	Positive	9 (1%)
Positive	Negative	11 (2%)
Positive	Positive	114 (17%)

Sampling within the broiler house was performed by sock samples in Paper IV. Sock sampling has not been a common practice in analysis of campylobacter.

However, it has been used for analysis of salmonella (Skov *et al*., 1999; Gradel *et al*., 2002; Buhr *et al*., 2007) and since 1 January 2007 it is being used for routine analyses of salmonella in poultry in Sweden (SJVFS, 2007). The results of sampling by socks and faecal droppings were compared in a small study during the high prevalence season (Table 4). The producers took swab samples in 10 faecal droppings at three different locations in the house and pooled them into three pooled samples with 10 samples in each pool. Sock samples were taken inside the house by walking at least four times on the longest distance from wall to wall, preferably during ordinary work in the broiler house, as described in Paper IV. In the comparison between sock samples and fresh dropping samples, campylobacter was found in faecal droppings in 58 (95%) of the 61 flocks with positive sock samples. In 3 (1%) of the flocks, campylobacter was isolated from the socks but not from the faecal droppings. This could be due to a recent introduction of campylobacter into the flock that only involved a small number of broilers, and the sampling by faecal droppings not covering that part of the house. However, in six flocks, campylobacter was isolated from the faecal droppings but was not found in the sock samples (Table 4). The number of positive samples from faecal droppings may indicate the spread of campylobacter in the broiler house. When campylobacter was found in only one or two of the faecal dropping samples, it was assumed that the bacteria had not spread through the whole broiler house.

Table 4. Campylobacter in sock samples compared with faecal droppings from broilers sampled at farm level within the Swedish Campylobacter Programme for broilers, 2005

	No. of positive faecal droppings				
	0 of 3	1 of 3	2 of 3	3 of 3	Total
Pos. sock samples	3	0	4	54	61 (16%)
Neg. sock samples	310	3	1	2	316 (84%)
Total no. of samples	313 (83%)	3 (1%)	5 (1%)	56 (15%)	377 (100%)

Sampling at farm level versus at slaughter

Within the Swedish Campylobacter Programme in 2005, four different types of samples from slaughter groups were compared, namely sock samples at farm level and cloacal, caecal and neck skin samples at slaughter. In 1489 slaughter groups with all four types of sample, campylobacter was found in 12.2% of the sock samples at farm level. However at slaughter, campylobacter was found in 15.7% of the cloacal samples, 13.6% of the caecal samples and in 20.6% of the neck skin samples (Fig. 5). All differences were statistically significant. Sampling by sock samples at farm level should be the sampling method that best reflects the current campylobacter level in a flock. According to Swedish results, the caecal sample is the type of sample at slaughter that best represents the situation at farm level. The differences in campylobacter incidence between the different samples are probably due to contamination during transport to slaughter and during the slaughter process (Hansson *et al*., 2007a; Papers I, II, III).

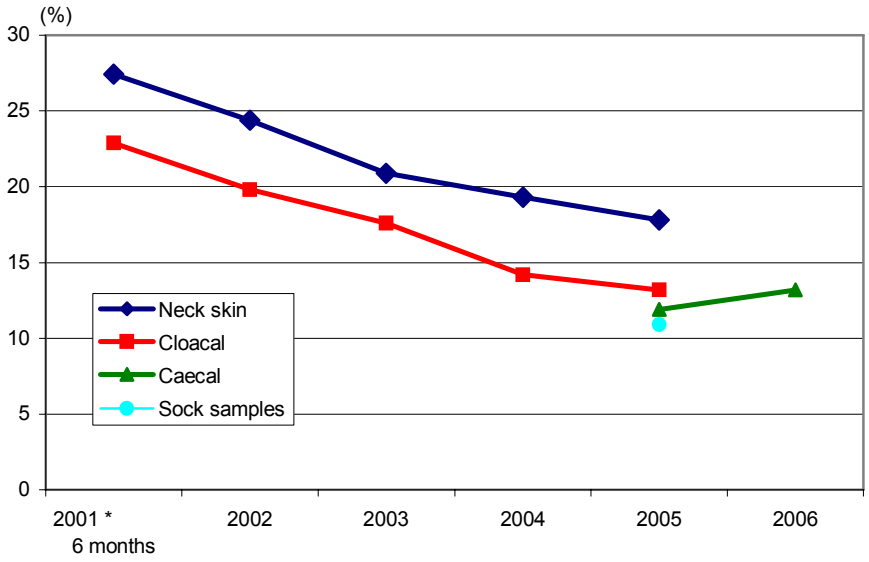


Figure 5. Annual incidence of campylobacter at farm level and at slaughter in the Swedish Campylobacter Programme in broilers, 2001-2006.

Quantification of Campylobacter

An enumeration of *Campylobacter* spp. in carcass rinse samples was performed in Paper III. One limitation of this study might be that only one carcass was analysed from each slaughter group. Hence, that sampled broiler may not have been representative of the whole slaughter batches of 2 000 - 50 000 broilers. In a minor study performed in 2006 (unpublished) within the Swedish Campylobacter Programme on carcass rinse samples, at least five carcasses from each slaughter group were sampled with the aim of determining differences in the load of campylobacter per carcass within a slaughter group.

In 22 of the 29 slaughter groups analysed, campylobacter was found prior to slaughter. In those 22 groups, campylobacter enumeration in the rinse samples per slaughter group showed that the within-group colonisation varied from 80% to 100% with a variation between 1.0-3.2 log(10) cfu per mL rinse sample within a slaughter group (Fig. 6). The results thus confirmed the large variation in campylobacter load within slaughter groups and this variation should be considered when analysing only one carcass in a flock.

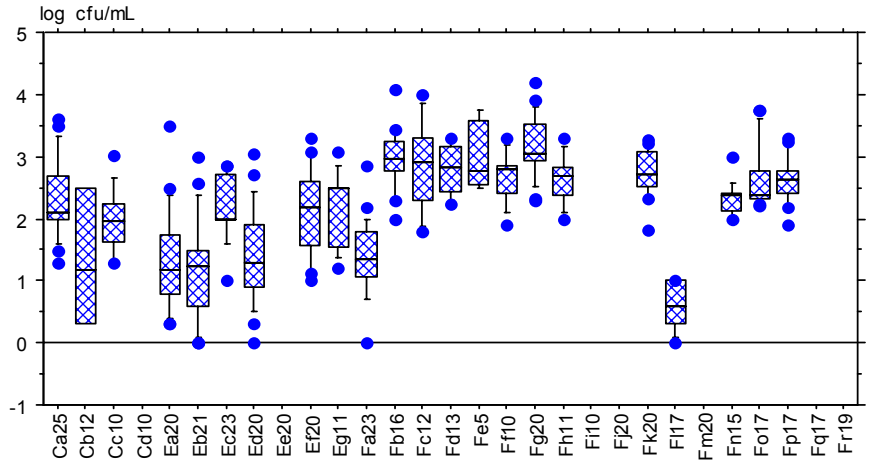


Figure 6. Variation in campylobacter load (cfu per mL) in carcass rinse samples within a flock. The capital letter denotes the same slaughter house as in Paper III. The number represents the number of carcass rinse samples taken within a flock. The boxes show values between the 25th and 75th percentiles. So-called extreme values (below the 10th and over the 90th percentile) are shown as circles in the figure.

Comments on subtyping by PFGE and 16S rRNA

One of the aims of this thesis was to investigate potential relationships between campylobacter presence in the environment, in the broiler flocks and at slaughter. The restriction enzyme used here to digest the DNA was *Sma*I. The use of more than one enzyme would have increased the discriminatory power of the technique. It is recommended by On *et al*. (1998) that a second enzyme be used before relatedness is established. Furthermore, by using additional subtyping techniques, for instance AFLP, flaA typing or MLST, the discriminatory power can be considerably increased. It should also be noted that as only one colony was routinely collected from each selective agar plate in Papers II and IV, colonisation by multiple campylobacter strains could have remained undetected.

Comparison of 16S rRNA sequences was performed in Paper VI. This technique is more commonly used in bacterial taxonomy to infer phylogenetic relationships. The diversity of the gene for 16S rRNA from *Campylobacter* spp. was evaluated to study whether the intraspecific variation in the 16S rRNA genes is sufficient to be used in subtyping of strains from broilers and to determine whether the 16S rRNA sequence types correlate to the different *Sma*I types.

Farm visit and questionnaire

The questionnaire used during the farm visits included questions about the surroundings of the farm concerning the presence of other animals nearby, the design of buildings, the house environment, the hygiene and working routines (Paper V). All 37 broiler producers were visited once by the author, who completed the questionnaire. The different factors were studied to find possible associations with the campylobacter incidence. Some questions were answered either yes/no, good/bad or countable, while the hygiene barrier and general tidiness were divided into three classes; poor, average and good. The aim was to identify risk factors for *Campylobacter* spp. in Swedish broiler flocks, in particular with regard to the broiler house environment, the design of buildings and the management of the farm.

A number of studies have been performed to explain transmission routes of *Campylobacter* spp. into broiler flocks (Kapperud *et al*., 1993; van de Giessen *et al*., 1996; Evans and Sayers, 2000; Refiegier-Petton *et al*., 2001; Bouwknegt *et al*., 2004; Cardinale *et al*., 2004; Barrios *et al*., 2006; Bull *et al*., 2006). Most of these studies have been performed by a questionnaire delivered to the farm manager, who completed it. This might result in a 'right answer' but not always a truthful answer, introducing a risk for misclassification bias. In direct interviews, the answers obtained may require some degree of interpretation. For example, one of the questions raised in Paper V concerned the use of coccidiostats. All farmers used coccidiostats as feed additives until 5-7 days before slaughter, in compliance with Swedish regulations. The coccidiostats were added to the feed by the feed manufacturer and 31 of the farmers said they used Monteban® and six said they used Narasin. A significant difference in campylobacter incidence at farm level was found between the producers using Monteban® compared with those using Narasin. However Narasin is the active ingredient in Monteban®, so it is actually the same coccidiostat. This illustrates the possibility of obtaining erroneous significant results when investigating a large number of possible risk factors due to chance or to confounding bias in a univariate analysis.

Results and Discussion

Variation in Campylobacter prevalence

Variation among broiler producers

It appears possible to produce campylobacter-free chickens in Sweden, as during the first year of the Swedish Campylobacter Programme, 24% of the producers did not deliver any campylobacter-positive slaughter groups. One of the main issues considered in this thesis is how some producers succeed in producing campylobacter-free broilers year after year. The results show that producers who frequently produced campylobacter-positive slaughter groups delivered significantly more slaughter groups compared with producers who never or sporadically produced campylobacter-positive slaughter groups (Paper I). Producing a higher number of slaughter groups means that more transport and movement of broilers, feed and litter take place around the farm. However, differences in the incidence of *Campylobacter* spp. in the surroundings outside the broiler houses were negligible between the producers that often/rarely deliver campylobacter-positive broilers (Paper IV). The factors identified through farm visits as carrying the highest risk of producing campylobacter-positive broilers in Sweden (Paper V) were (i) insufficient general tidiness and (ii) presence of other livestock such as cattle, pigs and poultry on the farm. The increased risk of *Campylobacter* spp. in broiler flocks with livestock on the farm or in the vicinity was not unexpected, as it has been found in several previous studies (Kapperud *et al*., 1993; van de Giessen *et al*., 1998; Bouwknegt *et al*., 2004). Farm animals such as cattle and pigs are often carriers of *Campylobacter* spp. (Gregory *et al*., 1997; Stanley *et al*., 1998). Paper V concluded that (iii) producers who applied split slaughter had a higher incidence of campylobacter-positive broilers; (iv) broiler houses designed with the doors between the outside and in to the broilers situated in-line instead of at an angle to each other were associated with a higher incidence of campylobacter-positive flocks; and (v) farms situated with a grove in the neighbourhood were associated with a higher campylobacter incidence than farms that had forest near the houses.

Variation within a flock

Most of the positive slaughter groups had a high within-flock prevalence, with four positive cloacal samples out of four (Paper I). The fraction of positive groups with one or two positive cloacal samples out of four (*i.e*. low within-flock prevalence) was 18% in Paper I, with a range of 6 - 38% between the different slaughter houses. Producers categorised as frequently delivering positive slaughter groups had a significantly higher ($P<0.001$) fraction (76%) of four positive cloacal samples out of four (high within-flock prevalence) than those with a low occurrence (58% had four positive samples out of four). The fraction of positive groups with a low within-flock prevalence in Paper I is in agreement with later years of the SCP, in which 18-19% of the positive slaughter groups had a low within-flock prevalence during 2002-2004 (Anonymous, 2006b). No obvious explanation could be found

for the findings in this study of low within-flock prevalence. However it is possible that a late introduction of campylobacter could spread less rapidly in a flock, or that part of a flock could have become contaminated during transport to the slaughter house. In previous studies (Jacobs-Reitsma, 1995; Berndtson *et al*., 1996a), it has been found that broiler flocks become colonised by campylobacter at about 3-4 weeks of age, with a within-flock prevalence close to 100%, and that affected chickens remain colonised until slaughter. However the results from this thesis and within the Swedish Campylobacter Programme indicate that a low within-flock prevalence exists in Sweden (Anonymous, 2006b).

Seasonal variation

A seasonal variation in campylobacter in broilers, with a peak in the summer, was observed in Paper I. This is in agreement with previous reports (Berndtson, 1996; Anonymous, 2004) and more recent reports of campylobacter in broilers in Sweden (Anonymous, 2006b; Hansson *et al*., 2007a) (Fig. 7). It seems that it is difficult to control campylobacter in broilers in the summer. This seasonal variation has also been observed in broilers in many other countries such as Norway (Hofshagen & Kruse, 2005), Denmark (Wedderkopp *et al*., 2000; Bang *et al*., 2003), the Netherlands (Bouwknegt *et al*., 2004), and in the number of human cases of campylobacteriosis both in Sweden and in other temperate countries (Nylen *et al*., 2002; Patrick *et al*., 2004; Kovats *et al*., 2005; Meldrum *et al*., 2005). Therefore factors relating to climate may be important for campylobacter infection of broiler flocks and humans.

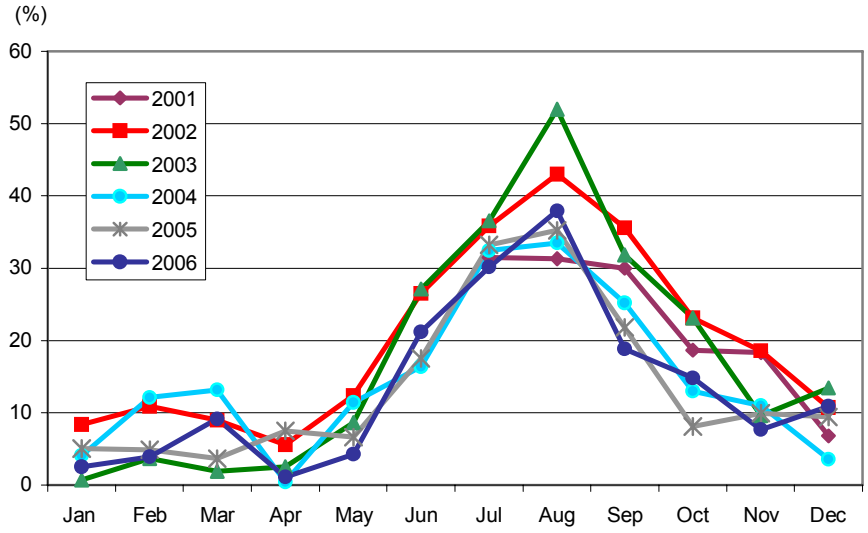


Figure 7. Seasonal variation in campylobacter prevalence in broilers slaughtered in Sweden (cloacal samples taken at slaughter 2001-2005, caecal samples 2006).

The producers recorded the weather conditions during sampling days in Paper IV. The environment outside the broiler houses was sampled by sock samples, which were analysed qualitatively but not quantitatively. The presence of campylobacter in environmental samples can be a sign of recent faecal contamination because *Campylobacter* spp. are unable to multiply outside the host animal and they survive for a shorter time than other bacteria found in the intestinal tract.

The month with the highest temperature during sampling days was August (Paper IV), which is also the month with the highest prevalence of campylobacter-positive slaughter groups (Fig. 7). This indicates a possible relationship between temperature and campylobacter survival and transmission of infection to broilers and humans. Further results from Paper IV indicate that the chances of finding campylobacter in the environment outside the houses were just as high in October-November as in July-September (Fig. 8).

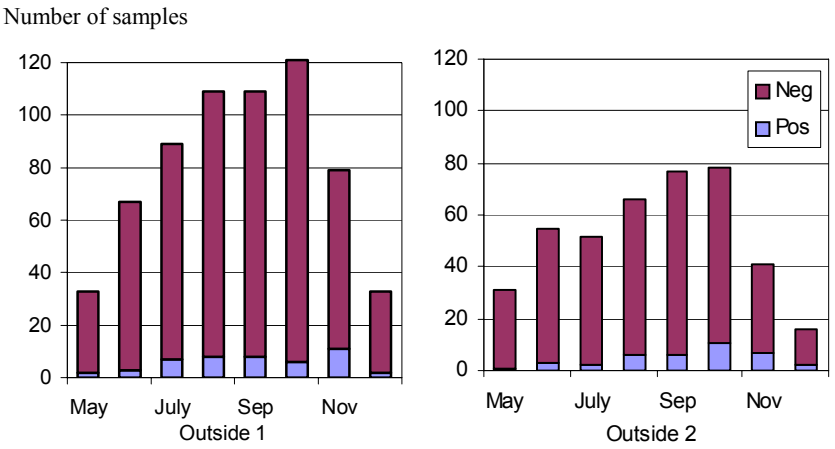


Figure 8. Number of positive and negative sock samples from the environment outside the broiler houses on 31 farms in Sweden, May - December 2004 (Paper IV).

Campylobacter is most likely to be found inside broiler houses in August (Fig. 9). The reason could be that in August the temperature is higher, which leads to the need for increased ventilation, which in turn increases the possibilities for transmission of *Campylobacter* spp. by vectors such as flies entering the broiler houses. Flies are more numerous in August compared with October and November. A seasonal fly activity has also been observed, as most flies are inactive when the temperature is less than 15ºC (Hald, B., pers. comm.)

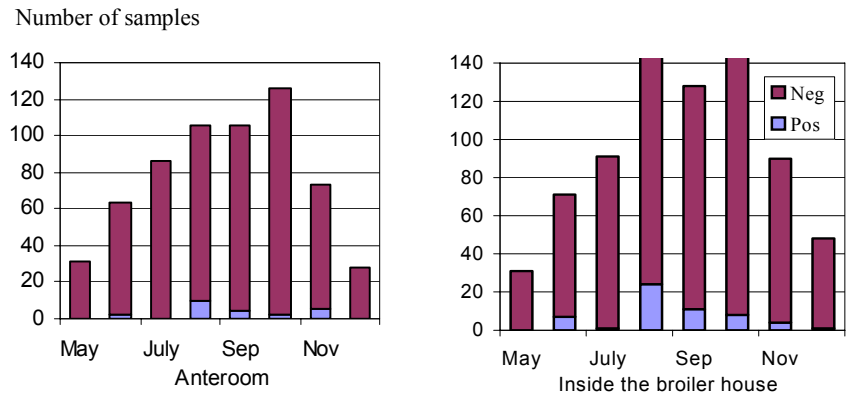


Figure 9. Number of positive and negative sock samples taken in the anteroom and in the broiler houses on 31 farms in Sweden, May - December 2004 (Paper IV).

Regional variation

Variations in campylobacter incidence were found between different slaughter houses within SPMA (Paper I). This variation was mainly due to the status of incoming broilers. The differences between slaughter houses have continued during the period 2002-2006 within the SCP (Fig. 10). Regional variations in campylobacter prevalence in broilers have also been found in other countries (Hofshagen & Kruse, 2005).

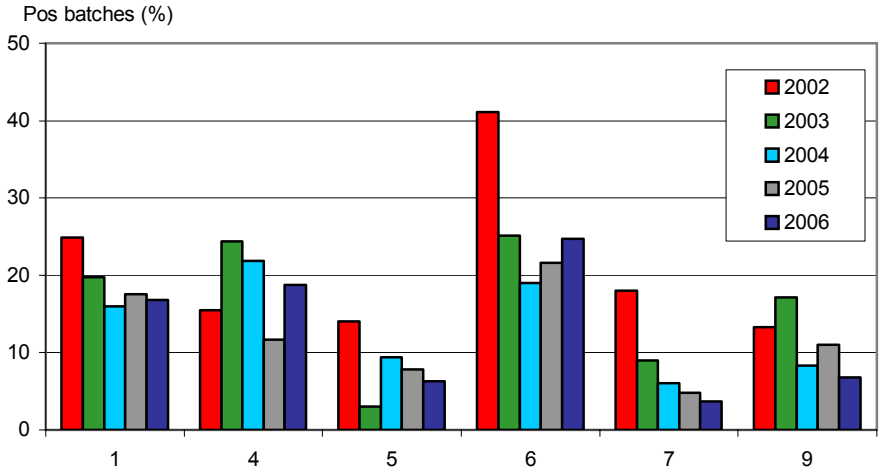


Figure 10. Variation in campylobacter incidence in broilers at slaughter during 2002-2006 at the six biggest slaughter houses in Sweden. The slaughter houses are those described in Paper I and are denoted here by the same numbers.

During farm visits, no obvious differences were found in terms of management, general tidiness or design of buildings that could explain the variation between the

different regions in Sweden (Paper V). However, farms were selected in order to include farms that frequently or that rarely delivered campylobacter-positive broilers from all regions.

The Swedish counties with broiler producers with the highest campylobacter incidence in Sweden are Skåne and Blekinge (Table 5), which are situated in the south of Sweden. This part of Sweden has a high level of livestock and the farms are situated closer to each other. Furthermore, the amount of forest is limited in both these counties compared with the rest of Sweden. The results from Paper V show that the presence of other livestock on-farm or in the vicinity was associated with a higher campylobacter incidence. Furthermore, the farms associated with a high campylobacter incidence were more frequently situated in groves in the neighbourhood rather than in forest.

Table 5. Regional variation in campylobacter incidence in different regions of Sweden. Analysis performed according to ANOVA, means with different superscript are significantly different according to the Scheffe procedure

County	No of farmers	Mean prevalence	P-value
			0.0004
Skåne-Blekinge	60	21.1 [a]	
Södermanland - Örebro -Västmanland	15	15.8 [ab]	
Halland-Kronoberg	25	14.6 [ab]	
Västra Götaland	21	9.0 [b]	
Östergötland-Kalmar	19	8.9 [b]	

There are other bacterial zoonoses that show an even higher regional variation in Sweden. A study by Eriksson *et al*. (2005) found that the prevalence of verotoxin-producing *E. coli* O157 in dairy herds was significantly higher (23.3%) in the county of Halland compared with the rest of Sweden (P<0.01).

Transmission routes for *Campylobacter* spp. at farm level

No significant statistical differences were found regarding occurrence in the environment between producers who often deliver campylobacter-positive slaughter groups (>30% positive slaughter groups per year) and those who rarely deliver campylobacter-positive slaughter groups (<10%) (Paper IV). A high level of general tidiness at the farm appeared to be an important factor linked to low prevalence of flock infection (Paper V). Adopting strict hygiene barriers could help to stop the transmission of campylobacter to chickens. Johnsen *et al*. (2006) observed that on farms with the poorest measures, broilers became infected at the youngest age and flocks on farms with the best hygiene barriers did not become infected. Further studies have found that farm workers are important in transmitting

campylobacter to broiler flocks (Humphrey *et al*., 1993; Berndtson *et al*., 1996a; van de Giessen *et al*., 1996; Gregory *et al*., 1997; van de Giessen *et al*., 1998; Gibbens *et al*., 2001; Nauta *et al*., 2005b). Some of the risk factors identified in an earlier farm study on 18 broiler farms in Sweden (Berndtson *et al*., 1996a) were also found ten years later in 2004-2005 (Paper V). These include split slaughter, other poultry in the vicinity and an insufficient hygiene barrier. However, some other risk factors found in the 1990s were not observed in 2004-2005. These include enlarged size of the flock, age at slaughter and empty period. Furthermore, in 2004-2005 ventilation through wall valves and surroundings with a grove close to the farm were associated with a higher campylobacter incidence, but not in the study ten years previously. Broiler production has changed during these years, *e.g*. the empty period has been shortened, the use of split slaughter has decreased, broilers are younger at slaughter, the maximum permissible flock density has increased from 25 to 36 kg/m^2 and the mean flock size were 17 900 in the study during the 1990s and 28 000 in 2004-2005.

The transmission of campylobacter at farm level could be due to insufficient hygiene measures between visits to the different livestock within the farm or by insects acting as vectors between different animals. Transmission via the air may also be important for spreading the organism between broilers and between different farm animals. It is well documented that flies can act as vectors and transmit *Campylobacter* spp. (Rosef & Kapperud, 1983; Shane *et al*., 1985; Khalil *et al*., 1994; Hald *et al*., 2004b; Nichols, 2005; Hald *et al*., 2005). The number of flies entering the broiler house increases when the ventilation rate is increased, and this has to occur during summer and as broilers enter the final stages of growth. Once campylobacter are present, they usually spread rapidly by feed, water, air and litter. Furthermore, coprophagy may partly explain the rapid transmission.

Campylobacter spp. have been found in groundwater (Jones *et al*., 1990; Hanninen *et al*., 1998; Rosef *et al*., 2001) and contaminated groundwater has been implicated as a source in the introduction of campylobacter into poultry (Pearson *et al*., 1993; van de Giessen *et al*., 1998). In the beginning of 2004, ultraviolet light irradiation of the water was introduced on one of the study farms with a campylobacter incidence above 60% between 2001 and 2003. During 2004-2006, campylobacter incidence on that farm decreased to less than 10% positive slaughter batches per year. Split slaughter, with a batch depletion of up to three slaughter batches during a maximum of four days due to thinning, is still in use in Sweden. Those producers that used split slaughter had a significantly higher incidence of campylobacter. It was concluded that campylobacter were introduced during depletion of broilers, when catching the first batch, and thereafter spread through the entire flock within a week (Hald *et al*., 2001).

A so-called 'Hawthorne effect' was observed after the farm visits in Paper V. The Hawthorne effect refers to the phenomenon that when people are observed in a study, their behaviour or performance temporarily changes (http://en.wikipedia.org/wiki/Hawthorne_effect#_ref-3). Others have broadened this definition to state that people's behaviour and performance change following any new or increased attention. The term gets its name from a factory called the Hawthorne Works,

where a series of experiments on factory workers were carried out between 1924 and 1932. During the farm visits in the present study, there was a discussion with producers on how to improve the hygiene barrier and other sources that might have an impact on campylobacter prevalence. The prevalence of campylobacter-positive slaughter batches delivered from the 37 broiler producers visited decreased significantly from 21.3% (1 July 2001- 30 June 2004) to 15.6% during 2005 ($p=0.0005$). In addition, 7 of the 37 farmers had a dramatic decrease (more than 20%) in positive slaughter batches, which could be referred to as the 'Hansson effect'. The 'Hansson effect' was also observed in September 2006, whereas a quantitative study was performed (Fig. 7). The author personally contacted the broiler producer before the study, and in 2006 the campylobacter incidence was below 20% for the first time in the month of September (Fig. 11).

The time of broiler colonisation by Campylobacter

Broilers are campylobacter-free on the day of hatching, as the bacteria are not vertically transmitted, in contrast to salmonella. Under normal commercial broiler production conditions, broilers are rarely colonised by *Campylobacter* spp. before two weeks of age (Jacobs-Reitsma *et al*., 1995; Berndtson *et al*., 1996b, Newell & Wagenaar, 2000; Bull *et al*., 2006; Hansson *et al*., 2007). Maternal antibodies may partly protect young broilers from campylobacter infection (Sahin *et al*., 2001). Vertical transmission from infected breeder flocks has not been proven and hence horizontal transmission from the environment is more likely (Shanker *et al*., 1986; van de Giessen *et al*., 1992; Humphrey *et al*., 1993; Jacobs-Reitsma, 1997; Petersen *et al*., 2001; Callicott *et al*., 2006). The proportion of colonised flocks increases gradually with age until the time of slaughter (Gregory *et al*., 1997; Herman *et al*., 2003). The results at farm level indicate that broilers often become colonised by campylobacter during the last week before slaughter (Paper IV), in agreement with two other studies performed within the SCP 2003 and 2006 (Anonymous, 2006b). In the study during 2003, cloacal samples from 648 flocks were taken at farm level 1-2 weeks before slaughter, at the same time as the producers took samples to be tested in the Swedish Salmonella Programme, and campylobacter were found in 8% of the flocks. In the same flocks sampled less than 24 hours before slaughter, campylobacter were found in 21% of these flocks. In the study within the SCP performed in 2006 on 220 flocks, campylobacter were found in 22% of the flocks sampled by sock samples one week before slaughter, and in 36% of the flocks sampled on the same day as transport to slaughter. These results confirm that campylobacter are often introduced during the last week prior to slaughter and logistic slaughter based on samples taken 1-2 weeks before slaughter is of limited value because most flocks become colonised during their last week. During this week, consumption of feed and water, growth rate and the amount of manure produced all increase and the ventilation rate also has to be increased.

Contamination during transport and processing

During processing, microorganisms can be transferred from contaminated broiler carcasses to water, equipment, workers' hands and between carcasses. A large amount of water creates a favourable environment for the dehydration-sensitive microbes, which could lead to contamination of carcasses at slaughter. The higher prevalence in neck skin samples compared with cloacal swabs, taken when the broilers are hanging on the slaughter line before scalding, represents process contamination. In Sweden, process contamination during 2001-2002 was 8% (Paper I) and during 2002-2005 between 6-7% (Hansson *et al*., 2007a). Hence, a higher campylobacter burden entering slaughter houses poses a risk of carcass contamination. During the slaughter process, bacterial levels may either increase or decrease in the various process steps (Bryan & Doyle, 1995; Rosenquist *et al*., 2006). It was found that slaughter groups with low within-group prevalence had significantly more negative neck skin samples compared with slaughter groups with high within-group prevalence (Paper I). Furthermore, the level of *Campylobacter* spp. on broiler carcass was higher in slaughter groups with a high degree of intestinal colonisation (high within-flock prevalence) (Paper III).

The risk for cross-contamination of campylobacter-free broilers transported in contaminated crates must be considered (Paper II). If transport containers are not adequately cleaned, faecal material from a previous campylobacter-positive flock could contaminate the following flock (Newell *et al*., 2001; Slader *et al*., 2002; Hansson *et al*., 2005). In addition, the delivery of contaminated crates from the slaughter house may contribute to the introduction of campylobacter onto farms, and this is of even higher importance when 'thinning' is practised. Thinning is split slaughter, in which a part of a flock is kept for further rearing. After delivery of broilers to slaughter, all transport crates are cleaned with hot water, sometimes with the addition of a disinfectant. In analyses of transport crates performed in Papers II and IV, *Campylobacter* spp. were isolated from 58% and 53% of crates respectively. However, even when *Campylobacter* spp. were found in 58% of the crates, the PFGE results were only able to confirm contamination via crates in a few flocks. This could be explained by the limited number of isolates analysed per transport crate sample; 10 crate samples were pooled to one and only one colony from each positive sample was analysed by PFGE subtyping. The number of corresponding strains between crates and samples at slaughter might have increased if the ten crates had been analysed individually, or if more than one isolate from each batch of crates had been analysed by PFGE.

In Sweden, producers earn a premium for delivering campylobacter-free flocks to slaughter. Until 2004, this premium was based on the results from the cloacal samples at slaughter, as this was thought to correspond to the campylobacter status at farm level. However, the results from the transport crate studies showed that the results of the cloacal samples could be due to contamination of the crates. Therefore, during 2005 the premium was based on the sock samples at farm level and since 2006 the premium has been based on results from the caecal samples at slaughter.

Campylobacter load in relation to the results at farm level and slaughter

Slaughter groups with a high within-flock prevalence (more than half of the pooled cloacal samples positive) had significantly higher campylobacter load in carcasses at slaughter compared with slaughter groups with a low degree of colonisation (Paper III). The average numbers of *Campylobacter* spp. on positive carcasses from slaughter groups with negative cloacal samples were relatively low, about 1,000 cfu per carcass, with a range of 2.6-5.0 log(10) cfu per carcass (Paper III). This should be compared with about 10,000 cfu per carcass with a range of 2.6-7.0 log(10) cfu per carcass from slaughter groups with positive cloacal samples. The results from Paper III were confirmed in a quantitative study performed during 2005 within SCP on neck skin and whole carcass rinse samples. In that study, a significant difference was found when comparing the results from the different sampling sites at slaughter. Those slaughter groups for which campylobacter had already been found in at least one of the samples (sock and faecal droppings) at farm level had a mean campylobacter load, which was 1,000-fold higher in the quantitative analyses compared with those slaughter groups where campylobacter was only found at slaughter. Furthermore, slaughter groups with positive caecal samples at slaughter also had a mean campylobacter load, which was 1,000-fold higher as compared with those slaughter groups where campylobacter was found only in the cloacal and/or neck skin samples (Hansson *et al*., 2007a).

In another study performed in 2006 in which one carcass rinse sample was analysed from 220 flocks, campylobacter were found in 22% of the flocks sampled by sock samples one week before slaughter (Fig. 11), and in 36% of the flocks sampled on the same day as transport to slaughter. Unfortunately, no difference was found in campylobacter load in carcass rinse samples between flocks that were colonised in the last days before slaughter compared with those colonised at least one week before slaughter. However, those flocks where campylobacter was only found in carcass rinse samples and not at farm level or caecal samples at slaughter had a significantly lower campylobacter incidence (Fig. 11). Similar results with a correlation between campylobacter concentrations in the intestinal contents and on chicken carcasses after defeathering have also been found in other studies (Nauta *et al*., 2005b; Rosenqvist *et al*., 2006; Allen *et al*., 2007). Hence the usefulness of logistic slaughter with the aim of lowering the risk for human consumption must be questioned.

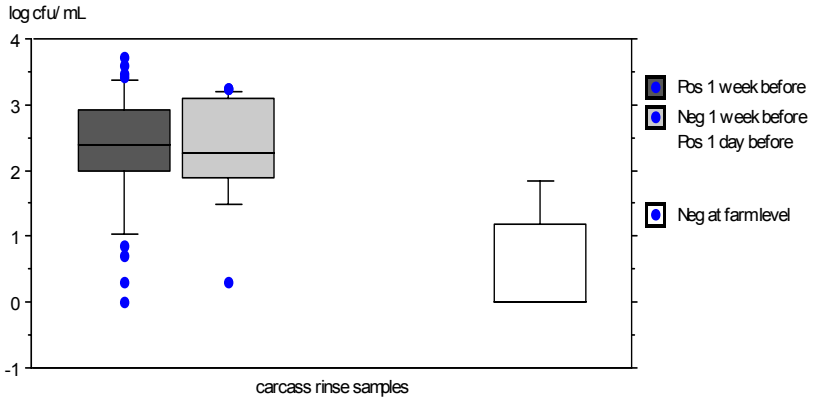


Figure 11. Carcass rinse samples from 220 flocks, compared with whether they were campylobacter-positive one week before slaughter and at slaughter, or negative one week before slaughter but campylobacter-positive on the day of slaughter at farm level, or campylobacter-negative at farm level on both sampling occasions, but campylobacter-positive in carcass rinse samples.

Subtyping by 16S rRNA and PFGE

Analysis of 16S rRNA gene sequences was used in identifying *Campylobacter* spp. This technique is more commonly used in bacterial taxonomy to infer phylogenetic relationships. Sequence analysis of the 16S rRNA genes of *C. jejuni* identified eight signature nucleotide positions (Paper VI) by which the studied strains of *Campylobacter* spp. could be differentiated into nine groups according to their 16S rRNA sequences. One sequence type (e) had the same 16S rRNA sequence type as strains from both *C. jejuni* and *C. coli*. This observation shows that phylogenetic analysis based on 16S rRNA cannot always be used to differentiate between *C. jejuni* and *C. coli*. Sequence type e was identical with the sequence type of 10 strains retrieved from the Ribosomal Database Project or GenBank, including one strain originating from a chicken in the US (Parker *et al*., 2006).

A possible correlation was found between the *Sma*I profiles and the 16S rRNA sequences, as a certain *Sma*I type only appeared in one of the two major phylogenetic groups (Paper VI). *Campylobacter* spp. have been associated with a genetic instability (Wassenaar & Newell, 2000). Instability is defined as a single or series of events leading to a change in the genetic organisation, but the frequency of such events is not well-known. Only a small portion of the genome may be involved in rearrangement, as the same PFGE profile was found despite five to nine years having elapsed between the different sampling occasions (Paper VI). Genotypic instability determined under laboratory conditions may be different under more natural conditions and reparation mechanisms for concerted evolution of rRNA genes seem to be very efficient, because all three rRNA genes were identical in the 47 isolates analysed (Paper VI).

As expected, macrorestriction profiling by PFGE was found to be a more discriminatory typing method, as analysis of 47 strains resulted in 22 different *Sma*I subtypes and 9 different 16S rRNA types (Paper VI). The discriminatory power of the PFGE for genotyping *Campylobacter* spp. has been previously demonstrated (Wassenaar et al., 1998; de Boer et al., 2000). In previous studies in Sweden (Olsson Engvall et al., 2005) common *Sma*I subtypes among broilers were identified. A study by Lindmark et al. (2004) found that Swedish retail chicken does not usually carry more than one isolate distinguishable by PFGE and some profiles occur frequently. That study included strains originating from humans, chicken, turkey, lamb and water, which resulted in 54% of the strains being divided into five clusters after digestion with *Sma*I (Lindmark et al., 2004). These clusters have a similar banding pattern to *Sma*I types 2, 6, 7 and 8 in Paper IV (Table 6). This observation raises the question whether certain clones are more common than others and whether these clones have a higher ability to survive in the environment around the broilers or are harboured by the animals around the broiler houses for a very long time. Ringoir & Korolik (2003) observed a range from immediate and sustained colonisation to complete non-colonisation using different phenotypes of *C. jejuni*, and the different phenotypes remained unchanged before and after passage in vivo.

Table 6. PFGE profiles frequently found in strains isolated from campylobacter-positive broilers within the Swedish Campylobacter Programme 2002-2003 (Olsson Engvall et al., 2005) and 2004 (Paper IV).

*Sma*I type	PFGE profile	No of isolates/farms 2002-2003	No of isolates/farms 2004
1		8/4	1/1
2		29/17	23/4
3		5/3	0/0
4		5/2	8/1
5		8/7	13/1
6		5/4	26/2
7		7/4	8/1
8		7/4	2/1
9		17/4	0/0
10		17/11	20/6

Bacteria with relatively small genomes such as *C. jejuni* may undergo genetic variation to increase their potential to adapt to new environments (Taylor et al.,

1992). Such genotypic variation could result in phenotypic changes. These variations are probably of importance in the route from broiler to man, where *Campylobacter* spp. must survive several hostile environments.

In epidemiology, the most interesting application of subtyping is in tracing an organism as it flows through a host of interest. Eight producers were found to deliver campylobacter-positive broilers from more than one grow-out in Paper IV. A grow-out is defined as the rearing period of approximately five weeks, starting at the entry of one-day-old broilers into the house and finishing at slaughter of all broilers. The broilers from the 21 positive grow-outs of the total of 89 grow-outs were often colonised by *Campylobacter* spp. of different *Sma*I types. This indicates that there is no environmental survival of *Campylobacter* spp. in broiler houses after adequate cleaning and disinfection, which is in agreement with a study by Evans & Sayers (2000). However, it has been proven that *Campylobacter* spp. survive in water and aquatic biofilms (Buswell *et al*., 1998; Trachoo *et al*., 2002) and protozoans can act as a potential reservoir for *C. jejuni* (Axelsson-Olsson *et al*., 2005). A study by Bull *et al*. (2006) found that in a colonised flock, one predominant campylobacter type was gradually superseded and sometimes replaced by another. In a Swedish study (Höök *et al*., 2005) eight different *Sma*I types were found in one grow-out, probably due to different sources of transmission and different times during the rearing process. Furthermore, in Paper IV different subtypes were identified in the same broiler house during the same grow-out, but on different sampling occasions. This observation does not exclude the possibility that infection with different subtypes is possible, as only one isolate from each sample was subtyped.

Preventive measures

Preventive measures at farm level

It should be noted that a third of broiler producers delivered campylobacter-free flocks consistently, while most of the positive flocks originated from a few broiler producers, some of whom had a flock prevalence approaching 60% per year. Reducing the proportion of infected poultry flocks and poultry carcasses will considerably lower the risk of consumers contracting campylobacteriosis. No association was found in isolation of campylobacter from the environment between producers that often delivered campylobacter-positive groups and those who rarely delivered campylobacter-positive groups (Paper IV). The best way of reducing the number of campylobacter-positive broilers after processing is to minimise the number of campylobacter-positive broilers at farm level.

Different measures have been proposed to prevent campylobacter colonisation in broilers, vaccination of chickens being one possible intervention. Cawthraw & Newell (2005) found that DNA vaccination of broilers with a purified plasmid of one strain provided significant protection. However, there are currently no commercial vaccines available as there are difficulties in providing protection up to

the time of slaughter and there is a lack of knowledge of the protective immunity. The effects of phage therapy have been tested (Wagenaar *et al*., 2005) as an alternative for reducing *C. jejuni* colonisation in broilers. A reduction in *C. jejuni* in caecal contents of up to 2-3 log(10) cfu/g was observed during the following days, so phage therapy may lower the amount of campylobacter entering the slaughter house. There could be difficulties in targeting the right time and phage therapy may not be sufficient when the total content of *Campylobacter* spp. exceeds 10^8 cfu/g faeces. Several studies have been published on the use of competitive exclusion to prevent colonisation in broilers. The effect of pre- or probiotics in feed varied in degree of control of campylobacter in broilers (Fernandez, 2005).

Preventive measures at slaughter

During evisceration, transfer of microorganisms continues from carcasses to hands of workers, equipment and utensils and back to the surfaces of other carcasses. A large amount of water creates a favourable environment for the dehydration-sensitive microbes, which could lead to contamination of carcasses at slaughter (Berndtson *et al*., 1996b). During processing, the level of campylobacter contamination on carcasses fluctuates, whereas the final steps in the process, washing and cooling, reduce campylobacter contamination (Rosenquist *et al*., 2006; Allen *et al*., 2007). Prevention of cross-contamination at slaughter might be achieved by cloacal plugging prior to scalding to minimise faecal leakage during processing (Musgrove *et al*., 1997), which significantly lowers the incidence of contamination of *Campylobacter* spp., but the use in large-scale broiler processing needs to be developed.

The use of logistic slaughter has been discussed in preventing contamination of broilers at slaughter. In this, broilers testing positive prior to slaughter or delivered by farmers who frequently produce campylobacter-positive broilers are slaughtered at the end of the day or preferably on Friday afternoon. Logistic slaughter may decrease the prevalence of broilers contaminated with campylobacter during processing. However, this is of limited value for the consumer, as those broilers that become contaminated during transport and processing have a low level of campylobacter after processing (Mead *et al*., 1995; Lindblad *et al*., 2006; Wagenaar *et al*., 2006; Hansson *et al*., 2007a).

The campylobacter load on broiler carcasses could also be decreased by decontamination by either chemical or physical measures. A study by Hendricks *et al*. (2000) describes reduction in campylobacter on carcasses by different antimicrobial treatments. However, if the campylobacter level is high on the carcasses, the use of decontamination may not be sufficient. One of the risk factors in contracting campylobacteriosis identified in case-control studies is consumption of fresh, unfrozen chicken (Wingstrand *et al*., 2006). The incidence of campylobacteriosis has increased during the same period as the consumption of fresh, unfrozen chicken has increased. Freezing of campylobacter contaminated chicken is an effective method to reduce the contamination (Georgson *et al*., 2006).

A study by Stern *et al*. (2003) suggested that carcass freezing contributed to the large reduction in campylobacteriosis in Iceland between 1999 and 2000.

Concluding remarks and future perspectives

The slaughter process eliminates some of the campylobacter on broiler carcasses. The best way of reducing the number of campylobacter-positive broilers after processing is to minimise the number of campylobacter-positive broilers entering the slaughter house. Three different chilling systems are used at the slaughter houses in Sweden, water chilling, air chilling and spray chilling. In a previous quantitative study in Sweden (Anonymous, 2006b), samples from two carcasses from each batch were taken, one before and one directly after chilling. An average reduction of campylobacter after chilling by 1-2 log(10) cfu per carcasses could be seen. The results indicate that water chilling was the most effective chilling system in reduction of campylobacter in comparison to the other chilling systems, however the difference was not significant and a big variation between the slaughter groups was identified in the enumeration before and after chilling (Anonymous 2006b, Hansson et al., 2007a). More studies are needed to determine the effect of different chilling systems and the importance of campylobacter in carcasses.

Some strains of *Campylobacter* spp. appear to have a higher ability to survive in the environment around broiler houses and to colonise broilers. They may also have a higher capacity for cross-contamination during the slaughter process and in the kitchen. More knowledge is needed about the biology of these strains and their ability to survive and multiply under different conditions.

This thesis identifies three key measures for reducing the campylobacter incidence in broilers at farm level in Sweden.

- A high level of general tidiness on the farm and a high standard of hygiene barrier protecting the broilers
- Avoidance of thinning, in which a part of a flock is kept for further rearing.
- Avoidance or minimisation of other livestock on the farm.

To identify the most useful and effective options for decreasing the load of *Campylobacter* spp. on chickens, a comparative evaluation is needed to cover efficacy, safety and cost-benefit relationships. The costs of any proposed measures in proportion to the benefit obtained and the nature of these measures have to be acceptable to both producers and consumers.

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