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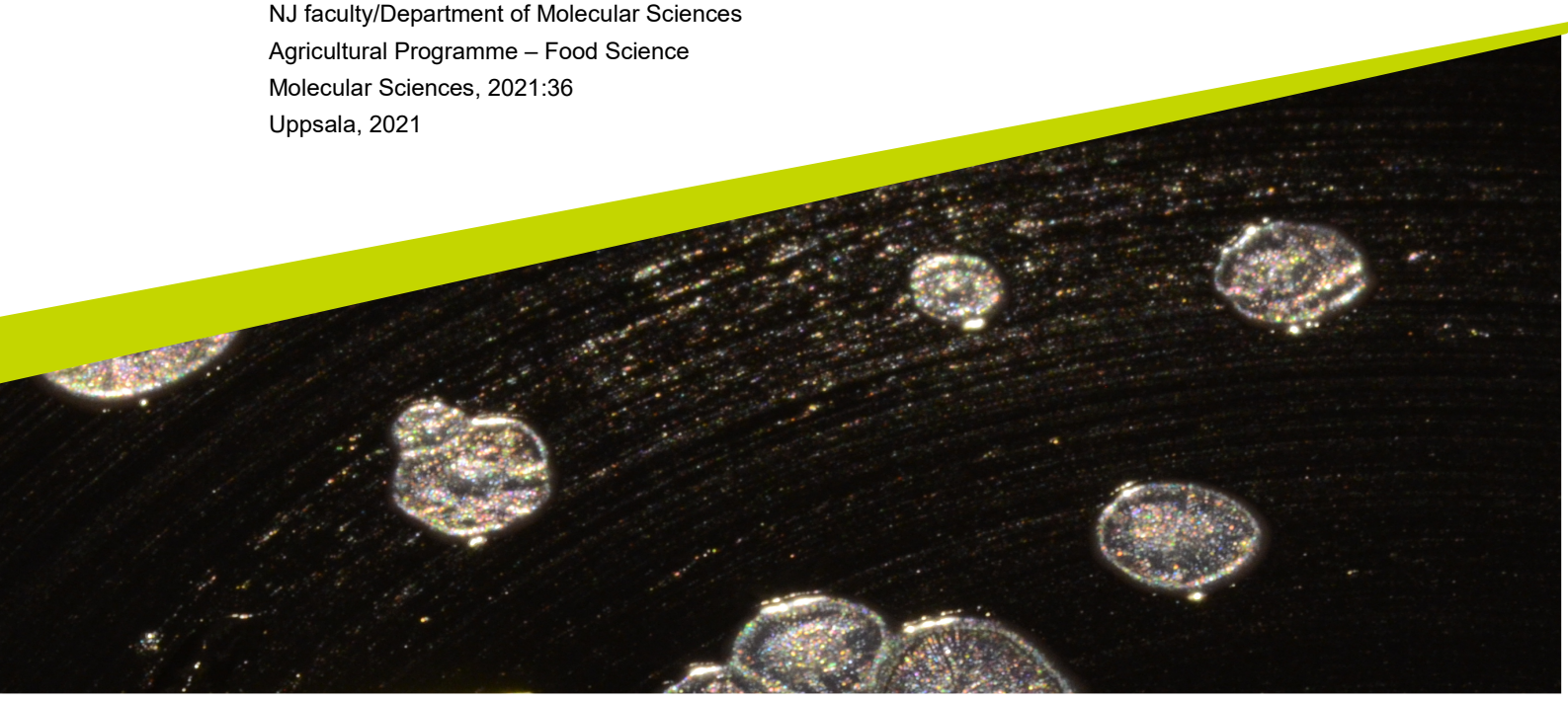
Överlevnad av Campylobacter jejuni i fryst kycklingfilé

Ella Råhlén

Independent project • 30 hp
Swedish University of Agricultural Sciences, SLU
NJ faculty/Department of Molecular Sciences
Agricultural Programme – Food Science
Molecular Sciences, 2021:36
Uppsala, 2021



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Supervisor:	**Ingrid Hansson, SLU, Department of Biomedical Science and Veterinary Public Health**
Assistant supervisor:	Lise-Lotte Fernström, SLU, Department of Biomedical Sciences and Veterinary Public Health
Assistant supervisor:	Emma Bergenkvist, SLU, Department of Biomedical Sciences and Veterinary Public Health
Examiner:	Hans Jonsson, SLU, Department of Molecular Sciences

Credits:	30 hp
Level:	Second cycle, A2E
Course title:	Master thesis in Food science
Course code:	EX0877
Programme:	Agriculture Programme – Food
Course coordinating dept:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2021
Title of series:	Molecular Sciences
Part number:	2021:36
Cover picture:	Colonies of ST-257 growing on a mCCDA plate, photo taken by Ingrid Hansson

Keywords: *Campylobacter jejuni*, broiler meat, frozen, survival, ST-257, ST-918

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences

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Abstract

This thesis aimed to study how two sequence types of *Campylobacter jejuni*, ST-257 and ST-918, survive freezing in broiler fillets. Campylobacteriosis is the most reported zoonosis in the EU, with over 200,000 reported cases annually. Broilers are considered the primary source of human exposure. Freezing reduces the concentration of bacteria and can be used as a post-harvest preventive measure. In this thesis, broiler meat was artificially contaminated and frozen together with meat juice, thereafter stored for 49 days. During the time, analysis was performed at ten different occasions. The quantification of *C. jejuni* in broiler meat and meat juice was made according to ISO 10272 part 2. Mean concentrations in the meat before freezing were 5.3 and 4.2 $\log_{10}$ CFU/g in ST-257, and in ST-918 5.2 and 4.2 $\log_{10}$ CFU/g. In the meat juice, the mean concentrations in ST-257 were 5.1 $\log_{10}$ CFU/ml for both concentrations, while for ST-918 concentrations were 5.2 and 4.6 $\log_{10}$ CFU/ml. The largest rate of decrease occurred the first two or four days after freezing, and the rate of decrease flattened after approximately one week. After 49 days, ST-257 decreased by a mean 1.6 and 2.0 $\log_{10}$ CFU/g in the meat and ST-918 decreased by 0.7 and 1.0 $\log_{10}$ CFU/g in the meat. The reduction in the meat juice was equal to or larger than the reduction in the meat. ST-918 survived freezing to a greater extent than ST-257, indicating different abilities to stand the stress from low temperatures.

Keywords: Campylobacter jejuni, *broiler meat, frozen, survival, ST-257, ST-918.*

Sammanfattning

Studiens syfte var att studera hur två olika sekvenstyper av *Campylobacter jejuni*, ST-257 och ST-918 överlever i frysen i kycklingkött. Campylobacterios är den mest rapporterade zoonosen i EU med över 200 000 fall årligen. Slaktkyckling anses vara den primära smittokällan. Nedfrysning reducerar antalet bakterier och kan användas som en förbyggande åtgärd efter slakt. Den här studien undersökte artificiellt kontaminerad kycklingfilé som frystes ned tillsammans med köttsaft och lagrades i frysen upp till 49 dagar. Under lagringstiden gjordes analys vid tio olika tillfällen. Kvantifieringen av *C. jejuni* i kycklingkött och köttsaft gjordes enligt ISO 10272 del 2. Medelvärdet av koncentrationerna innan nedfrysning var 5.3 och 4.2 $\log_{10}$ CFU/g i ST-257, och i ST-918 5.2 och 4.2 $\log_{10}$ CFU/g. I köttsaften var koncentrationerna i ST-257 5.1 $\log_{10}$ CFU/ml i båda koncentrationerna, medan i ST-918 var koncentrationerna 5.2 och 4.6 $\log_{10}$ CFU/ml. Störst minskning av antalet *Campylobacter* skedde under de första två till fyra dagarna i frysen och minskningen planade ut efter en vecka. Efter 49 dagar minskade ST-257 i genomsnitt med 1.6 och 2.0 $\log_{10}$ CFU/g i köttet och ST-918 minskade 0.7 och 1.0 $\log_{10}$ CFU/g i köttet. Reduceringen i köttsaften var likamed eller större än reduceringen i köttet. ST-918 överlevde frysningen bättre än ST-257, vilket tyder på olika förmåga att tåla nedfrysning.

Nyckelord: Campylobacter jejuni, *kycklingkött, fyst, överlevnadl, ST-257, ST-918.*

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Abbreviations

BHI	Brain heart infusion
BPW	Buffered peptone water
CFU	Colony forming units
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
GBS	Guillain-Barré Syndrome
HACCP	Hazard Analysis and Critical Control Points
IBS	Irritable bowel syndrome
LOS	Lipooligosaccharides
MALDI-TOF	Matrix Assisted Laser Desorption Ionization – Time of Flight
mCCDA	Modified Charcoal-Cefoperazone-Deoxycholat agar
PHC	Process Hygiene Criterion
RT-PCR	Real-time polymerase chain reaction
ST	Sequence type

1. Introduction

1.1. The genus *Campylobacter*

1.1.1. Historical aspects

In 1906, *Campylobacter* was first isolated and described as *Vibrio* (now *Campylobacter*) *fetus*, causing abortions in bovine and ovine (McFadyean & Stockman 1913). Fifty years later, the bacterium was renamed, and the genus *Campylobacter* was established (Sebald & Veron 1963). In humans, *Campylobacter* spp. was first isolated in 1938, connected to an outbreak of milk-borne gastroenteritis in Illinois, USA (Levy 1946). King (1957) isolated the bacteria from blood samples in the late 1950s, originating from children suffering diarrhoea. King made three essential observations. She found that the optimal temperature for growth was 42°C, the bacteria was isolated from patients suffering acute diarrhoea, and the strains isolated from humans were not possible to distinguish from those recently isolated from chicken. In Belgium, at the beginning of the 1970s, *Campylobacter* spp. was isolated from faeces origin from a previously healthy woman now suffering acute haemorrhagic enteritis (Dekeyser et al. 1972). This finding did not receive a response until a few years later when Skirrow (1977) isolated the bacteria from a baby suffering acute diarrhoea.

1.1.2. Taxonomy

The name *Campylobacter* origins from the Greek words *kampulos,* meaning curved and *bacter,* meaning rod. The genus *Campylobacter* is included in the family *Campylobacteraceae*, which belongs to the order *Campylobacterales*, belonging to the *Epsilonproteobacteria* class in the phylum *Proteobacteria* (Kaakoush et al. 2015). The genus *Campylobacter* includes 34 species (https://lpsn.dsmz.de/genus/campylobacter 19-April-2021).

Campylobacter jejuni subsp. *jejuni* (henceforth called *Campylobacter jejuni*) is considered the most important concerning food-borne gastroenteritis (WHO 2020;

EFSA & ECDC 2021). The name *jejuni* originates from the Latin word *jejunum*, also the anatomic name for the second part of the small intestine (Nationalencyklopedin 2021). Four species, *C. jejuni, C. coli, C. lari,* and *C. upsaliensis,* grow as good at 42°C as in 37°C and are often referred to as thermotolerant.

1.1.3. Morphology

Campylobacter spp. are gram-negative, oxidase-positive, and catalase-positive bacteria. No species form spores nor ferment carbohydrates but instead gain energy from amino acids or intermediates from the tricarboxylic acid cycle (Kaakoush et al. 2015). The rods are spiral or curved and reach motility through one or several polar or bipolar flagella (Figure 1). The size varies from 0.2-0.8 µm in width and 0.5-5 µm in length.

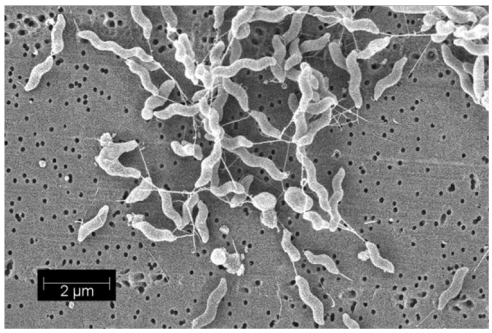


Figure 1. C. jejuni in a scanning electron microscope of. Photo taken by Ingrid Hansson (BVF, SLU),Tapio Nikkilä (BVF, SLU) & Leif Ljung (UU).

Members of the genus *Campylobacter* are known to be sensitive to desiccation, both high and low temperatures, and have specific requirements on the atmosphere's composition (Bolton & Coates 1983). Most of the species are microaerophilic, meaning sensitive to oxygen. Nevertheless, some show tolerance to higher levels of oxygen, and some are roughly anaerobic. The composition of the atmosphere for optimal growth for most of the species is 5-10% oxygen and 1-10% carbon dioxide (ibid).

1.1.4. Culture

All *Campylobacter* spp. grow at 37 °C. The thermotolerant, where *C. jejuni* is included, can grow at 42 °C (King 1957). The incubation of *C. jejuni* is performed at 42 °C to minimize the risk for contamination by other bacteria. *Campylobacter* grow slowly, and if stressed, the bacteria can undergo coccal transformation, losing their ability to grow on media. However, viable bacteria are still present and the states are not necessarily connected since the coccal state can be cultivated (Rollins & Colwell 1986). In temperatures below 30 °C, thermotolerant *Campylobacter* do not multiply (Hazeleger et al. 1998).

Thermotolerant *Campylobacter* are historically considered to be sensitive and fastidious in the environment outside the host. *C. jejuni* is sensitive to dehydration, temperatures over 72 °C, UV, freezing, high and low pH,

disinfectants and survive poorly in dry conditions (Humphrey et al. 1995; Hansson et al. 2018). In contrast, according to Humphrey, O'Brien, and Madsen (2007) their sensitivity outside the host is a myth since approximately 1% of the population in western Europe gets infected by *Campylobacter*, primarily from contaminated foods.

Standard methods for the analysis of other bacteria, such as aerobic atmosphere, are usually not suitable for the cultivation of *Campylobacter* (Luechtefeld et al. 1981). Cultivation of *Campylobacter* is most frequently performed by direct plating on solid media. If necessary, enrichment is applied before direct plating (Hansson et al. 2018). Three main groups of media are dominating; chromogenic agar, charcoal-containing media, and blood-containing media (ibid). Media containing blood or charcoal removes toxic oxygen derivates formed when the media is exposed to light (Corry et al. 1995).

Commonly used standards for analysis of *Campylobacter* are provided by different organizations such as the International Standard Organisation 10272 (ISO 2017), Nordic Committee on Food Analysis 119 (NMKL 2007), World Organization for Animal Health (OIE 2004), and United States Department of Agriculture (National Advisory Committee on Microbiological Criteria for Foods 2007). The colonies of *C. jejuni* cultured on mCCD agar have a characteristic grey colour and irregular shape with smooth edges (Figure 2). Other detection methods are real-time polymerase chain reaction (RT-PCR) (Lund et al. 2004) and enzyme immunoassay (Endtz et al. 2000). For isolation of *C. jejuni*, membrane filters can be used (Steele & McDermott 1984).

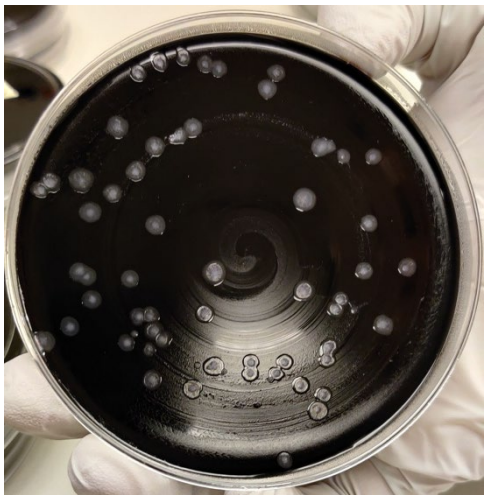


Figure 2. mCCDA plate with colonies of C. jejuni after incubation.

1.1.5. Sources

Campylobacter spp. are widely spread in the environment. *Campylobacter* can asymptotically colonize most of the animals bred for food and many pets. *C. jejuni* is found in pigs, cattle, sheep, dogs, wild birds, rabbits, and insects (Stanley & Jones 2003; Humphrey et al. 2007). *Campylobacter* can colonise all poultry species. The broiler is the most consumed of the poultry species and thereby stands for the most significant risk to cause human campylobacteriosis. (Cody et al. 2019).

Water, such as streams and groundwater, can be contaminated with *Campylobacter*. A Swedish study investigated the associations between sporadic campylobacteriosis and environmental risks and found indications of associations between drinking water contaminated from livestock and human infection, and it might be an essential factor for explaining parts of the sporadic cases (Nygård et al. 2004). In dark and moist conditions with low temperatures, 4-10 °C, *Campylobacter* can survive in stream water for more than four months (Rollins & Colwell 1986).

1.1.6. Subtypes

Characterization below the species level is called sub-typing. Sub-typing is used in epidemiological studies to track transmission routes and identify infection sources (Hansson 2007). *C. jejuni* is traditionally considered sensitive to oxygen. However, in a study by Oh et al. (2015), strains of *C. jejuni* were isolated from retail chicken, and some proved to be aerotolerant. Aerotolerance and therethrough survival among strains of *C. jejuni* is one reason for an increased risk for food-borne transmission to humans via aerobic packaging. Oh et al. (2018) found ST of *C. jejuni* tolerant to freezing and thawing, oxygen, disinfectants, heat treatment, and osmotic stress to play a vital role in causing human infection. Also, aerotolerant and hyper-aerotolerant ST of *C. jejuni* are less sensitive to freezing than oxygen-sensitive ST (Oh et al. 2019). Other factors, such as sialyation of LOS might be beneficial to colonize the host and survive in the environment, giving certain strains beneficial properties (Habib et al. 2009). Diversity among strains of *C. jejuni* is critical to consider regarding human infection due to different properties on species level.

1.1.7. Virulence

Even though *C. jejuni* is of great importance as a human pathogen, knowledge gaps occur in the understanding of its pathogenicity mechanisms (Hansson et al. 2018). *C. jejuni* is contrary to other pathogens causing diarrhoea and do not express classic virulence factors (Dasti et al. 2010). Some established virulence factors are motility caused by flagella, proteins, and genes linked to adhesion and invasion of the host cells, chemotaxis, lipooligosaccharides on the surface of the cell and excretion of cytolethal distending toxin (Bolton 2015). Identification of the virulence mechanisms requires appropriate animal models of disease. However, most model animals become colonized but show no symptoms and are therefore not helpful in investigating the virulence mechanisms in *C. jejuni* (Newell 2001).

1.2. Campylobacteriosis

1.2.1. Number of cases

In the EU, campylobacteriosis is the most reported gastrointestinal infection in humans since 2005. *Campylobacter* spp. is estimated to yearly cause 1.5 million cases in the United States (CDC 2021) and nine million cases in the EU (EFSA 2011). In 2019, 220 682 cases and 47 deaths from human campylobacteriosis were reported in the EU, corresponding to 59.7 cases per 100 000 population and case fatality of 0.03%. A decrease by 6.9% of reported cases was observed between 2018 and 2019. However, the trend for cases of campylobacteriosis between 2015 and 2019 was maintained flat. Campylobacteriosis represents half of the reported cases of human zoonoses in the EU, and salmonellosis was the second most reported human zoonosis with 87 923 confirmed cases and 140 reported deaths, 0.22% case fatality (EFSA & ECDC 2021).

Campylobacteriosis affects humans worldwide. In high-income countries reporting of campylobacteriosis occurs, while in low-income countries, the rate of human campylobacteriosis is not well categorized (Hansson et al. 2018). Reported cases of campylobacteriosis in 2019 in the EU was 220 682, however, it is estimated to cause over nine million cases of food-borne illness annually (EFSA & ECDC 2021). The cost of campylobacteriosis in the EU is estimated by the European Food Safety Authority (EFSA) to be 2.4 billion Euros every year due to costs to the public health system and loss in productivity (EFSA 2021a).

1.2.2. Underreporting

According to the Zoonoses Directive 2003/99/EC (European Commission 2003), all member states in the EU are obligated to monitor the prevalence of *Campylobacter*. Member states are obliged to collect data linked to campylobacteriosis, including the occurrence of zoonosis, zoonotic agents, the prevalence of antimicrobial resistance, prevalence in animal populations, plus food-borne outbreaks of campylobacteriosis. In Sweden, infection with *Campylobacter* is compulsory to report since 1989 according to the Communicable Disease Act SFS 2004:168.

Despite the regulations with notification requirements, many cases of campylobacteriosis are not reported. Underreporting is a significant problem in the mapping of the infection, thereby underestimation of the true prevalence can appear. No cohort system for surveillance is present in the EU. Instead, the member states are obligated to report data from their national surveillance system. Passive surveillance to collect data is used to the greatest extent. A fraction, 0.6%, of data of human campylobacteriosis is received by food-borne outbreak

investigations (EFSA & ECDC 2021). Differences among countries in health care systems and laboratory capacity contribute to a variation of underreporting rates between countries (Haagsma et al. 2013). In the UK, Tam et al. (2012) estimated the actual number of cases to be 9.3 times greater than the reported number of cases.

1.2.3. Seasonal variation

Campylobacteriosis in humans shows a seasonal variation with annual peaks in reported cases during the summer (Williams et al. 2015; Friedrich et al. 2016; EFSA & ECDC 2021). Yearly from 2012, a minor peak in January is observed in Europe (EFSA & ECDC 2021). The seasonality in the number of reported cases is a distinguishing characteristic of campylobacteriosis (Humphrey et al. 2007). Seasonality has been investigated and resulted in several theories. Among these, changed eating behaviour with outdoor barbecues and activities, higher prevalence of *Campylobacter* in the environment during summer, flies, warmer temperatures, higher prevalence in poultry, and migration of wild birds are suggested causative factors (Humphrey et al. 2007; Strachan et al. 2013; Hansson et al. 2018). Nevertheless, the reasons for seasonality remain uncertain.

1.2.4. Sources of human infection

Poultry meat is considered the primary source of human campylobacteriosis, with considerable supporting evidence (EFSA 2011; Cody et al. 2019). A Belgian study by Vellinga and Van Loock (2002) reported a reduction of 40% of human cases of campylobacteriosis in June 1999 in Belgium, mainly due to the withdrawal of native poultry due to dioxins in the feed. Another study from Iceland showed a correlation between human infection of *Campylobacter* and the prevalence of *Campylobacter* in broilers. In 1999, the rate of campylobacteriosis was 116/100 000, and at the time, 62% of the broilers were tested positive (Stern et al. 2003). With increased biosecurity on farms, freezing of *Campylobacter*-positive flocks, and public education, the rate of campylobacteriosis one year later dropped to 33/100 000, and the prevalence in tested broilers was 15%.

Multiple species of *Campylobacter* can cause human disease, with the main species being *C. jejuni, C. coli, C. lari, C. fetus*, and *C. upsaliensis*. The three species last mentioned are responsible for relatively few human cases though. Most common in the EU was *C. jejuni* with 83.1% and *C. coli* with 10.8% of the confirmed cases when the strain was identified (EFSA & ECDC 2021). The majority of human cases are transmitted via the faecal-oral route, and most frequently people get infected by eating undercooked meats or unwashed vegetables and drinking unpasteurized milk (EFSA & ECDC 2021). The EFSA Panel on Biological Hazards (2011) estimated that handling, preparing, and

consuming broiler meat accounts for 20-30% of human cases, and chicken reservoirs as a whole may account for 50-80% of human campylobacteriosis. Cool, dark and moist conditions are occurring in chilled retail poultry meat. Consequently, chilled poultry meat is an ideal environment for the survival of *Campylobacter* (Sandberg et al. 2005; Georgsson et al. 2006).

1.2.5. Symptoms

Symptoms of campylobacteriosis are often clinically undistinguishable from those of other enteric pathogens like *Shigella* and *Salmonella*. Patients often suffer diarrhoea, fever, abdominal pain, malaise, and headache (Adams et al. 2016). Although, symptoms vary from absence of them to sepsis and death. *C. jejuni* causes inflammatory enteritis in the small intestine and colon, with symptoms lasting for one week and up to three weeks in approximately 20% of ill persons. The incubation time is usually 3-5 days, and the infection is often self-limiting. Severe abdominal pain is associated with infection of *C. jejuni*. The infection usually lasts for one week, though it can last for up to three weeks in approximately 20% of ill persons (Allos & Blaser 1995).

Elderly, young children and persons with suppressed immune systems are at higher risk of severe or fatal infection (WHO 2020). The infectious dose is low, but only a few studies have been done in this field when investigating *C. jejuni*. One experimental study by Robinson (1981) showed that the author got ill after drinking milk containing 500 CFU. Another study with 111 adult volunteers done by Black et al. (1988) showed that the lowest investigated concentration, 800 CFU, caused illness in the participants.

1.2.6. Secondary diseases

Campylobacteriosis can lead to secondary diseases. Infection of *C. jejuni* can cause Guillain-Barré Syndrome (GBS), affecting the peripheral nervous system, leading to paralysis in limbs (Hughes & Rees 1997). A study in Sweden estimated the risk to 30.4 cases of GBS per 100 000 cases of *C. jejuni* infection, which is 100 times more than in the uninfected people (McCarthy & Giesecke 2001). The disease might be triggered by lipooligosaccharides (LOS), on the surface of *C. jejuni*, mimicking gangliosides in the nervous tissue leading to cross-reactive antibodies causing GBS (Godschalk et al. 2004).

Another secondary disease after infection of *C. jejuni* is reactive arthritis. In southern Sweden, 45% of patients with recent-onset arthritis showed evidence of a previous infection of *Campylobacter* (Söderlin et al. 2003). Furthermore, in the group of patients suffering reactive arthritis, patients with recent *Campylobacter*-infection dominated. In the study, 24% of all patients tested positive for a

previous infection of *C. jejuni*. An additional secondary disease is irritable bowel syndrome (IBS). A study from the USA showed an IBS incidence rate of 33.1 cases per 1000 patients diagnosed with *Campylobacter* infection (Scallan Walter et al. 2019).

1.2.7. Outbreaks

Campylobacter was in 2019 the third most frequently reported agent to cause food-borne outbreaks in the EU, causing 319 outbreaks and 1254 illnesses linked to the outbreaks (EFSA & ECDC 2021). The definition by EFSA of a food-borne outbreak is "two or more people developing the same food-borne illness after eating or drinking the same food" (EFSA 2021b). In total, 3101 outbreaks were reported in 2019; however, the causative agent was, to a great extent, 40%, not identified (EFSA & ECDC 2021). In 72 outbreaks, *C. jejuni* was identified, though two-thirds of the food-borne outbreaks lacked species information. Eight outbreaks were linked to broiler meat and three to milk, which is correspondent to strong evidence sources over the last decade (EFSA & ECDC 2021). Also, consumption of poultry meat was in strong-evidence outbreaks, associated with many cases of campylobacteriosis (ibid).

1.2.8. Outbreak 2016-2017 in Sweden

During the summer of 2016, extraordinary high numbers of domestic human campylobacteriosis cases were reported in Sweden. The prevalence of *Campylobacter*-positive broiler flocks increased in July 2016 and remained high through October (Swedish Poultry Meat Association 2021b). For this reason, the Swedish Food Agency and Public Health Agency of Sweden (2018) exhibited an investigation to identify the source of infection. By comparing whole-genome sequencing of isolates from retail chicken and patients, the source of human infection was primarily correlated to consumption of domestic broiler meat.

The sequence type of *C. jejuni* ST-918 was found to a large extent and ST-257 to some extent (Swedish Food Agency & Public Health Agency of Sweden 2018). This outbreak was the largest food-borne outbreak in the EU in 2016, with more than 3000 cases caused by domestic poultry meat contaminated with *Campylobacter* (EFSA & ECDC 2017).

1.3. Legislation

1.3.1. Consumer

Legislation regarding thermotolerant *Campylobacter* has not been in valid for long. For *Campylobacter* in food, no official surveillance programme exists. The Swedish Food Agency have controls once a year in retail broilers (Swedish Food Agency 2020). However, neither regulation nor surveillance system are in practice for private households. Most reported cases of campylobacteriosis are considered sporadic and underreported to a great extent since the infection is often self-limited and not diagnosed (EFSA & ECDC 2021). The primary vehicle for introducing *Campylobacter* into the kitchen is broiler meat (ibid). Cross-contamination and undercooked broiler meat play a significant role in transmitting food-borne infection of *C. jejuni* (de Jong et al. 2008; EFSA 2010).

According to EFSA (2011), a 100% risk reduction can be achieved after slaughter of broiler if they are cooked on an industrial level and not re-contaminated. To prevent food-borne diseases, the World Health Organization (2006) states five keys;

- Keep clean
- Separate raw and cooked
- Cook thoroughly
- Keep food at safe temperatures
- Use safe water and raw materials.

The awareness and knowledge about food safety in consumers are to a lesser extent investigated, and few studies have been performed. However, studies on this topic found urgent knowledge gaps in consumers' handling of pathogens, cleaning, storage temperatures, and handling risk foods (Marklinder et al. 2013; Lange et al. 2016). The prevalence of *C. jejuni* in broilers can be regulated by legislation to reduce the risk of exposure at the consumer level.

1.3.2. The Swedish *Campylobacter* programme

Since 1991, the Swedish Poultry Meat Association has been operating the voluntary Swedish monitoring programme for broilers (Swedish Poultry Meat Association 2021b), since 2001 financed mainly by the Swedish Board of Agriculture thru regulation SJVFS 2015:17, K152 (National Veterinary Institute et al. 2019). The programme aims to reduce the incidence of *Campylobacter* in the food chain by preventive measures starting with the primary production, leading to a *Campylobacter*-free production (Hansson 2007). Today the target is to achieve an overall annual prevalence of less than 10% of *Campylobacter* in

slaughtered broilers, before 2017, the goal was 5% (National Veterinary Institute et al. 2019).

Nearly all broiler flocks, 99% slaughtered in Sweden, is covered by the programme. Sampling is since 2006 performed by pooling ten cecum samples from the batch, in turn, analysed according to ISO-10272 part 1. The prevalence of thermotolerant *Campylobacter*-positive broiler flocks has been reduced since the programme started. In 2019 *Campylobacter* was isolated from 5.3% of the broiler flocks, which is the lowest prevalence since the start of the programme (National Veterinary Institute et al. 2019). In 2020, the *Campylobacter* prevalence of broiler produced by members of the Swedish Poultry Meat Association was 4.6%, whereas broiler produced by non-members (producers of organic and other free range broilers) had a *Campylobacter* prevalence of 36% of the flocks (Swedish Poultry Meat Association 2021b). Previously the prevalence of conventional broilers was 20% in 2001 and 13% in 2005 (Hansson et al. 2007).

1.3.3. Baseline survey

In 2008, the first baseline survey investigating foodstuffs was performed in the European Union to monitor the prevalence of thermotolerant *Campylobacter* (EFSA 2010). It was conducted in 26 member states, as well as in Norway and Switzerland. The survey included 10 132 broiler batches, in every batch the analysis consisted of 10 caecal samples pooled into one sample, plus neck skin and breast skin from one chilled carcass.

The prevalence of *Campylobacter*-contaminated carcasses was 75.8%, ranging from the lowest 4.9% in Estonia to 100% prevalence in Luxembourg. Norway and Finland declared 5.1% and 5.5% prevalence, respectively. Countries exceeding 80% prevalence were Poland, Austria, the United Kingdom, and France. Spain, Malta, Ireland, and Luxembourg declared over 90% prevalence. Overall, in the *Campylobacter*-positive flocks, 5.8% of the chilled carcasses had a prevalence of more than 4 $\log_{10}$ CFU/g, and 15.8% had levels between 3-4 $\log_{10}$ CFU/g. Almost half of the positive, 47%, had counts below 1 $\log_{10}$ CFU/g. *C. jejuni* was most commonly isolated in the caecal samples and the skin samples.

1.3.4. Process Hygiene Criterion

A microbiological criterion for foodstuffs was first introduced in 2005 for pathogens like *Salmonella* and *Listeria monocytogenes* (European Commission 2005). Thirteen years later, *Campylobacter* was added. Since 2018, the microbiological process hygiene criterion (PHC) for *Campylobacter* in broilers is set by the European Commission, No 2017/1495 (2018). The PHC aims to limit the number of bacteria in the food chain, improve food safety, and reduce cases of

human campylobacteriosis linked to handling or consumption of broiler meat. The food business operators, abattoirs, are required to use the PHC to validate and verify their food safety management procedures. In turn, these procedures should be based on Hazard Analysis Critical Control Point (HACCP) principles and Good Manufacturing Practices (GMP). If the PHC target is exceeded, the plant must take action (EFSA & ECDC 2021).

Sampling is performed on the neck skin on chilled carcasses, where 50 pooled samples derived from 10 consecutive sampling occasions are evaluated. The reference method for analysis is ISO 10272 part 2. The criterion requires interventions from the food business operator if the regulatory limit of 3 $\log_{10}$ CFU/g is exceeded (EFSA & ECDC 2021). However, in 2019, 40% of the samples were allowed to exceed the limit, reduced to 30% in 2020 (Emanowicz et al. 2021). In 2011, EFSA estimated a risk reduction for the public health to be >50% if all batches comply with the limit of 3 $\log_{10}$ CFU/g on neck skin, and a risk reduction of >90% if complying with a limit of 2.5 $\log_{10}$ CFU/g (EFSA 2011). This report also stated that 15% of the tested batches would not comply with the limit of 3 $\log_{10}$ CFU/g, and 45% of the batches would not comply with the criterion if the limit was 2.5 $\log_{10}$ CFU/g.

The zoonosis report declared results from seven member states (Denmark, Estonia, Germany, Ireland, Latvia, Romania, and Sweden) reporting PHC monitoring results from food business operators. In 15,323 samples from chilled carcasses' neck skin, 13% tested positive for *Campylobacter*. Half of the positive, 7% of 15,323 samples, exceeded the limit of 3 $\log_{10}$ CFU/g. Results collected from the slaughterhouses in seven countries (Bulgaria, Croatia, Cyprus, Estonia, Latvia, Romania, and Spain) were reported. Fewer samples, 3,346 neck skins, were analysed, and 41% tested positive. Of the positive, 37% exceeded the PHC limit corresponding to 15% of the total number of samples (EFSA & ECDC 2021).

1.4. Broilers

1.4.1. Production

Members of the Swedish Poultry Meat Association produced 106.9 million broilers corresponding to 161 900 tonnes in 2020, representing 99% of the production in Sweden (Swedish Poultry Meat Association 2021a). For comparison, in 2018, the EU produced 15,2 million tonnes of poultry meat (Eurostat 2019), out of that, 83% were broilers (EFSA Panel on Biological Hazards et al. 2020). Total production in 2017 worldwide was estimated to 118.1

million tonnes, where the largest producer of poultry meat is the US, followed by China and the EU (EFSA Panel on Biological Hazards et al. 2020). In the EU, Poland is the largest producer, followed by the UK and France (Eurostat 2019).

1.4.2. Consumption

On average, the consumption of poultry meat per year in the world is 14.7 kg per capita. Israel is on top, consuming 64 kg per capita (OECD 2021). The average consumption in the EU was 23.4 kg per capita in 2020 (European Commission. Directorate General for Agriculture and Rural Development 2020), and in Sweden, the poultry meat consumption was 21.5 kg (Swedish Board of Agriculture 2021a). To put into context, the total meat consumption in Sweden 2020 was 78.6 kg per person, poultry included (Swedish Board of Agriculture 2021a). The market share for Swedish poultry in Sweden was 75.9% in 2020, and import was mainly from Denmark, followed by the Netherlands and Germany (Swedish Board of Agriculture 2021b). Approximately half of the Swedish broiler meat is bought as frozen meat at the consumer level, the other half as fresh meat (Maria Donis, Swedish Poultry Meat Association, personal message).

1.4.3. *Campylobacter* in broiler flocks

It is possible to produce *Campylobacter*-free broilers in Sweden (Hansson et al. 2007). However, broilers are quickly colonized, and the infectious dose is low, reported as 40 CFU (Cawthraw et al. 1996). Consequently, the whole flock is rapidly asymptotically colonized. Despite the absence of symptoms in the bird, the intestines of broilers can be colonized with *Campylobacter* with concentrations up to 8 $\log_{10}$/g faeces (Stern & Robach 2003; Hansson et al. 2010). It is, however, difficult to prevent colonization (Wagenaar et al. 2013). A risk factor for colonisation is seasonality, but the mechanisms are not clearly understood (EFSA Panel on Biological Hazards et al. 2020).

1.4.4. Slaughter

Thinning is used to achieve maximum capacity over a more extended period, and it is when part of the flock is removed for slaughter earlier. Thinning is an issue since *Campylobacter* is introduced to the house with crates and trucks visiting multiple farms, spreading rapidly to the flock (EFSA Panel on Biological Hazards et al. 2020). On the abattoir, *Campylobacter* can spread during evisceration, as *Campylobacter* can be found on work surfaces, scalding water, and the defeathering machine (Perez-Arnedo & Gonzalez-Fandos 2019). Allen et al. (2007) found cross-contamination on the slaughterhouse to negative flocks. García-Sánchez et al. (2017) and Peyrat et al. (2008) found *C. jejuni* to be able to

survive in the plant after cleaning and disinfection, also that the abattoir can act as a source of contamination.

Due to air chilling on the plant, the prevalence of *Campylobacter* is reduced on beef, pork, and lamb (Oosterom et al. 1983). In the slaughter of broilers, water is introduced during scalding, rinsing, and chilling, causing a moist environment on the carcasses. *C. jejuni* can survive for long periods in 4-10 °C, dark and humid conditions (Rollins & Colwell 1986). Vacuum packaged pork was stored in a refrigerator for 28 days, and the levels of *C.* jejuni was not dramatically reduced (Wen & Dickson 2012). This indicates that *C. jejuni* might survive in chilled retail chicken for more extended periods.

1.5. Preventive measures

To reduce the risk of exposure at consumer level, different preventive measures can be adopted. EFSA's Panel of Biological Hazards estimated a public health reduction of > 50% if all broiler batches comply with the limit of 3 $\log_{10}$ CFU/g, and a risk reduction of >90% if all batches comply with a limit of 2.7 $\log_{10}$ CFU/g (EFSA 2011). An updated report estimated that a 3 $\log_{10}$ reduction in caecal concentrations would lead to a risk reduction of 58% for human campylobacteriosis connected to broiler meat (EFSA Panel on Biological Hazards et al. 2020). Rosenquist et al. (2003) estimated that a reduction of 2 $\log_{10}$ on broiler carcasses would result in a decrease by 30-fold in the chance of human campylobacteriosis.

1.5.1. Methods for reduction

Chlorine, alkaline, and acids can be used to reduce the levels of *C. jejuni*, however the effectiveness is uncertain or varies (EFSA 2011). Peracetic acid reduces the counts of *Campylobacter* without affecting the quality of the carcass (Nagel et al. 2013). Treatment with hot water reduces the numbers of *Campylobacter*, though the appearance is affected with flaws appearing in the skin (Corry et al. 2007). The same problem with the appearance was found when using steam, causing the skin to shrink and change in colour (James et al. 2007).

The steam-ultrasound treatment combines steam and ultrasound, reducing the bacterial count (Moazzami et al. 2021). More studies are needed to optimize the treatment and reduce the visual flaws. Irradiation effectively reduces bacterial counts, however, it is not legal in most countries in the EU. Ultraviolet light and high hydrostatic pressure processing are two methods that need more research to conclude if effective or not. Crust freezing is a technique when freezing the crust rapidly and then thaw the meat. This method reduces the number of

Campylobacter between 0.4-0.9 $\log_{10}$, but more research needs to be done on an industrial scale (EFSA 2011).

1.5.2. Freezing

Freezing is effective in reducing the count of *Campylobacter* and thereby reduce the risk for consumers. During freezing, the formation of ice crystals, ice nucleation, and dehydration are factors injuring the bacteria (Alter & Reich 2021). In Iceland, Georgesson et al. (2006) found a reduction of 0.65-2.87 $\log_{10}$ in naturally contaminated carcasses after freezing and storing for 31 days, and the reduction after one day was 1 $\log_{10}$. Sampers et al. (2010) also found one $\log_{10}$ reduction after one day in the freezer and thereafter a reduction but not significant in naturally contaminated meat.

In Norway, *Campylobacter*-positive broiler flocks are slaughtered at the end of the workday and selected to be frozen products, resulting in fewer *Campylobacter*-positive products on the market (Hofshagen & Kruse 2005). The authors connect the lower incidence of domestic campylobacteriosis to the action plan. In a Danish study, freezing of carcasses on the plant reduced the numbers of *Campylobacter* by 1.44 $\log_{10}$ (Rosenquist et al. 2006) and a reduction of 1.3 $\log_{10}$ have been observed in inoculated chicken wings stored at -20 °C (Zhao et al. 2003).

Great benefits in reduced number of cases of campylobacteriosis can be achieved by a reduced concentration in the broiler meat reaching consumers. The routine of freezing *Campylobacter*-positive carcasses in Iceland contributed to the reduction from 116 cases of campylobacteriosis per 100,000 people in 1999 to 33 per 100,000 people, the following year (Stern et al. 2003). Elimination of the highest contaminated meat products would decrease the number of human cases of campylobacteriosis (Wagenaar et al. 2013). EFSA (2011) stated, that a risk reduction of 50-90% would be achieved by freezing the carcass for 2-3 day, while a 90% risk reduction is achieved if freezing for 2-3 weeks. According to EFSA, freezing for a few days reduce the prevalence of 0.9-1.4 $\log_{10}$ and freezing for three weeks gives a reduction of 1.8-2.2 $\log_{10}$.

1.6. Aim

The purpose of the study was to analyse the survival of *C. jejuni* ST-257 and ST-918, in frozen chicken meat and thereby the use of freezing as a measure to reduce the risk for consumers to get campylobacteriosis. Previous studies indicate differences between sequence types in the rate of survival of freezing. However, different oppinions exists regarding the effectiveness of freezing *C. jejuni*.

2. Materials and methods

2.1. Bacterial culture preparation

The sequence types used in the study were isolated at farm level during the time of the *Campylobacter* outbreak in Sweden 2016-2017, they can therefore be concidered as the same sequence types involved in the outbreak. Two sequence types of *C. jejuni* were used, ST-257, and ST-918. Both sequence types were isolated within an in-depth analysis of Swedish broiler producers delivering *Campylobacter* positive flocks. ST-257 was isolated from the water pipes in a broiler house where *C. jejuni* ST-257 had been isolated from chickens during several rotations. ST-918 was isolated from transport crates after cleaning and disinfection and before loading of chicken to slaughter.

Both sequence types were removed from the storage in the freezer (-80°C), to blood agar plates (SVA, Uppsala, Sweden) incubated in microaerophilic conditions using CampyGen^{TM} (Oxoid, Basingstoke, UK) for 48 hours at 37°C. One colony was transferred to Brain Heart Infusion (BHI) with horse serum. The species of the isolates were confirmed by Matrix Assisted Laser Desorption Ionization -Time Of Flight (MALDI-TOF). A viable count was performed for the cultures. A 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared and 0.1 ml from dilution 10^{-2} - 10^{-7} was plated onto mCCDA the first time (high) and onto blood agar plates (low). The viable count in the first and second batch of contamination was for ST-257 $\log_{10}$ 7.5 and $\log_{10}$ 8.9, and for ST-918 $\log_{10}$ 6.3 and $\log_{10}$ 8.8.

2.2. Sample preparation and quality control

Deep frozen breast fillets of chicken, originating from Sweden, were bought in a supermarket. Fillets were thawed in a refrigerator (7 °C) overnight and thereafter cut into pieces of approximately 50 g. The pieces were placed in separate buckets with its meat juice, buffered peptone water (BPW), and culture of *C. jejuni* ST-257, respectively ST-918. In high, 2 litres of BPW and 45 ml culture were used

for each ST, and in low, 1 litre BPW and 1 ml culture were used (Picture A in Figure 3). Every sampling was performed in five duplicates. The mixture of breast fillets, BPW, and culture of *C. jejuni* was left one hour at room temperature. Thereafter, one piece of breast fillet and 5 ml suspension were placed in a stomacher bag, sealed, and stored at -22 °C (Picture B in Figure 3).

Before contamination, the chicken fillets were analysed according to ISO 10272 part 2 (ISO 2017) to ensure that the broiler fillet were not naturally contaminated with *Campylobacter*. A total of 10g meat were taken from the surface of several fillets and placed in a stomacher bag together with 90 ml Bolton enrichment broth (Oxoid, Basingstoke, UK). Thereafter the sample were homogenized and incubated at 41.5°C ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere generated by the use of CampyGen^{TM} (Oxoid, Basingstoke, UK). Thereafter, the enriched culture was plated on mCCDA and incubated at 41.5°C ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere. All packages of fillets tested negative for thermotolerant *Campylobacter*.

2.3. Quantitative analysis

Five pieces of breast fillet were taken from the freezer and placed in refrigerator to thaw overnight before analysis. The quantitative analysis was performed according to ISO 10272 part 2 (ISO 2017). Briefly, 10g was removed from the piece of meat (Picture C in Figure 3), placed in a stomacher bag together with 90 ml BPW. A stomacher (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA) homogenized the sample for 1 min at 240 rpm (Picture D in Figure 3). A 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared and 0.1 ml from dilution 10^{-1} - 10^{-3} were plated onto modified Charcoal Cephoperazone Desoxycholat Agar (mCCDA) (Oxoid, Basingstoke, UK) plates (90 mm diameter) (Picture E in Figure 3). For estimation of low numbers of *Campylobacter* 1,0 ml of inoculum from dilution 10^{-1} were distributed on three regular plates (90 mm) of mCCDA.

The plates were incubated at 41.5°C ± 0.5°C for 44 ± 4 h in a microaerobic atmosphere. After incubation, colonies characteristic of *C. jejuni* were quantified and the number of *C. jejuni* is expressed as $\log_{10}$ CFU/g, the detection limit for meat juice is 1 CFU/ml and for meat 1 $\log_{10}$ CFU/g (Picture F in Figure 3).

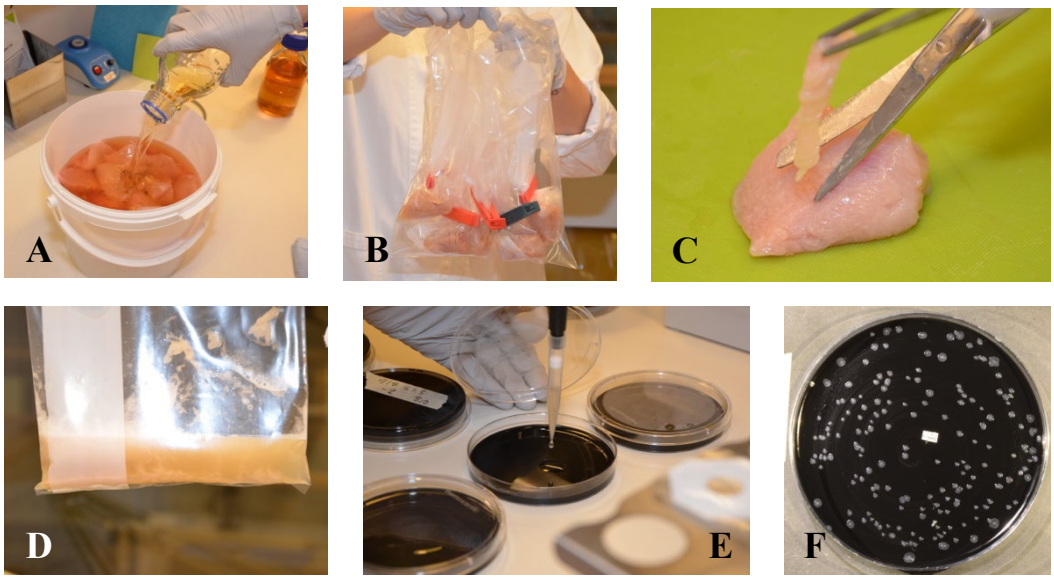


Figure 3. Sample preparation and quantitative analysis. Picture A shows the contamination of the meat. Picture B shows the sealed stomacher bags ready to be stored in freezer. Picture C shows how the surface meat was removed. Picture D shows the meat and BPW after stomacher. Picture E shows spreading 1 ml on three mCCDA plates. Finally, picture F shows the mCCDA plate after incubation..

2.4. Statistical analysis

The CFU was calculated from the dilution series with the formula from ISO 7218 (2014):

$$N = \sum C/(V\ x\ 1.1\ x\ d)$$

- N: number of organisms
- ∑ C: the sum of CFU on two plates from successive dilution within the countable range
- V: volume spread on the plate in ml
- 1.1: a factor to weigh the mean from two plates, if only one plate is counted, the factor is 1.0
- d: first countable dilution retained

The calculated CFU was used to create a boxplot in Excel (version 16.19, Microsoft, Redmond, Washinton, USA). Associations between findings were assessed using T-test in comparison of mean values and p-values of ≤ 0.05 was considered significant.

3. Results

3.1. Meat

The broiler meat contaminated with a high level of ST-257, had a mean of 5.3 $\log_{10}$ CFU/g before freezing (day 0), whereas the broiler meat contaminated with a low concentration of ST-257 had a mean of 4.1 $\log_{10}$ CFU/g. The high and low concentrations decreased significantly ($p < 0.05$) after freezing (day 2) with a drop in mean values of 1.4 $\log_{10}$ CFU/g and 0.8 $\log_{10}$ CFU/g, respectively. After storage for 49 days, the decrease was 2.0 $\log_{10}$ CFU/g and 1.6 $\log_{10}$ CFU/g, high and low, respectively. The decrease between day 0 and day 4 was significant ($p < 0.05$) in both high and low concentrations. However, the mean values were not significantly different on day 2 compared to day 4.

The mean concentrations of ST-918 in the meat were identical to ST-257 before freezing (day 0), 5.2 $\log_{10}$ CFU/g in high and 4.1 $\log_{10}$ CFU/g in low. After two days of freezing, the fillet contaminated with a high level of ST-918 decreased significantly ($p < 0.05$) 0.8 $\log_{10}$ CFU/g. Whereas meat contaminated with a low level of ST-918 had a decrease of 0.2 $\log_{10}$ CFU/g, which was not significant ($p > 0.05$). When comparing day 0 with day 4, a significant ($p < 0.05$) decrease was found in meat contaminated with a low level of ST-918, but not in the meat contaminated with a high level of ST-918. Comparison between day 2 and day 4 showed a significant decrease in low but not in meat contaminated with a high level. After 49 days, the concentration had decreased with 1.0 $\log_{10}$ CFU/g in the meat contaminated with a high concentration and 0.7 $\log_{10}$ CFU/g in meat contaminated with a low concentration (Figure 4).

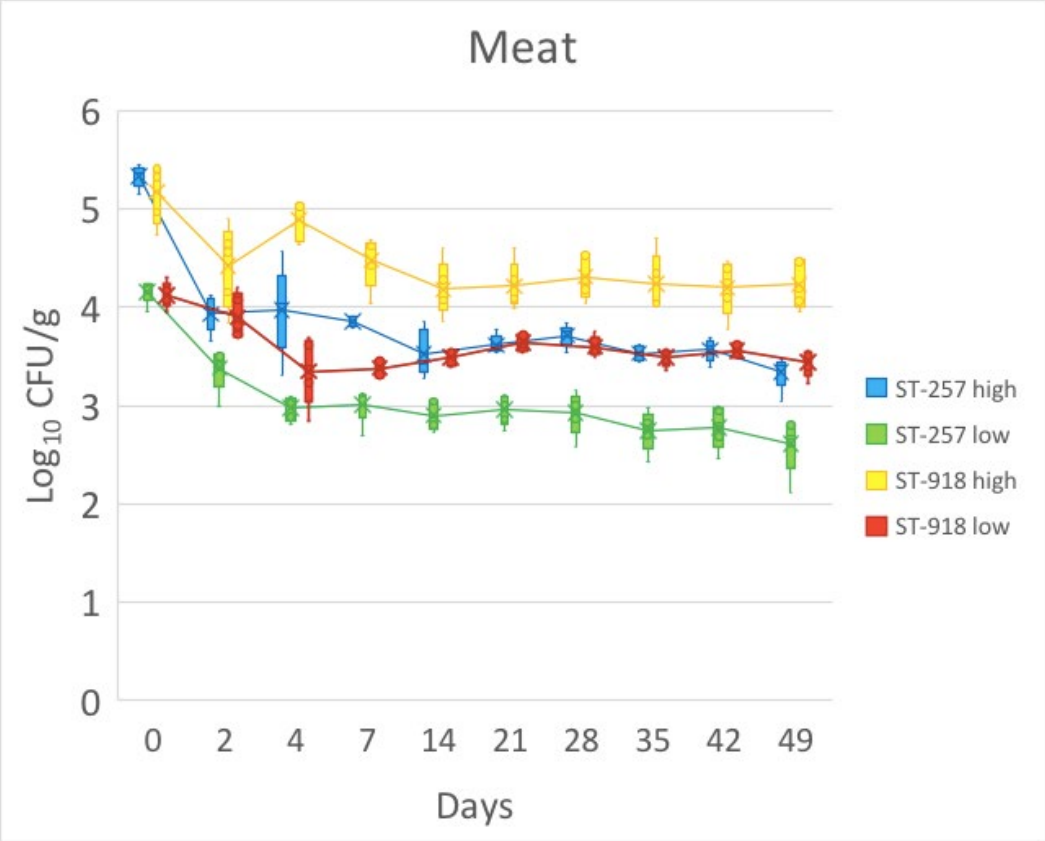


Figure 4. Amount of C. jejuni, ST-918 and ST-257 in contaminated meat and analysed continuously during 49 days of storage at -22°C.

3.2. Meat juice

The mean initial concentration of ST-257 in the meat juice was the same for high and low concentrations (Figure 5). Both decreased significantly by 0.7 $\log_{10}$ CFU/ml in high and 1.6 $\log_{10}$ CFU/ml in low the first day after freezing (day 2), whereas no significant ($p > 0.05$) decrease was observed between day 2 and day 4. After 49 days in the freezer, the mean values in broiler meat contaminated with a high level of *C. jejuni* ST-257 decreased 1.9 $\log_{10}$ CFU/ml and meat contaminated with a low level decreased 2.4 $\log_{10}$ CFU/ml.

The meat juice from broiler fillet contaminated with ST-918 at high level was 5.2 $\log_{10}$ CFU/ml day 0 and juice from meat contaminated with a low level at 4.6 $\log_{10}$ CFU/ml day 0. The mean concentration after freezing decreased significantly ($p < 0.05$) by 1.1 $\log_{10}$ CFU/ml in high and by 0.8 $\log_{10}$ CFU/ml in low. The total decrease in high and low, respectively, after 49 days was 1.3 $\log_{10}$ CFU/ml and 1.6 $\log_{10}$ CFU/ml meat juice (Figure 5).

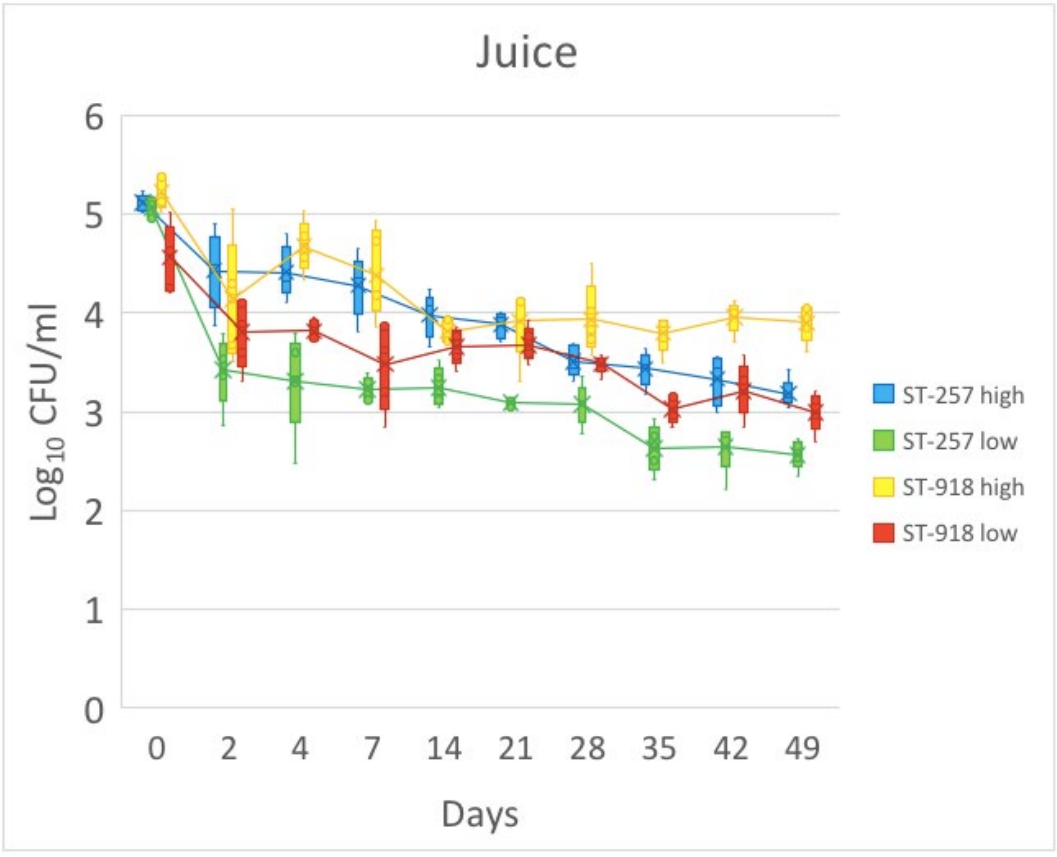


Figure 5. Amount of C. jejuni ST-918 and ST-257, with initially two different concentrations, in meat juice and analysed continuously during 49 days of storage at –22°C.

4. Discussion

The objective of this thesis was to investigate how two ST of *C. jejuni* survive freezing in broiler meat. The concentration of ST-257 and ST-918 decreased in the freezer, while ST-918 had a higher level of survival compared with ST-257. The rate of decrease in *C. jejuni* was largest after two to four days of freezing in both ST. Thereafter, the concentration of *C. jejuni* decreased to a lesser extent during storage in -22°C. In the meat, ST-918 had a mean decrease of 0.7 and 1.0 $\log_{10}$ CFU/g and ST-257 of 1.6 and 2.0 $\log_{10}$ CFU/g after 49 days, despite almost the same concentrations before freezing. A similar decrease in viable cells short after freezing was observed in the meat juice. The decrease in juice in $\log_{10}$ units was either equal to or greater than the reduction in the meat. Moreover, the reduction was higher for ST-257 than ST-918, suggesting different abilities to survive freeze storage.

No previous studies have been performed regarding the decrease of *C. jejuni* ST-257 and ST-918 during freezing. However, EFSA (2011) states in the scientific opinion that freezing for a few days reduces the prevalence of *C. jejuni* in broiler meat with 0.9-1.4 $\log_{10}$ CFU/g and freezing for three weeks gives a reduction of 1.8-2.2 $\log_{10}$ CFU/g. This study observed differences in the rate of decrease between the STs during storage. After four days of storage, ST-257 decreased with 1.3 $\log_{10}$ CFU/g in meat both high and low concentrations, corresponding to the predicted reduction by EFSA. ST-918 decreased with 0.3 $\log_{10}$ CFU/g in high and 0.8 $\log_{10}$ CFU/g in low after four days, decreasing less than predicted by EFSA. The concentration after 49 days in the ST-257 meat was reduced by 2.0 $\log_{10}$ in high and 1.6 $\log_{10}$ in low. While in ST-918, the reduction in high concentration was 1.0 $\log_{10}$ and 0.7 $\log_{10}$ in the low concentration. Compared to the reduction estimated in the report by EFSA, 1.77-2.18 $\log_{10}$ CFU/g after three weeks, the viable cells in ST-257 meat sample, it comprehended with the reduction stated by EFSA. Contrariwise, the reduction in ST-918 was minor, meaning ST-918 had a higher survival in the freezer compared with ST-257.

Several studies have investigated the reduction of *C. jejuni* in naturally and artificially contaminated meat (Bhaduri & Cottrell 2004; Sandberg et al. 2005; Georgsson et al. 2006; Ritz et al. 2007; Maziero & de Oliveira 2010; Huang et al. 2012). The studies show a variance in the effect of reducing cell counts by

freezing. Most studies declare a more considerable decline short after freezing followed by a phase when the number of viable cells remains at stable levels (Sandberg et al. 2005; Georgsson et al. 2006; Ritz et al. 2007; Sampers et al. 2010), complying with the results in this thesis. Naturally contaminated broiler meat, with on average 3.1 $\log_{10}$ CFU/g initial concentration of *C. jejuni,* observed a mean reduction in frozen meat after 28 days to be 2.3 $\log_{10}$ CFU/g (Maziero & de Oliveira 2010). Another study of naturally contaminated meat found a 1 $\log_{10}$ reduction after ten days and 2 $\log_{10}$ reduction after 21 days of storage in the freezer (Sandberg et al. 2005). Georgsson et al. (2006) found, in naturally contaminated carcasses a reduction of 0.7 to 2.9 $\log_{10}$ CFU/g after freezing for 31 days. More extended storage did not significantly reduce the counts. A study found the reduction in naturally contaminated ground chicken meat to be 1 $\log_{10}$ after one day, thereafter no significant reduction was observed (Sampers et al. 2010).

In artificially contaminated chicken meat, 8 $\log_{10}$ CFU/cm, the most significant reduction occurred within the first 24 hours of freezing, with a reduction of 1 to 1.5 $\log_{10}$ (Ritz et al. 2007) Thereafter, gradual or no decline in viable cells was observed. Bhaduri and Cottrell (2004) found a reduction of 0.6-1.5 $\log_{10}$ CFU/g after 14 days of frozen storage in artificial contaminated ground chicken meat. The reduction of three ST originating from chicken meat was 2.9, 3.1 and 3.2 $\log_{10}$ CFU/g after 55 days in the freezer (Huang et al. 2012). In contrast to this thesis' results, the rate of decrease in the first 20 days of storage was low, and after 25 days of storage the concentration of viable cells dropped rapidly until day 45, when the rate of decrease flattened.

The importance of using ST found in the broiler meat and at a reasonable concentration is pointed out by Boysen et al. (2013). They found the mean reduction after freezing for seven days to be greater in high concentration, 7 $\log_{10}$ CFU/g, than in 3 $\log_{10}$ CFU/g. The mean reduction in $\log_{10}$ ranged from 1.2-1.1 in 7 $\log_{10}$ CFU/g and 1.1-0.9 in 3 $\log_{10}$ CFU/g after 24 hours of freezing. The corresponding reduction after seven days was 1.8-1.2 in 7 $\log_{10}$ CFU/g and 1.4-1.0 in 3 $\log_{10}$ CFU/g in that study. It is important to investigate the reduction in lower concentrations and not overestimate the reduction through high bacterial concentrations. In ST-257, the total reduction in the meat was 0.4 $\log_{10}$ units larger in high compared to low. The same trend was observed in ST-918, with 0.3 $\log_{10}$ unit larger reduction in the high concentration. In the meat juice, the opposite of the trend in reduction in the meat was observed. For both ST, the total reduction in the juice after 49 days was greater in the low concentrations. Differing 0.5 $\log_{10}$ in ST-257 and 0.3 $\log_{10}$ in ST-918.

The initial mean concentrations ranged from 4 to 5 $\log_{10}$ CFU/g in both meat and meat juice. However, the initial concentrations in this thesis were slightly high to reflect the situation in Swedish broiler meat. In the baseline study 2008, *Campylobacter* was quantified in 9% of the neck skin samples and most of them in a level beween 2-3 $\log_{10}$ CFU/g. However, the initial concentration of 4 $\log_{10}$ CFU/g exists in Europe. In the baseline study nearly 6% exceeded 4 $\log_{10}$ CFU/g, and nearly 16% had counts between 3-4 $\log_{10}$ CFU/g among the 28 participating countries (EFSA 2010). In the latest zoonosis report, half of the *Campylobacter*-positive samples, corresponding to 7% of the total neck skin samples, reported via monitoring programs at food business operators, exceeded the limit of 3 $\log_{10}$ CFU/g (EFSA & ECDC 2021). The zoonosis report and the baseline survey do not declare the rate of how much the sample exceeds the limit.

The ST-257 and ST-918 were chosen due to their role in causing human campylobacteriosis. During 2016-2017, the outbreak of campylobacteriosis in Sweden was related to domestic poultry production. Every fourth sequenced isolate from patients during the outbreak was ST-918, while ST-257 was isolated in a few patients (Swedish Food Agency & Public Health Agency of Sweden 2018). Moreover, ST-918 was the most frequently isolated sequence type in fresh retail broiler meat during the investigation of the outbreak. ST-257 was the most frequent ST isolated from patients in Sweden week 34 in 2019 (Public Health Agency of Sweden & Swedish Food Agency 2020) and has been significantly associated with hospitalisation (Harvala et al. 2016). ST-918 is probably more resistant to oxidative stress and has the ability to adhere and endure on surfaces on transport crates and at the slaughterhouse.

The variation between sequence types' tolerance to stresses, including freezing, contributes to the risk of human infection (Oh et al. 2018, Oh et al. 2019). The differences in their ability to survive freezing and other physical tests are therefore of interest. Comparing the results of ST-257 and ST-918 with a ST that have not been associated with human infection would be of interest to state the efficiency of freezing in other ST. The virulence factors involved in human infection might be related to the ability to stand freezing, either due to the higher dose or in combination with other mechanisms of pathogenicity like the ability to attach to surfaces. The ability to survive stress might explain the outbreak in 2016, primarily caused by ST-918.

In future studies, the initial concentration in the contaminated meat should be lower, 2 to 3 $\log_{10}$ CFU/g, to analyse if the reduction of viable cells corresponds to the reduction in higher concentrations. In this thesis, the meat was frozen together with the culture of *C. jejuni*. In future studies, it would be of interest to use contaminated fresh meat instead of frozen meat. Since the fresh meat will lose

water after freezing, this will give meat juice to analyse which will be more similar to the juice found in the store-bought frozen broiler meat.

5. Conclusion

Freezing reduced the concentration of *C. jejuni* in broiler meat and meat juice. However, freezing did not eliminate the presence of the bacteria. The rate of decrease was most significant the first two to four days, thereafter the rate of decrease flattens. The reduction in the meat juice was equal or higher than in the meat after storage for 49 days. A difference in the ability to stand the stress was observed, with ST-918 decreasing to a lesser extent than ST-257, indicating ST-918 surviving better in frozen broiler meat. For the consumer, the meat juice probably poses a greater risk than an undercooked core in the meat, since the concentrations of *C. jejuni* in the juice was rather similar to the meat, and the juice can spread to other surfaces in the kitchen.

References

Adams, M.R., Moss, M.O. & McClure, P.J. (2016). *Food microbiology*. 4th edition. Cambridge, UK: Royal Society of Chemistry.

Allen, V.M., Bull, S.A., Corry, J.E.L., Domingue, G., Jørgensen, F., Frost, J.A., Whyte, R., Gonzalez, A., Elviss, N. & Humphrey, T.J. (2007). *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. *International Journal of Food Microbiology*, 113 (1), 54–61. https://doi.org/10.1016/j.ijfoodmicro.2006.07.011

Allos, B.M. & Blaser, M.J. (1995). *Campylobacter jejuni* and the Expanding Spectrum of Related Infections. *Clinical Infectious Diseases*, 20 (5), 1092–1101. https://doi.org/10.1093/clinids/20.5.1092

Alter, T. & Reich, F. (2021). Management Strategies for Prevention of *Campylobacter* Infections Through the Poultry Food Chain: A European Perspective. I: Backert, S. (red.) *Fighting Campylobacter Infections*. Cham: Springer International Publishing, 79–102. https://doi.org/10.1007/978-3-030-65481-8_4

Bhaduri, S. & Cottrell, B. (2004). Survival of Cold-Stressed *Campylobacter jejuni* on Ground Chicken and Chicken Skin during Frozen Storage. *Applied and Environmental Microbiology*, 70 (12), 7103–7109. https://doi.org/10.1128/AEM.70.12.7103-7109.2004

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. & Blaser, M.J. (1988). Experimental *Campylobacter jejuni* Infection in Humans. *Journal of Infectious Diseases*, 157 (3), 472–479. https://doi.org/10.1093/infdis/157.3.472

Bolton, D.J. (2015). *Campylobacter* virulence and survival factors. *Food Microbiology*, 48, 99–108. https://doi.org/10.1016/j.fm.2014.11.017

Bolton, F.J. & Coates, D. (1983). A study of the oxygen and carbon dioxide requirements of thermophilic campylobacters. *Journal of Clinical Pathology*, 36 (7), 829–834. https://doi.org/10.1136/jcp.36.7.829

Boysen, L., Wechter, N.S. & Rosenquist, H. (2013). Effects of decontamination at varying contamination levels of *Campylobacter jejuni* on broiler meat. *Poultry Science*, 92 (5), 1425–1429. https://doi.org/10.3382/ps.2012-02889

Cawthraw, S.A., Wassenaar, T.M., Ayling, R. & Newell, D.G. (1996). Increased colonization potential of *Campylobacter jejuni* strain 81116 after passage through chickens and its implication on the rate of transmission within

flocks. *Epidemiology and Infection*, 117 (1), 213–215. https://doi.org/10.1017/s0950268800001333
CDC (2021). *Campylobacter (Campylobacteriosis) | Campylobacter | CDC*. https://www.cdc.gov/campylobacter/index.html [2021-04-15]
Cody, A.J., Maiden, M.C., Strachan, N.J. & McCarthy, N.D. (2019). A systematic review of source attribution of human campylobacteriosis using multilocus sequence typing. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, 24 (43). https://doi.org/10.2807/1560-7917.ES.2019.24.43.1800696
Corry, J.E., Post, D.E., Colin, P. & Laisney, M.J. (1995). Culture media for the isolation of *campylobacters*. *International Journal of Food Microbiology*, 26 (1), 43–76. https://doi.org/10.1016/0168-1605(95)00044-k
Corry, J.E.L., James, S.J., Purnell, G., Barbedo-Pinto, C.S., Chochois, Y., Howell, M. & James, C. (2007). Surface pasteurisation of chicken carcasses using hot water. *Journal of Food Engineering*, 79 (3), 913–919. https://doi.org/10.1016/j.jfoodeng.2006.03.018
Dasti, J.I., Tareen, A.M., Lugert, R., Zautner, A.E. & Groß, U. (2010). *Campylobacter jejuni*: A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology*, 300 (4), 205–211. https://doi.org/10.1016/j.ijmm.2009.07.002
Dekeyser, P., Gossuin-Detrain, M., Butzler, J.P. & Sternon, J. (1972). Acute Enteritis Due to Related Vibrio: First Positive Stool Cultures. *Journal of Infectious Diseases*, 125 (4), 390–392. https://doi.org/10.1093/infdis/125.4.390
EFSA (2010). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008 - Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal*, 8 (3). https://doi.org/10.2903/j.efsa.2010.1503
EFSA (2011). Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, (2011;9(4):2105). https://doi.org/10.2903/j.efsa.2011.2105
EFSA (2021a). *Campylobacter*. *European Food Safety Authority*. https://www.efsa.europa.eu/en/topics/topic/campylobacter [2021-03-18]
EFSA (2021b). *Foodborne outbreak*. *European Food Safety Authority*. https://www.efsa.europa.eu/en/glossary/foodborne-outbreak [2021-04-15]
EFSA & ECDC (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, 15 (12). https://doi.org/10.2903/j.efsa.2017.5077
EFSA & ECDC (2021). The European Union One Health 2019 Zoonoses Report. *EFSA Journal*, 19 (2). https://doi.org/10.2903/j.efsa.2021.6406

EFSA Panel on Biological Hazards, Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Alter, T., Crotta, M., Ellis-Iversen, J., Hempen, M., Messens, W. & Chemaly, M. (2020). Update and review of control options for *Campylobacter* in broilers at primary production. *EFSA Journal*, 18 (4). https://doi.org/10.2903/j.efsa.2020.6090

Emanowicz, M., Meade, J., Bolton, D., Golden, O., Gutierrez, M., Byrne, W., Egan, J., Lynch, H., O'Connor, L., Coffey, A., Lucey, B. & Whyte, P. (2021). The impact of key processing stages and flock variables on the prevalence and levels of *Campylobacter* on broiler carcasses. *Food Microbiology*, 95, 103688. https://doi.org/10.1016/j.fm.2020.103688

Endtz, H.P., Ang, C.W., van den Braak, N., Luijendijk, A., Jacobs, B.C., de Man, P., van Duin, J.M., van Belkum, A. & Verbrugh, H.A. (2000). Evaluation of a new commercial immunoassay for rapid detection of *Campylobacter jejuni* in stool samples. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology*, 19 (10), 794–797. https://doi.org/10.1007/s100960000368

European Commission (2005). *COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs*. *OJ L*. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32005R2073 [2021-01-22]

European Commission (2018). *COMMISSION REGULATION (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005 as regards Campylobacter in broiler carcases*. https://eur-lex.europa.eu/eli/reg/2017/1495/oj

European Commission. Directorate General for Agriculture and Rural Development. (2020). *EU agricultural outlook for markets and income 2020-2030.* LU: Publications Office. https://data.europa.eu/doi/10.2762/252413 [2021-04-30]

Eurostat (2019). *Poultry meat production in EU at new high in 2018*. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20190325-1 [2021-05-02]

Friedrich, A., Marshall, J.C., Biggs, P.J., Midwinter, A.C. & French, N.P. (2016). Seasonality of *Campylobacter jejuni* isolates associated with human campylobacteriosis in the Manawatu region, New Zealand. *Epidemiology and Infection*, 144 (4), 820–828. https://doi.org/10.1017/S0950268815002009

García-Sánchez, L., Melero, B., Jaime, I., Hänninen, M.-L., Rossi, M. & Rovira, J. (2017). *Campylobacter jejuni* survival in a poultry processing plant environment. *Food Microbiology*, 65, 185–192. https://doi.org/10.1016/j.fm.2017.02.009

Georgsson, F., Þorkelsson, Á.E., Geirsdóttir, M., Reiersen, J. & Stern, N.J. (2006). The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. *Food Microbiology*, 23 (7), 677–683. https://doi.org/10.1016/j.fm.2005.10.003

Godschalk, P.C.R., Heikema, A.P., Gilbert, M., Komagamine, T., Ang, C.W., Glerum, J., Brochu, D., Li, J., Yuki, N., Jacobs, B.C., van Belkum, A. & Endtz, H.P. (2004). The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barré syndrome. *Journal of Clinical Investigation*, 114 (11), 1659–1665. https://doi.org/10.1172/JCI200415707

Haagsma, J.A., Geenen, P.L., Ethelberg, S., Fetsch, A., Hansdotter, F., Jansen, A., Korsgaard, H., O'Brien, S.J., Scavia, G., Spitznagel, H., Stefanoff, P., Tam, C.C. & Havelaar, A.H. (2013). Community incidence of pathogen-specific gastroenteritis: reconstructing the surveillance pyramid for seven pathogens in seven European Union member states. *Epidemiology and Infection*, 141 (8), 1625–1639. https://doi.org/10.1017/S0950268812002166

Habib, I., Louwen, R., Uyttendaele, M., Houf, K., Vandenberg, O., Nieuwenhuis, E.E., Miller, W.G., van Belkum, A. & De Zutter, L. (2009). Correlation between Genotypic Diversity, Lipooligosaccharide Gene Locus Class Variation, and Caco-2 Cell Invasion Potential of *Campylobacter jejuni* Isolates from Chicken Meat and Humans: Contribution to Virulotyping. *Applied and Environmental Microbiology*, 75 (13), 4277–4288. https://doi.org/10.1128/AEM.02269-08

Hansson, I. (2007). *Bacteriological and epidemiological studies of campylobacter spp. in Swedish broilers*. Dept. of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences. https://pub.epsilon.slu.se/1461/1/Avhandling_nr_63.2007_tryckfil_Ingrid_Hansson_reserv.pdf

Hansson, I., Forshell, L.P., Gustafsson, P., Boqvist, S., Lindblad, J., Engvall, E.O., Andersson, Y. & Vågsholm, I. (2007). Summary of the Swedish *Campylobacter* Program in Broilers, 2001 through 2005. *Journal of Food Protection*, 70 (9), 2008–2014. https://doi.org/10.4315/0362-028X-70.9.2008

Hansson, I., Pudas, N., Harbom, B. & Engvall, E.O. (2010). Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. *International Journal of Food Microbiology*, 141 (1–2), 51–55. https://doi.org/10.1016/j.ijfoodmicro.2010.04.019

Hansson, I., Sandberg, M., Habib, I., Lowman, R. & Engvall, E.O. (2018). Knowledge gaps in control of *Campylobacter* for prevention of campylobacteriosis. *Transboundary and Emerging Diseases*, 65 Suppl 1, 30–48. https://doi.org/10.1111/tbed.12870

Harvala, H., Rosendal, T., Lahti, E., Engvall, E.O., Brytting, M., Wallensten, A. & Lindberg, A. (2016). Epidemiology of *Campylobacter jejuni* infections

in Sweden, November 2011-October 2012: is the severity of infection associated with C. jejuni sequence type? *Infection Ecology & Epidemiology*, 6, 31079. https://doi.org/10.3402/iee.v6.31079

Hazeleger, W.C., Wouters, J.A., Rombouts, F.M. & Abee, T. (1998). Physiological Activity of *Campylobacter jejuni* Far below the Minimal Growth Temperature. *Applied and Environmental Microbiology*, 64 (10), 3917–3922. https://doi.org/10.1128/AEM.64.10.3917-3922.1998

Hofshagen, M. & Kruse, H. (2005). Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. *Journal of Food Protection*, 68 (10), 2220–2223. https://doi.org/10.4315/0362-028x-68.10.2220

Huang, J., Jiang, F., Hu, Y., Zhou, X., Gu, S. & Jiao, X. (2012). An Inactivation Kinetics Model for *Campylobacter jejuni* on Chicken Meat Under Low-Temperature Storage. *Foodborne Pathogens and Disease*, 9 (6), 513–516. https://doi.org/10.1089/fpd.2011.1070

Hughes, R.A.C. & Rees, J.H. (1997). Clinical and Epidemiologic Features of Guillain-Barré Syndrome. *The Journal of Infectious Diseases*, 176 (s2), S92–S98. https://doi.org/10.1086/513793

Humphrey, T., Mason, M. & Martin, K. (1995). The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *International Journal of Food Microbiology*, 26 (3), 295–303. https://doi.org/10.1016/0168-1605(94)00135-s

Humphrey, T., O'Brien, S. & Madsen, M. (2007). *Campylobacters* as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, 117 (3), 237–257. https://doi.org/10.1016/j.ijfoodmicro.2007.01.006

ISO (2014). *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations AMENDMENT 1*. (ISO 7218:2007/Amd.1:2013(E)). https://www.iso.org/standard/52204.html [2021-05-24]

ISO (2017). *Microbiology of the food chain– Horizontal method for detection and enumeration of Campylobacter spp. – Part 1: Detection method—Part 2: Colony count technique (2017).* https://www.iso.org/standard/63228.html

James, C., James, S.J., Hannay, N., Purnell, G., Barbedo-Pinto, C., Yaman, H., Araujo, M., Gonzalez, M.L., Calvo, J., Howell, M. & Corry, J.E.L. (2007). Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *International Journal of Food Microbiology*, 114 (2), 195–203. https://doi.org/10.1016/j.ijfoodmicro.2006.09.019

de Jong, A.E.I., Verhoeff-Bakkenes, L., Nauta, M.J. & de Jonge, R. (2008). Cross-contamination in the kitchen: effect of hygiene measures. *Journal of Applied Microbiology*, 105 (2), 615–624. https://doi.org/10.1111/j.1365-2672.2008.03778.x

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global Epidemiology of *Campylobacter* Infection. *Clinical Microbiology Reviews*, 28 (3), 687–720. https://doi.org/10.1128/CMR.00006-15

King, E.O. (1957). Human infections with Vibrio fetus and a closely related vibrio. *The Journal of Infectious Diseases*, 101 (2), 119–128. https://doi.org/10.1093/infdis/101.2.119

Lange, M., Göranzon, H. & Marklinder, I. (2016). Self-reported food safety knowledge and behaviour among Home and Consumer Studies students. *Food Control*, 67, 265–272. https://doi.org/10.1016/j.foodcont.2016.03.014

Levy, A.J. (1946). A gastro-enteritis cutbreak probably due to a bovine strain of vibrio. *The Yale Journal of Biology and Medicine*, 18, 243–258

Luechtefeld, N.W., Wang, W.L., Blaser, M.J. & Reller, L.B. (1981). Evaluation of transport and storage techniques for isolation of *Campylobacter fetus* subsp. *jejuni* from turkey cecal specimens. *Journal of Clinical Microbiology*, 13 (3), 438–443

Lund, M., Nordentoft, S., Pedersen, K. & Madsen, M. (2004). Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR. *Journal of Clinical Microbiology*, 42 (11), 5125–5132. https://doi.org/10.1128/JCM.42.11.5125-5132.2004

Marklinder, I., Magnusson, M. & Nydahl, M. (2013). CHANCE: a healthy lifestyle in terms of food handling and hygiene. *British Food Journal*, 115 (2), 223–234. https://doi.org/10.1108/00070701311302203

Maziero, M.T. & de Oliveira, T.C.R.M. (2010). Effect of refrigeration and frozen storage on the *Campylobacter jejuni* recovery from naturally contaminated broiler carcasses. *Brazilian Journal of Microbiology: [publication of the Brazilian Society for Microbiology]*, 41 (2), 501–505. https://doi.org/10.1590/S1517-838220100002000034

McCarthy, N. & Giesecke, J. (2001). Incidence of Guillain-Barré syndrome following infection with *Campylobacter jejuni. American Journal of Epidemiology*, 153 (6), 610–614. https://doi.org/10.1093/aje/153.6.610

McFadyean, J. & Stockman, S. (1913). *Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire into Epizootic Abortion.* (Part III. Abortion in Sheep). HMSO London.

Moazzami, M., Bergenkvist, E., Fernström, L.-L., Rydén, J. & Hansson, I. (2021). Reducing *Campylobacter jejuni*,Enterobacteriaceae, *Escherichia coli*, and Total Aerobic Bacteria on Broiler Carcasses Using Combined Ultrasound and Steam. *Journal of Food Protection*, 84 (4), 572–578. https://doi.org/10.4315/JFP-20-395

Nagel, G.M., Bauermeister, L.J., Bratcher, C.L., Singh, M. & McKee, S.R. (2013). *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *International Journal of Food Microbiology*, 165 (3), 281–286. https://doi.org/10.1016/j.ijfoodmicro.2013.05.016

National Advisory Committee on Microbiological Criteria for Foods (2007). Analytical utility of *Campylobacter* methodologies. *Journal of Food Protection*, 70 (1), 241–250. https://doi.org/10.4315/0362-028x-70.1.241

National Veterinary Institute, Swedish Board of Agricultur, Public Health Agency of Sweden, & Swedish Food Agency (2019). *Surveillance of infectious diseases in animals and humans in Sweden*. https://www.sva.se/media/fpodqpau/surveillance_2019.pdf

Nationalencyklopedin (2021). *jejunum - Uppslagsverk - NE.se*. https://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/jejunum [2021-04-14]

Newell, D.G. (2001). Animal models of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar Helicobacter pylori models. *Journal of Applied Microbiology*, 90 (S6), 57S-67S. https://doi.org/10.1046/j.1365-2672.2001.01354.x

NMKL (2007). *Nordisk Metodikkomite for Næringsmidler – Nordic Committee on Food Analysis, NMKL 119. Thermotolerant Campylobacter. Detection, semi-quantitative and quantitative determination in foods and drinking water.* https://www.nmkl.org/index.php/en/publications/item/termotolerante-campylobacter-pavisning-semi-kvantitativ-og-kvantitativ-bestemmelse-i-levnedsmidler-og-drikkevand-nmkl-119-3-utg-2007

Nygård, K., Andersson, Y., Røttingen, J.A., Svensson, Å., Lindbäck, J., Kistemann, T. & Giesecke, J. (2004). Association between environmental risk factors and *campylobacter* infections in Sweden. *Epidemiology and Infection*, 132 (2), 317–325. https://doi.org/10.1017/S0950268803001900

OECD (2021). Meat consumption. OECD. https://doi.org/10.1787/fa290fd0-en

Oh, E., Andrews, K.J., McMullen, L.M. & Jeon, B. (2019). Tolerance to stress conditions associated with food safety in *Campylobacter jejuni* strains isolated from retail raw chicken. *Scientific Reports*, 9 (1), 11915. https://doi.org/10.1038/s41598-019-48373-0

Oh, E., Chui, L., Bae, J., Li, V., Ma, A., Mutschall, S.K., Taboada, E.N., McMullen, L.M. & Jeon, B. (2018). Frequent Implication of Multistress-Tolerant *Campylobacter jejuni* in Human Infections. *Emerging Infectious Diseases*, 24 (6), 1037–1044. https://doi.org/10.3201/eid2406.171587

Oh, E., McMullen, L. & Jeon, B. (2015). High Prevalence of Hyper-Aerotolerant *Campylobacter jejuni* in Retail Poultry with Potential Implication in Human Infection. *Frontiers in Microbiology*, 6, 1263. https://doi.org/10.3389/fmicb.2015.01263

OIE (2004). *Manual of diagnostic tests and vaccines for terrestrial animals Part 2, Section 2.10, chapter 2.10.8. Campylobacter jejuni and Campylobacter coli, pp. 1072–1081.* https://www.oie.int/standard-setting/terrestrial-manual/

Oosterom, J., De Wilde, G.J.A., De Boer, E., De Blaauw, L.H. & Karman, H. (1983). Survival of *Campylobacter jejuni* during Poultry Processing and

Pig Slaughtering. *Journal of Food Protection*, 46 (8), 702–706. https://doi.org/10.4315/0362-028X-46.8.702
Perez-Arnedo, I. & Gonzalez-Fandos, E. (2019). Prevalence of *Campylobacter* spp. in Poultry in Three Spanish Farms, A Slaughterhouse and A Further Processing Plant. *Foods (Basel, Switzerland)*, 8 (3). https://doi.org/10.3390/foods8030111
Peyrat, M., Soumet, C., Maris, P. & Sanders, P. (2008). Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology*, 124 (2), 188–194. https://doi.org/10.1016/j.ijfoodmicro.2008.03.030
Public Health Agency of Sweden & Swedish Food Agency (2020). *Campylobacter* från butik och klinik 2019. 18
Ritz, M., Nauta, M.J., Teunis, P.F.M., van Leusden, F., Federighi, M. & Havelaar, A.H. (2007). Modelling of *Campylobacter* survival in frozen chicken meat. *Journal of Applied Microbiology*, 103 (3), 594–600. https://doi.org/10.1111/j.1365-2672.2007.03284.x
Robinson, D.A. (1981). Infective dose of *Campylobacter jejuni* in milk. *BMJ*, 282 (6276), 1584–1584. https://doi.org/10.1136/bmj.282.6276.1584
Rollins, D.M. & Colwell, R.R. (1986). Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology*, 52 (3), 531–538. https://doi.org/10.1128/AEM.52.3.531-538.1986
Rosenquist, H., Nielsen, N.L., Sommer, H.M., Nørrung, B. & Christensen, B.B. (2003). Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *International Journal of Food Microbiology*, 83 (1), 87–103. https://doi.org/10.1016/S0168-1605(02)00317-3
Rosenquist, H., Sommer, H.M., Nielsen, N.L. & Christensen, B.B. (2006). The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. *International Journal of Food Microbiology*, 108 (2), 226–232. https://doi.org/10.1016/j.ijfoodmicro.2005.12.007
Sampers, I., Habib, I., De Zutter, L., Dumoulin, A. & Uyttendaele, M. (2010). Survival of *Campylobacter* spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. *International Journal of Food Microbiology*, 137 (2–3), 147–153. https://doi.org/10.1016/j.ijfoodmicro.2009.11.013
Sandberg, M., Hofshagen, M., Østensvik, Ø., Skjerve, E. & Innocent, G. (2005). Survival of *Campylobacter* on Frozen Broiler Carcasses as a Function of Time. *Journal of Food Protection*, 68 (8), 1600–1605. https://doi.org/10.4315/0362-028X-68.8.1600
Scallan Walter, E.J., Crim, S.M., Bruce, B.B. & Griffin, P.M. (2019). Postinfectious Irritable Bowel Syndrome After *Campylobacter* Infection.

American Journal of Gastroenterology, 114 (10), 1649–1656. https://doi.org/10.14309/ajg.0000000000000408
Sebald, M. & Veron, M. (1963). [BASE DNA CONTENT AND CLASSIFICATION OF VIBRIOS]. *Annales De l'Institut Pasteur*, 105, 897–910
Skirrow, M.B. (1977). *Campylobacter* enteritis: a "new" disease. *BMJ*, 2 (6078), 9–11. https://doi.org/10.1136/bmj.2.6078.9
Stanley, K. & Jones, K. (2003). Cattle and sheep farms as reservoirs of *Campylobacter*: *CAMPYLOBACTER* ON CATTLE AND SHEEP FARMS. *Journal of Applied Microbiology*, 94, 104–113. https://doi.org/10.1046/j.1365-2672.94.s1.12.x
Steele, T.W. & McDermott, S.N. (1984). The use of membrane filters applied directly to the surface of agar plates for the isolation of campylobacter jejuni from feces. *Pathology*, 16 (3), 263–265. https://doi.org/10.3109/00313028409068535
Stern, N.J., Hiett, K.L., Alfredsson, G.A., Kristinsson, K.G., Reiersen, J., Hardardottir, H., Briem, H., Gunnarsson, E., Georgsson, F., Lowman, R., Berndtson, E., Lammerding, A.M., Paoli, G.M. & Musgrove, M.T. (2003). *Campylobacter* spp. in Icelandic poultry operations and human disease. *Epidemiology and Infection*, 130 (1), 23–32. https://doi.org/10.1017/s0950268802007914
Stern, N.J. & Robach, M.C. (2003). Enumeration of *Campylobacter* spp. in Broiler Feces and in Corresponding Processed Carcasses. *Journal of Food Protection*, 66 (9), 1557–1563. https://doi.org/10.4315/0362-028X-66.9.1557
Strachan, N.J.C., Rotariu, O., Smith-Palmer, A., Cowden, J., Sheppard, S.K., O'Brien, S.J., Maiden, M.C.J., Macrae, M., Bessell, P.R., Matthews, L., Reid, S.W.J., Innocent, G.T., Ogden, I.D. & Forbes, K.J. (2013). Identifying the seasonal origins of human campylobacteriosis. *Epidemiology and Infection*, 141 (6), 1267–1275. https://doi.org/10.1017/S0950268812002063
Swedish Board of Agriculture (2021a). *Konsumtion av kött*. [text]. https://jordbruksverket.se/mat-och-drycker/hallbar-produktion-och-konsumtion-av-mat/konsumtion-av-kott [2021-04-15]
Swedish Board of Agriculture (2021b). *Priser och marknadsinformation för livsmedel*. [text]. https://jordbruksverket.se/mat-och-drycker/handel-och-marknad/priser-och-marknadsinformation-for-livsmedel [2021-04-30]
Swedish Food Agency (2020). *Campylobacter i färsk kyckling från butik — augusti 2019*. https://www.livsmedelsverket.se/bestall-ladda-ner-material/sok-publikationer/artiklar/2020/l-2020-nr-12-campylobacter-i-farsk-kyckling-fran-butik
Swedish Food Agency & Public Health Agency of Sweden (2018). *Campylobacter från butik och klinik – jämförelser under och efter*

utbrottet 2016–2017. https://www.livsmedelsverket.se/bestall-ladda-ner-material/sok-publikationer/artiklar/2018/2018-nr-10
Swedish Poultry Meat Association (2021a). Fågel i siffror. *Svensk Fågel*. https://svenskfagel.se/fagel-i-siffror/ [2021-04-30]
Swedish Poultry Meat Association (2021b). *Årsrapport 2020*. https://svenskfagel.se/wp-content/uploads/2021/04/arsrapport-2020-popular-slutlig.pdf
Söderlin, M.K., Kautiainen, H., Puolakkainen, M., Hedman, K., Söderlund-Venermo, M., Skogh, T. & Leirisalo-Repo, M. (2003). Infections preceding early arthritis in southern Sweden: a prospective population-based study. *The Journal of Rheumatology*, 30 (3), 459
Tam, C.C., Rodrigues, L.C., Viviani, L., Dodds, J.P., Evans, M.R., Hunter, P.R., Gray, J.J., Letley, L.H., Rait, G., Tompkins, D.S., O'Brien, S.J., & IID2 Study Executive Committee (2012). Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. *Gut*, 61 (1), 69–77. https://doi.org/10.1136/gut.2011.238386
Vellinga, A. & Van Loock, F. (2002). The dioxin crisis as experiment to determine poultry-related *campylobacter* enteritis. *Emerging Infectious Diseases*, 8 (1), 19–22
Wagenaar, J.A., French, N.P. & Havelaar, A.H. (2013). Preventing *Campylobacter* at the source: why is it so difficult? *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 57 (11), 1600–1606. https://doi.org/10.1093/cid/cit555
Wen, X. & Dickson, J.S. (2012). Survival of *Campylobacter jejuni* and Salmonella enterica Typhimurium in vacuum-packed, moisture-enhanced pork. *Journal of Food Protection*, 75 (3), 576–579. https://doi.org/10.4315/0362-028X.JFP-11-343
WHO (2006). *Five keys to safer food manual.* France: World Health Organization.
WHO (2020). *Campylobacter*. https://www.who.int/news-room/fact-sheets/detail/campylobacter [2021-03-25]
Williams, M.S., Golden, N.J., Ebel, E.D., Crarey, E.T. & Tate, H.P. (2015). Temporal patterns of *Campylobacter* contamination on chicken and their relationship to campylobacteriosis cases in the United States. *International Journal of Food Microbiology*, 208, 114–121. https://doi.org/10.1016/j.ijfoodmicro.2015.05.018
Zhao, T., Ezeike, G.O.I., Doyle, M.P., Hung, Y.-C. & Howell, R.S. (2003). Reduction of *Campylobacter jejuni* on Poultry by Low-Temperature Treatment. *Journal of Food Protection*, 66 (4), 652–655. https://doi.org/10.4315/0362-028X-66.4.652

Acknowledgements

I want to thank my supervisor Ingrid for all the time you spent on helping me with the laboratory work and the writing. I am greatful for the opportunity to write and learn about *Campylobacter*. I also want to thank Lise-Lotte for the help in the laboratory and Emma for helping with the thesis.

Appendix 1

Survival of *Campylobacter jejuni* in frozen chicken fillet

CONCLUSION

Freezing reduces the concentration of viable *Campylobacter*. The reduction is largest the first two to four days, thereafter the rate of decrease flattens. ST-918 survived the freezing better than ST-257.

Background

Campylobacteriosis is the most reported zoonosis in the EU, with over 200,000 reported cases annually. Many cases are not reported and the true number of cases is estimated to nine million, leading to an estimated yearly cost of 2.4 billion euro. *C. jejuni* causes over 80% of the human infections and broilers are considered to be the major source of human exposure.

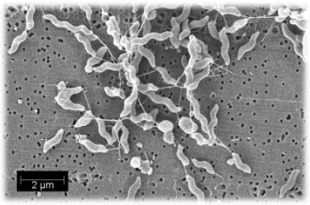
Objective

The purpose of the study was to analyse the survival of *C. jejuni* ST-257 and ST-918, in frozen chicken meat and thereby the use of freezing as a measure to reduce the risk for consumers to get campylobacteriosis.

Method

This thesis analysed artificially contaminated breast fillet with two human pathogenic sequence types of *C. jejuni*, ST-257 and ST-918. The fillets were frozen with added meat juice and stored up to 49 days. Analysis was performed on ten occasions and made in five duplicates. The analysis was performed according to ISO 10272, part 2.

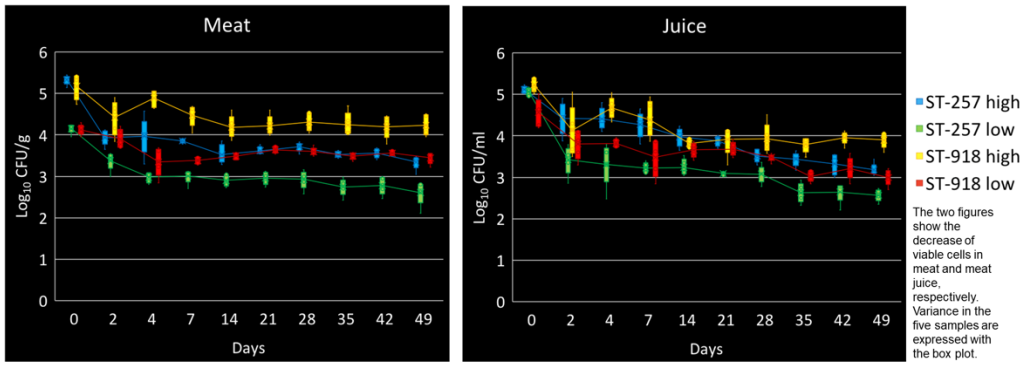
Meat from the surface was removed with a scissor and a tweezer.



C. jejuni in a scanning electron microscope of. Photo taken by Ingrid Hansson (BVF, SLU),Tapio Nikkilä (BVF, SLU) & Leif Ljung (UU).

Results

The largest rate of decrease occurred during the first two to four days of freezing, thereafter the rate of decrease flattened. ST-257 decreased in the meat in mean by 1.6 and 2.0 $\log_{10}$ CFU/g, and ST-918 decreased by 0.7 and 1.0 $\log_{10}$ CFU/g, low and high, respectively. The reduction in the juice was equal to or larger than the reduction in the meat.



The two figures show the decrease of viable cells in meat and meat juice, respectively. Variance in the five samples are expressed with the box plot.

Ella Råhlén
Agricultural Programme – Food Science
elrn0002@stud.slu.se

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