Longitudinal Transmission of *Campylobacter jejuni* Subtypes throughout the Broiler Chicken Production Continuum

by

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Abstract

The broiler chicken production continuum (including three commercial broiler farms, abattoir and retail poultry stream) was sampled longitudinally over a 542-day period in Southwestern Alberta (SWA) as a model agroecosystem. Furthermore, fecal samples collected from beef cattle in a confined feeding operation adjacent to one of the broiler farms, and stools from diarrheic human beings in SWA were collected during the sampling period. *Campylobacter jejuni* isolates were recovered from chickens using two methods (i.e. direct plating and enrichment/membrane filtration). Presumptive *Campylobacter* isolates from chickens, beef cattle, and diarrheic human beings were identified by taxon-specific polymerase chain reaction, and *C. jejuni* isolates were genotyped using a high resolution comparative genome fingerprinting method. The results showed that *C. jejuni* outbreaks were uncommon events in broiler farms. When infections occurred, they happened late in the production cycle, and typically in the spring and summer. When a flock was exposed to *C. jejuni*, the entire flock rapidly become colonized by a limited number of subtypes. Subtype diversity increased from farm to abattoir and retail. In many instances, birds deemed free of *C. jejuni* in the farms became contaminated during transport to and within the abattoir. Only a subset of *C. jejuni* subtypes isolated from broilers and poultry meat were deemed to represent a significant risk to human health. A high prevalence of beef cattle adjacent to one of the poultry farm sampled frequently shed diverse *C. jejuni* subtypes in their feces, including subtypes that infect people. Furthermore, *C. jejuni* subtypes responsible for outbreaks in chickens were observed in feces from steers in the feedlot, implicating beef cattle as an important reservoir of *C. jejuni* infecting broiler chickens. In conclusion, study findings showed that a relatively small number of birds are able to contaminate the abattoir with diverse *C. jejuni* subtypes, and clean birds are infested with these subtypes within the abattoir, which are subsequently transferred to retail meat. Importantly, only a subset of *C. jejuni* strains pose a risk to human health, and efforts to reduce the burden of foodborne *C. jejuni* on human beings should focus on mitigation of high risk subtypes within the abattoir.

Preface

This thesis is an original work by Nahal Ramezani and Dr. G. D. Inglis' group. All of the research presented in the thesis was conducted at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre (LeRDC). The research project, of which this thesis is a part, received research ethics approval from the LeRDC Animal Care Committee (ACC) for the application entitled "Mitigation of *Campylobacter jejuni* in chickens" (ACC Protocol #1615, February 2016- February 2018).

Dedication

This thesis is dedicated to my family for their unwavering confidence in me and their support to overcome another challenge in my life. Furthermore, I dedicate this thesis to my best friends, who continuously motivate me to be the best version of myself.

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List of Abbreviations

C. jejuni	*Campylobacter jejuni*
C^3GFdb	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database
CGF	Comparative Genomic Fingerprint
CRS	Clinically-Relevant Subtype
CC	Clonal Complex
CDT	Cytholethal Distending Toxin
CFA	Campyfood agar
CGF	Comparative Genome Fingerprinting
DC	Dendritic Cells
GBS	Guillain-Barre Syndrome
IBS	Irritable Bowel Syndrome
ISO	International Organization for Standardization
LOS	Lipooligosaccharides
LPS	Lipopolysyccharides
mCCDA	Modified Charcoal Cefaperazone Desoxycholate Agar
MCGH	Microarray Comparative Genomic Hybridization
MLST	Multi-Locus Sequence Typing
NGS	Next Generation Sequencing
NLR	Node-Like Receptor
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
ST	Sequence Type
SWA	Southwestern Alberta
TLR	Toll-Like Receptor
WGS	Whole genome sequencing

Chapter 1. Literature Review

1.0 Introduction

Campylobacter jejuni is the most common cause of food-borne disease around the world (Sheppard et al., 2009b; Taboada et al., 2008) and campylobacteriosis has been reported as one of the highest prevalent bacterial enteric illnesses in Canada, especially Alberta (Goverment-of-Alberta, 2009). A recent estimation of campylobacteriosis is ≈447 cases/100,000 per year in Canada, not including under-reporting of the illness (Thomas et al., 2013). This rate is more than three times higher than the number of combined cases of disease caused by *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* (Agunos et al., 2014; Sheppard et al., 2009b; Skarp et al., 2016). Campylobacteriosis is a self-limiting disease and typically develops 1 to 5 days after exposure. The clinical symptoms vary from watery diarrhea to dysentery with abdominal cramping, vomiting, and fever lasting for ≈5 to 7 days. It is also known that *C. jejuni* is capable of causing secondary post-infectious diseases such as chronic-active arthritis and Guillain-Barre syndrome (GBS), and is a risk factor for development of Irritable Bowel Syndrome (IBS) (Ohishi et al., 2017; Skarp et al., 2016).

Campylobacter jejuni is genetically diverse and is also part of the gut microbiota of many farm animals, wild animals and birds populations (Sheppard et al., 2010) and as such controlling campylobacteriosis is challenging as it occurs sporadically within many different reservoirs. It is well-established that poultry is considered as a major reservoir of *C. jejuni* in the livestock sector (Kaakoush et al., 2015; Williams et al., 2015). Unfortunately, determining the transmission routes of *C. jejuni* within the chicken production continuum is complicated (Williams et al., 2015), and this makes any further understanding of the disease even more difficult. Moreover, an increase in global poultry production (i.e. 95.5 million tonnes in 2014) has put more pressure on the poultry industry and public health services to develop effective disease prevention protocols and mitigation strategies to control poultry-associated *Campylobacter* infections in people (Skarp et al., 2016). Even with improved global control methods, the prevalence of *Campylobacter* infections has markedly increased especially among developed nations. A major challenge is a lack of detailed information in understanding different aspects of the molecular epidemiology of *C. jejuni*. These aspects include identification of pathogen subtypes or CRS of *C. jejuni*, elucidation in transmission routes of CRS between different species by using agroecosystem model, a complete understanding of the genomic diversity of *C. jejuni*, accurate and cost-effective detection methods, and comprehensive understanding of the mechanism that induce diseases in human by using animal models (Kaakoush et al., 2015). For instance, a study done by Inglis et al. in SWA has indicated that conventional diagnostic methods are not able to effectively detect *C. jejuni* infection (Inglis et al., 2011). Another study has shown that more than two-fold increase in culture-positive diarrheic stools samples have been detected by specialized isolation methods compared to the previous diagnostic methods (≈10% vs. 4.5%) (Inglis et al., 2019). Due to the unique characteristic of SWA such as having the highest rate of *Campylobacter* infection (Public Health Agency of Canada, 2010), high densities of livestock (Hannon et al., 2009; Inglis et al., 2010) and an ≈40:60 rural:urban population distribution, SWA

is an ideal agroecosystem model to investigate the molecular epidemiology of *C. jejuni* by using a high-throughput and cost-effective subtyping method. In this literature review, I will present an overview of the most current information of the molecular epidemiology of *C. jejuni* regarding recognition of main reservoirs and transmission routes of *C. jejuni*, evaluating current microbial and molecular laboratory detection methods of *C. jejuni* and outlining the pathogenesis of the disease.

1.1 General characteristic of *C. jejuni*

Campylobacter jejuni, a member of the *Campylobacter* genus, is a gram-negative, spiral curved and non-spore forming rod bacteria, with a single or bipolar flagellum and has a morphology ranging from 0.2 to 0.9 μm to 0.5 to 5 μm in width and length respectively (Epps et al., 2013; Ketley, 1997) (Figure 1.1). *Campylobacter jejuni* is a thermophilic bacterium which grows in temperatures that range between 37 to 42°C and preferentially grows in a microaerobic environment, at 5% O_2, 10% CO_2, and 85% N_2 (Altekruse et al., 1999; Penner, 1988). The organism is exquisitely fastidious, and its growth is affected by freezing, drying, acidic conditions (pH ≤ 5.0), and high salinity (Altekruse et al., 1999; Epps et al., 2013). *Campylobacter jejuni* can utilize various protective mechanisms to survive in harsh environments such as reduced oxygen tension and increased amounts of UV radiation (Hazeleger et al., 1998). The bacterial survival strategies include the transformation of a spiral curved formed to a non-culturable coccoid bacteria, biofilm formation, and horizontal gene transformation (Altekruse et al., 1999; Jang et al., 2007; Shin et al., 1998).

1.2 Genomic variation and natural transformation of *C. jejuni*

The genetic sequence of *C. jejuni* was determined in 2006; it contains 1,641,481 nucleotides with various polymorphic regions (Gundogdu et al., 2007) and is considered a genetically diverse organism (Pearson et al., 2003). These genetic variations include encoding genes involved with carbohydrate surface structures, flagella proteins and enzymes involved in energy metabolism. Interestingly, there is an attribution between gene transcription of these structures and effects the virulence of *C. jejuni* and the capacity to survive in the environment (Duong and Konkel, 2009; Vegge et al., 2012). However, these observations are not supported by all research as one study using a large patient population and applying multilocus sequence typing (MLST) showed no specific genetic marker of virulence factors between the major *C. jejuni* clonal complexes (Havelaar et al., 2009) . It should be noted that MLST subtyping genotypes the conserved house-keeping genes which are likely not attributed to the genes coding virulence factors. According to the findings, more studies are needed to genotype a large number of *C. jejuni* isolates within different sources and reservoirs using high-throughput and high resolution subtyping method to investigate the strain-specific differences in genes coding virulence factors in *C. jejuni*.

Prominent genomic rearrangement and hypervariable sequences within the bacterium are responsible for the genetic diversity of *C. jejuni* and horizontal gene transfer between different strains of *C. jejuni* also contribute to the gene diversity of *C. jejuni* (Leonard et al., 2003; Nuijten et al., 2000; Young et al., 2007). Several *in vitro* and *in vivo* studies have shown the ability of *C. jejuni* to uptake DNA from the environment and horizontally transfer both plasmid and chromosomal DNA between different *C. jejuni*

strains (Boer et al., 2002; Vegge et al., 2012; Young et al., 2007). This natural genetic transformation has also been reported in wide range of taxonomic groups of *C. jejuni,* and has a vital role in genome plasticity and transference of antibiotic resistance between bacteria (Claverys and Havarstein, 2002; Young et al., 2007). Although natural transformation frequently occurs in *C. jejuni*, the genetic mechanisms involved in the process remains unknown. It has been suggested however, that environmental carbon dioxide concentrations, bacterial cell density and changes in nutrients levels during the growth phase can affect the natural transformation process (Wang and Taylor, 1990; Young et al., 2007). As an example, one study showed that natural transformation of *C. jejuni* occurs in both the growth phase and in growth limiting conditions that include low temperature or in aerobic environments (Vegge et al., 2012). Therefore, this information suggests that *C. jejuni* could adapt itself within the microenvironment of the gastrointestinal tract (GIT) of the different hosts and external natural environment using strategies such as horizontal gene transfer. The mechanisms of these strategies however, have not been completely understood (Vegge et al., 2012).

1.3 Molecular epidemiology of *C. jejuni*

Epidemiology is the study of development, distribution and risk factors of disease in different groups of populations in time and space, and it also evaluates strategies and methods that prevent disease and reduce illness. Molecular epidemiology, is the integration of molecular biology with epidemiological studies. Molecular epidemiology applies genetic markers to measure various risk factors of disease, identify animal and environmental reservoirs of the agent, determine the mode of transmission, and trace evolutionary changes of bacteria within a specific demographic of species, or across diverse groups of species within large populations. The goal of the molecular epidemiology is to link a disease to a source of infectious agents and subsequently design mitigation methods. Molecular epidemiological studies that focused on subspecies or at the strain levels of a pathogen are more effective at studying the disease, as pathogenicity of the bacterium can be strain-dependent. Furthermore, in the context of molecular epidemiology investigations, subspecies or strain of infectious agents are referred to microorganisms within a species (intra-specific level) with distinct genotyping variation. Previous investigations indicated that some bacterial strains might not be associated with induction of disease in some host species and as such identifying. Specific bacterial strains within a species can lead to develop more effective and targeted mitigation strategies (Fouts et al., 2005; Hofreuter, 2014).

Campylobacteriosis, caused by *C. jejuni*, as a food-borne disease can potentially have a marked adverse impact on the economic viability of the livestock sector. Indeed, *C. jejuni* has been isolated from the environment and intestine of wild animals, farm animals and birds (Figure 1.2), and these *C. jejuni* isolates are also present in various facets of the livestock industry (Sheppard et al., 2009b). Understanding the prevalence and distribution of *C. jejuni* at the subtype level in human campylobacteriosis would be important for evaluating the level of risk posed by subtypes in circulating within environmental and animal reservoirs. In addition, understanding and comparing circulating *C. jejuni* pathogenic subtypes across various environmental and animal reservoirs could help elucidate the

transmission routes of human campylobacteriosis and provide needed information to potentially decrease the transmission of the organism from livestock species. Due to the sporadic nature of human campylobacteriosis, and substantive levels of genetic, antigenic, and reservoir diversity of *C. jejuni*, a good understanding of the environmental ecology and molecular epidemiology of this bacterium is still required (Dingle et al., 2002). Furthermore, most studies have been limited to cross-sectional investigations on the transmission routes of *C. jejuni* at the species level, and few studies have examined the transmission mechanism of the *C. jejuni* subtypes within the chicken production continuum; from the producer (farm) to retail market (Damjanova et al., 2011; Hakkinen et al., 2009; Lienau et al., 2007; Thakur et al., 2010). Therefore, these observations suggest that a more comprehensive understanding of the molecular epidemiology of *C. jejuni* at the subtype level is required to fully ascertain the routes of transmission of the bacterium and prevalence of the disease within the livestock sector and human population.

1.4 Host specificity of *C. jejuni*

The significant advancement in the molecular subtyping techniques of *C. jejuni* has improved the understanding of the molecular epidemiology of *C. jejuni* (Dingle et al., 2002; Sheppard et al., 2008; Taboada et al., 2013). These molecular subtyping investigations have shown host-association in *C. jejuni* which indicates that subtypes of *C. jejuni* are varied within different hosts and environmental niches (Dingle et al., 2001; McCarthy et al., 2007). For instance, genetic variation of *C. jejuni* has shown species-specific differences in *C. jejuni* subtypes isolated from farmed cattle and chickens (McCarthy et al., 2007). Interestingly, it has been shown that horizontal gene transfer also occurs more often between different subtypes of *C. jejuni* in similar animal species than within the same geographical location (Sheppard et al., 2009a). This horizontal gene transfer predominantly happens between homologous sequences of DNA present in closely related donor lineages of *C. jejuni* (McCarthy et al., 2007; Sheppard et al., 2011).

Importantly, the information observed in these studies was not comprehensive analysis, and this is likely due to the lack of extensive longitudinal studies from the different sources of infection, the lack of a universally accepted genotyping method, and data acquisition analysis system (Sheppard et al., 2010). These observations suggest that more robust studies that apply better subtyping methods in concert with large-scale studies are required to determine accurate population structures of *C. jejuni* within different hosts accurately.

1.5 Establishment and transmission of *C. jejuni* in/among human beings

Globally, campylobacteriosis is the most commonly reported human gastrointestinal infection (Frost, 2001; Kaakoush et al., 2015). Most large-scale outbreaks of human campylobacteriosis occur sporadically, and the identification of specific interactions between reservoirs and human *Campylobacter* infection is complex (Kaakoush et al., 2015; Schouls et al., 2003; Wassenaar and Blaser, 1999; Wilson et al., 2008). For instance, the potential risk factors of human campylobacteriosis include consumption of unpasteurized milk, uncooked meat, and untreated water, and direct contact with livestock and non-

livestock animal species (Altekruse et al., 1999) (Figure 1.2). Similarly, travel between distant locations is also considered to be a risk factor for *Campylobacter* infections.

It is well-recognized that contaminated chicken meat is the primary source of human campylobacteriosis, particularly in industrialized countries (Kaakoush et al., 2015). There is increasing evidence that more than 80% of cases of human infection might be attributed to handling and/or consumption of contaminated broiler chicken meat due to a high number of *C. jejuni* strains present in chicken meat and other than chicken-associated foodstuffs (García-Sánchez et al., 2017; Hermans et al., 2012; Sheppard et al., 2009b). Importantly, the risk factors and transmission routes vary depending on the geographical region and may not be only associated with the poultry industry (Mullner et al., 2010). As an example, one longitudinal study in SWA by Webb et al. (2018) has shown that high numbers of human campylobacteriosis cases in SWA are associated with *C. jejuni* subtypes associated with feedlot cattle.

It has been suggested that the prevalence of campylobacteriosis within populations varies and is correlated to age and gender of the human population, as well as season (i.e. more incidence of disease during the summer and autumn) and geographic location (Ferreira et al., 2014; Kaakoush et al., 2015). For instance, one study has shown a higher infection rate in children (18.5%) and adult males (16.9%) as compared to the general population (Ferreira et al., 2014). Moreover, a study by Inglis et al. (unpublished data) found a correlation between the diversity of *C. jejuni* subtypes and the age of diarrheic patients in SWA. In this study, more subtype diversity of *C. jejuni* were observed in patients greater than 60-years-of-age as compared to people under 21-years-of-age. As stated earlier, *C. jejuni* is considered as a multi-host microorganism, and geographical regions and the population of people can affect the epidemiological pattern of disease (Mullner et al., 2010; Webb et al., 2018). In this context, Inglis et al. (unpublished data) and Mullner et al. (2010) have shown that urban and rural areas possess different epidemiological patterns of human campylobacteriosis with potentially different modes of transmission of *C. jejuni*. For instance, some surveys have suggested that chicken-associated campylobacteriosis is considerably more common in urban areas, whereas ruminant-associated campylobacteriosis is mostly found in rural areas (Kaakoush et al., 2015; Skarp et al., 2016) and as such these findings suggest that urban populations are mainly exposed to *C. jejuni* from the contaminated retail chicken meat. It has been noted that human campylobacterosis has different epidemiological pattern within human populations. As an example, young children (< 10 years) are the age group most affected by ruminant-associated human campylobacteriosis in rural areas, while poultry-associated strains of *C. jejuni* were present across all age groups in urban areas, suggesting that young children may have closer or more frequent contact with cattle as compared to adults, although the reason for that closer interaction with cattle is unknown. Furthermore, another study found a higher rate of asymptomatic campylobacteriosis among children in both developed and under-developed countries (Kaakoush et al., 2015). The authors suggested that asymptomatic infections may be attributed to either infection with non-pathogenic strains of *C. jejuni* which do not incite pronounced enteritis or individuals having a prominent adaptive immune response to different strains of *C. jejuni* (Kaakoush et al., 2015). Importantly, these asymptomatic infections can

inadvertently and negatively affect accurate estimations of exposure and infection rates of *C. jejuni* in people (Hannon et al., 2009).

Collectively, these data show that the prevalence of human campylobacteriosis is a complicated process and depends on various factors. These factors include age and immune status of individuals, the seasonality and time of year of infection and the subtype diversity of the bacterium (Damjanova et al., 2011). Further investigations into the epidemiology of *C. jejuni* are still required to obtain a more comprehensive understanding of campylobacteriosis in people.

1.6 Pathogenicity of *C. jejuni*

As mentioned earlier, enteric campylobacteriosis in humans can present as a mild to severe enteritis with watery to bloody diarrhea associated with acute abdominal pain and fever. The oral infective dose of *C. jejuni* in people is 500 to 800 bacteria (Ketley, 1997; Young et al., 2007) and the incubation period of *C. jejuni* in people varies from one to seven days. In the more common clinical cases, the illness can extend beyond seven days, but it is often self-limiting. Notably, *C. jejuni* can also be shed from people several weeks following the resolution of clinical disease (Ketley, 1997). The clinical symptoms of campylobacteriosis are diverse; because of either differences between the pathogenicity of *C. jejuni* strains or differences in host susceptibility (innate and acquired immunities) to the infection. There is no conclusive evidence that comprehensively describes various immune responses in human populations and this could be due to the lack of animal models and undefined genetic markers of virulence factors in *C. jejuni* pathogenic strains (Havelaar et al., 2009)

The molecular mechanisms involved in pathogenicity of this bacterium are also poorly understood. It is known that molecular mechanisms for inducing disease differ between different strains of *C. jejuni* (Young et al., 2007). Following ingestion of contaminated animal products or contaminated environmental products such as water; the bacterium colonizes the distal ileum and colon of human intestine (Kaakoush et al., 2015) (Figure 1.2). It has been speculated that flagella, lipopolysaccharides (LPS), lipooligosaccharides (LOS), and toxin production (cytolethal distending toxin) have significant roles in adhesion and invasion of *C. jejuni* into enterocytes (Young et al., 2007). For instance, the bacterium flagellum and curved shape of *C. jejuni* facilitate the motility of the bacterium and directs the organism by chemo-attractants products towards macromolecules within mucus (Young et al., 2007). During the colonization process within the intestinal mucus and including adhesion to the enterocyte apical membrane, this bacterium directly disrupts the normal absorptive capacity of the intestine by damaging epithelial cell function through either direct cell invasion and the release of toxin(s) (e.g. cytolethal distending toxin), or indirectly by injuring enterocytes following a robust host pro-inflammatory response (Ketley, 1997; Rollins and Colwell, 1986). In addition, it is well-established that *C. jejuni* can translocate the host polarized enterocytes through various routes. These include: a transcellular route, which is endocytic uptake of the bacterium followed by intracellular trafficking, a paracellular route that occurs by disrupting tight junctions of the intestinal barrier, or by classical M cells translocation pathways (Ketley, 1997; Wassenaar and Blaser, 1999). Notably, it has been recently found that lipid rafts, which are part of

the plasma membrane and is composed of cholesterol and sphingolipid, play an important role in translocating of *C. jejuni* via a transcellular route. It seems that the lipid rafts of *C. jejuni* prevent the lysosomal fusion through the intracellular translocation, although the molecular mechanism is not clearly understood (Kalischuk et al., 2009). Although several studies have shown some of the pathogenic pathways of *C. jejuni* involved in people and animals, research is still required to further identify the molecular mechanism of the pathogenicity of *C. jejuni* and host immune responses to the infection.

1.7 Immunity against *C. jejuni*

The innate and adaptive immune systems play important roles in mitigating enteric disease by *C. jejuni*. Toll-liked receptors (TLRs) and Node-like receptors (NLRs) are important recognition receptors and are involved in innate immunity within the intestine. The receptors are associated with host protection by inducing production of an array of antimicrobial peptides, pro-inflammatory cytokines and chemokines (Havelaar et al., 2009; Young et al., 2007). For instance, β-defensins are produced in the presence of *C. jejuni* and will disrupt the bacterial cell wall and damage the bacteria (Zilbauer et al., 2005). *Campylobacter jejuni* also causes upregulation of pro-inflammatory chemokines and cytokines as well as activation of phagocytosis (Havelaar et al., 2009). Other components of *C. jejuni* such as LOS (Hu et al., 2006), Cytolethal distending toxin (CDT) (Hickey et al., 2000) and bacterial DNA (Dalpke et al., 2006) can also activate a robust innate immune response in the host. In addition, it is well-documented that LOS, flagellum, and CDT are present within hypervariable sequences in different *C. jejuni* subtypes, and can influence the effects of the host-pathogen interaction and clinical manifestation of the disease in individuals (Havelaar et al., 2009; Young et al., 2007). As an example, the continuous variation (i.e. antigenic variation) of flagellin primary structure prevents TLR5 activation, which can postpone the innate host responses (Johanesen and Dwinell, 2006; Watson and Galán, 2005; Young et al., 2007). *Campylobacter jejuni*, however is also able to stimulate a TLR5 independent mechanism (NOD1), an important pathogen-recognition receptor and activator of innate immunity, which helps to induce the innate immunity and assists in protection against *C. jejuni* infection (Young et al., 2007).

The adaptive immune responses such as antibody production and activation of the complement pathway are essential in protection against *C. jejuni* (Jones et al., 1980; Jones et al., 1981). As an example, Abuoun et al. (2005) demonstrated that CDT of *C. jejuni* could initiate strong neutralizing IgA and IgG antibody responses that can reduce infections in mammals (Abuoun et al., 2005). Moreover, an *in vitro* study examining anti-*Campylobacter* antibodies has found various immunity responses in terms of sensitivity and functionality within serum that likely associate with the remarkable antigenic diversity of *C. jejuni* strains component such as bacterium capsule or LOS (Havelaar et al., 2009) . It has also been confirmed that the *C. jejuni*-specific humoral immunity can be generated from previous exposure of different strains of *C. jejuni*, and this can lead to the different clinical presentations of the disease (Altekruse et al., 1999; Everest et al., 1993; Young et al., 2007). People with previous exposure to specific *C. jejuni* strains are less likely to show severe clinical symptoms following the second exposure of the same *C. jejuni* strains; even though the bacterium still colonizes within the intestine (asymptomatic

infection) (Figure 1.3) (Ketley, 1997). For instance, a study investigating an outbreak of campylobacteriosis associated with drinking unpasteurized milk showed that asymptomatic infections were recorded in individuals with previous *C. jejuni* exposure, whereas 73% people who were not previously exposed to *C. jejuni* developed severe clinical manifestation following infection, (Blaser et al., 1987). Collectively, these studies suggest that previous exposure to *C. jejuni* may lead to partial protection, asymptomatic infection and enhance the development of the adaptive immunity in humans. In contrast, naïve populations or people infected with different strains of *C. jejuni* can result in symptomatic disease (Havelaar et al., 2009; Oberhelman, 2000). In addition, the mechanisms of expression of virulence bacterial factors in different *C. jejuni* strains that lead to varied clinical symptoms (acute to asymptomatic infection) are still unknown.

Interestingly, the manifestation of enteric disease is not uniform across animal species, and different animal species exhibit different susceptibilities or immunity responses to *C. jejuni* infection. There is a notable difference in the clinical manifestations of the disease between people and chickens with people having a more pronounced presentation of the disease (Ruiz-Palacios et al., 1981; Sanyal et al., 1984). Although unknown, it was speculated that the weakness of humoral responses in chickens (i.e. reduced T helper-type II response), the lack of neutralizing the antibody against CDT in chickens, and the non-invasive behaviour of *C. jejuni* in the chicken intestine may moderate host responses and reduce the manifestation of disease in poultry (Young et al., 2007).

In conclusion, the numbers of bacteria within the intestine, the exposure of the specific subtypes of *C. jejuni*, the level of immunity, the age of individuals, the molecular biology and virulence mechanisms of various *C. jejuni* subtypes (Black et al., 1988; Black et al., 1993) are all crucial factors that are involved in the induction of campylobacteriosis in various people and animal species.

1.8 Clinical-relevant subtypes of *C. jejuni*

Several *Campylobacter* species including *C. jejuni, C. coli, C. lari* and *C. upsaliensis* are considered to be public health concerns as they are able to induce mild to severe disease in people (http://www.antimicrobe.org/b91.asp). These *Campylobacter* species are referred to 'pathogenic campylobacteria' (Inglis et al. unpublished). A study by Inglis et al. (unpublished) showed that many *C. jejuni* subtypes found in the environment and animals are not pathogenic, however a few confirmed *C. jejuni* subtypes are pathogenic to people, and these are known as CRS. These subtypes were isolated from cattle, poultry operations, environment and wild animals. In this study, Inglis et al. (unpublished data) found subsets of CRS of *C. jejuni* in SWA that were mostly associated with cattle feedlots and poultry operations, inferring that cattle and chickens could be considered as primary reservoirs of CRS of *C. jejuni* in SWA. These CRS are likely able to enter and survive within chicken production continuum, and to cause infection. Although many investigations have been conducted to elucidate the molecular epidemiology of *C. jejuni,* no study has investigated at the subtype level of *C. jejuni* and the transmission routes of *C. jejuni* CRS in the context of season and geographical location; as such this area requires further investigations.

1.9 Tracing Clinically-relevant subtypes of *C. jejuni*

1.9.1 Broiler chickens

1.9.1.1 Production farms

Understanding the modes of transmission of *C. jejuni* in the poultry industry is an essential consideration for mitigating human campylobacteriosis. Vertical transmission of *C. jejuni* in chickens, which involves the transfer of *C. jejuni* from hens (broiler breeders) to progeny (commercial broilers), is rare. In contrast, horizontal transmission is the most common form of transmission within flocks (Callicott et al., 2006; Cox et al., 2012). For instance, one study isolated different subtypes of *C. jejuni* from parent birds (broiler breeders) as compared to their offspring (commercial broilers), confirming the rarity of vertical transmission of *C. jejuni* contamination (Bull et al., 2006).

The colonization of commercial broilers by *C. jejuni* commonly occurs at 3 to 4 weeks-of-age, likely due to the presence of maternal antibody in the egg yolk, which protects the chickens from *C. jejuni* infection for the first few weeks of life (Bull et al., 2006). Once a chicken flock is exposed to *C. jejuni*, the entire flock is rapidly colonized by the bacterium, and *C. jejuni* can remain within the flock until slaughter. Notably, the source of the initial infection of the flock is often varied (Bull et al., 2006; Johnsen et al., 2006; Newell and Fearnley, 2003), and studies have documented *C. jejuni* contamination in chicken flocks can derive from many different sources. As an example, *C. jejuni* contamination can occur following exposure to insects (beetles and flies), rodents, wild birds, unchlorinated drinking water, nearby farm animals and farm workers (Altekruse et al., 1999; Jonsson et al., 2012; Kaakoush et al., 2015; Ogden et al., 2007; Strother et al., 2005). Other studies have demonstrated the probability of *Campylobacter* contamination increasing in chicken farms with inefficient ventilation, *C. jejuni* contaminated farm equipment, longer production cycles, and flock thinning (reduce the size of the flock by removal of some birds part away through the cycle) (Cokal et al., 2011; Kaakoush et al., 2015; Ridley et al., 2011b). Interestingly, not all the sources of the bacterium have the same ability to spread within chicken flocks. For instance, Skov et al. (2004) revealed that many different species of beetles were incapable of transferring *C. jejuni* to chickens. This information suggests that only specific species of beetles, such as darkling beetle, are able to carry *Campylobacter* bacteria and infecting flocks (Skov et al., 2004). It also been documented that both avian and human feces are potential transmission sources of *C. jejuni.* This bacterium can survive in feces and persist within the environment for up to six days and as such *C. jejuni* survived in feces are potential sources of *C. jejuni* transmission between people and poultry flocks. *Campylobacter* infection can also have a seasonal prevalence of contamination in broilers (Kaakoush et al., 2015). Several studies have shown increased infection rates between May to October and it has been suggested there is a correlation between increased temperatures and exposure to the environmental sources of *C. jejuni* (i.e. migratory birds, rodents and darkling beetles) that leads to increased prevalence of *Campylobacter* contamination in poultry during those months (Hermans et al., 2012; Huneau-Salaün et al., 2007). Not all studies have supported the seasonality of *C. jejuni* infections. For example, Griekspoor et al. (2015) and Williams et al. (2015) found no evidence for the seasonal prevalence *C. jejuni* in poultry

operations despite the presence of the confirmed sessional pattern in local campylobacteriosis outbreaks in human beings.

Identifying main reservoirs of human campylobacteriosis as the source of *C. jejuni* contamination is crucial to mitigate *C. jejuni* within chicken production continuum. However, due to the horizontal gene transfer and recombination events of this bacterium, lack of high-resolution and high-throughput subtyping methodologies and longitudinal epidemiological studies, identifying different clusters of *C. jejuni* involved in disease transmission is challenging. Some studies have demonstrated the role of eating contaminated chicken meats in the prevalence of human campylobacteriosis outbreaks (Stafford et al., 2007; Studahl and Andersson, 2000). According to the European Food Safety Authority (2010), 80% of diarrheic human isolates were attributed to chicken contamination. Interestingly, 40% of the chicken-associated isolates were associated with direct contact with live chicken and chicken meat within farms and abattoirs (EFSA, 2010). Similarly, a case-control study (Gras et al., 2012) applied Multi-Locus Sequence Typing (MLST) profile as a genotyping method to identify source attribution of human camplybacteriosis in the Netherlands. Their results have shown that 66% of human campylobacteriosis cases were associated with consumption of contaminated chicken meats. Their findings were also consistent with previous studies (Deckert et al., 2014; Mullner et al., 2010; Strachan et al., 2009) that showed chickens were considered as a primary reservoir of *C. jejuni* in campylobacteriosis of young children residing in urban setting, whereas cattle were the primary source of infection of *C. jejuni* in young people in rural areas (Gras et al., 2012; Strachan et al., 2009). A study which analyzed source attribution of human campylobacteriosis in Canada, has demonstrated that 65% to 70% of cases are attributed to poultry (Ravel et al., 2017). A recent Canadian baseline study was also consistent with the previous findings and reported that 40% of retail chicken were infected by *C. jejuni* (Canadian-Food-Insepction-Agency, 2016).

Most molecular epidemiological studies have genotyped *C. jejuni* only at the species level (Berndtson et al., 1996; Perko-Mäkelä et al., 2002), and there are contradictory findings of strain/subtype diversity of *C. jejuni* contamination in chicken farms. Several studies have isolated a single serotype of *C. jejuni* within contaminated broiler flocks (Berndtson et al., 1996; Perko-Mäkelä et al., 2002; Shreeve et al., 2002). A study by Rivoal et al. (2005) revealed that a single subtype of *C. jejuni* was present on different farms at different times of sampling. The source of birds at these farms was from a single operation and as such the contamination was likely from a single source of birds. Other investigations however, have isolated different highly genetically diverse strains of *C. jejuni* among poultry flocks, and this could be attributed to the horizontal genetic transfer of *C. jejuni* within different subtypes present at farm (Manning et al., 2001; Pokamunski et al., 1986; Rivoal et al., 2005; Wassenaar and Blaser, 1999).

From the above observations, many investigations have examined the prevalence and risk factors of *C. jejuni* in chicken flocks; unfortunately the precise mechanisms of *C. jejuni* subtypes transmission are still unclear. These studies also raise several critical questions concerning colonization of *C. jejuni* in the

chicken intestine, inter- and intra-flock transmission of the CRS, and identifying other potential transmission vectors of the CRS of *C. jejuni* in poultry operations.

1.9.1.2 Processing plants

A steady increase in the amount of *C. jejuni* contamination of chicken meat along the food chain is a well-known phenomenon. As the prevalence of *C. jejuni* in broiler farms can be low, it suggests other sources of *C. jejuni* maybe causing contamination of chicken meat. The identification of the source(s) of transmission of *Campylobacter spp*. within epidemiological studies has been challenging (Damjanova et al., 2011; Gruntar et al., 2015; Melero et al., 2012). Several investigations have proposed that abattoir transport trucks before slaughter could be considered as a possible source of *C. jejuni* contamination by examining obtained samples from chicken feathers, coop washing equipment, crates, truck wheels and from drivers and catcher boots (Franchin et al., 2005; Ramabu et al., 2004). The intestinal content (*Campylobacter*–contaminated fecal material) of *C. jejuni* infected chickens is also considered the other primary source of carcass contamination in the poultry processing plant. This contamination could be transmitted to the equipment of the processing plant during the process of slaughtered infected flocks (García-Sánchez et al., 2017). Consequently, the contaminated abattoir equipment act as fomites and cross-contamination of chicken meat might subsequently occur between slaughtered chickens from different infected farms (Arsenault et al., 2007; Pacholewicz et al., 2016; Rasschaert et al., 2007; Seliwiorstow et al., 2016). For instance, Elvers et al. (2011) has shown that *C. jejuni* could be transmitted to clean flocks by exposure to the cecal content of *C. jejuni* positive birds of contaminated flocks. Furthermore, several investigations have recorded identical *C. jejuni* subtypes between farm and slaughterhouse samples, suggesting that some subtypes of *C. jejuni* can survive during processing and act as a source of cross-contamination during carcass processing (Allen et al., 2007; García-Sánchez et al., 2017; Gruntar et al., 2015). Notably, not all studies support these observations. Several contradictory studies have also reported a variation of *Campylobacter* cell numbers on carcasses upon entry in the processing plant and after the carcass processing (i.e. plucking and evisceration) (Nauta et al., 2009a; Nauta et al., 2009b; Seliwiorstow et al., 2016). Moreover, Melero et al. (2012) and Zweifel et al. (2015) have suggested that the level of subtype diversity of *C. jejuni* in abattoirs can vary markedly and this is affected by pre-slaughter antibiotic treatment and different specialized carcass processing procedures in different abattoirs (Zweifel et al., 2015). These various processing procedures make an accurate identification of source(s) of *C. jejuni* contamination challenging.

From the above observations, it is apparent that the collected information can be contradictory, suggesting more longitudinal large-scale sampling surveys are required to definitively elucidate the source and mechanism of transmission of *C. jejuni* subtypes within poultry processing operation.

1.9.1.3 Retail poultry

As noted above, one of the most common bacterial species isolated from poultry meat is *C. jejuni* (Altekruse et al., 1999; Guévremont et al., 2006; Williams and Oyarzabal, 2012; Zhao et al., 2001). Transmission along the food chain; from broiler farms through the abattoir to the retail store, is the major

source of *C. jejuni* contamination of chicken products (Damjanova et al., 2011). The rate of *C. jejuni* contamination in retail chicken meat can vary from 49.5% to 93.2% of tested meats (Guyard-Nicodème et al., 2015; Little et al., 2008; Pointon et al., 2008; Taremi et al., 2006; Whyte et al., 2004; Williams and Oyarzabal, 2012; Wong et al., 2007). It has been suggested that this wide variation of the prevalence of *C. jejuni* contamination could be associated with using different isolation and genetic subtyping methods for *C. jejuni*, yearly seasonality and the geographical region of the samples collected, and the sample size of chicken population being tested (Bohaychuk et al., 2006; Guévremont et al., 2006; Taremi et al., 2006; Whyte et al., 2004; Williams and Oyarzabal, 2012; Zhao et al., 2001). As stated previously, cross-contamination during processing is considered to be an important route of carcasses contamination (Corry and Atabay, 2001). The significant subtype diversity of *C. jejuni* contamination in retail chicken meats suggests that isolated *C. jejuni* could be attributed to either different farm flocks contaminated by different *C. jejuni* strains passing through the slaughterhouse process or resident subtypes of *C. jejuni* already present within the abattoir and retail store (Guyard-Nicodème et al., 2015; Oyarzabal et al., 2013; Sheppard et al., 2010).

There are critical gaps in knowledge regarding *C. jejuni* in the chicken supply chain. This includes the source of CRS entering to production systems and factors that promote the amplification and dissemination of CRS that lead to the contamination of flocks that had remained *C. jejuni* free at the farm level.

1.9.2 Cattle

Cattle are considered asymptomatic carriers of *Campylobacter* spp. and can intermittently shed *C. jejuni* in their feces (Altekruse et al., 1999; Inglis et al., 2005). The prevalence of *C. jejuni* varied from 0.8 to 46.7% in the cattle population, and this difference in prevalence is based on various factors including different bacterial isolation methods, herd size, cattle diet, husbandry practices, sample size, sampling frequency, geographic location, yearly seasonality of sampling and age of the animal (Bae et al., 2005; Hermans et al., 2012; Stanley and Jones, 2003). Several risk factors for *C. jejuni* contamination in feedlot cattle have been proposed. There is an increasing prevalence of *C. jejuni* within cattle feedlots as compared to the cattle (i.e. cow-calf operation) transported to the feedlot for processing and this is associated with transmission of fecal bacteria between individual feedlot cattle (Webb et al., 2018). It has also been suggested that cattle are more likely to be exposed to *C. jejuni* by naturally-sourced drinking water and pasture grazing, especially during spring runoff, rather than cattle provided water and food within indoor shelters (Allen et al., 2007; Kaakoush et al., 2015).

Cattle are considered to be one of the important animal reservoirs of *C. jejuni*, although the transmission of *C. jejuni* between cattle and human is not fully understood (Bae et al., 2005; Clark et al., 2003; Schouls et al., 2003; Webb et al., 2018). A recent study has observed highly diverse *C. jejuni* subtypes in feedlot cattle in SWA, however many subtypes of *C. jejuni* isolated from livestock, environments, and wildlife in this area are not CRS (Webb et al., 2018). Other studies have also shown identical *C. jejuni* clones isolated from cattle, chickens, and diarrheic human beings, confirming the role of

cattle as a *C. jejuni* reservoir and a potential link between cattle, chickens and human beings (Hermans et al., 2012). As an example, a longitudinal epidemiological study found that identical strains of *C. jejuni* isolated in dairy cattle were also present in an adjacent chicken farm, indicating that horizontal transmission can occur between different animal species (Ridley et al., 2011a). This study has also demonstrated that *C. jejuni* contamination in cattle could increase the likelihood of contaminating chicken flocks near cattle operations, although the mechanism is still unclear. Additionally, it is possible that reverse transmission can occur, with *C. jejuni* positive poultry flocks contaminating cattle facilities, suggesting bidirectional transmission of *C. jejuni* between the two different animal species (Ridley et al., 2011a). To conclude, these findings indicate that cattle could be considered as a potential reservoir for certain *C. jejuni* subtypes and there is a potential link between cattle, chickens and diarrheic humans.

1.9.3 Environment

It is unclear whether the role of poultry, to induce human campylobacteriosis, has been overrepresented in epidemiological surveys, as only a limited number of potential non-chicken sources have been observed (Skarp et al., 2016). For instance, Champion et al. (2005) has categorized *C. jejuni* positive samples isolated from various sources into two clusters; livestock clade and non-livestock clade. Based on this investigation, 55.7% of human isolates were included into the non-livestock clade, which was phylogenetically related to the subtypes found persisting in the environment and not present in poultry meat or poultry operations. These findings coincide with other studies that environmental sources of infection such as water, are likely an underestimated transmission sources for *C. jejuni* in animals and human beings (Champion et al., 2005; Kaakoush et al., 2015). It has been suggested that natural and untreated municipal water (i.e. runoff) is a major source of *C. jejuni* infection in human beings. It has also been identified as the main source of infection for *C. jejuni* outbreaks, in various regions throughout the world (Altekruse et al., 1999; Kaakoush et al., 2015). Contamination of runoff by livestock (poultry and cattle operations), wild animals and human sewage have also been considered as primary sources of outdoor contaminated water (Jacobs-Reitsma et al., 2008; Kaakoush et al., 2015; Wilson et al., 2008). This data is supported by other studies that *C. jejuni* isolated through environmental reservoirs (water, litter, mouse, insects, flies) were similar to *C. jejuni* subtypes observed in local chicken flocks (Hiett et al., 2007). Moreover, there is a higher likelihood of *C. jejuni* contamination from farms which utilize private water supplies (i.e. wells and dugouts) as their main source of water (Champion et al., 2005). Together, these observations indicate that the persistence of *C. jejuni* subtypes from various sources within the environment could be responsible for some *C. jejuni* outbreaks in human beings.

1.9.4 Non-domesticated animals

Due to the host adaptive capability and high genomic diversity of *C. jejuni,* wild mammals and many wild birds (such as gulls, geese, ducks, and cranes) carry *C. jejuni* as part of their normal intestinal microbiota (Adhikari et al., 2004; Altekruse et al., 1999; Griekspoor et al., 2015; Kaakoush et al., 2015). There are, however, inconsistencies in the transmission of *C. jejuni* between different species, and a uniform mode of transmission between animal groups has not been identified. As an example, a study in

Norway tested 540 birds of 40 different species for the presence of *C. jejuni*. In these birds, 28.4% tested positive for *C. jejuni* with a high prevalence in crows, puffins, and gulls (Kapperud and Rosef, 1983). Similarly, another study by Griekspoor et al. (2015) showed 15% and 4% similarities of genotyping *C. jejuni* isolated from wild birds correlated to human beings and chicken respectively, indicating the potential for transmission of *C. jejuni* between species. Furthermore, wild birds and rodents are the major vector for transmission of *C. jejuni* to dairy cattle and broiler chickens through fecal contamination of water or feed (Adhikari et al., 2004; Adhikari et al., 2002; Johnsen et al., 2006). Interestingly, most *C. jejuni* isolates from wild animals were not isolated from diarrheic human beings, which indicate that these animals are likely not carrying human pathogenic subtypes of *C. jejuni* or CRS (Colles et al., 2011; Kaakoush et al., 2015; Petersen et al., 2001). Finally, *C. jejuni* subtypes isolated from both chickens and ruminants were not closely genetically related as compared to different *C. jejuni* subtypes isolated in wild birds (Sheppard et al., 2011). Collectively, the inconsistencies of information suggest that further large-scale studies are required to determine the role of wild animals and wild bird populations in the transmission of *C. jejuni* pathogenic subtypes between animal species and people.

1.10 Methods to elucidate the epidemiology of campylobacteriosis

1.10.1 Isolation and detection

As discussed earlier, the prevalence of *C. jejuni* is likely underestimated in human and animal infections. There are several possibilities for this observation including isolation procedures that favor the recovery of the most abundant and common *Campylobacter* species, and under-reporting of disease patients not receiving medical intervention and as such were potentially unreported positive samples (MacDougall et al., 2008).

This section will describe some of the major methods used to isolate *C. jejuni*. Bacteriological culturing of *C. jejuni* is the most common procedure for the isolation of bacteria, but this isolation method is challenging as the fastidious nature of the *C. jejuni* makes growing the bacteria difficult (Park, 2002). The International Organization for Standardization (ISO) has indicated that direct plating on selective agar, which includes modified charcoal cefoperazone desoxycholate agar (mCCDA) or Campyfood Agar (CFA) in microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) at 42°C as a standard procedure required to isolate the organism (Gharst et al., 2013). Other methods can also be used to culture *C. jejuni*. As examples, the enrichment method is another effective culture-based technique, which facilitates the growth of low bacterial numbers or morphologically and biochemically damaged bacteria; a common observation in samples present in the food chain (Gharst et al., 2013; Musgrove et al., 2001). Several enrichment methods have been specifically developed to isolate *Campylobacter.* Bolton broth and Preston broth are enrichment methods that composed of a basal medium supplemented with antimicrobials and used for isolation of *Campylobacter* from food samples (Baylis et al., 2000; Bolton and Robertson, 1982). Bolton broth is a better enrichment method for reducing non-specific (i.e. non-*Campylobacter* spp.) microbial growth as compared to Preston broth (Baylis et al., 2000). Bolton broth however, showed significant false negative results, due to the overgrowth of beta-lactamase producing

Escherichia coli and *Pseudomonas* bacteria present in poultry meat (Jasson et al., 2009). Additionally, Ugarte-Ruiz et al. (2012) showed excellent recovery of *Campylobacter* bacteria after using a combination of Preston broth (which contains antibiotics including rifampicin and polymixin) with mCCDA plating for meat samples compared to the standard ISO method. From the information, it appears that both enrichment methods have specific advantages and disadvantages for isolating bacteria. According to a revision of ISO 10272 Part 1 and Part 2:2006 by The EURL (The European Union Reference Laboratory for *Campylobacter,* National Veterinary Institute, SVA, Uppsala, Sweden) isolating *C. jejuni* with Bolton broth is considered the best isolation technique for samples with low numbers of *Campylobacter*. In contrast, Preston broth is a better enrichment method for samples with high background flora of multi-resistant *E.coli*. Finally, some studies have combined enrichment methods with filtration. This technique is effective for isolation of *C. jejuni* in samples with low numbers of bacteria (<0.8 CFU per/g), a common occurrence in retail chicken meats (Speegle et al., 2009; Williams and Oyarzabal, 2012). Another advantage is the ability to obtain large number of pure *Campylobacter* colonies while reducing the amount of antimicrobials added to the membrane filter to improve *C. jejuni* isolation (Baylis et al., 2000). One of the potential problems of the enrichment-filtration however, is the presence of other fast growing microorganisms during isolation. In this method, there is a lack of selective antibacterial agents in the media, and the overgrowth of non-specific bacteria can inhibit the growth of some *Campylobacter* subtypes. This disadvantage leads to both the reduction in the isolation of low numbers of bacteria and the isolation of specific subtypes of *Campylobacter* bacteria (Gharst et al., 2013; Ugarte-Ruiz et al., 2012). Another disadvantage of the enrichment-filtration method is the number of live and motile *Campylobacter* cells in a sample needs to be sufficiently large to be able to pass through the filter in a large numbers and grow on the culture media. There are numerous studies directly comparing the efficacy of various direct plating and enrichment methods to obtain better isolation of *Campylobacter* samples. For instance, Ugarte-Ruiz et al. (2012) compared two direct plating methods (mCCDA and CFA) with four combinations of enrichment media and standard plating media (Bolton broth or Preston broth enrichment combined with mCCDA or CFA plates) on different carcasses samples (neck skin, feces, and meat). The results indicated that direct plating is the fastest and most cost-effective isolation method for detection of *Campylobacter* in the skin of the neck and fecal samples. Additionally, CFA plating was the better method of selective agar direct plating, owing to easier colony identification relative to mCCDA plating, specifically for plating highly contaminated samples isolated from feces and skin of the neck. In comparison, Preston broth was recommended as a good enrichment method for detection of low-level organisms or stressed bacteria present in other food samples. A study by Inglis et al. (unpublished data) has compared the conventional *Campylobacter* isolation methods with specific isolation methods that include direct plating, enrichment, membrane filtration and immunobead within a high hydrogen microaerobic atmosphere. The results have shown a substantively higher growth rate in the specific isolation methods as compared to the conventional methods. There was however, no significant difference between the specialized isolation method and current culture method (CVA agar). Moreover,

Inglis et al. (2011) also demonstrated that conventional culture-based methods (i.e. Campy CVA agar) failed to isolate cryptic campylobacteria, and this is an important observation as cryptic *Campylobacter* species can induce campylobacteriosis in people. Furthermore, in a survey of a large number of samples, isolation conventional culture methods were unable to isolate *C. jejuni*, but were found positive by PCR-based method (Inglis et al., 2011). This information demonstrates that culture-based methods of bacterial isolation cannot always detect *Campylobacter* species, and other identification methods of *C. jejuni* should be considered (Inglis et al., 2011). Presently, a culture-independent method such as Polymerase Chain Reaction-based (PCR) is commonly applied for *Campylobacter* detection due to its faster performance and the detection of very low numbers of bacteria. Some potential limitations have been observed in non-cultural based detection methods including the lack of fidelity for identifying some specific subtypes of *C. jejuni,* or testing for antimicrobial resistance in bacterial species (Ugarte-Ruiz et al., 2012).

1.10.2 Subtyping

Genotyping is a DNA-based subtyping method that classifies a bacterial species into distinct strains or subtypes based on genetic variation within the DNA (Van Belkum et al., 2007). The technique plays a significant role in molecular epidemiology, as it can identify etiological agents of disease, and the mode of transmission during potential outbreaks of bacteria (Fitzgerald et al., 2001). Molecular subtyping methods or DNA-based subtyping methods provides sensitive discrimination between bacterial strains, and has a higher level of standardization and reproducibility when compared with phenotype-based identification methods such as colorimetric analysis and culture-based isolation techniques. Investigations using molecular subtyping methods can detect clusters and lineage of the bacteria (a group of bacteria derived from common descent) isolated in both outbreaks and sporadic cases of disease (Muellner et al., 2013; Sheppard et al., 2010). An ideal molecular subtyping method should be accessible, inexpensive, easily interpreted and have high resolution and high throughput genotyping capacity for large outbreak investigations (Taboada et al., 2013). Current molecular subtyping methods have been directed at different molecular approaches to distinguish the genetic difference in bacterial strains. These approaches have been developed based on restriction fragment of bacterial DNA, genotyping a single highly variable locus of bacteria or methods based on PCR amplification of targeted fragment DNA (Olive and Bean, 1999).

Many molecular typing methods have developed a better understanding of molecular epidemiology of *C. jejuni* and some of these methods are discussed below. *Campylobacter jejuni* was initially typed based on the surface antigen (O antigen) in 1980 by Penner and Hennssy (Penner and Hennessy, 1980). They found various serotypes in human fecal isolates based on the lipopolysaccharide component of the outer membrane. The expense of sera analysis, the difficulties in standard preparation and inability to identify cryptic strains of *C. jejuni* are disadvantages of the Penner serotyping method (Dorrell et al., 2001). Pulsed-field gel electrophoresis (PFGE), one of the molecular methods, cuts large DNA sequences into diagnostic DNA fragments using restriction enzymes. These small fragments generate a fingerprint by

migrating within a gel under direction of the electric field (Taboada et al., 2012). Pulsed-field gel electrophoresis is currently used in a national surveillance system called PulseNet surveillance to investigate outbreaks and subtype isolates within the epidemiological follow-up of cases. The PulseNet surveillance is a national laboratory network and critical surveillance system that utilizes DNA fingerprinting of bacteria and food-borne bacteria patterns to identify and respond to foodborne disease outbreaks in Canada. PFGE also has the potential to improve sensitivity and specificity of *Campylobacter* detection (Gilpin et al., 2012; Hedberg et al., 2001). However, to gather enough epidemiological information from follow-up cases, more samples are needed to be collected from different infectious sources, to build up a comprehensive public health database. It is now known that PFGE is practical technique with high discriminatory power of different bacterial species (Hedberg et al., 2001; Taboada et al., 2013). Based on previous studies, PFGE has been successfully applied in genotyping of *C. jejuni* in the poultry production continuum (Miller et al., 2010; Oyarzabal et al., 2013). Although some investigations have shown that PFGE is an effective method to differentiate varied clusters in *C. jejuni* (Lorenz et al., 1998; Olsen et al., 2001; Wassenaar and Newell, 2000), some studies have suggested that this method is unable to effectively discriminate *C. jejuni* subtypes required for epidemiological investigation due to the low number of DNA bands in each profile, chromosomal rearrangement of the bacteria and high genetic diversity of *C. jejuni* (Barton et al., 2007; Champion et al., 2002; Michaud et al., 2005). In this regard, genome instability of *C. jejuni* can dramatically affect the PFGE results of closely related strains and identify highly clonal lineages of *C. jejuni* within sporadic cases of campylobacteriosis, which is particularly important for *Campylobacter* investigations (Champion et al., 2002). Furthermore, PFGE is considered a labour intensive method and it requires specialized equipment (Ribot et al., 2001). From these observations, it is not surprising that PFGE might not be well-suited for large-scale investigations (Taboada et al., 2013). Microarray comparative genomic hybridization (MCGH) is another genotyping method that compares inter-strain and intra-specific variations in bacteria at the genomic level. Microarray comparative genomic hybridization is based on the differential fluorescent labeling of the bacterial DNA of an isolate, which is designed on co-hybridization of microarray template. The presence or absence of target genes in a single experiment defines the variability of different strains (Taboada et al., 2013). Some studies have used the MCGH method for genotyping the diversity of *C. jejuni* subtypes at the whole-genome level (Champion et al., 2005; Dorrell et al., 2001; Pearson et al., 2003), and one study has proved transmission of *C. jejuni* between cattle and diarrheic human beings by this method (Hannon et al., 2009). Based on previous studies, the MCGH is able to distinguish the significant difference in gene content of isolates that were not recognized by other subtyping methods. Also, this molecular technique has improved resolution of data collected in both global clustering and gene association studies (Gripp et al., 2011; Taboada et al., 2008). The MCGH method has limited genome coverage of the bacteria as the availability of the genome sequences is restricted to the genes in the array. This method is not a cost-or time-effective technique and these are considered disadvantages of MCGH. Moreover, the low number of throughput data makes it less useful for performing intra-species

comparative genomic analysis compared to the other subtyping methods (Hannon et al., 2009; Taboada et al., 2007). Another method of molecular typing is the single locus genotyping method. This includes PCR amplification of sequence short regions of a highly variable gene of the bacteria which represents significant heterogeneity of the bacteria in many epidemiological studies. As an example, the Flagellin (*flaA*) gene in *C. jejuni* has provided a prominent marker gene to differentiate different *C. jejuni* isolates using a method called short variable of region the *flaA* gene *(flaA*-SVR) (Meinersmann et al., 1997; Nachamkin et al., 1993). Unfortunately, due to the capability of *C. jejuni* to take up exogenous DNA by either recombination and horizontal intra-species transfer, this method may not be reliable for long-term epidemiological investigations, even though *flaA*-based typing methods are relative simplicity and inexpensive (Dingle et al., 2005; Taboada et al., 2013).

Whole Genome Sequencing (WGS) is another molecular fingerprinting technique that characterizes bacterial isolates which differentiate strains by a single base pair. An advantage of this epidemiologic subtyping method is the high level of discriminatory power, as each nucleotide base pair of every isolated bacterium can be compared. Whole Genome Sequencing is referred to as a gold standard subtyping technique due to the comprehensive identification of phylogenetic genetic relationships (Laing et al., 2011; Taboada et al., 2013). Presently, the WGS method only differentiates a few limited numbers of *C. jejuni* strains, due to the required technical expertise and equipment for the procedure (Pendleton et al., 2013). To improve the usage of WGS, a next-generation sequencing (NGS) platforms has been employed with WGS to examine *Campylobacter* subtyping in large outbreaks. Improvement in the NGS subtyping method will make WGS an excellent, cost-effective methodology to analyze outbreaks of *Campylobacter* on a global scale (Gardy et al., 2011; Gilmour et al., 2010; Taboada et al., 2013). Some strains of *C. jejuni* have been recently sequenced by NGS method in several studies (Gripp et al., 2011; Lefebure et al., 2010). Multi-locus sequence typing (MLST) is another molecular subtyping approach that sequence short DNA fragments within seven stable loci that are considered as housekeeping genes. Each locus is numbered specifically and importantly the number matches an allele in the global PubMLST database. The seven allele set is considered a sequence type (ST) based on the database. Sharing four or more allele profiles in sequence types is determined to be the same clonal complex (CC) or lineage of bacteria (Dingle et al., 2001; Taboada et al., 2013). Major advantages of this approach are that MLST provides both phylogenetic and a population genetic analysis with an improved level of discriminatory power; and it is also able to provide more reproducible subtyping and consistent genome analysis than PFGE. Moreover, due to the constantly expanding and global accessibility of database, MLST is also able to provide electronic portability and inter-laboratory comparison of data without the requirement of reference isolates, unlike PFGE requirement (Ahmed et al., 2012). Several studies have used this method for elucidation of transmission dynamics of *C. jejuni* populations in different geographical areas or specific hosts (Muellner et al., 2013; Sheppard et al., 2009b). This method also allows deeper investigation in reconstructing ancestral and evolutionary relationships among *Campylobacter* populations (Sheppard et al., 2011). Despite these advantages of MLST, high cost, low input, lengthy running time and the

specialized equipment requirement are considered disadvantages of this method (Taboada et al., 2012). Furthermore, previous studies have found unrelated STs due to the large number of horizontal gene transfer, intergenic recombination and mutation events in *C. jejuni.* The recombination in different loci may enable *C. jejuni* to adapt itself to harsh conditions such as high oxygen environment or new antibiotics (Schouls et al., 2003; Suerbaum et al., 2001). It is also noteworthy that MLST is not a promising method for short-term studies or studies with large numbers of isolates such as outbreaks, as MLST can be costly and its data output is relatively low (Sails et al., 2003). From these observations, it is clear that even though MLST is a highly discriminatory analytic method; high cost, time-consumption and need for better allele determination due to the increased number of loci required, make this method challenging. In comparison, WGS, which is more comprehensive analyses for more alleles, costs less than before, which may make a huge effect on the discriminatory power of MLST (Taboada et al., 2013).

As previously discussed, an excellent genotyping technique to identify *C. jejuni* subtypes should provide high discriminatory power at a reasonable price, present data in a minimum time, and provide high throughput data that is accessible by most researchers. It should also be able to overcome all the ongoing challenges facing conventional subtyping methods for *C. jejuni* genotyping. Some of these challenges include high diversity in genome, fastidious growth requirements, and increasing rate of interspecies recombination (Clark et al., 2012). Taboada et al. (2012) identified accessory genes in *C. jejuni* subtypes by using WGS method. These observations allow the development of a new molecular subtyping technique called CGF. The accessory genes were exploited to develop a high resolution PCR-based fingerprinting method based on 40 loci expressed phylogenetic characteristics such as iron acquisition, capsule and lipooligosaccharide biosynthesis, and flagellar modification of the bacteria (Clark et al., 2012; Gundogdu et al., 2007; Taboada et al., 2012). These 40 loci are promising candidates for representing genetic variation throughout the *Campylobacter* genome and intra-species relationships (Webb et al., 2015). Some studies have documented a consistency between phylogenetic properties in accessory genome content variation and genetic variation in bacteria (Deng et al., 2010; Taboada et al., 2008). As such CGF method identifies *C. jejuni* isolates based on a binary distribution (presence or absence) of the bacterial genome (i.e. accessory genes). As a PCR-based method, the CGF is more easily applied by researchers lacking the specialized equipment and expertise required for sequence-based methods such as MLST. Moreover, the CGF assay, as a cost-effective and high-throughput method, discriminates closely related strains of *Campylobacter* more than other subtyping methods such as MLST. This discriminatory power is critical for the surveillance of genetically diverse species such as *C. jejuni* (Clark et al., 2012; Taboada et al., 2012; Webb et al., 2015). A study completed by Taboada et al. (2013) demonstrated that CGF method could be applied in a large scale *Campylobacter* surveillance programme to understand better the population structure and strain dynamics of *C. jejuni* circulating in Canada. In this investigation more than 28,000 *C. jejuni* isolates subtyped from various sources including human, livestock, wildlife, and environmental samples obtained across Canada. Indeed, these data are all housed within the C^3GFdb (Taboada et al., 2013). Examination of data within the C^3GFdb has identified a

remarkable high genetic diversity within *C. jejuni*, which includes ≈5,000 distinct subtypes across Canada. Although, only a small number of these subtypes (≈80) are frequently observed in diarrheic human beings, indicating that only a subset of *C. jejuni* subtypes represent a significant health risk (i.e. they are CRS) (E. Taboada, pers. comm.).

Currently, although many molecular typing techniques are applied to investigate subtypes of different *C. jejuni* clusters to elucidate the similarities between species, some challenges remain (Taboada et al., 2013). As an example, some subtyping methods such as PFGE are not commonly used for *C. jejuni* isolations due to inadequate resources for data acquisition. Furthermore, all the molecular studies have identified *C. jejuni* at the species level; while it has been shown that a single sample may contain many genotypes of this bacterium (Gilpin et al., 2012; Hedberg et al., 2001). To date, only apparent clusters from large outbreaks are detected by the common methods such as MLST which are mostly restricted to specific times and locations of events. To evaluate the level of risk presented by subtypes, it is essential to identify the prevalence and distribution of CRS in human population. In addition, comparing the CRS across different environmental/animal reservoirs extremely lighten up the transmission routes of those pathogenic subtypes within various species. The WGS method is a promising subtyping method in *C. jejuni* human outbreaks, and help in understanding the transmission mechanisms of campylobacteriosis (Joensen et al., 2018). Therefore, it has shown that techniques based on the analysis of multiple loci, such as MLST and CGF, are more reliable for distinguishing *C. jejuni* strains as compared to single-locus methods (Taboada et al., 2013).

1.10.3 A model agroecosystem approach

Previous studies have shown that chicken flocks become contaminated throughout chicken production continuum, and the resulting risk of human campylobacteriosis from ingesting contaminated products is substantive (Hermans et al., 2012). However, these studies are often cross-sectional and do not account for genetic variation within the *C. jejuni* population. To elucidate the dynamic of *C. jejuni* population structure in the poultry industry, future studies should be designed in a manner that facilitates identification and tracking of *C. jejuni* subtypes from initial contamination of chicken flocks, to transmission of *C. jejuni* to retail chicken. An accurate sampling strategy should include potential sources of initial infection at chicken farms, possible persistence of *C. jejuni* in farms and abattoirs, and the source of contamination of retail meat during slaughter processing. Interestingly, a recent study of Inglis et al. (unpublished) concluded that some non-human sources (animals and environment) of *C. jejuni* subtypes were not present in diarrheic human beings, suggesting that some of these subtypes are not CRS. Therefore, the agro-ecosystem model describes a potential link between major reservoirs of *C. jejuni* CRS (i.e. poultry industry, cattle farms and the local environment) and *C. jejuni* CRS associated with diarrheic events in human that occur at the same time and same place. Indeed, this model can provide a large number of samples within different spatially and temporally association to host species, identifying important reservoirs and transmission mechanisms of CRS that induce disease in people. The CGF is a promising high-resolution subtyping method, more than twice the resolution of MLST, which is able to

examine high numbers of *C. jejuni* isolates from different sources at the specific time and space. Importantly, CGF can directly compared the data within the C^{3}GFdb as a foundational tool. As discussed earlier, studies have indicated that chicken is not the only primary reservoir of CRS. Cattle and the environment also play an important role as sources of CRS, though the transmission mechanism is not clearly delineated. Developing an accurate agro-ecosystem model could help to elucidate the modes of transmission of *C. jejuni* (De Haan et al., 2010; Webb et al., 2018). SWA could be considered as an appropriate agroecosystem model to elucidate the molecular epidemiology of *C. jejuni* because of high rates of campylobacteriosis (Public-Health-Agency-of-Canada, 2010), high densities of livestock (including poultry and feedlot cattle) (Hannon et al., 2009; Inglis et al., 2010) (Figure 1.4), a single and public diagnostic facility, an ≈40:60 rural:urban distribution of human population, a single prominent watershed, and spatial gradation of human activity from west to east.

Chicken should be considered the best model to study the transmission of *C. jejuni* CRS between individuals of a host species, and the pathogenicity-associated factors such as host-species preference and gut colonization (Dasti et al., 2010). The ideal candidates of *C. jejuni* subtypes to study the disease could be identified from regional surveillance studies. Identified *C. jejuni* positive chicken flocks could then, be used to determine methods of subtype-specific colonization, competitive exclusion of colonization, and dominance of *C. jejuni* subtypes within individuals and across the chicken flocks. These factors will further improve our understanding of the clinical relevance of *C. jejuni* subtypes, and the induction of the disease in people. Therefore, further investigations should be designed to include longitudinal intra- and inter-species experimental models accompanied with high-performance genotyping techniques (i.e. insensitive to genetic recombination). Notably, these investigations could determine the molecular epidemiology of *C. jejuni* including identification of *C. jejuni* reservoirs and modes of transmission of CRS. This could subsequently lead to creating effective mitigation strategies to present human campylobacteriosis.

1.11 Summary

Despite many investigations of *C. jejuni* as a human intestinal pathogen, there is still paucity of knowledge of the molecular epidemiology of the organism. The sporadic nature of campylobacteriosis, genetic plasticity, high genetic diversity of *C. jejuni*, the infrequent association with disease outbreaks, widespread reservoirs, and lack of detailed knowledge in pathogenicity of this bacterium limit effort to control human campylobacteriosis. It is generally accepted that *C. jejuni* is widespread within the environment and is found in many domesticated and wild animals and birds, with poultry being considered as one of the most important reservoir of pathogenic *C. jejuni* in human beings. Regardless of various subtypes of *C. jejuni* found in different animals and environment, there are only some subtypes of *C. jejuni* which are considered to be a public health concern and can induce disease in people (i.e. CRS). For a better understanding of the sources and routes of transmission of these CRS, a universal high-resolution subtyping method and an accurate agro-ecosystem approach are required. These should provide valuable insight into the complex epidemiology of campylobacteriosis.

1.12 Hypotheses

The hypotheses of my study were: (1) *C. jejuni* subtypes are associated with broilers and poultry meat, and not all subtypes will be CRS; (2) infection of broilers by *C. jejuni* is an uncommon event, and infections primarily occur in the spring, summer, and fall; (3) broilers will be infected by a limited number of *C. jejuni* subtypes late in the production cycle; (4) once a *C. jejuni* subtypes established, the subtypes will become widely distributed within the farm (e.g. in air, litter, fomites, arthropods), and the subtypes will be rapidly disseminated horizontally throughout the flock; (5) *C. jejuni* subtypes infecting birds will be transmitted to poultry meat within the abattoir, and the contamination of poultry meat will be maintained within retail stream (including by *C. jejuni* subtypes that are clinically relevant); (6) resident populations of *C. jejuni* subtypes exist within the abattoir, serving as a source of bacterium contaminating poultry meat from birds coming from production farms that are free of bacterium; and (7) *C. jejuni* subtypes frequently shed in beef cattle feces are present in broilers, and beef cattle are an important reservoir of CRS of *C. jejuni* infecting poultry.

1.13 Objectives

To test the hypotheses, the objectives of the study were: (1) longitudinally sampling of broilers, and the farm environment (three farms) on a weekly basis in SWA over a 1-year period; (2) sample poultry meat from the processed birds at the abattoir; (3) meat obtained from the abattoir sampled in a retail setting; (4) sample feces from feedlot cattle that are adjacent to one of the broiler farms weekly over a 1-year period; (5) comprehensively isolate and identify *C. jejuni* from collected samples, and subtypes large numbers of representative isolates using the high-throughput and high-resolution comparative genome fingerprinting method; (6) analyse subtype data to identify reservoirs and transmission mechanisms of *C. jejuni*, and to ascertain the risk of human campylobacteriosis following consumption of poultry meat; and (7) gain insight on presented strategies to mitigate the presence of clinically-relevant subtypes of *C. jejuni*, and reduce the burden of campylobacteriosis in Canadians.

1.7 Tables and Figures

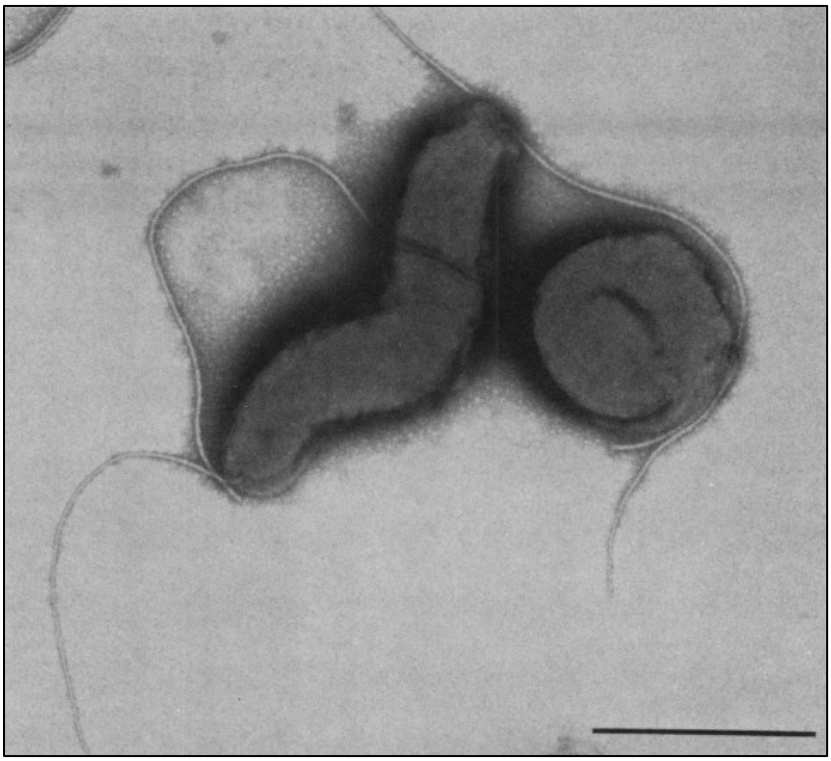

Figure 1.1 Electron micrograph of negative-stained *Campylobacter jejuni* bacteria with polar flagella. The image represents both spirallum and coccoid forms of the bacterium. Bar=0.5 µm. (Ng et al., 1985).

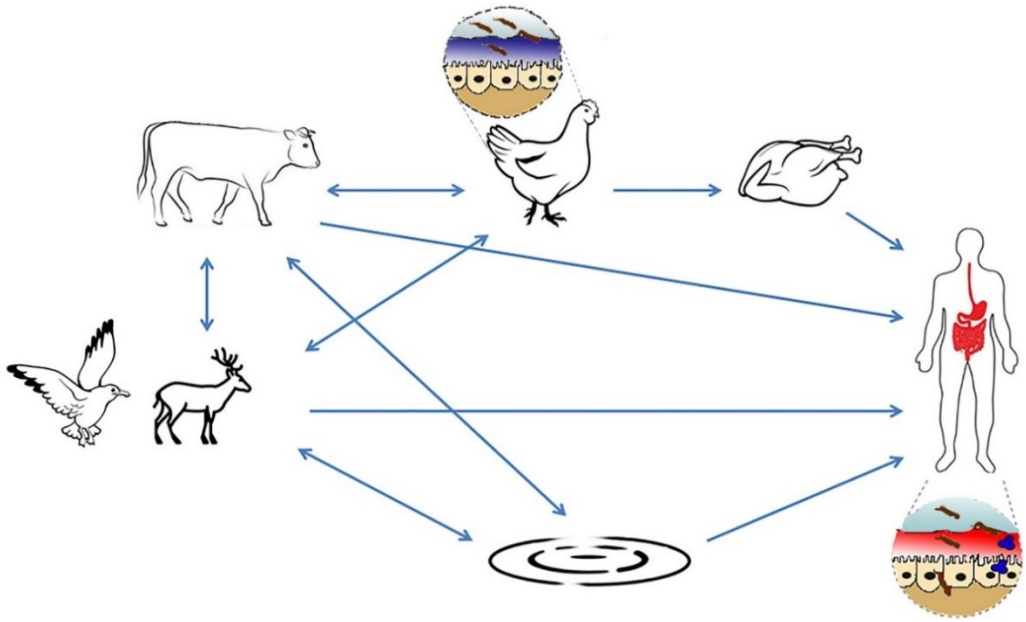

Figure 1.2 Primary reservoirs and transmission pathways of *Campylobacter jejuni* (Young et al., 2007). Uncooked meat, unpasteurized milk, untreated water and contact with wild animals, and domestic animals and birds constitute risk factors of campylobacteriosis in human beings. High numbers of *Campylobacter jejuni* colonize in the mucus layer of the chicken GIT, and are transferred horizontally to other birds through the faecal–oral route. In human beings, *Campylobacter jejuni* translocates across the intestinal epithelial layer and this leads to inflammation and diarrhea.

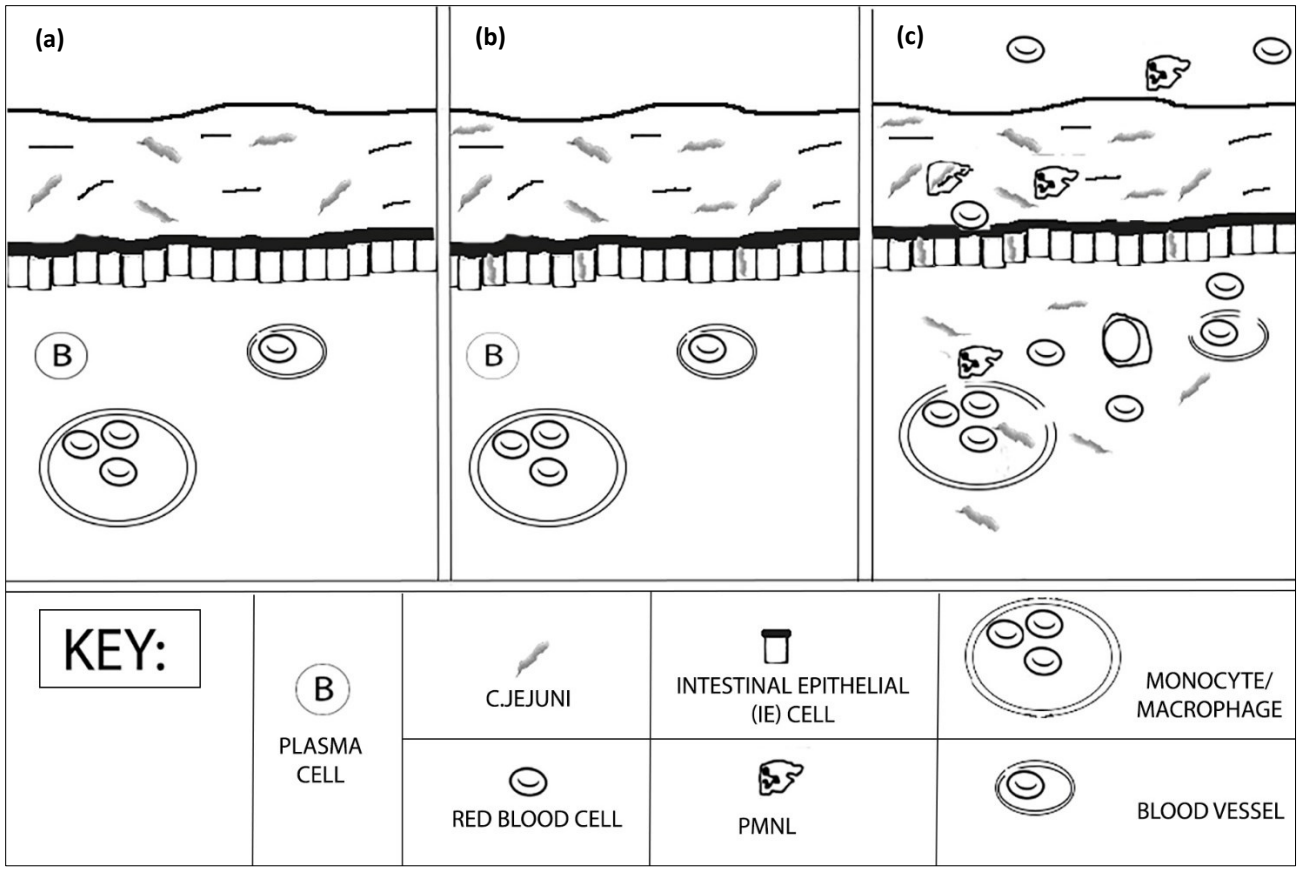

Figure 1.3 Different levels of immunity in human challenging with *Campylobacter jejuni* (Ketley, 1997). (a) An immune host. The bacterium may colonize the mucus, but there is relatively infrequent invasion of enterocytes with a small amount of toxin production. (b) A partially susceptible host. Colonization, tissue invasion and toxin secretion are restricted to the epithelial cell layer, which leads to minor loss of fluid absorption and subsequent watery diarrhea. (c) A fully susceptible host. Colonization, invasion, toxin secretion and tissue injury within the epithelium and lamina propria resulting in inflammation and hyper secretion of fluid and dysentery.

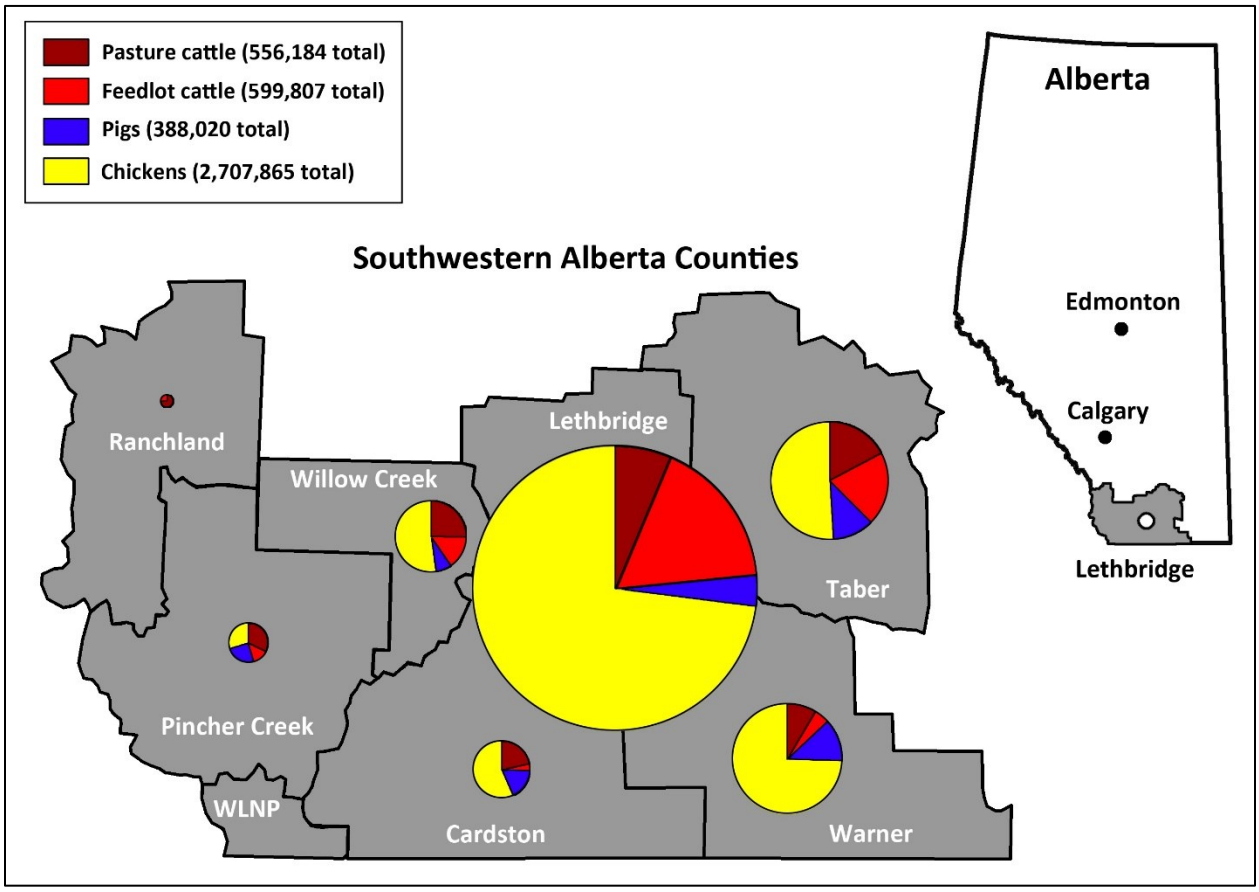

Figure 1.4 Livestock distribution and densities in Southwestern Alberta by county. Figure generated and provided by G.D. Inglis.

Chapter 2. Materials and Methods

2.1 Ethics statement

Approval to obtain cloacal samples from birds and trapping mice was obtained from the Lethbridge Research and Development Centre (LeRDC) Animal Care Committee (ACC) before commencement of sample collection (Animal Use Protocol Review 1615).

2.2 Sample collection

A longitudinal sampling strategy was employed (Figure 2.1). Samples were obtained weekly from three commercial broiler farms (all cycles) located in SWA over a *ca.* 1.5 year period (i.e. Farm A, B, and C). Each chicken farm was located in different area in SWA, and they were representative of broiler production in Alberta (Figure 2.2). Samples were obtained from a single barn at each farm, and the barns sampled at Farm A and C had cement floors, whereas the barn sampled at Farm B had a soil floor. The three barns contained ≈30,000 birds per cycle. A similar diet was provided to broilers at all three farms (corn-based diet from a same feed company), and drinking water was chlorinated . A single and local hatchery operation located adjacent to the abattoir (Figure 2.2) provided day-old chicks to the three farms. To collect the environmental samples (i.e. air, floors, walls, feed, water, litter, insects, and mice), the interior of each farm was divided into six sections. *Campylobacter jejuni* on the surfaces of floors and walls was determined weekly. Sterile sponges within the Whirl-Pak® Speci-Sponge® bags (Sigma-Aldrich, Markham, ON) moistened with 13 ml sterile Columbia broth (Oxoid Canada, Nepean, ON) were used to swab arbitrarily-selected 100 cm^2 areas of the wall and floor (litter removed) in each of the six sampling sections. Sponges were replaced in Whirl-Pak® Speci-Sponge® bags (Sigma-Aldrich) for transport to the laboratory. Litter (≈5 g) was collected weekly from the six sampling sections, and placed in sterile bags for transport (Figure 2.3). Additional environmental samples (e.g. feed, beetle larvae and adults, flies, and air) were obtained from each of three sections weekly (far left, center, and far right). Feed (≈5 g) was obtained from arbitrarily-selected feeding trays. Beetle larvae and adults were exposed by manually removing litter, and collecting larvae and adults with sterile forceps and placing them collectively in separate tubes by location for transport; a maximum of 10 individuals were obtained per subsample (Figure 2.3). Adult flies were collected in six containers (250 ml) filled with ham, tomato juice and water. Flies were individually removed with forceps and placed in sterile tubes for transport; a maximum of 10 individual flies were obtained per subsample. *Campylobacter jejuni* in air was determined using an inertia-type microbial air sampler (MAS 100; Millipore Canada Ltd, Etobicoke, ON) operated at 100 L of air per min for a 10 min test period (Figure 2.3). Particles in air were deposited directly onto Karmali agar (KA; Oxoid Canada) with selective supplement SR0167 (KSA; Oxoid Canada) placed in the sampler. A single feed sample (≈5 g) was also obtained before distribution to birds, and an individual water sample (2 L) was collected from the end of one pipeline weekly. Air and fly samples were also obtained from three designated locations outside and adjacent to each farm. Mice in the farm and adjacent feed room were trapped using live traps (Victor brand multi-catch live mouse trap; model #M333) baited with peanut butter. Samples from floors, walls, litter, air, beetle adults, and beetle larvae were

obtained from farms after they were sanitized, and before they were populated with birds. The total number of environmental samples collected per farm per week was ≥38. (Table 2.1)

At the time of population of the farms with chicks, 20 arbitrarily-selected soiled chick transport papers were obtained. Samples were also obtained each week from 75 arbitrarily-selected live birds via cloacal mini-swabs (Cat#22029571, Fisher Scientific Company, Ottawa, ON). To obtain cloacal swabs, birds were humanely immobilized by one worker, and another worker gently inserted the mini-swab moistened with sterile Columbia broth into the vent and rotated it until it was covered with feces (Figure 2.3). The mini-swab was then placed in an individual tube containing 3 ml of Columbia broth for transport. For some of the cycles in Farm A and Farm B, a subsample of excreted feces was collected from the surface of the litter with a sterile spatula, and placed in a tube for transport. Digesta from ceca was obtained from a maximum of 15 cull birds each week. Cull birds were placed in bags, transported to the necropsy facility at AAFC LeRDC, the abdominal cavity was opened with sterile instruments to expose the ceca, individual ceca were aseptically removed and incised, a subsample of digesta was removed, placed in a sterile tube, and maintained on ice for transport to the laboratory. During the last 2 weeks of each production cycle, feather samples were collected from a maximum of 15 cull birds. The total number of samples obtained from birds per farm per week was ≥146 (Table 2.1).

At the end of each production cycle, birds from the farms sampled in the current study were followed to the abattoir at which they were processed, and subsamples of feathers, ceca and skin were obtained (Figure 2.4). Skin samples were collected from salvage parts of the abattoir after sanitation with chlorine. In addition, feather and cecal digesta samples were obtained from birds that died during transport from the farms to the abattoir and were processed; to obtain cecal digesta the same procedure described above for cull birds was employed (Figure 2.5). The total number of samples obtained from the abattoir per farm per week was ≥180. At ten sample times throughout the study period, skin samples from the cloacal region (n=25) and breast (n=25) of carcasses were provided by a retailer who obtained chicken from the abattoir at which the birds in the study were processed (Figure 2.6).

A subsample of feces (≈10 g) was obtained from fresh fecal pats of beef cattle housed in a confined feeding operation adjacent to Farm C; sample times were concurrent with those from Farm C, and different cattle pens were sampled. Samples of feces (n=25) from individual pats were placed in tubes, and maintained on ice for transport to the laboratory. In addition, *C. jejuni* in air adjacent to feedlot pens (two samples per week) was evaluated using the MAS 100 air sampler as described previously.

2.3 Sample processing

Samples were processed the same day as collection (i.e. typically within 4 hr of collection). For floor and wall samples, sponges within the Whirl-Pak® Speci-Sponge® bags (Sigma-Aldrich) were filled with 50 ml of sterile Columbia broth, homogenized using a Smasher® (BioMérieux Canada, Inc., St-Laurent, QC) for 1 min (normal speed), and the homogenate was centrifuged for 10 min at 14,900 x g. Immediately after centrifugation, the supernatant was removed to a final volume of 3 ml, and the remaining liquid was vortexed to re-suspend bacterial cells within the pellet. Feed samples and litter samples (5 g) were

individually placed into a BagPage bag containing a microperforated filter (Cat#122025, Interscience, Woburn, MA), and sterile Columbia broth (25 ml) was added to the sample (i.e. 1:5 dilution). The sample was then homogenized using the Smasher® (BioMérieux Canada) for 1 min (normal speed), and broth on the non-feed/non-litter size of the micropeforated filter was collected. Drinking water samples (2 L) were individually filtered through a Whatman glass microfiber filter (Cat#1827055, 55-mm-diam; Whatman Inc., NJ) under vacuum to remove large particulate matter, and then through a Supor® 200 PES membrane disc filter (47-mm-diam, 2 µm pore size; Pall Corporation, Port Washington, NY). The Supor® filters were vigorously vortexed (high setting for 3 min) in 15 ml Columbia broth to release any bacterial cells on the filter surface. The Columbia broth was centrifuged at 14,900 x g for 10 min to sediment any bacterial cells, and immediately after centrifugation, the supernatant was removed to a final volume of 3 ml. The remaining liquid was vortexed to re-suspend bacterial cells within the pellet. Beetle larvae and adults, and flies were each placed into a 2 ml tube, homogenized with a polypropylene mini-mortar and pestle (Fisher Scientific Company) in 1.5 mL of Columbia broth. Mice were euthanized with isoflurane (Isoflurane USP, Fresenius Kabi, Toronto, ON), and humanely euthanized under anesthesia by cervical dislocation. The cecum was exposed by laparotomy, removed, incised, cecal contents were collected, weighed, Columbia broth was added at a 1:10 dilution, and samples were vortexed for 1 min (high setting).

Individual soiled chick transport papers (42.5 cm by 59.5 cm) were folded and placed into the BagPage filter bag (Interscience). For every 16 cm^2 surface area of the transport papers, 100 ml of Columbia broth was added, the paper was homogenized using the Smasher® (BioMérieux Canada) for 2 min (high speed), and a subsample of the liquid on the non-paper side of the micropeforated filter was collected. Cloacal mini-swabs in Columbia broth were vortexed for 1 min (high setting). For cecal digesta, and feces from chickens and cattle, samples were weighed, Columbia broth was added at a 1:10 dilution, and samples were vortexed for 1 min (high setting). Feathers (5 g) were weighed, placed in a BagPage filter bag (Interscience), Columbia broth was added at a 1:5 dilution, the samples were homogenized with the Smasher® (BioMérieux Canada) for 1 min (normal speed), and liquid on the non-feather side of the micropeforated filter was collected. For skin samples, 25 g of each sample was placed into the BagPage filter bag (Interscience), Columbia broth was added at a 1:4 dilution, samples were homogenized with the Smasher® (BioMérieux Canada) for 1 min (normal speed), and liquid on the non-skin side of the micropeforated filter was collected.

2.4 Isolation of *C. jejuni* from chicken and beef cattle

Two isolation methods were applied for most samples, which included non-selective enrichment followed by membrane filtration, and direct plating onto Karmali Agar Supplement (KAS) (Figure 2.7). For enrichment (all samples with exception of air and cattle fecal samples), 500 µl of each sample was added to 4.5 ml of an enrichment broth containing Bolton broth (Oxoid Canada) with 5% laked horse blood, and 10 mg/L a Amphotericin (10 mg/L) and Trimethoprim (5 mg/L) (BAT) in 100 x 16 mm culture tubes (Inglis et al., 2018). Tubes were incubated for 48 hr at 37°C, and 200 µl of each enrichment broth was spread centrally onto a sterile 47-mm-diam filter with 0.45 µm pores positioned on the surface of Karmali Agar

(KA). After 15 min, the filter was aseptically removed taking care to ensure that enrichment broth remained on the filter, and KA cultures were incubated at 37°C in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) for 48 hr. For direct plating (all samples with the exception of air samples, for which air was impacted directly onto KSA), 25 µl of each sample was streaked onto KSA in duplicate, and cultures were maintained in the microaerobic atmosphere at 42°C for 48 hr. For air samples, KSA from the MAS 100 air sampler were placed directly in the microaerobic atmosphere at 42°C for 48 hr. Biomass from presumptive *Campylobacter* colonies (maximum of five colonies per culture) was streaked for purity on KA, and cell size, morphology, and motility characteristic of *Campylobacter* was used to select isolates for biomass generation. Biomass was stored in 40% glycerol at -80°C (Figure 2.8).

2.5 *Campylobacter jejuni* isolates from diarrheic people

All isolates of *C. jejuni* isolated from stools of diarrheic human beings in SWA by Chinook Regional Hospital staff during the study period were transferred to AAFC LeRDC under an existing transfer agreement. Information provided with the isolates was limited to date of collection. In addition, *C. jejuni* isolates infecting people in SWA provided by the Chinook Regional Hospital outside of the study period from 2004 to 2017 were included reference strains (e.g. to identify clinically-relevant subtypes associated with poultry).

2.6 Identification of *C. jejuni*

Genomic DNA from presumptive *Campylobacter* isolates was extracted using an AutoGen 740 robot (Holliston, MA) according to the manufacturer's protocol. Extracted DNA was subjected to diagnostic PCR for *Campylobacter* genus targeting the 16S rRNA gene using the primers (C412F:5'-GGA TGA CAC TTT TCG GAG C-3' and C1228R: 5'-ATA AAA GAC TAT CGT CGC GTG-3') (Linton et al., 1996). DNA was also subjected to species-specific PCR using two of primer sets, targeting the IpxA gene (IpxAF:5'- ACA ACT TGG TGA CGA TGT TGT A-3' and lpxARKK2mR:5'-CAATCATGDGCDATATGASAATAHGCCAT-3') (Klena et al., 2004), and the HipO gene (C. jejunihipOF:5'-AAA TAG GAA AAA CAG GCG TTG T-3' and C. jejunihipOR:5'-TAT CAT TAG CCT GTG CAA GAC C-3') (Inglis et al., 2018) . Amplification reactions consisted of 2.0 µl of ten times PCR Buffer, 0.4 µl of 25 mM MgCl2 (Qiagen Inc., Montreal, QC), 2.0 µl of BSA (1.0 mg ml^{-1}; Ambion, Life Technologies Inc., Burlington ON), 0.4 µl of 10 mM dNTP (Bio Basic Canada Inc., Markham, ON), 0.1 µl of HotStar Taq Plus DNA Polymerase (5.0 U $µl^{-1}$; Qiagen Inc.), 1.0 µl each of forward and reverse primer (10 µM); (Integrated DNA Technologies, Coralville, IA), 2.0 µl DNA template, and 11.1 µl nuclease free water (Qiagen Inc.). The PCR conditions were as follows: one initial denaturation cycle at 95°C for 5 min; 35 cycles of 30 sec at 94°C for denaturation, 1 min 30 sec at 60°C and 1 min at 72°C for annealing, and a final 10 min extension cycle at 72°C for extension. Amplicons were run on a 1% TAE agarose gel to detect positive samples relative to negative and positive controls. For confirmed *Campylobacter* isolates in which taxon-specific PCR was indefinite, the near complete 16S rRNA gene of these isolates was sequenced, and sequence data was compared to reference sequences within GenBank (National Center for Biotechnology Information, Bethesda MD) using BlastN.

2.7 Genotyping

Representative isolates of *C. jejuni* were fingerprinted using a 40 locus CGF method (Taboada et al., 2012); this method targets accessory genes that are distributed throughout the *C. jejuni* chromosome to generate a binary fingerprint. Eight-multiplex PCR reactions were utilized for every *C. jejuni* isolate. Each five-multiplex reaction mix consist of 1 U Fisherbrand Taq DNA polymerase (Fisher Scientific, Nepean, ON), 1X buffer, 2.5 mM MgCl2, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.12 to 0.74 µM of the 10 primers (of note, primer concentration was optimized to produce a robust amplicon for each primer set in the multiplex), and 1 µl of DNA template (20 to 100 ng) in a 25 µl reaction mix. An EP Gradient Mastercycler (Eppendorf, Mississauga, ON) was used for the PCR reactions. The PCR conditions were: an initial denaturation at 94^{o}C for 5 min; 35 cycles of denaturation at 94^{o}C for 30 sec, annealing at 55^{o}C for 30 sec, and extension at 72^{o}C for 30 sec; and a final extension step at 72^{o} C for 5 min. Amplicons were resolved using a QIAxcel high-throughput capillary electrophoresis system (Qiagen Inc., QC) with DNA Screening Cartridges (Qiagen Inc., QC) using the AM320 separation method and a 20 sec injection time. The 15 to 3000 base pair alignment marker and a 100 to 2.5 kb size ladder were used as size standards (Qiagen Inc.).

2.8 Data Analysis

Most analyses were conducted using Statistical Analyses Software (SAS; Cary, NC). In order to determine if significant count shifts occurred among the two sample times, the Genmod non-parametric procedure from SAS was used. When a significant treatment effect was observed, the least square means method was used to evaluate differences among means of interest. To analyze subtype diversity of *C. jejuni*, isolates were assigned to CGF subtype clusters using the simple matching analysis coefficient with unweighted pair group method with arithmetic mean (UPGMA) clustering in Bionumerics (version 6.6, Applied Maths, Austin, TX). Randomized resampling was performed to normalize sample size, and cluster richness and abundance were used to calculate the Shannon diversity index. Hutcheson's t-test was used to test the significance of differences in subtype diversity (Hutcheson, 1970). Population structures were visualized as Minimum Spanning Trees (MSTs) using Bionumerics (version 6.6, Applied Maths). Venn diagrams of subtypes between sample types were generated using pivot tables at a 95% level of resolution, including subtypes recovered from diarrheic human beings, chickens, and beef cattle isolated in SWA outside of the study period and accessioned within the C^{3}GFdb. For comparisons with all isolates within the C^{3}GFdb, CGF profiles were queried against those in the database C^{3}GFdb (i.e. *C. jejuni* isolates recovered nationally).

2.9 Tables and Figures

Table 2.1 Sample category and type, and numbers of samples obtained per week for each of twenty two production cycles in Southwestern Alberta.

Category/type	Number
Barn: pre-sampling	
Floors	6
Walls	6
Litter	3
Beetle adults	3
Beetle larvae	3
Air-inside	3
Air-outside	3
Barn: weekly sampling	
Walls	6
Feed	4
Water	1
Litter	6
Flies	6
Mice	V[b]
Air-inside	3
Air-outside	3
Beetle adults	3
Beetle larvae	3
Chick transport paper[a]	20
Cloaca	75
Feces	6
Ceca[c]	≥15
Feathers[c]	≥15
Transport	
Ceca[a]	≥40
Feathers[a]	≥40
Abattoir	
Skin[a]	≥100
Retail	
Skin-breast[a]	25
Skin-cloaca[a]	25
Beef cattle[d]	
Feces	25
Air	2

[a]Obtained once per production cycle. [b]Variable number and not for all production cycles. [c]Obtained from birds that died from natural causes (i.e. within *ca.* 24 hours). [d]Adjacent to one of the three farms sampled (i.e. Farm C).

Table 2.2 Numbers of samples by category collected over a 542-day period in Southwestern Alberta (2016-2017).

	Broiler barn			
Category/type	A	B	C	Total
Barn				
Floors	50	42	42	134
Walls	222	241	238	701
Feed	118	132	132	382
Water	30	36	32	98
Litter	197	212	218	627
Flies	9	9	10	28
Mice	2	4	0	6
Air-inside	111	94	114	319
Air-outside	45	60	49	154
Beetle adults	60	119	55	234
Beetle larvae	24	67	27	118
Chick transport paper	158	131	140	429
Cloaca	2,686	2,605	2,448	7,739
Feces	235	90	0	325
Ceca	375	211	262	848
Feathers	89	54	78	221
Transport				
Ceca	80	68	216	364
Feathers	72	68	171	311
Abattoir				
Skin	500	415	795	1,710
Retail				
Skin-breast	--	--	--	219
Skin-cloaca	--	--	--	221
Beef cattle				
Feces	0	0	791	791
Air	0	0	18	18
Total	5,063	4,658	5,027[a]	15,997[b]
Percent	34.3	31.6	34.1	

[a]Total for Farm C does not include samples from cattle. [b]Grand total includes samples from all sources.
Note: 5,751 total *Campylobacter jejuni* isolates recovered, 1,052 *C. jejuni* positive samples, (1,052/15,997)*100 = 6.58% samples overall positive for *Campylobacter jejuni.*

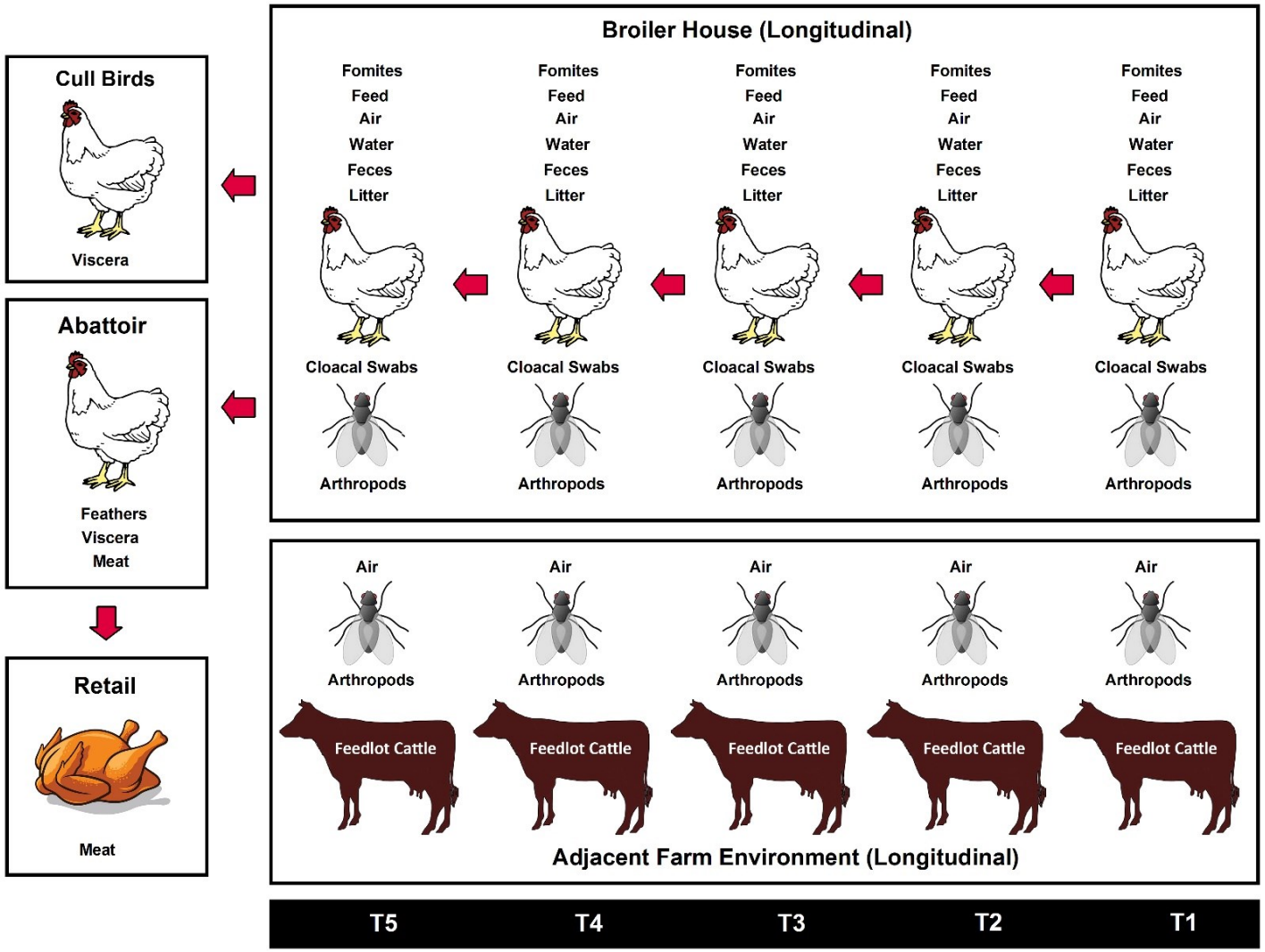

Figure 2.1 Graphical schematic of longitudinal sampling strategy applied. Figure generated and provided by G.D. Inglis.

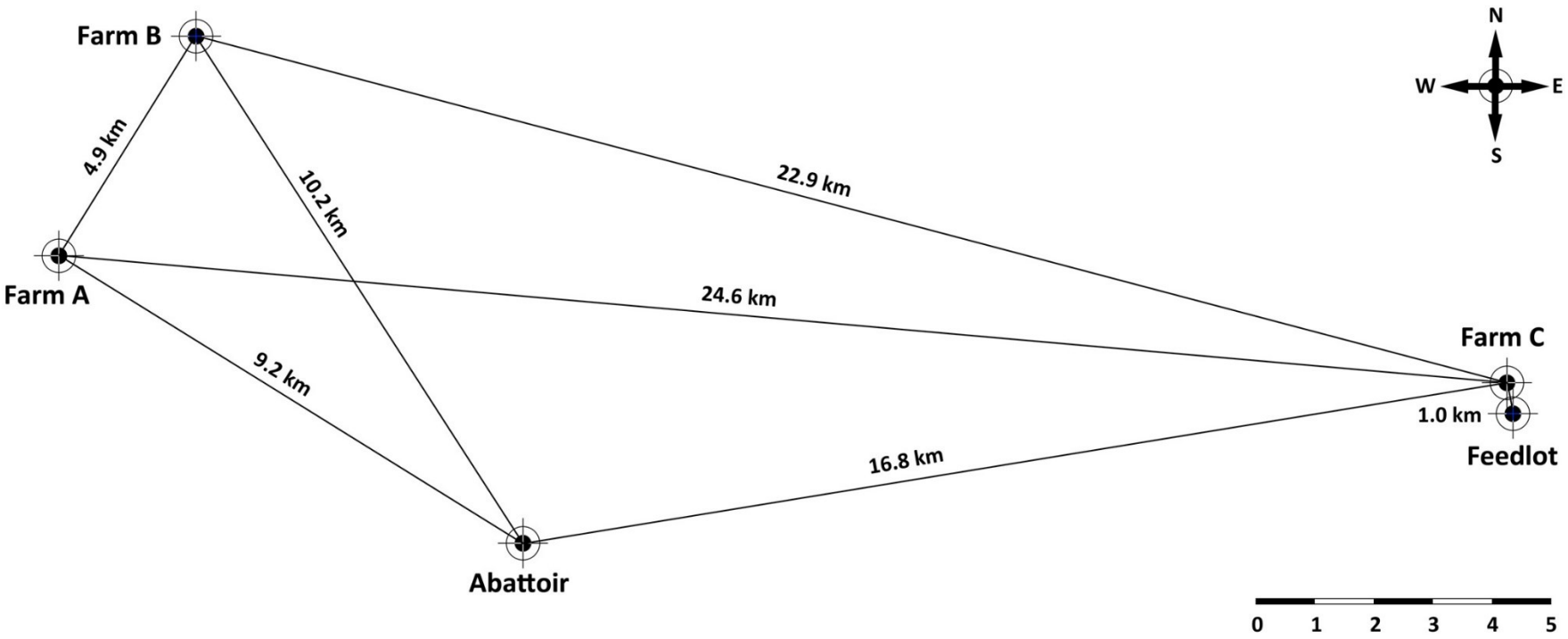

Figure 2.2 Relative position of the three farms included in the study and the abattoir at which birds were processed in Souwestern Alberta. In addition, the position of the feedlot sampled relative to Farm C is shown. The scale bar and distances are in km. Figure generated and provided by G.D. Inglis.

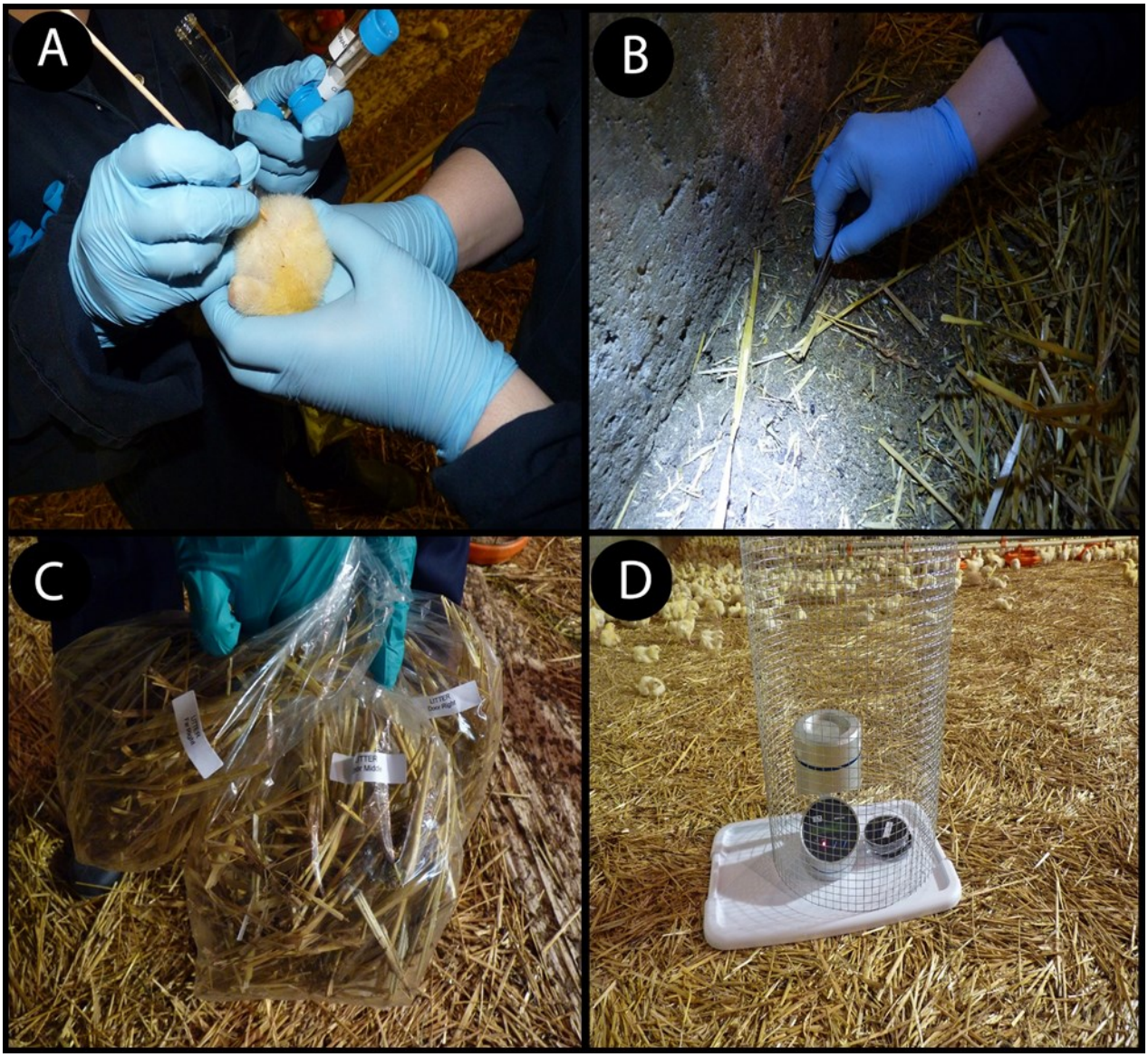

Figure 2.3 Representative sample types obtained per week for each of twenty two production cycles in SWA chicken farms. (A) Cloacal swab. (B) Beetles. (C) Litter. (D) Air sample.

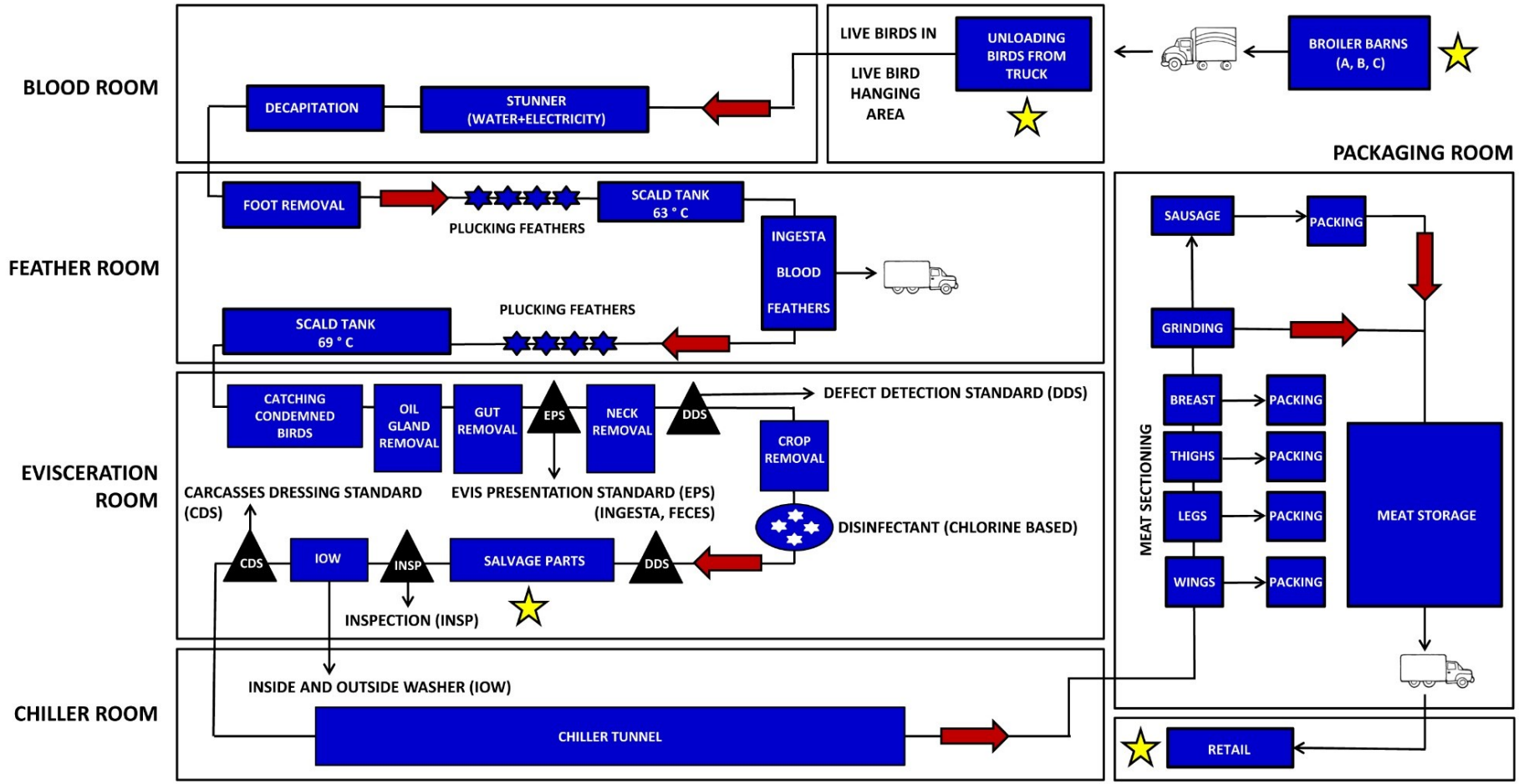

Figure 2.4 Graphical schematic of the longitudinal sampling strategy at employed in the current study at broiler farms, the abattoir at which the birds were processed, and a local retailer. Sample points throughout the production continuum are indicated with a star.

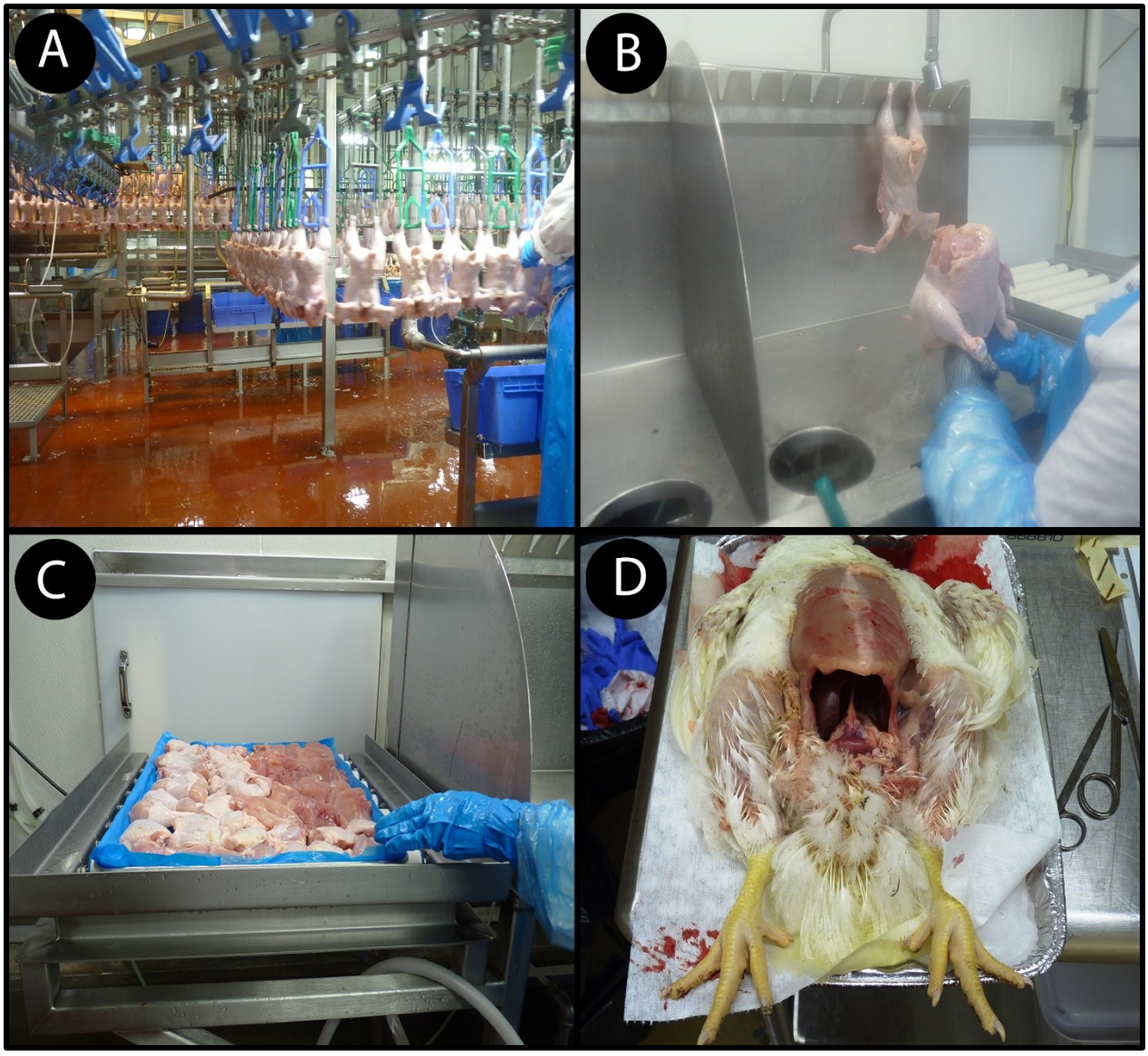

Figure 2.5 Representative sample types and different parts of chicken abattoir from which samples were collected at end of each production cycle in SWA. (A) Production line of abattoir. (B) Skin samples from chicken in the salvage room of abattoir. (C) Chicken meats after cutting off at salvage part, (D) Cecal digesta sample was aseptically obtained.

Figure 2.6 Processed chicken meats at a retail chicken store in Southwestern Alberta. (A) A box of chicken meats arrived from abattoir. (B) & (C) Cutting board and a knife and the process of slicing and dividing chicken meats. (D) Packing the meat slices.

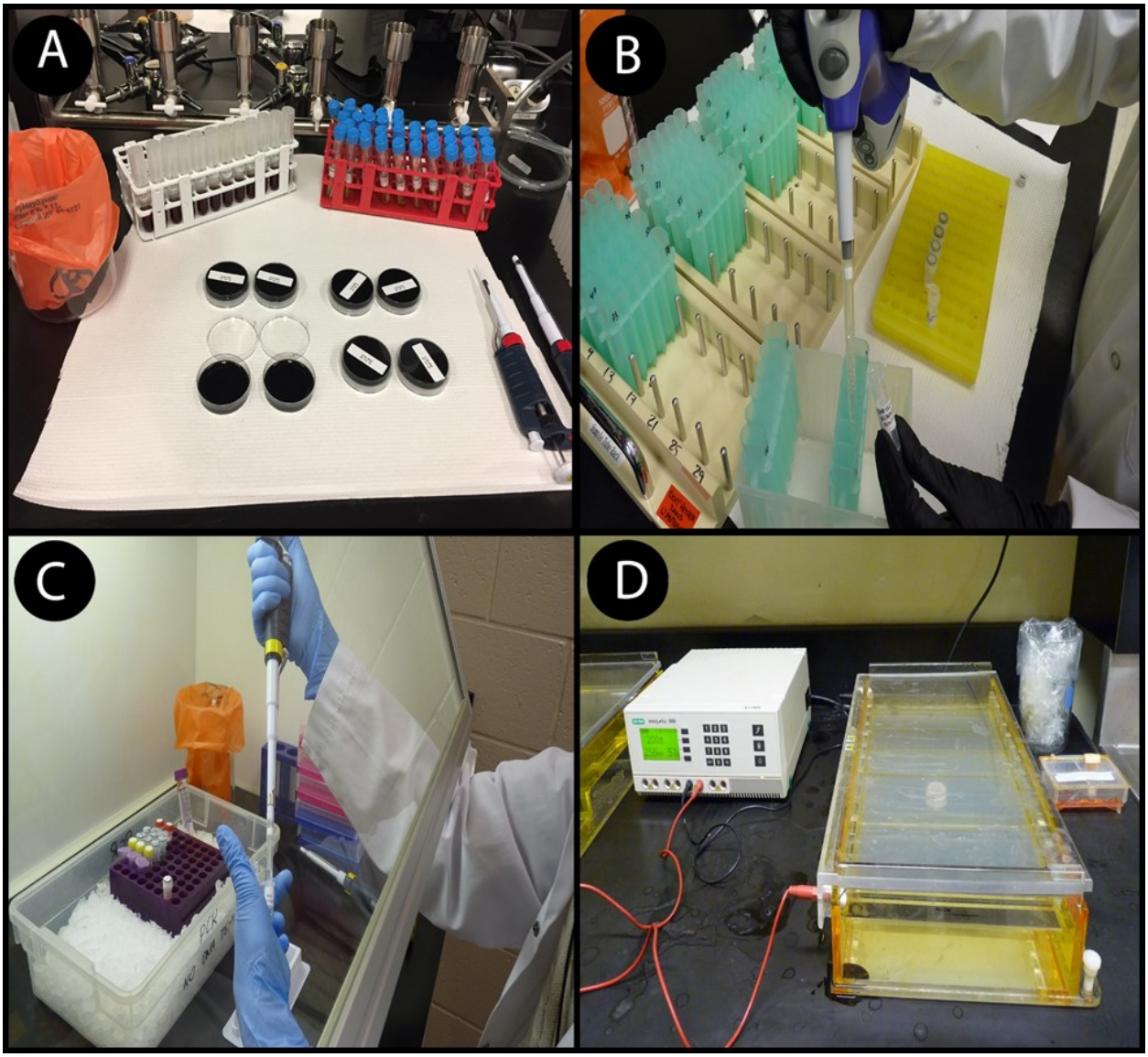

Figure 2.7 Laboratory techniques for isolating and identifying *Campylobacter jejuni*. (A) Direct plating and enrichment methods. (B) DNA extraction. (C) Making master mix for end-point PCR. (D) Gel electrophoresis.

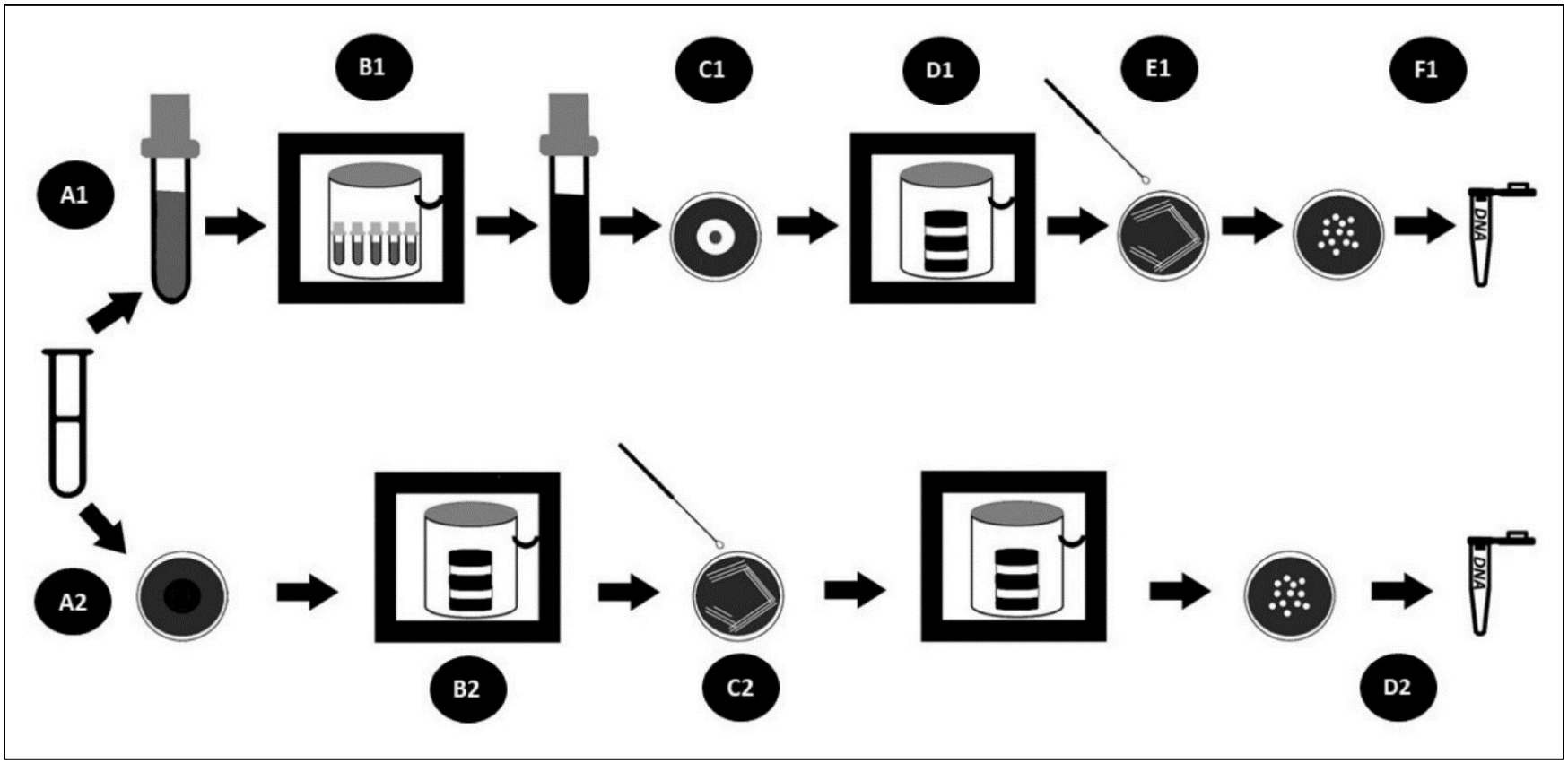

Figure 2.8 Isolation methodology. Non-selective enrichment: (A1) 500 µl of sample was added to 4.5 ml of an enrichment broth containing Bolton broth. (B1) Tubes were incubated for 48 hr at 37°C. (C1) 200 µl of the enrichment broth was spread centrally onto a sterile filter (0.22 µm pores) positioned on the surface of Karmali agar. (D1) After 15 min, the filter was aseptically removed and the KA was incubated in a microaerobic atmosphere at 37°C for 48 hr. (E1) Biomass from a presumptive *Campylobacter* colony was streaked for purity on Karmali agar, and the culture incubated at 37°C for 48 hr. (F1) Biomass from the pure culture on Karmali agar was collected and stored in 40% glycerol at -80°C for subsequent DNA extraction. Selective direct plating: (A2) 25 µl of sample was streaked onto Karmali Agar Supplement. (B2) the Karmali Agar Supplement culture was maintained in the microaerobic atmosphere at 42°C for 48 hr. (C2) Biomass from a presumptive *Campylobacter* colony was streaked for purity on Karmali agar, and the culture incubated at 37°C for 48 hr. (D2) Biomass from the pure culture on Karmali agar was collected and stored in 40% glycerol at -80°C for subsequent DNA extraction.

Chapter 3. Results

3.1. Total samples collected and processed

Fifteen thousand, nine hundred, and ninety seven samples were longitudinally collected and processed over the 542-day sampling period (Table 2.2). This included 12,363 samples associated with three broiler farms, 675 samples from broilers after transport from the farms to the abattoir, 1,710 samples from the abattoir at which the birds were processed, 440 retail samples from the abattoir, and 809 samples associated with beef cattle housed in a confined feeding operation adjacent to broiler Farm C. Samples were obtained from eight, seven, and seven cycles from Farms A, B, and C, respectively (Figure 3.1A). SWA is located in a semi-arid environment, and ambient conditions fluctuated over the sample period (Figure 3.1B-F); air temperatures and relative humidity ranging from -29.8 to 36.7^{o}C and 8.7-100%, respectively.

3.2 Differentially recovery by direct plating and non-selective enrichment

Twenty seven thousand, five hundred and fifty cultures were processed, and 5,751 *C. jejuni* isolates were recovered by enrichment (i.e. in a non-selective enrichment broth at 37^{o}C followed by membrane filtration) and direct plating (i.e. on Karmali agar with selective supplement at 37^{o}C), and identified by taxon-specific PCR. For the majority of sample types, a higher prevalence of cultures ($P<0.001$) were positive by enrichment than by direct plating (Figure 3.2A-B). Direct plating was more effective only for feathers obtained from birds after transport to the abattoir ($P<0.001$) and from cattle feces ($P<0.001$). Notably, enrichment was conspicuously more effective than direct plating for isolating *C. jejuni* from chicken cloacal swabs and ceca digesta, and from retail samples. Although *C. jejuni* was detected statistically more frequently by enrichment from abattoir skin samples, the difference between the two isolation methods for this substrate was nominal.

3.3 *Campylobacter jejuni* recovery from broiler farm samples

The majority of 15,997 (93.4%) samples analyzed were negative for *C. jejuni*. In only two instances was *C. jejuni* detected in farms before placement of chicks; in Farm A (A4) and Farm B (B6) the bacterium was detected in litter. All three farms provided chlorinated drinking water for birds, and none of the 98 water samples examined throughout the 22 broiler cycles were positive for *C. jejuni*. One of the 382 feed samples examined within the farm (i.e. within a feeding hopper) was positive for *C. jejuni* (i.e. cycle B1 at week 6). The bacterium was isolated from farm walls (n=701) on three instances (i.e. cycle B1 at week 7, and C7 at weeks 4 and 5), but not from farm floors. In only one instance, was *C. jejuni* recovered from air samples (n=319) with a poultry farm (i.e. B1 at week 6), and never from air samples outside and adjacent to farms (n=154). *Campylobacter jejuni* was recovered from farm litter on eight occasions (n=627) from all three farms (i.e. A4 at week 0, B1 at week 6, B6 at week 0, B7 at week 2, and C7 at week 5). From composite samples of darkling beetle larvae (n=118) and adults (n=118), *C. jejuni* was recovered from beetle larvae as well as adults on two occasions (i.e. A3 at week 4, and C7 at week 5). Fly adults were infrequently observed in the farms, and none of the 28 composite fly samples

examined during the summer and fall were positive for *C. jejuni*. Furthermore, none of the mice (n=8) examined were positive for the bacterium.

3.4 Isolation of *C. jejuni* from chickens in broiler farms

None of the 429 soiled paper liners from transportation of chicks to the broiler farms were positive for *C. jejuni*. Of the 325 excreted fecal samples collected from Farm A and Farm B, *C. jejuni* was frequently observed in Farm B during an outbreak within the flock (i.e. cycle B1 and week 7). *Campylobacter jejuni* was detected from 2.7% (n=212) of the 7,739 cloacal swabs obtained from birds, and from 1.8% (n=15) of 848 ceca and 1.4% (n=3) of 221 composite feather samples from cull broilers within farms. As well, the bacterium was isolated from 14.6% (n=53) of 364 ceca and from 11.6% (n=36) of 311 feather samples after transport to the abattoir. Occurrence of *C. jejuni* recovered from ceca after euthanization of the birds at the abattoir was considered to represent infections that occurred in the farm before transport, whereas *C. jejuni* recovered from feathers may have represented occurrences of the bacterium within the farms and/or contamination of birds during transport to the abattoir (e.g. passive contamination from transport trucks).

3.5. Occurrence of *C. jejuni* outbreaks in broiler farms

Campylobacter jejuni was isolated from birds in one of eight cycles in Farm A, one of seven cycles in Farm B, and five of seven cycles in Farm C (Figure 3.3-3.5). One outbreak (>10% of samples from birds positive for *C. jejuni*) was observed in Farm A (cycle A3), one outbreak in Farm B (cycle B1), and two outbreaks in Farm C (i.e. cycle C1 and C7) (Figure 3.3-3.6). Notably, all outbreaks occurred late in the production cycle, and the outbreak in Farm C (i.e. cycle C1) was not detected in birds within the farm (i.e. only in 23.6% of birds sampled after transport to the abattoir) (Figure 3.5). When infections were observed on two consecutive sample times within a farm, the vast majority of birds sampled (≥95.9%) were infected at the latter week (e.g. cycles B1 and C7). In two instances in Farm C (i.e. cycles C1 and C6), *C. jejuni* was detected in a small number of birds early in the production, but an outbreak of *C. jejuni* did not occur within the flock on these occasions. *Campylobacter jejuni* was detected on feathers of birds from all three farms after transport to the abattoir (Figure 3.3-3.5), including in instances where the farm was deemed free of the bacterium (i.e. cycles A4, A6, A8, B4, and B5). Overall, *C. jejuni* was recovered from 11.6% (n=198) of 1,710 skin samples obtained from birds at the abattoir at which they were processed; at least one sample at the abattoir was positive for 85.7% to 100% of the cycles examined (Figure 3.6). The bacterium was readily isolated from skin samples in the abattoir from birds originating from a farm in which a *C. jejuni* outbreak was detected (i.e. cycles C1 and C7) (Figure 3.3-3.5). However, the bacterium was also recovered from birds originating from farms that were deemed free of *C. jejuni* (i.e. cycles A4, A5, A6, A7, A8, B3, B5, B6, B7 C1, C2, C4, C5, C6, and C7), including from birds that were deemed negative after transport (i.e. cycles A5, A7, B3, B6, B7, C2, and C5). On retail meat, *C. jejuni* was isolated from 21.0% (n=46) and 16.7% (n=37) of 219 skin samples from the breast and 221 skin samples from the cloacal region, respectively. The prevalence of infestation of retail poultry ranged from 0% to 52.0% (Figure 3.7).

3.6 Isolation of *C. jejuni* from beef cattle adjacent to poultry farm C

Campylobacter jejuni were readily detected in excreted feces from beef cattle housed in a confined feeding operation adjacent to Farm C (Figure 3.8). The prevalence of fecal pats that were positive for *C. jejuni* ranged from 0% to 100.0% (overall mean of 52.1%).

3.7 Temporal occurrence of *C. jejuni* in broilers

Although chickens, and abattoir and retail samples were positive for *C. jejuni* throughout the year, all four outbreaks detected in the current study occurred in the spring and summer, and a trend for higher rates of contamination of abattoir and retail samples similarly occurred during this period (Figure 3.9).

3.8 *C. jejuni* subtype isolation bias by direct plating versus enrichment

Considerable diversity of *C. jejuni* strains were recovered from poultry by both enrichment and direct plating (Figure 3.10). However, the diversity of recovered by enrichment ($H=4.33 \pm 0.16$) was higher ($P<0.001$) than by direct plating ($H=3.13 \pm 0.17$).

3.9 *C. jejuni* subtypes associated with outbreaks in broiler farms

The outbreak that was observed in Farm A (cycle three) was incited by a single prominent subtype of *C. jejuni* (Figure 3.11; grey shading), although other subtypes were present at low frequencies in some instances (Figure 3.11; black arrows). Similarly, in Farm B (cycle one) and Farm C (cycle one and cycle seven), outbreaks were incited by a predominant subtype, and a limited number of other subtypes were present (Figure 3.12-3.13). The subtypes responsible for the outbreak in Farm A (i.e. A3) primarily belonged to CGF subtype 0957.001 and 0957.004 (Table 3.1). Examination of metadata within the C^3GFdb indicated that this subtype cluster is primarily associated with cattle, but is also infects people and chickens; CGF subtype 0957.001.001 is particular prevalent within the C^3GFdb. Although this subtype cluster has been previously observed in Alberta, it is primarily observed in Ontario. For the outbreak in Farm B (i.e. B1) and in Farm C (i.e. C1), the CGF subtypes responsible were 0817.001 and 0817.006. This subtype cluster is common in Alberta, and associated with people, chickens, and cattle. For the second outbreak observed in Farm C (i.e. C7), the CGF subtypes responsible were 0735.001, 0735.003, 0735.004, and 0735.009. This subtype cluster is most commonly associated with chickens in Alberta, and is also found in people and cattle. Subclades of isolates associated with the outbreak were observed (i.e. C7b and C7c); both constituted novel subtypes that were most closely related to 0901.001.002, and 0731.006.002 and 0.735.003.004, respectively.

Although relatively rare, in some instances *C. jejuni* subtypes observed in farms, including early in the cycle period did not incite outbreaks (Figure 3.11-3.13; white arrows). With a few exceptions (0044.005.002, 0082.001.001, 0253.004.001, 0811.008.001, 0811.009.002, 891.001.001, and 0960.007.001) the subtypes observed in farms that were not associated with outbreaks were novel or low ranking with regard to frequency within the C^3GFdb; only three CGF subtypes (0082.001.004, 0253.004.001, and 0960.007.001) were commonly associated in chickens (Table 3.2-3.4).

3.10 Detection of *C. jejuni* subtypes associated with outbreaks in the abattoir

Subtypes of *C. jejuni* that incited outbreaks in all three farms were subsequently isolated from birds within the abattoir providing evidence that *C. jejuni* from infected chickens entering the abattoir were transferred to meat during processing within the plant (Figure 3.11-3.13). Furthermore, subtypes that incited outbreaks in Farm B and C were observed on retail meat at the same time period (Figure 3.12-3.13).

3.11 Detection of non-outbreak *C. jejuni* subtypes in the abattoir

Sixty four, 92, and 47 subtypes were recovered from birds in farms/meat in farms, the abattoir, and at retail, respectively (Figure 3.14). Adjusted to equivalent numbers isolates by random subsampling, the diversity of *C. jejuni* subtypes associated with birds in the abattoir was substantially higher ($P<0.001$) than observed in farms (Figure 3.15). Consistent with this observation, meat from birds that entered the abattoir from farm cycles that were deemed free of *C. jejuni* (i.e. before transport to the plant) subsequently became infested with a diversity of subtypes of the bacterium, indicating that there is a resident population of *C. jejuni* subtypes within the abattoir (Figure 3.11-3.14). Subtype diversity remained higher ($P<0.001$) on retail meat relative to that in farms. There was no difference ($P=0.432$) in subtype diversity of *C. jejuni* isolated from birds in the abattoir and from retail meat.

3.12 Contamination of birds during transport to the abattoir

In some instances, *C. jejuni* subtypes not observed within the farms were recovered from feathers of birds at the abattoir (Figure 3.12-3.13; red arrows), suggesting that these birds were infested during transport from the farm to the abattoir.

3.13 CRS associated with chickens

A number of *C. jejuni* subtypes associated with chickens were also recovered from diarrheic people in SWA during the study period (Figure 3.16; black arrows). Of the 176 subtypes of *C. jejuni* recovered from chickens, 29 (16.5%) were recovered from human beings during the study period (Figure 3.17). The primary subtypes of *C. jejuni* associated with chickens and diarrheic human beings in SWA during the study period are shown in Table 3.5. Notably, some *C. jejuni* subtypes isolated from diarrheic people in the current study had not previously been detected in chickens (i.e. 0092.001.004, 0735.001.002, 0811.012.002, and 0853.008.001), whereas other subtypes were commonly associated with chickens throughout Canada (i.e. 0933.004.002, 0957.001.001, and 0960.007.001).

An examination of all *C. jejuni* isolates within the C^3GFdb that were recovered from chickens and human beings since 2004 in SWA indicated that a 45.5% of the subtypes recovered from chickens were also observed in diarrheic people (Figure 3.18).

3.14 Beef cattle as reservoir of *C. jejuni* subtypes infecting poultry

A high prevalence of beef cattle shed *C. jejuni* in their feces over the 1.5 year sampling period (Figure 3.9). Considerable diversity of *C. jejuni* isolates were recovered from beef cattle housed in a confined feeding operation adjacent to Farm C (Figure 3.16). In several instances, subtypes recovered from cattle were also recovered from chickens, including from chickens in Farm C adjacent to the cattle confined

feeding operation sampled (Figure 3.16; white arrows). Of the subtypes of *C. jejuni* recovered from beef cattle and chickens, 24 (11.8%) were recovered from both livestock species during the study period (Figure 3.17). Three subtypes (i.e. 0238.007.002, 0735.001.002 and 0853.008.001 were associated with cattle and chicken samples within or associated with Farm C (Table 3.6). Isolates belonging to subtype 0735.001.002 were observed in the farm (bird cloaca, ceca, feathers, farm wall, and darkling beetles), at the abattoir, and from feedlot cattle during the same time period; this subtype is commonly associated with cattle in Alberta, it has been recovered from diarrheic people, but it has not previously been associated with chickens anywhere in Canada.

3.15 Tables and Figures

Table 3.1 *Campylobacter jejuni* subtype clusters linked to outbreaks, and associated with broilers and meat longitudinally sampled in Southwestern Alberta.

Subtype Cluster[a]	No of Subtypes (100%)	No of Isolates	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database (C^3GFdb) Information												
			CGF Subtype	Rank	No	Hu[b]	Ch[c]	Ca[d]	BC[e]	AB	SK	MB	ON	QC	AP
A3		12	0957.004.007	453	5	0	0	5	0	1	0	0	0	3	0
A3		36	0957.004.002	173	17	0	0	14	3	0	0	0	6	0	6
A3		1	0957.004.011	3121	1	0	0	1	0	0	0	0	0	0	0
A3		4	0957.004.001	70	53	2	0	35	3	3	0	0	24	10	4
A3		10	0957.001.001	7	320	20	23	134	40	46	0	0	191	13	5
A3		1	0631.002.005	1160	2	0	0	1	1	0	0	0	1	0	0
A3		28	Novel (n=15)	--	--	--	--	--	--	--	--	--	--	--	--
Total	21	92	--	--	398	5.5%	5.8%	47.7%	12.9%	13.7%	0.0%	0.0%	61.0%	7.1%	4.1%
B1/C1/R4b		70	0817.001.008	1160	2	0	0	1	0	1	0	0	1	0	0
B1/C1/R4b		109	0817.001.004	87	41	0	2	4	2	18	0	1	15	2	0
B1/C1/R4b		1	0817.001.002	3121	1	0	0	0	0	1	0	0	0	0	0
B1/C1/R4b		22	0817.006.002	3121	1	0	0	0	0	0	0	0	1	0	0
B1/C1/R4b		2	0817.001.003	1160	2	1	0	0	0	2	0	0	0	0	0
B1/C1/R4b		33	Novel (n=18)	--	--	--	--	--	--	--	--	--	--	--	--
Total	23	237	--	--	47	2.1%	4.3%	10.6%	4.5%	50.0%	0.0%	2.3%	38.6%	4.5%	0.0%
R4a		8	0609.006.004	21	157	17	118	10	2	73	0	0	76	1	0
R4a		7	0611.001.002	159	19	1	10	6	0	1	0	0	18	0	0
R4a		1	0609.008.001	751	3	2	1	0	0	3	0	0	0	0	0
R4a		3	0609.013.002	54	71	4	61	5	0	62	0	0	7	0	0
R4a		2	0609.004.002	118	30	2	27	1	0	29	0	0	1	0	0
R4a		1	0609.003.002	229	12	2	4	6	0	7	0	0	0	0	0
R4a		2	Novel (n=2)	--	--	--	--	--	--	--	--	--	--	--	--
Total	8	24	--	--	292	9.6%	75.7%	9.6%	0.7%	62.5%	0.0%	0.0%	36.4%	0.4%	0.0%
R6		2	0960.009.001	554	4	0	0	4	0	0	0	0	4	0	0
R6		19	0960.007.001	14	210	34	18	117	15	40	1	1	101	24	1
R6		1	0960.008.001	3121	1	0	0	0	0	1	0	0	0	0	0
R6		5	Novel (n=3)	--	--	--	--	--	--	--	--	--	--	--	--
Total	6	27	--	--	215	15.8%	8.4%	56.3%	7.8%	21.2%	0.5%	0.5%	54.4%	12.4%	0.5%
C7a		8	0735.001.001	3121	1	1	0	0	0	1	0	0	0	0	0
C7a		135	0735.001.002	48	76	4	65	0	0	71	0	0	5	0	0
C7a		9	0731.004.004	179	16	1	8	0	0	16	0	0	0	0	0
C7a		1	0731.001.005	13	212	30	133	37	0	108	0	0	100	0	0
C7a		37	0735.003.004	453	5	0	5	0	0	5	0	0	0	0	0
C7a		1	0735.009.002	3121	1	0	0	0	0	1	0	0	0	0	0
C7a		26	Novel (n=12)	--	--	--	--	--	--	--	--	--	--	--	--
Total	18	217	--	--	311	11.6%	67.8%	11.9%	0.0%	65.8%	0.0%	0.0%	34.2%	0.0%	0.0%
C7b		36	Novel (n=7)	--	--	--	--	--	--	--	--	--	--	--	--
C7b		0	0901.001.002[f]	3121	1	0	0	0	0	1	0	0	0	0	0
Total	7	36	--	--	--	--	--	--	--	--	--	--	--	--	--
C7c		16	Novel (n=16)	--	--	--	--	--	--	--	--	--	--	--	--
C7c		0	0731.006.002[f]	751	3	2	1	0	0	2	0	0	1	0	0
C7c		0	0735.003.004[f]	453	5	0	5	0	0	5	0	0	0	0	0
Total	5	16	--	--	8	25.0%	75.0%	0.0%	0.0%	87.5%	0.0%	0.0%	12.5%	0.0%	0.0%
Total	88	649	--	--	1264	121	475	381	66	491	1	2	551	53	16

[a]Nomenclature corresponds to clusters shown in Figures 12-14 (≥37/40 loci; ≥92.5%). [b]Human beings. [c]Chickens. [d]Cattle. [e]Canadian provinces, where BC is British Columbia, AB is Alberta, SK is Saskatchewan, MB is Manitoba, ON is Ontario, QC is Quebec, and AP is Atlantic Provinces (i.e. New Brunswick, Prince Edward Island, Nova Scotia, and Newfoundland and Labrador). [f]Closest CGF subtype (39/40 loci; 97.5%).

Table 3.2 *Campylobacter jejuni* subtype clusters not linked to outbreaks, and associated with broilers and meat longitudinally sampled in Farm A.

Isolate	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database (C^3GFdb) Information													
	CGF Subtype	Similarity	Rank	No	Hu[a]	Ch[b]	Ca[c]	BC[d]	AB	SK	MB	ON	QC	AP
A234	Novel	≤0.900	--	--	--	--	--	--	--	--	--	--	--	--
A285	Novel	≤0.625	--	--	--	--	--	--	--	--	--	--	--	--
A495	0957.004.011	0.950	3121	1	0	0	1	0	0	0	0	0	0	0
	0957.004.008	0.950	3121	1	0	0	1	0	0	0	0	0	1	0
A497	0821.001.002	0.950	388	6	0	0	0	0	6	0	0	0	0	0
	0812.002.003	0.950	3121	1	0	0	0	0	1	0	0	0	0	0
A561	0954.001.002	0.925	3121	1	0	0	0	1	0	0	0	0	0	0
A844	891.001.001	1.000	43	81	15	12	23	3	6	0	0	55	10	5
A848	Novel	≤0.825	--	--	--	--	--	--	--	--	--	--	--	--
A1239	0811.009.002	0.975	19	177	8	0	13	19	80	0	0	68	6	0
	0811.003.004	0.975	751	3	0	0	0	1	0	0	0	2	0	0
	0811.002.003	0.975	1160	2	0	0	0	0	2	0	0	0	0	0
	0812.002.006	0.975	3121	1	0	0	1	0	0	0	0	0	0	0
	0827.001.001	0.975	3121	1	0	0	0	0	0	0	0	1	0	0
A1243	0773.001.001	0.950	3121	1	0	0	0	0	1	0	0	0	0	0
A1856	0196.002.002	0.975	751	3	3	0	0	0	3	0	0	0	0	0

[a]Human beings. [b]Chickens. [c]Cattle. [d]Canadian provinces, where BC is British Columbia, AB is Alberta, SK is Saskatchewan, MB is Manitoba, ON is Ontario, QC is Quebec, and AP is Atlantic Provinces (i.e. New Brunswick, Prince Edward Island, Nova Scotia, and Newfoundland and Labrador).

Table 3.3 *Campylobacter jejuni* subtype clusters not linked to outbreaks, and associated with broilers and meat longitudinally sampled in Farm B.

Isolate	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database (C^3GFdb) Information													
	CGF Subtype	Similarity	Rank	No	Hu[a]	Ch[b]	Ca[c]	BC[d]	AB	SK	MB	ON	QC	AP
B8	0810.001.002	0.950	3121	1	0	0	0	1	0	0	0	0	0	0
B11	0061.001.007	0.975	3121	1	1	0	0	1	0	0	0	0	0	0
B14	Novel	≤0.875	--	--	--	--	--	--	--	--	--	--	--	--
B19	0824.003.001	0.950	3121	1	0	0	0	0	1	0	0	0	0	0
B619	Novel	≤0.875	--	--	--	--	--	--	--	--	--	--	--	--
B743	0824.001.002	0.950	751	3	0	0	0	0	0	0	0	0	3	0
	0824.001.004	0.950	3121	1	0	0	1	0	0	0	0	0	0	1
B1501	Novel	≤0.850	--	--	--	--	--	--	--	--	--	--	--	--
B1534	0811.008.001	1.000	43	81	1	0	6	4	30	0	0	35	8	1
B1538	0333.001.001	0.925	3121	1	1	0	0	0	1	0	0	0	0	0
B1545	0811.009.002	1.000	19	177	8	0	13	19	80	0	0	68	6	0
B1562	0811.008.001	0.950	43	81	1	0	6	4	30	0	0	35	8	1
	0810.001.001	0.950	3121	1	0	0	0	1	0	0	0	0	0	0
B1564	0960.007.006	0.975	3121	1	0	1	0	0	0	0	0	1	0	0
	0960.007.019	0.975	3121	1	1	0	0	0	1	0	0	0	0	0
	0960.007.001	0.950	14	210	34	18	117	15	40	1	1	101	24	1
B1570	0960.007.001	1.000	14	210	34	18	117	15	40	1	1	101	24	1
B1592	Novel	≤0.850	--	--	--	--	--	--	--	--	--	--	--	--
B1594	0478.001.001	0.950	3121	1	0	1	0	0	1	0	0	0	0	0
B1811	0044.005.002	0.950	127	28	28	0	0	0	28	0	0	0	0	0
	0044.003.008	0.950	554	4	4	0	0	0	4	0	0	0	0	0
	0044.003.002	0.950	554	4	1	1	2	0	1	0	0	3	0	0
	0063.001.001	0.950	3121	1	1	0	0	0	1	0	0	0	0	0

[a]Human beings. [b]Chickens. [c]Cattle. [d]Canadian provinces, where BC is British Columbia, AB is Alberta, SK is Saskatchewan, MB is Manitoba, ON is Ontario, QC is Quebec, and AP is Atlantic Provinces (i.e. New Brunswick, Prince Edward Island, Nova Scotia, and Newfoundland and Labrador).

Table 3.4 *Campylobacter jejuni* subtype clusters not linked to outbreaks, and associated with broilers and meat longitudinally sampled in Farm C.

Isolate	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database (C^3GFdb) Information													
	CGF Subtype	Similarity	Rank	No	Hu[a]	Ch[b]	Ca[c]	BC[d]	AB	SK	MB	ON	QC	AP
C953	0817.001.004	1.000	87	41	0	2	4	2	18	0	1	15	2	0
C1174	Novel	≤0.900	--	--	--	--	--	--	--	--	--	--	--	--
C1202	0082.001.001	0.975	33	97	30	0	60	1	3	0	0	63	16	0
	0044.003.002	0.975	554	4	1	1	2	0	1	0	0	3	0	0
	0082.002.003	0.975	1160	2	2	0	0	0	1	0	0	1	0	0
C1210	0061.002.001	0.925	261	10	1	0	9	0	1	0	0	6	3	0
C2141	0260.005.003	1.000	751	3	0	2	1	0	2	0	0	1	0	0
C2148	0238.010.006	0.975	3121	1	0	1	0	0	1	0	0	0	0	0
	0238.010.004	0.975	3121	1	0	1	0	0	1	0	0	0	0	0
C2673	Novel	≤0.850	--	--	--	--	--	--	--	--	--	--	--	--
C3665	0253.004.001	1.000	15	208	48	0	150	7	16	0	1	113	25	11
C4505	0853.008.001	1.000	216	13	4	6	0	1	7	0	0	0	0	0
C4606	0853.015.003	0.975	1160	2	0	2	0	0	2	0	0	0	0	0
C5761	0695.017.001	0.925	245	11	1	8	0	0	11	0	0	0	0	0
C6085	0238.007.002	0.975	55	70	14	46	6	1	47	0	0	16	3	0
	0238.002.001	0.975	751	3	1	2	0	0	3	0	0	0	0	0
	0238.009.001	0.975	3121	1	0	1	0	0	1	0	0	0	0	0
	0238.008.002	0.975	3121	1	1	0	0	0	1	0	0	0	0	0
C6937	Novel	≤0.900	--	--	--	--	--	--	--	--	--	--	--	--
C7319	0437.001.001	0.950	3121	1	0	1	0	0	1	0	0	0	0	0
C7403	0670.001.002	0.975	3121	1	0	1	0	0	1	0	0	0	0	0
	0670.001.001	0.975	3121	1	0	1	0	0	1	0	0	0	0	0
C8335	0735.003.004	1.000	453	5	0	5	0	0	5	0	0	0	0	0
C8775	0092.001.004	1.000	173	17	6	4	0	0	17	0	0	0	0	0

[a]Human beings. [b]Chickens. [c]Cattle. [d]Canadian provinces, where BC is British Columbia, AB is Alberta, SK is Saskatchewan, MB is Manitoba, ON is Ontario, QC is Quebec, and AP is Atlantic Provinces (i.e. New Brunswick, Prince Edward Island, Nova Scotia, and Newfoundland and Labrador).

Table 3.5 Primary *Campylobacter jejuni* subtype clusters (100% level of resolution) associated with chickens and diarrheic human beings in Southwestern Alberta during the study period.

Clade	Number	Canadian *Campylobacter* Comparative Genomic Fingerprinting Database (C^3GFdb) Information											
		CGF Subtype	Rank	No	Hu[a]	Ch[b]	Ca[c]	BC[d]	AB	SK	MB	ON	QC	AP
26	11	0957.001.001	7	320	20	134	23	40	46	0	0	191	13	5
49	3	0933.004.002	35	94	24	45	11	15	25	0	0	46	0	3
55	4	0933.007.005	245	11	1	10	0	3	2	0	0	2	2	1
83	20	0960.007.001	14	210	34	117	18	15	40	1	1	101	24	1
216	5	0811.012.002	554	4	1	0	0	0	1	0	0	3	0	0
241	12	0853.008.001	216	13	4	0	6	1	7	0	0	0	0	0
321	136	0735.001.002	48	76	4	0	65	0	71	0	0	5	0	0
405	14	0695.006.001	2	612	86	22	454	2	490	0	0	103	9	0
432	3	0982.007.003	1160	2	0	1	1	0	1	0	0	0	0	0
441	2	0982.007.002	129	27	3	2	22	2	25	0	0	0	0	0
523	10	0609.006.004	21	157	17	10	118	2	73	0	0	76	1	0
526	4	0609.013.002	54	71	4	5	61	0	62	0	0	7	0	0
533	2	Novel												
534	2	Novel												
605	15	0092.001.004	173	17	6	0	4	0	17	0	0	0	0	0
683	9	0061.001.002	78	47	7	15	23	2	23	0	0	17	3	0
804	52	0238.007.002	55	70	14	6	46	1	47	0	0	16	3	0
849	18	0269.004.001	23	144	53	25	66	2	84	0	0	53	0	0

[a]Human beings. [b]Chickens. [c]Cattle. [d]Canadian provinces, where BC is British Columbia, AB is Alberta, SK is Saskatchewan, MB is Manitoba, ON is Ontario, QC is Quebec, and AP is Atlantic Provinces (i.e. New Brunswick, Prince Edward Island, Nova Scotia, and Newfoundland and Labrador).

Table 3.6 *Campylobacter jejuni* subtype clusters observed in chickens and abattoir (Farm C), an adjacent beef cattle confined feeding operation, and diarrheic human beings in Southwestern Alberta.

Study (Number of Isolates, and Isolation Cycle and Week)						C³GFdb Information						
Subtype	Barn C	Abattoir C	Cattle C	Human	Total	CGF Subtype	Rank	No	Hu[a]	Ch[b]	Ca[c]	AB[d]
A	1 (C6W1)	1 (C5)	6 (C1W5) (C7W1-2)	4	12	0853.008.001	216	13	4	0	6	7
B	87 (C7W4-5)	43 (C7)	1 (C7W4)	0	131	0735.001.002	48	76	4	0	65	71
C	1 (C6W6)	3 (C1,C6,C7)	43 (C1W3-5) (C2W3) (C7W1-5)	4	51	0238.007.002	55	70	14	6	46	47

[a]Human beings. [b]Chickens. [c]Cattle. [d]Alberta.

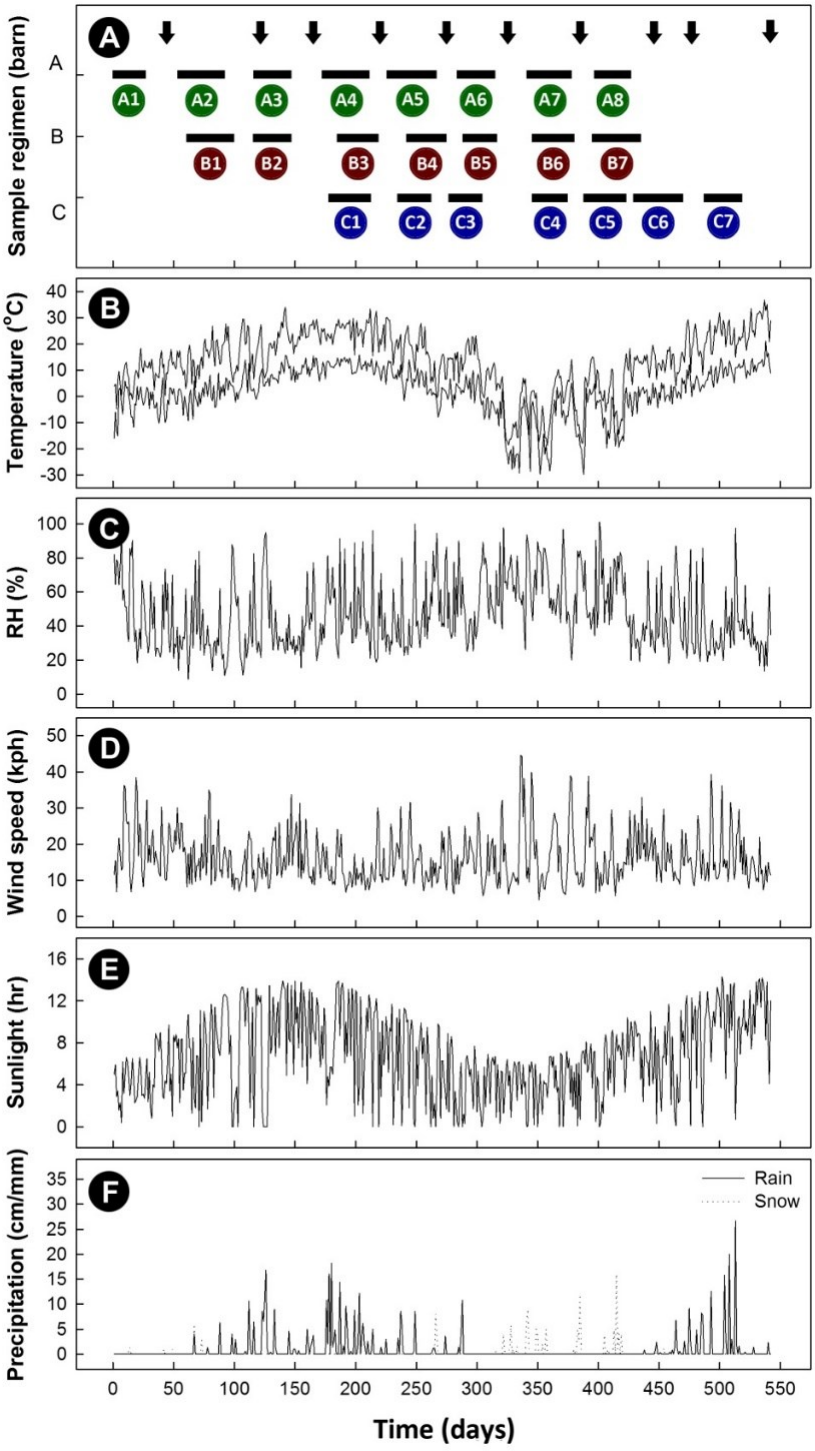

Figure 3.1 Temporal sampling regimen and weather parameters recorded at Lethbridge Research and Development Centre during the 542-day duration of the study, where day 1 is January 18 2017. (A) Sampling regimen, where A, B, and C within circles refer to the three broiler farms sampled and numbers to the production cycles, and arrows show retail sampling times. (B) Daily minimum and maximum air temperatures (°C). (C) Daily average relative humidity (%). (D) Mean daily wind speed (kph). (E) Hours of sunlight. (F) Precipitation, where solid lines represent rain and dotted lines snow; rain is in mm and snowfall amounts are in cm.

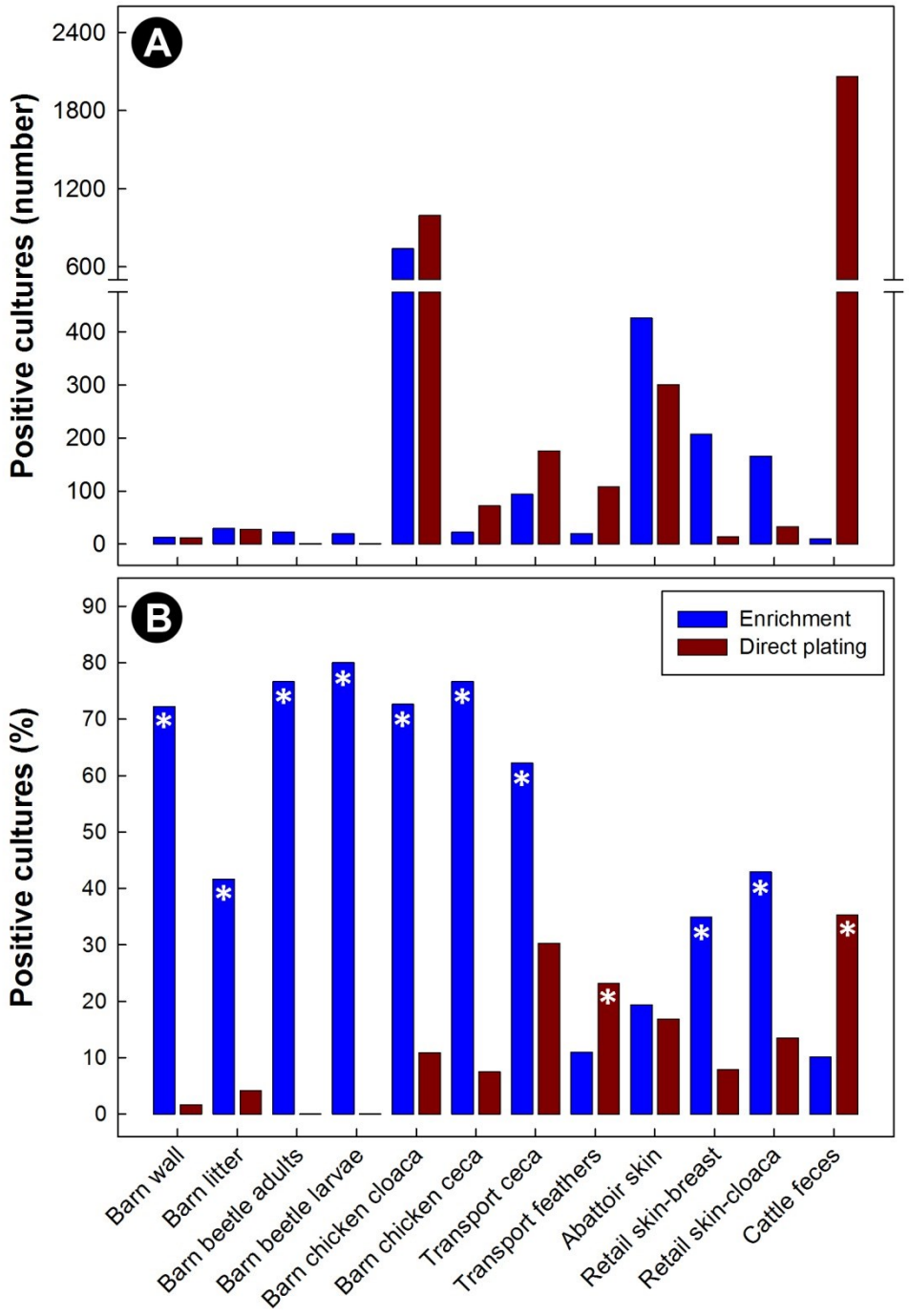

Figure 3.2 Enrichment and direct plating cultures positive for *Campylobacter jejuni*. (A) Total number of positive cultures. (B) Prevalence of positive cultures (%). Bars indicated with an asterisk represent a higher prevalence of isolation (P≤0.036) by sample type.

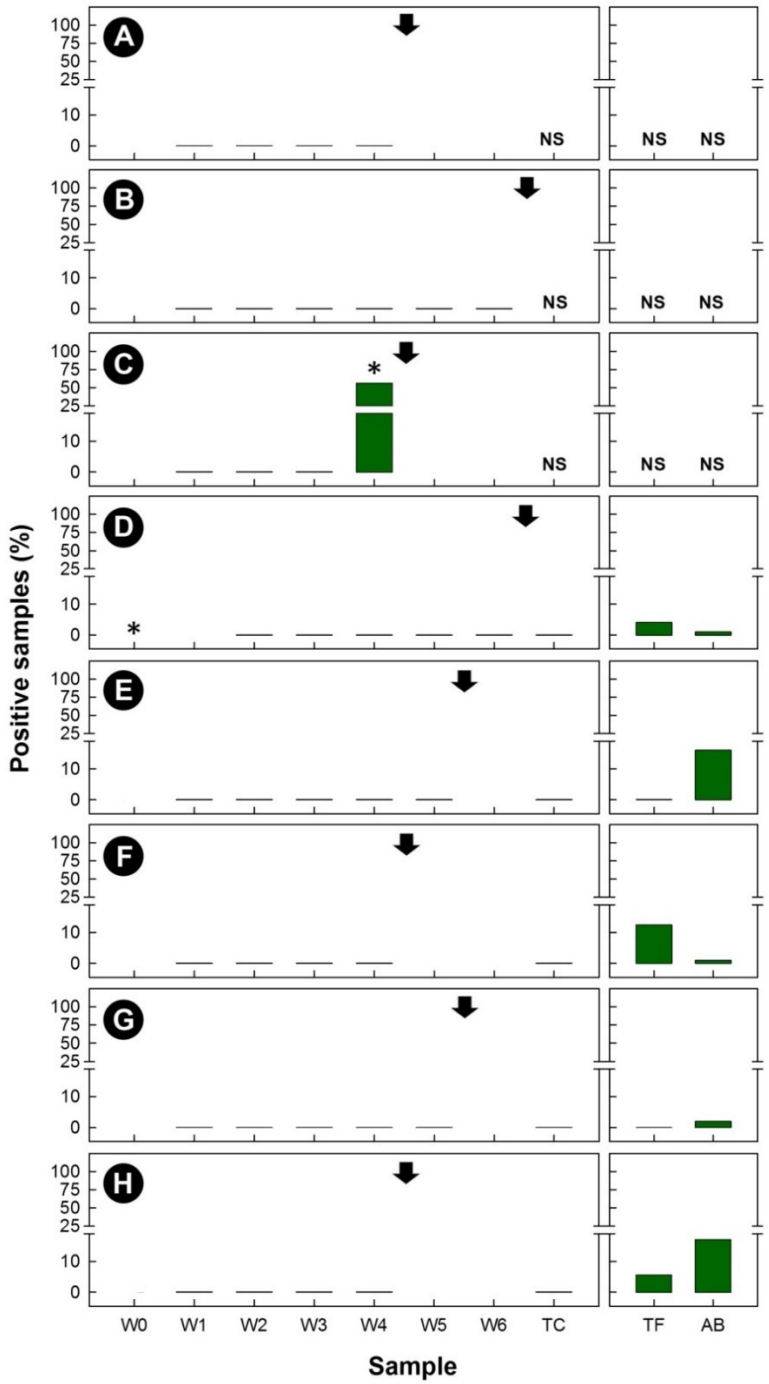

Figure 3.3 Samples positive for *Campylobacter jejuni* (%) in Farm A over a *ca.* 1.5 year period. (A) Cycle 1. (B) Cycle 2. (C) Cycle 3. (D) Cycle 4. (E) Cycle 5. (F) Cycle 6. (G) Cycle 7. (H) Cycle 8. W0 to W6 is week 0 to week 6, TC is transport ceca, TF is transport feather, AB is abattoir skin, and NS is not sampled. Arrows indicate the when birds were shipped to the abattoir. Asterisks indicate points where non-cloacal samples from birds were positive for *C. jejuni*. See Figure 3.1 for sampling times.

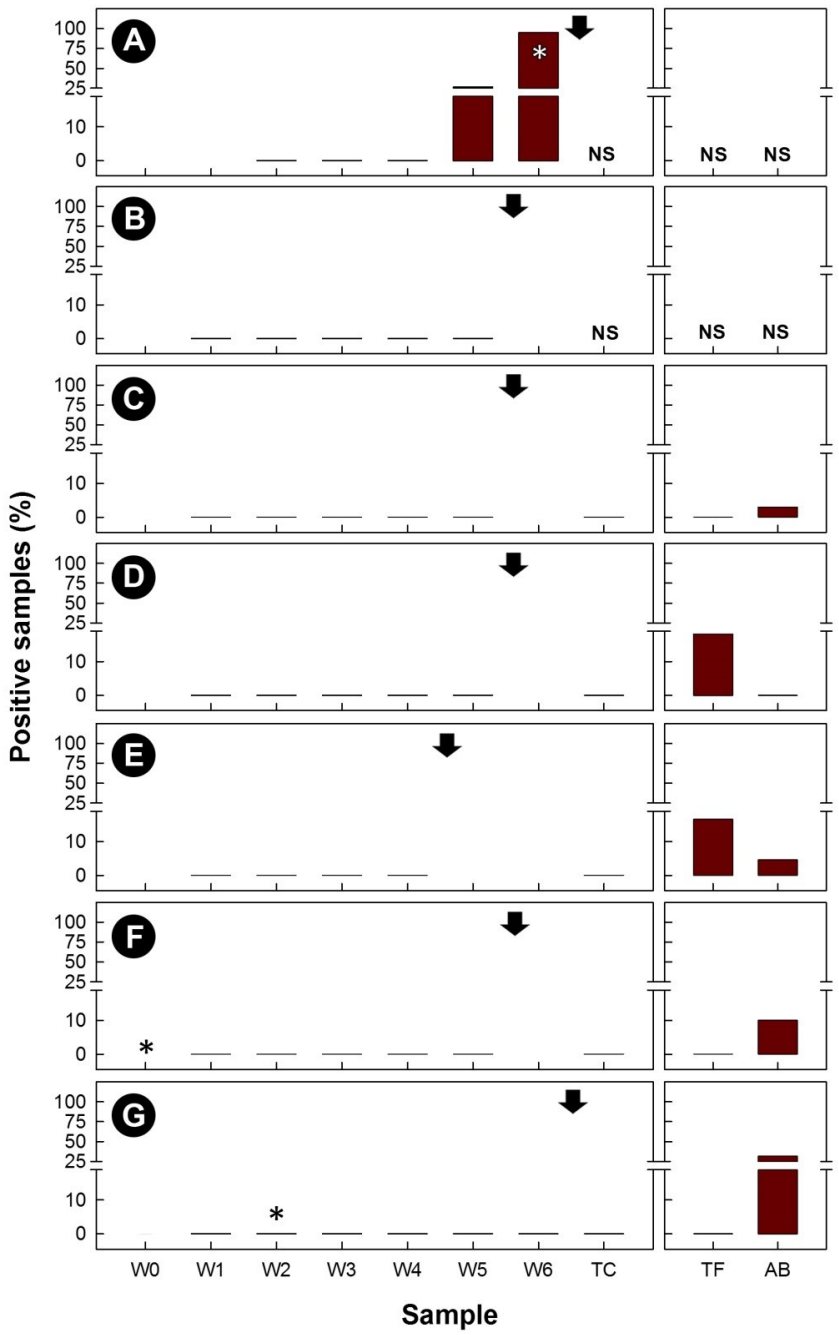

Figure 3.4 Samples positive for *Campylobacter jejuni* (%) in Farm B over a *ca.* 1.5 year period. (A) Cycle 1. (B) Cycle 2. (C) Cycle 3. (D) Cycle 4. (E) Cycle 5. (F) Cycle 6. (G) Cycle 7. W0 to W6 is week 0 to week 6, TC is transport ceca, TF is transport feather, AB is abattoir skin, and NS is not sampled. Arrows indicate the when birds were shipped to the abattoir. Asterisks indicate points where non-cloacal samples from birds were positive for *C. jejuni*. See Figure 3.1 for sampling times.

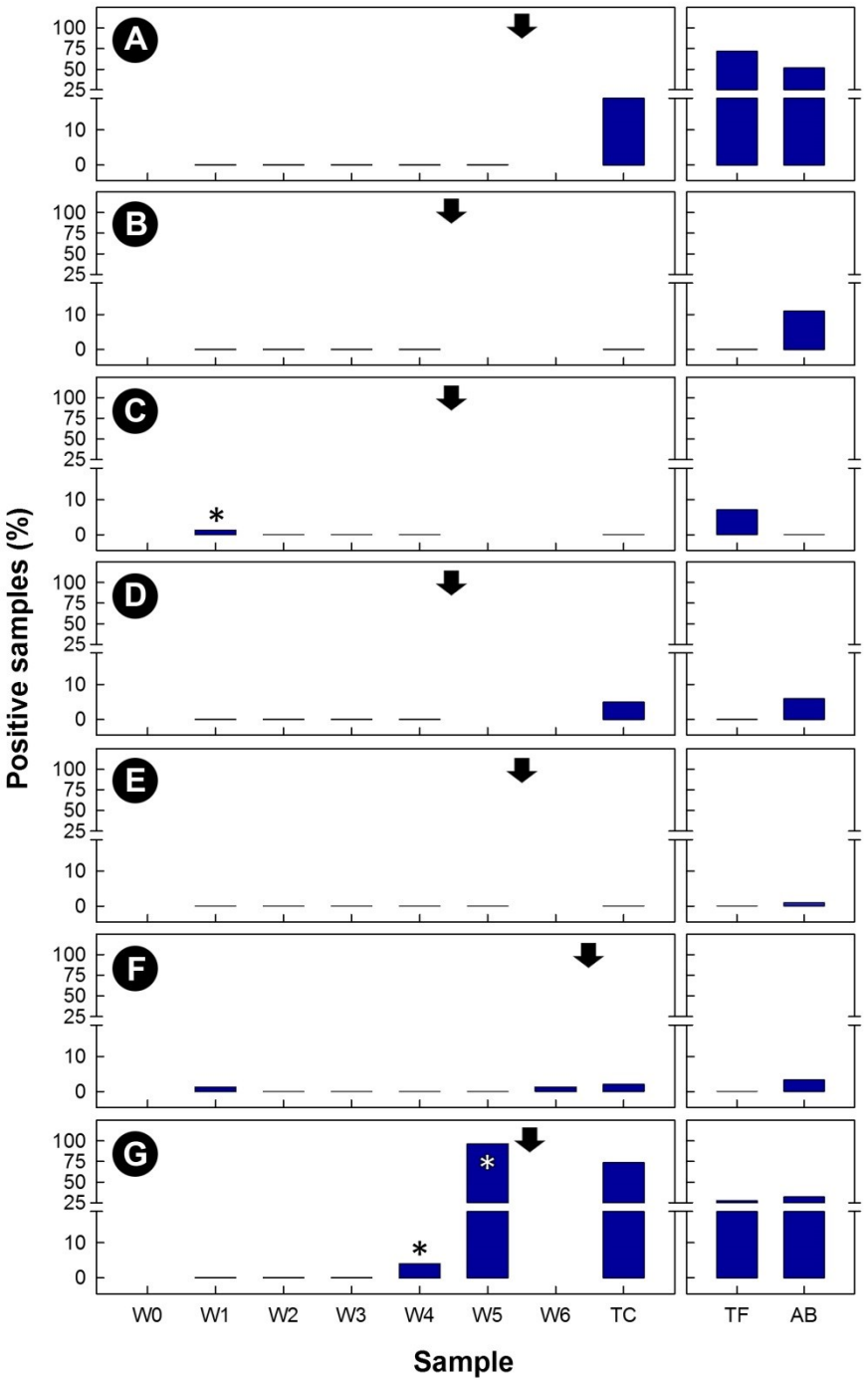

Figure 3.5 Samples positive for *Campylobacter jejuni* (%) in Farm C over a *ca.* 1.5 year period. (A) Cycle 1. (B) Cycle 2. (C) Cycle 3. (D) Cycle 4. (E) Cycle 5. (F) Cycle 6. (G) Cycle 7. W0 to W6 is week 0 to week 6, TC is transport ceca, TF is transport feather, and AB is abattoir skin. Arrows indicate the when birds were shipped to the abattoir. Asterisks indicate points where non-cloacal samples from birds were positive for *C. jejuni*. See Figure 3.1 for sampling times.

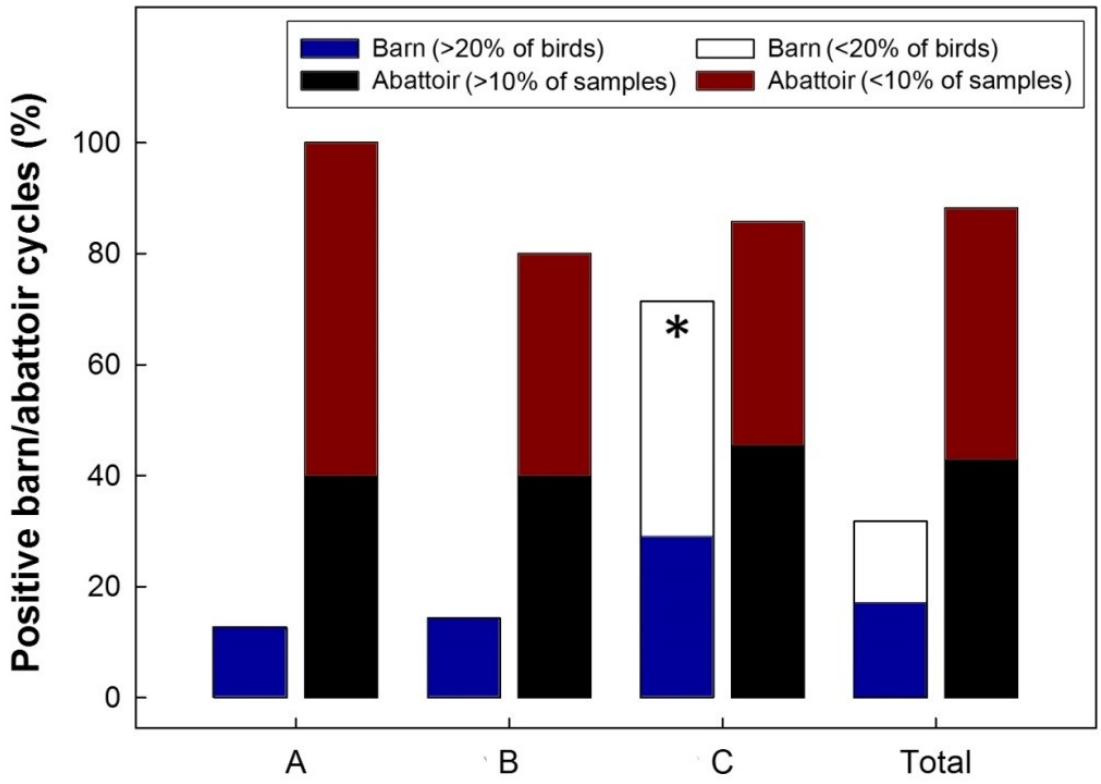

Figure 3.6 Overall prevalence of farm/abattoir cycles positive for *Campylobacter jejuni* (%) in/from Farm A, B, and C. Instances where greater than 20% of birds in farms and greater than 10 of samples in abattoirs are indicated within the graph. The histogram bar indicated with the asterisk indicates that the prevalence of samples positive for *C. jejuni* at Farm C was higher than for Farms A and B (P=0.051).

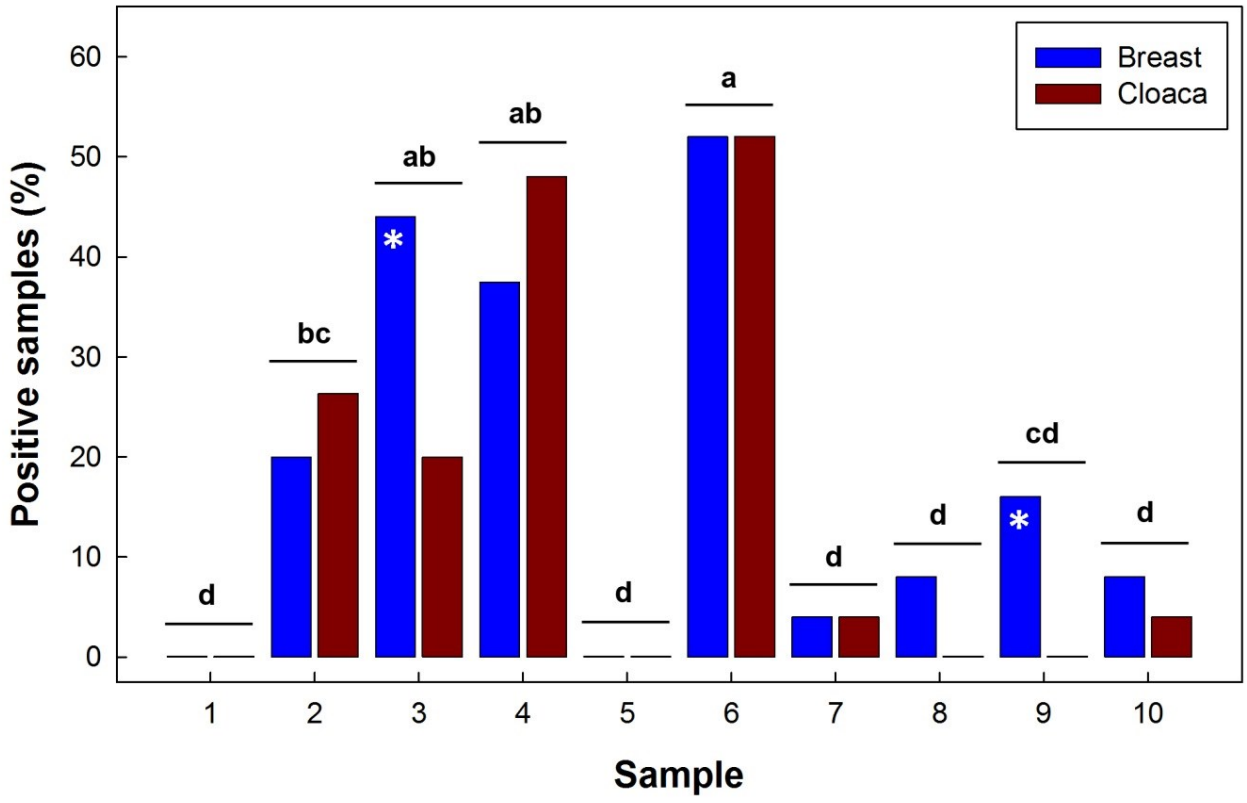

Figure 3.7 Prevalence of retail samples positive for *Campylobacter jejuni* (%) at ten times over the 542-day duration of the study. Asterisks indicate instances where the prevalence of samples positive for *Campylobacter jejuni* differed (P<0.050) between skin samples from breasts versus the cloacal region at individual sample times. Histograms at individual sample times not followed by the same letter differ (P≤0.050). See Figure 2 for sampling times.

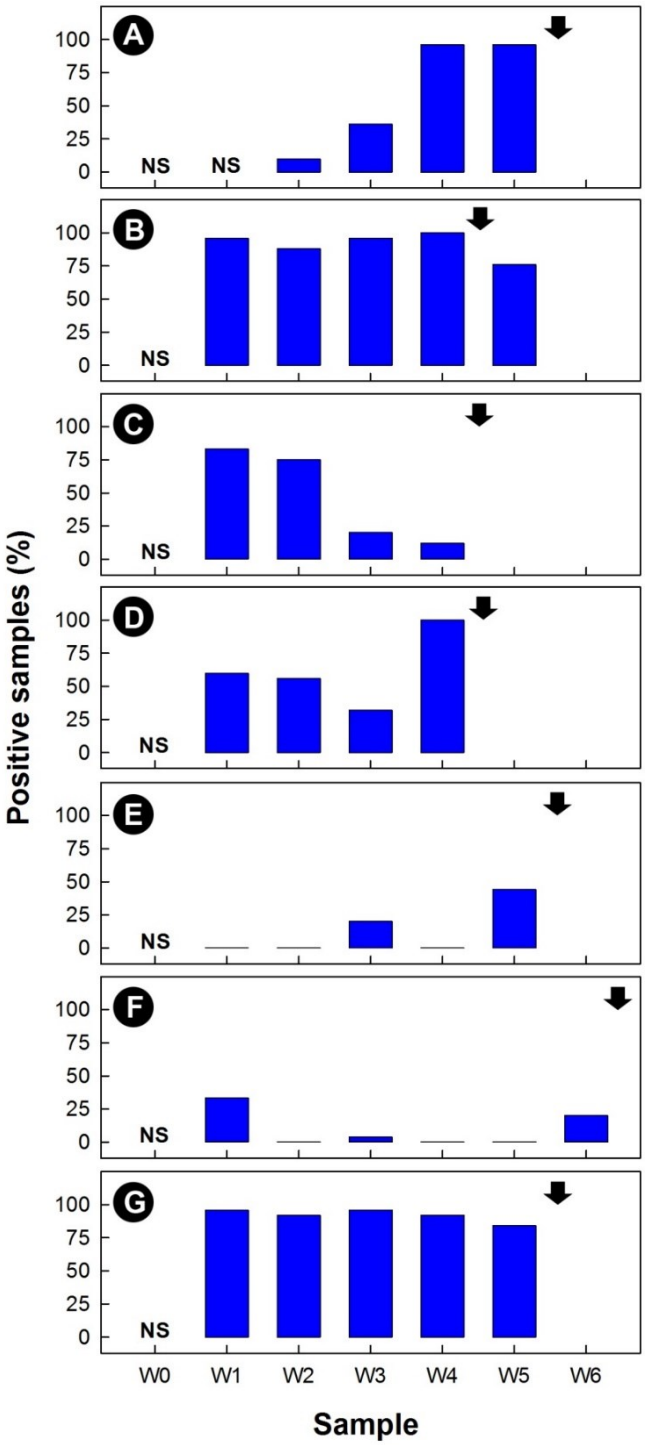

Figure 3.8 Feces from beef cattle positive for *Campylobacter jejuni* (%). Cattle were housed in a confined feeding operation adjacent to Farm C, and sampling corresponded to the poultry farm. (A) Cycle 1. (B) Cycle 2. (C) Cycle 3. (D) Cycle 4. (E) Cycle 5. (F) Cycle 6. (G) Cycle 7. W0 to W6 is week 0 to week 6, and NS is not sampled. Arrows indicate the when birds from Farm C were shipped to the abattoir. See Figure 3.1 for sampling times.

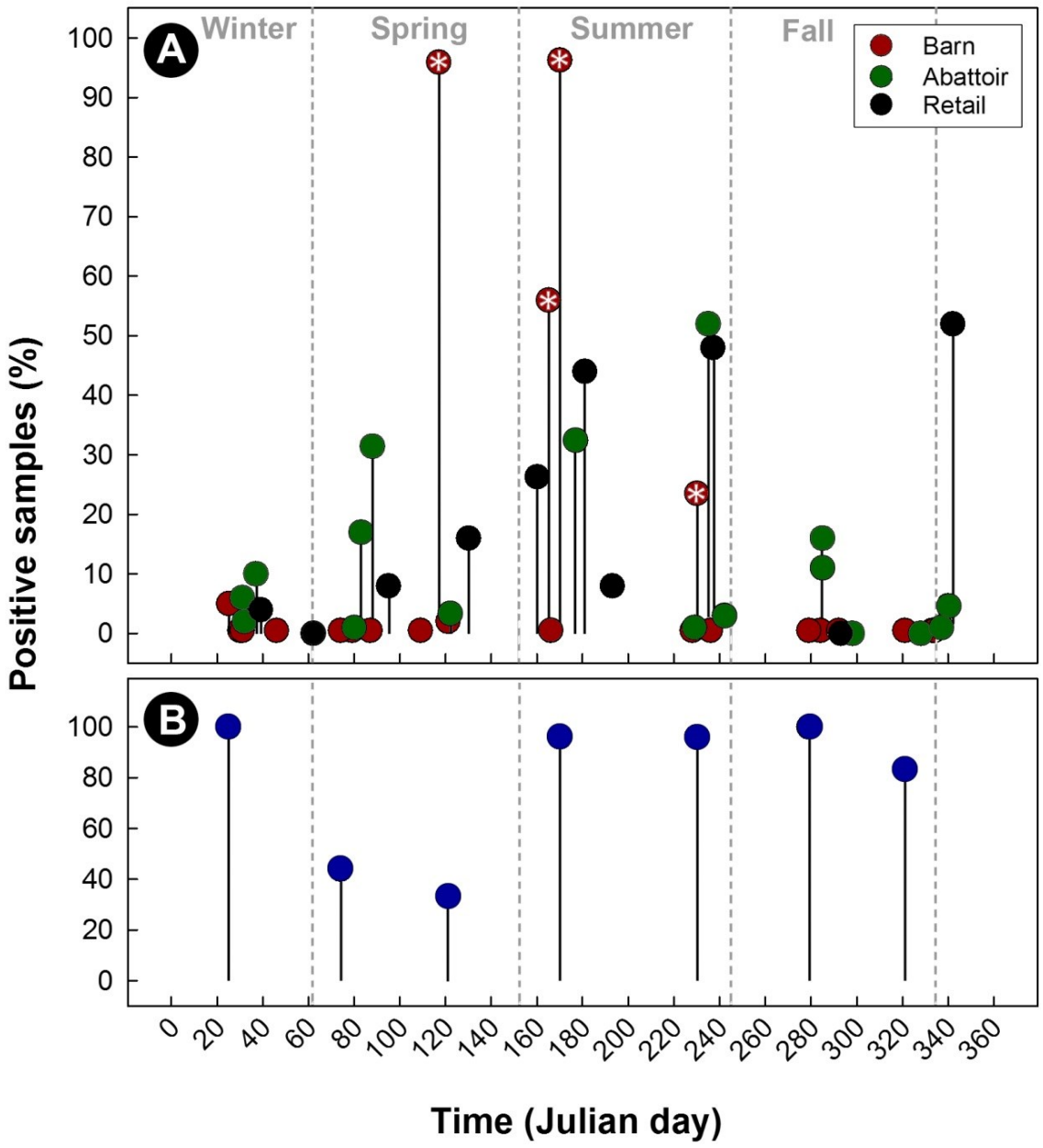

Figure 3.9 Seasonal prevalence of samples positive for *Campylobacter jejuni* (%). (A) Poultry samples in poultry farms, the abattoir at which the birds were processed, and at retail. (B) Beef cattle feces. Markers with asterisks indicate outbreaks of *C. jejuni* within poultry farms (i.e. >20% of birds infected with the bacterium). Outbreaks were detected in four of the 22 cycles examined (18.2%). Julian day 1 is January 1. Winter: December 1st to Feburary 28th, Spring: March 1st to May 30th, Summer: June 1st to August 31st, Fall: September 1st to November 30th.

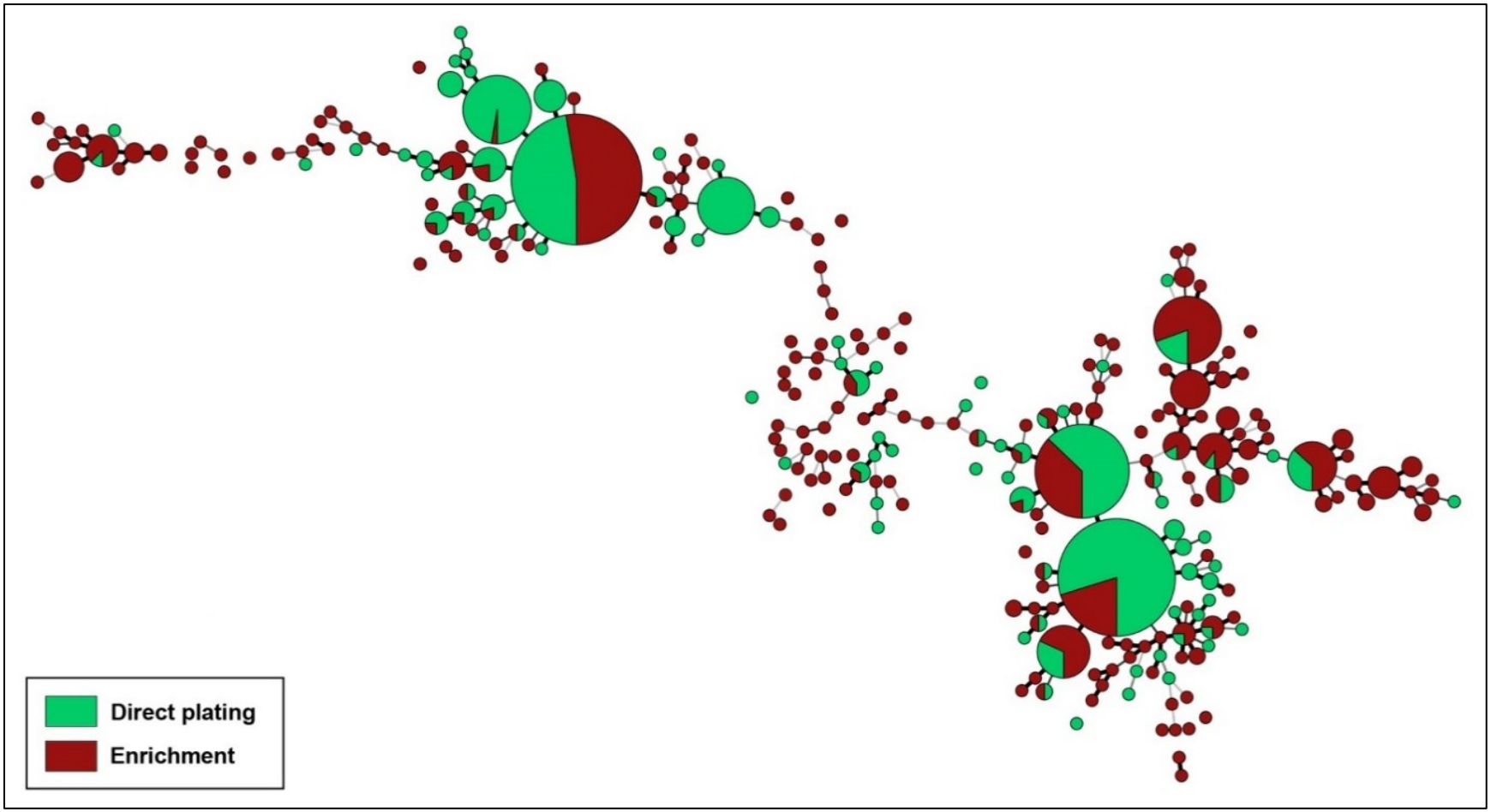

Figure 3.10 *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from poultry samples by direct plating and non-selective enrichment. The minimum spanning tree was generated in Bionumerics (version 6.6, Applied Maths), and samples were combined across sample type. The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes.

Figure 3.11

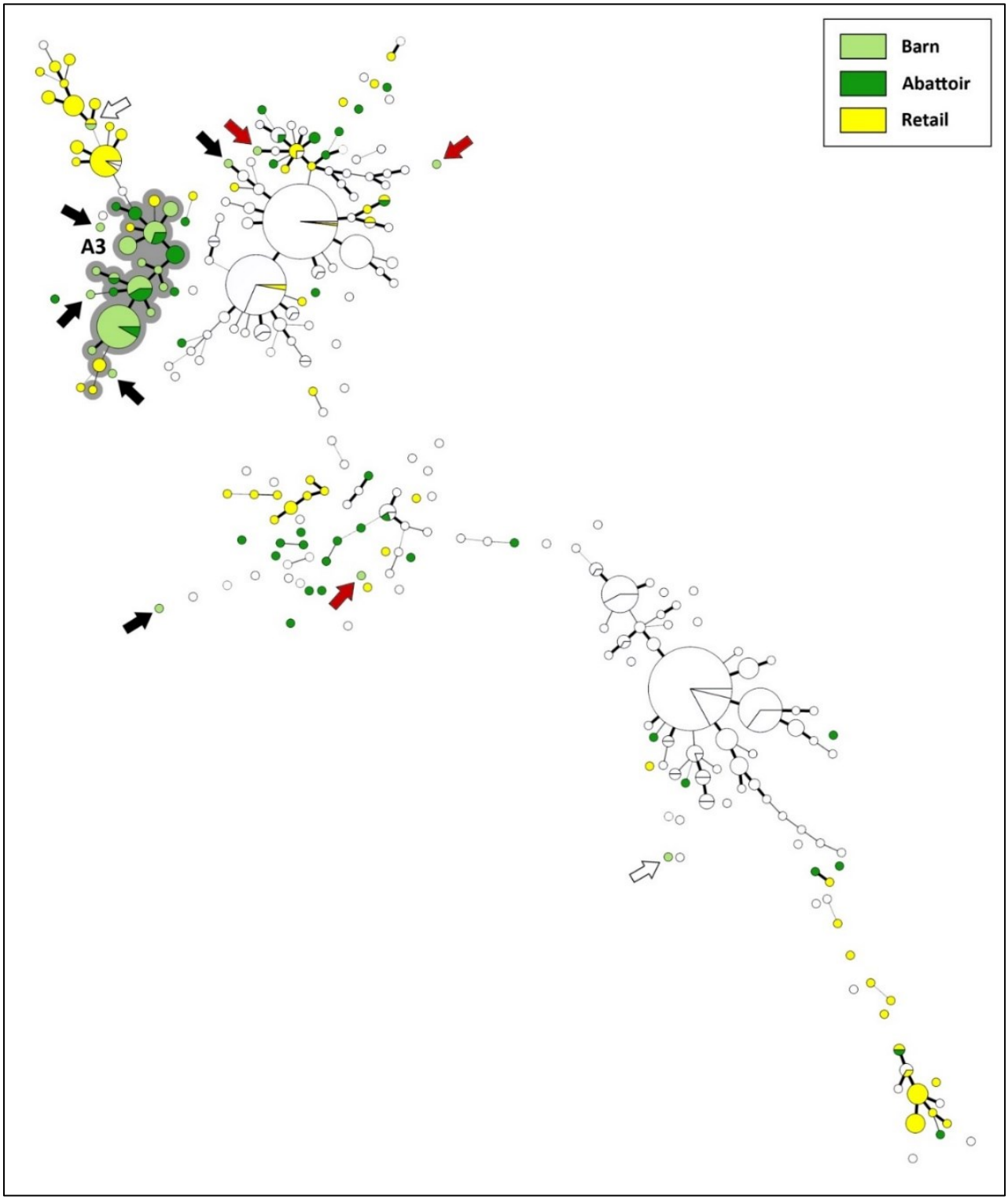

Figure 3.11 *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from poultry samples from Farm A, the abattoir at which the birds were processed, and retail poultry during the sample period. The minimum spanning tree was generated in Bionumerics (version 6.6, Applied Maths). The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes. The grey highlighted clusters marked A3 shows an outbreak within the farm during cycle 3 (≥92.5% similarity). Black arrows show isolates from the outbreaks in farms that differed from outbreak subtypes, red arrows show isolates that were likely obtained during transport of the birds to the abattoir, and the white arrow shows isolates that were detected in farms for which an outbreak did not occur.

Figure 3.12

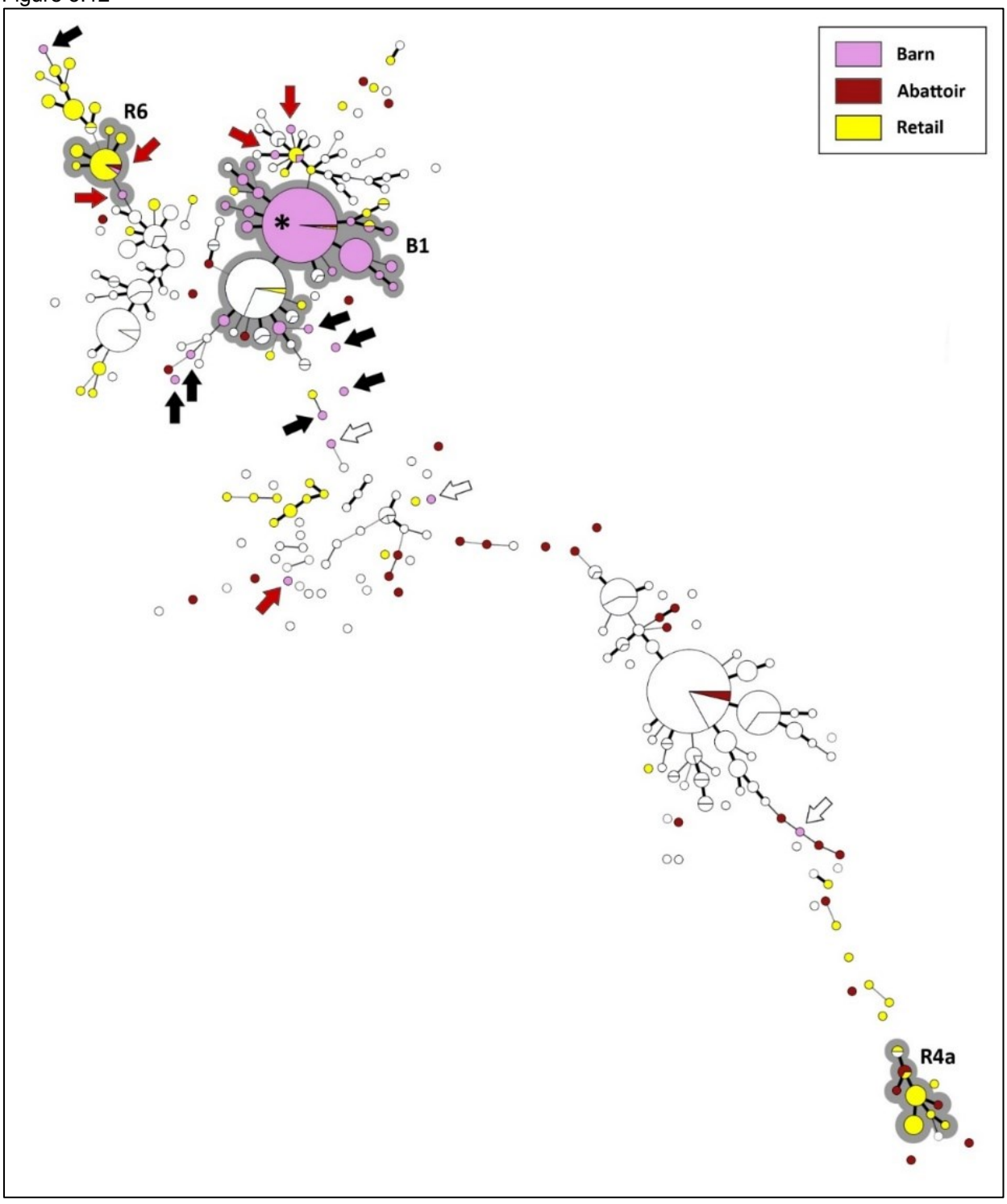

Figure 3.12 *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from poultry samples from Farm B, the abattoir at which the birds were processed, and retail poultry during the sample period. The minimum spanning tree was generated in Bionumerics (version 6.6, Applied Maths). The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes. The grey highlighted isolate cluster marked B1 shows an outbreak within the farm during cycle 1 (≥92.5% similarity). Grey highlighted clusters marked R4 and R6 show instances where the same subtype was obtained from abattoir and corresponding retail samples (≥92.5%). Black arrows show isolates from the outbreaks in farms that differed from outbreak subtypes, red arrows show isolates that were likely obtained during transport of the birds to the abattoir, white arrows show isolates that were detected in farms for which an outbreak did not occur, the asterisk shows that the same subtype responsible for the outbreak was recovered from skin samples from the birds within the abattoir.

Figure 3.13

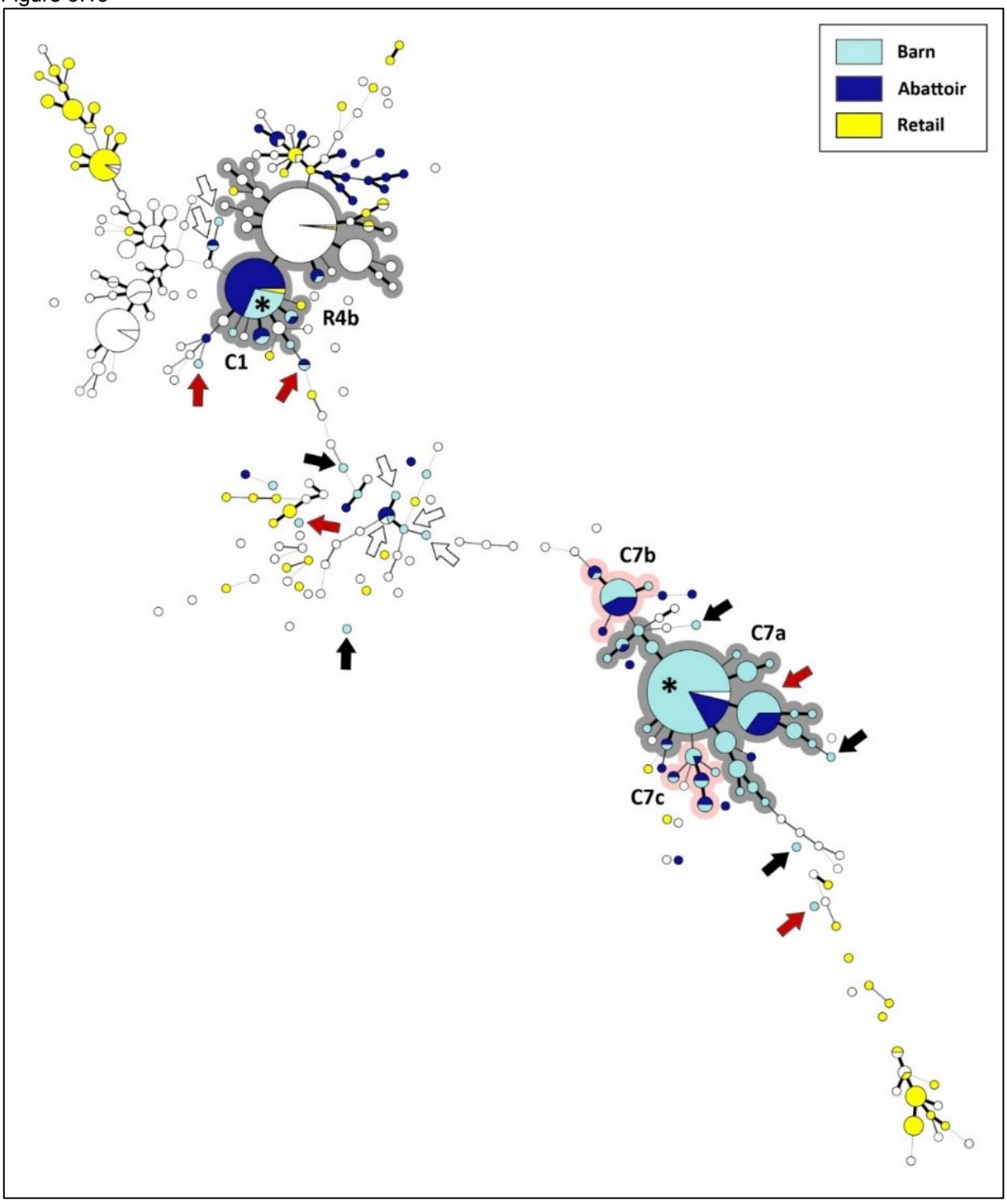

Figure 3.13 *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from poultry samples from Farm C, the abattoir at which the birds were processed, and retail poultry during the sample period. The minimum spanning tree was generated in Bionumerics (version 6.6, Applied Maths). The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes. The grey and pink highlighted clusters marked C1 and C7 (C7a, C7b, and C7c) show outbreaks within the farm during cycles 1 and 7 (≥92.5% similarity). The grey highlighted isolate cluster marked R4 shows an instance where the same subtype was obtained from abattoir and corresponding retail samples (≥92.5%). Black arrows show isolates from the outbreaks in farms that differed from outbreak subtypes, red arrows show isolates that were likely obtained during transport of the birds to the abattoir, white arrows show isolates that were detected in farms for which an outbreak did not occur, the asterisk shows that the same subtype responsible for the outbreak was recovered from skin samples from the birds within the abattoir.

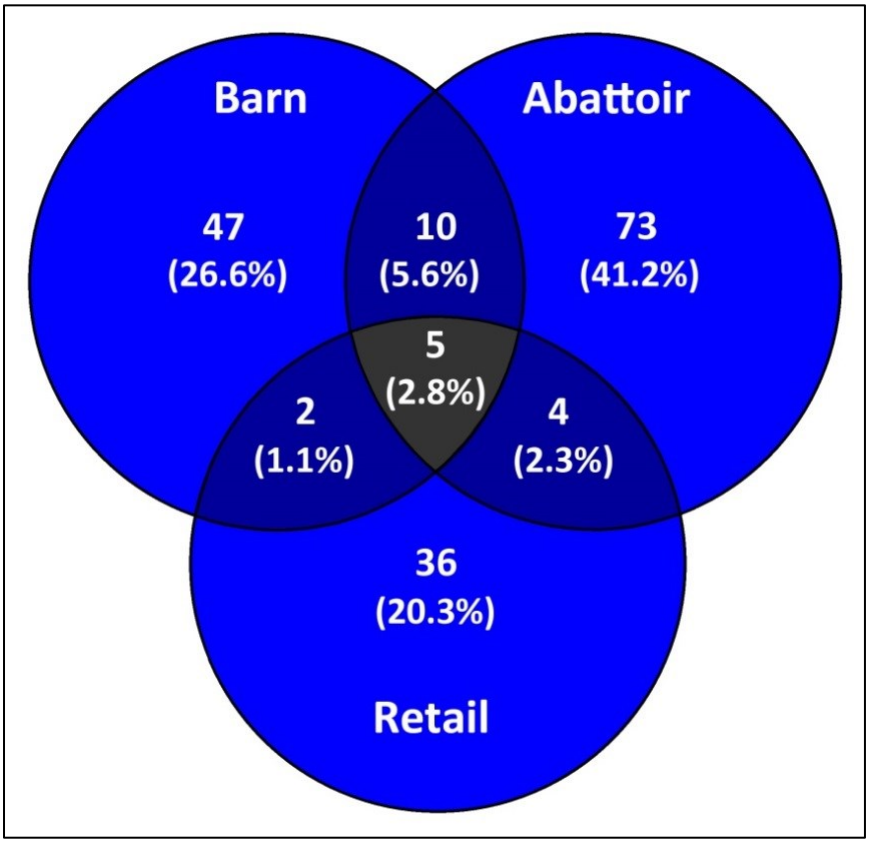

Figure 3.14 Venn diagram of subtype similarly for *Campylobacter jejuni* isolates longitudinally recovered from farm samples, the abattoir at which the birds were processed, and retail poultry during the study period. Subtypes were resolved at a 95% level of comparative genomic fingerprinting similarity.

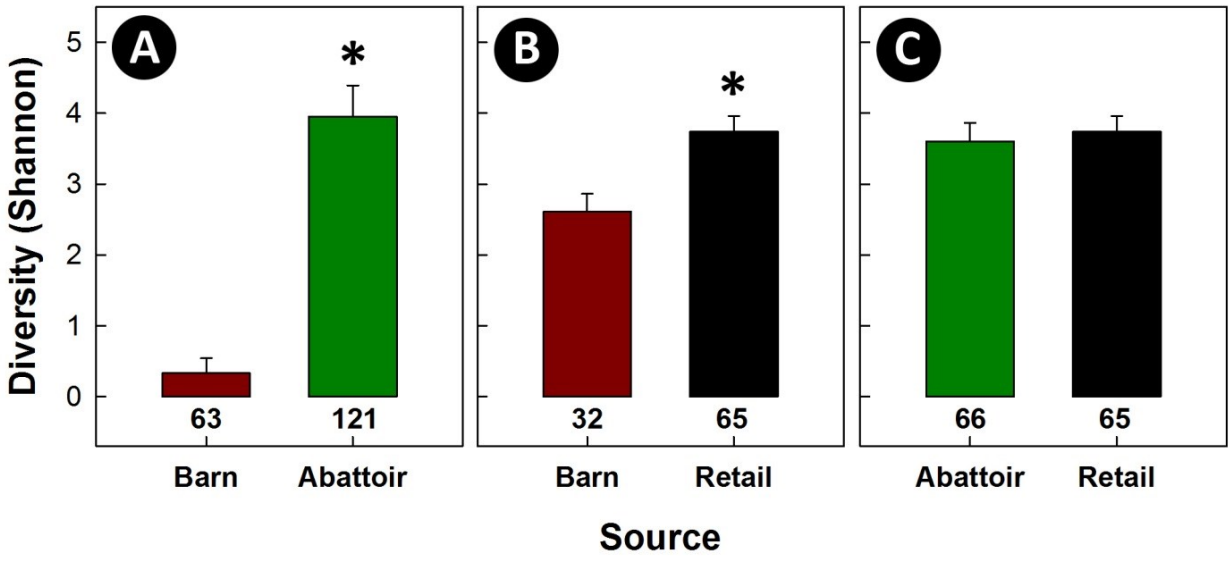

Figure 3.15 Comparative genomic fingerprinting subtype richness and diversity (Shannon H) of *Campylobacter jejuni* isolates longitudinally recovered from farms, the abattoir at which the birds were processed, and retail poultry. (A) Isolates from farms and the abattoir; (B) isolates from farms and retail poultry; and (C) isolates from the abattoir and retail poultry. Histogram bars for Shannon H diversity within individual graphs indicated with an asterisk differ ($P<0.001$) from the farm source. There was no difference ($P=0.432$) in diversity of isolates recovered from the abattoir and retail poultry. In all instances, random subsampling was applied to ensure that the number of isolates examined per source were equal; subtype diversity for 482, 234, and 234 isolates were examined for A, B, and C, respectively.

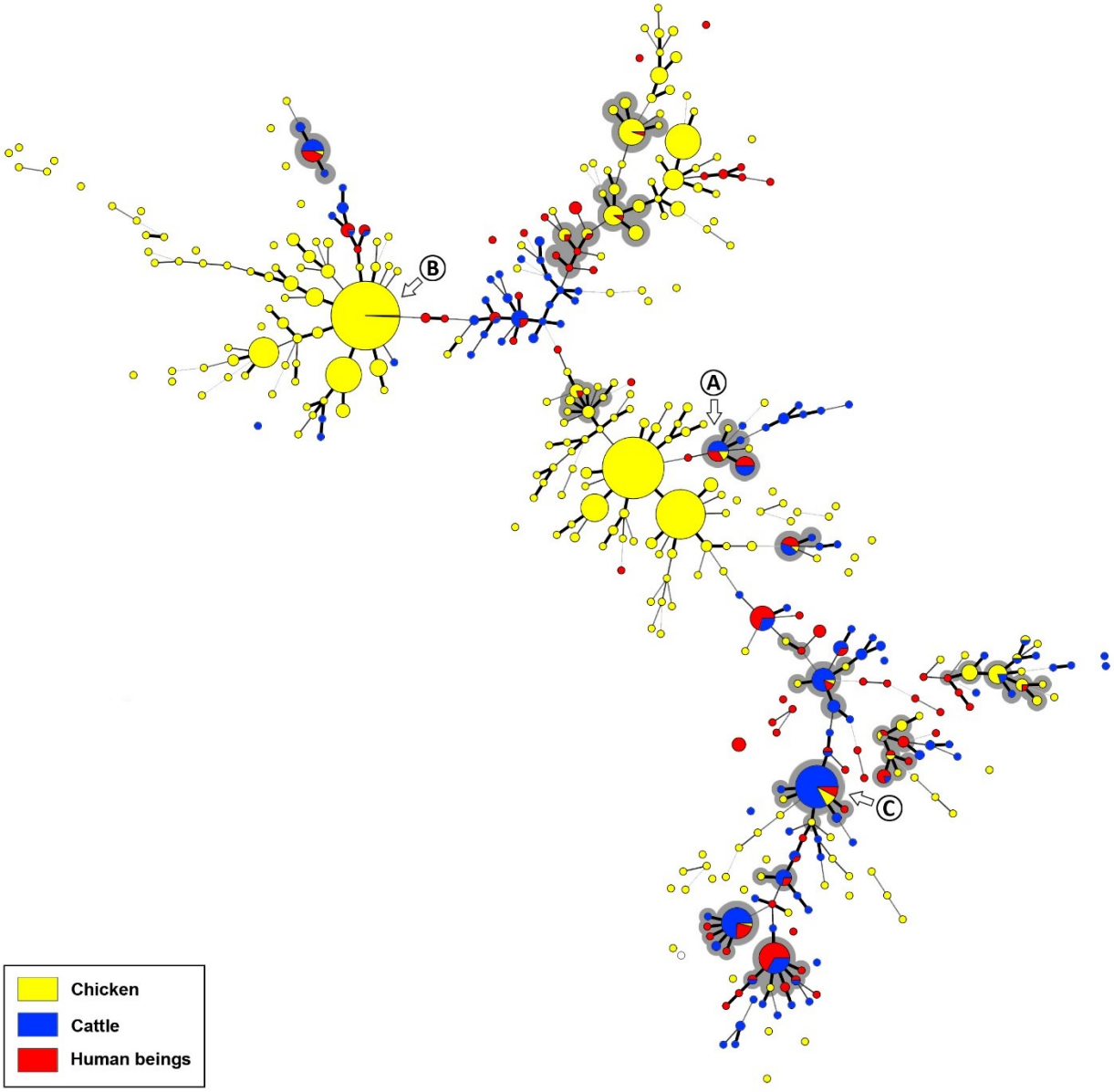

Figure 3.16 *Campylobacter jejuni* comparative genomic fingerprinting subtypes recovered from chicken samples (all three broiler farms, the abattoir, and retail poultry), from beef cattle, and from diarrheic human beings during the study period. The minimum spanning tree was generated in Bionumerics (version 6.6, Applied Maths). The thickness of lines connecting subtypes represent mismatched loci (i.e. one to three loci), and subtypes with no line represent ≥ four mismatched loci between respective subtypes. Grey highlighted clusters indicate prominent subtypes recovered from chicken samples and diarrheic people (95% similarity), and white arrows indicate subtypes recovered from poultry and from beef cattle housed in a confined feeding operation adjacent to the poultry farm (letters correspond to clades in Table 3.6).

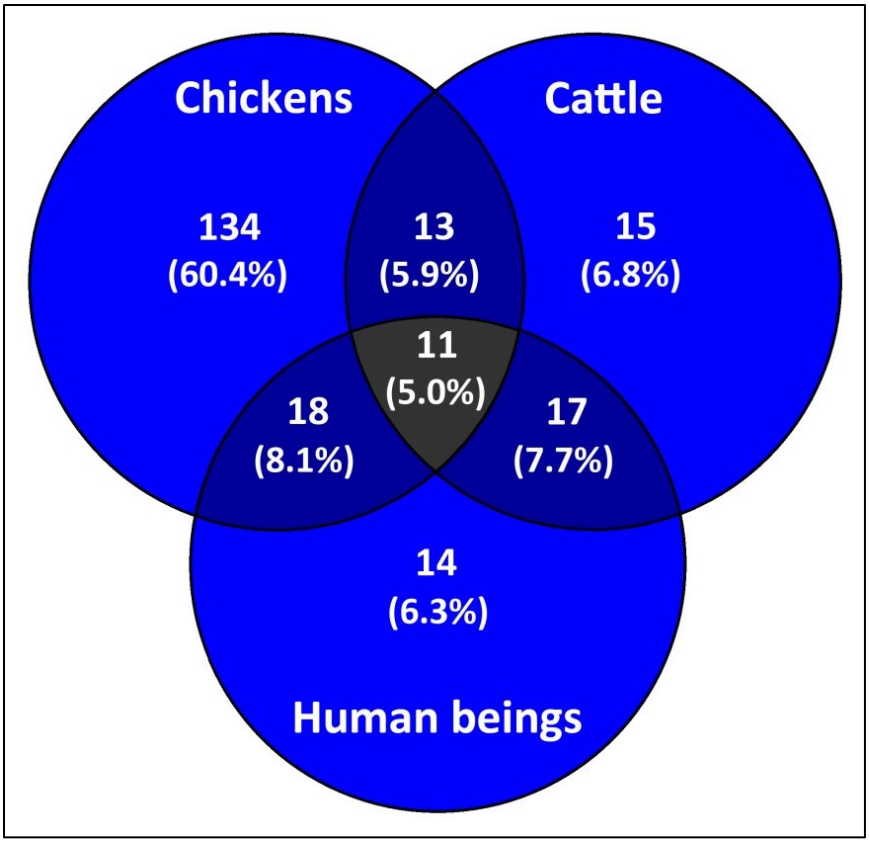

Figure 3.17 Venn diagram of subtype similarly for *Campylobacter jejuni* isolates recovered from chicken samples, from beef cattle, and from diarrheic people during the study period. Subtypes were resolved at a 95% level of comparative genomic fingerprinting similarity.

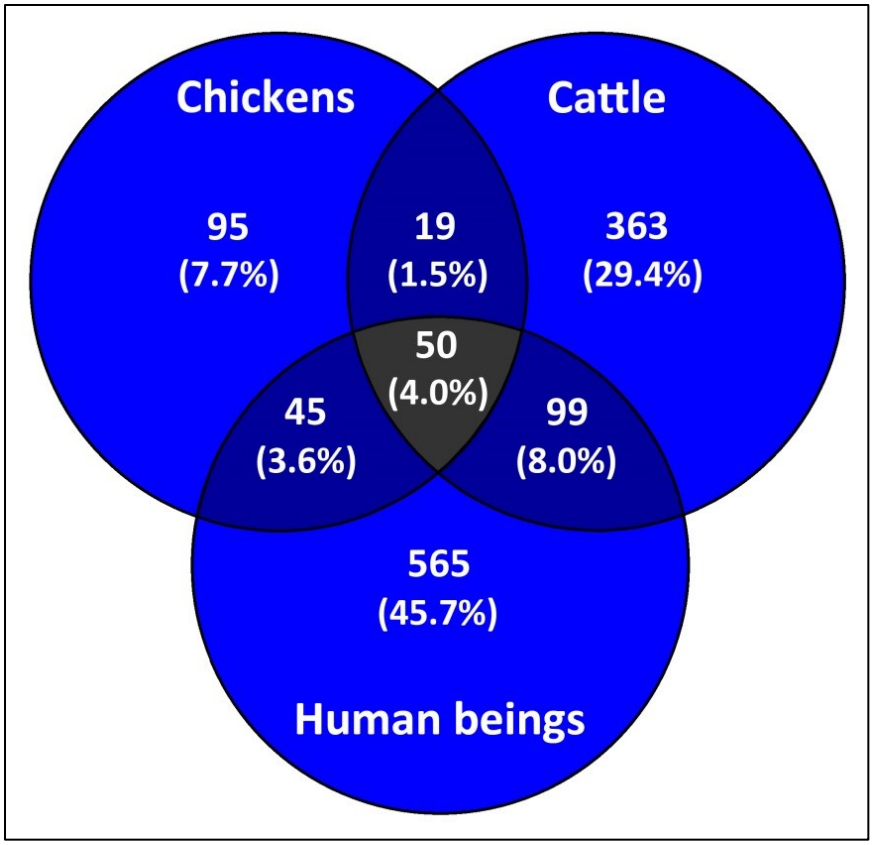

Figure 3.18 Venn diagram of subtype similarly for *Campylobacter jejuni i*solates recovered from chicken samples, from beef cattle, and from diarrheic people in Southwestern Alebrta from 2004 to 2017. The total number of isolates examined were 1096, 2904, and 2815 from chickens, cattle, and people, respectively. Subtypes were resolved at a 95% level of comparative genomic fingerprinting similarity.

Chapter 4. Discussion

4.1 Research goals

Livestock species are considered a potential reservoir for CRS of *C. jejuni*, and it is important to determine the presence of CRS within livestock production systems to ascertain risks and develop effective mitigation strategies. As such, the primary goal of my thesis was to measure CRS of *C. jejuni* within chicken production continuum using SWA as a model agroecosystem. To identify CRS, a novel high throughput and high resolution DNA fingerprinting method (i.e. CGF) was used, and recovered *C. jejuni* subtypes were compared to subtypes, including CRS within the C^3GFdb.

4.2. *Campylobacter jejuni* associated with broiler chickens

4.2.1 Farms

The poultry production system is considered to be the primary source of *C. jejuni* inciting campylobacteriosis in human beings (Hermans et al., 2012; Kaakoush et al., 2015). However, important reservoirs, how *C. jejuni* enters the farms and infects birds, transmission mechanisms, and risk to human beings are poorly understood. To address these issues requires that *C. jejuni* transmission be assessed at a subtype level of resolution. The findings of the current study showed that only four *C. jejuni* outbreaks were detected among the 22 production cycles that were sampled over a year period (Farm A: one, Farm B: one, and Bran C: two), indicating that *C. jejuni* contamination of broiler chickens is a rare event. During the outbreaks, *C. jejuni* was detected in both environmental sources (i.e. litter, farm walls, insects, feed) and was associated with broilers themselves (i.e. cloacal swabs, ceca, and feathers). My findings are in line with other studies that showed *C. jejuni* is present in the farm environment, suggesting that chicken farm environment likely an important source of *C. jejuni* contamination (Kaakoush et al., 2015; Ogden et al., 2007). Although many investigations conducted to date have reported samples from the farm environment that are positive for *C. jejuni*, the mechanisms by which birds become infected by *C. jejuni* remain unknown. In the current study, there were several occasions where litter samples were positive *C. jejuni* in early weeks of the production cycle, but outbreaks did not occur. This observation may be associated with either a paucity of detectable bacterial cells in the samples or inability of various *C. jejuni* subtypes to effectively colonize the chicken intestine and incite outbreak within the flocks. All the air samples with the exception of one outbreak (Farm B) were negative for *C. jejuni* within the farms. This is contrary to other studies that isolated the bacterium in air samples and showed airborne *C. jejuni* transmission within chicken production continuum (Wilson, 2004; Zhao et al., 2001). The reasons for not detecting *C. jejuni* in the collected air samples in this study may be attributed to continuous low level *C. jejuni* within the air, the presence of non-culturable bacterial cells, or the sampling of air during periods of low transmission.

The outbreaks of *C. jejuni* that were observed occurred primarily at the late stages in the production cycles. This observation is consistent with previous reports that demonstrated chickens younger than 3-4 weeks of age do not become infected with *C. jejuni*, which may be due to the presence of maternal antibody (Johnsen et al., 2006; Newell and Fearnley, 2003). Two instances, where elevated amounts of

C. jejuni, were also observed in Farm C (cycle C1 and C6).This occurred at the early weeks of the production cycle and in a small number of birds, but importantly did not lead to an outbreak of *C. jejuni* within the farm. This may be attributed to the presence of maternal antibody within birds, but it does not exclude the possible inability of various *C. jejuni* subtypes to colonize the chicken intestines. *Campylobacter jejuni* was detected in two consecutive sample periods within Farms B and C (cycle B1 and C7). Notably, in the first instance, few birds were infected with *C. jejuni* and this did not result in an outbreak, whereas in the second instance, an outbreak resulted with more than 95.9% of birds rapidly becoming infected (i.e. within a 7-day period) These data confirmed horizontal transmission and the rapid dissemination of *C. jejuni* within the flocks in birds, an the observation demonstrated previously by others (Bull et al., 2006; Callicott et al., 2006; Cox et al., 2012).

Campylobacter jejuni was also detected in transported ceca and feather samples from both contaminated and uncontaminated production cycles in the farms. These positive samples isolated from birds from production cycles deemed to be free of the bacterium were unexpected, and may be attributed to either late contamination of birds with low levels of *C. jejuni*, or contamination of the birds during the transportation to the abattoir. That latter possibility is unlikely as *C. jejuni* was isolated from the ceca of birds suggesting that they were infected in the broiler farm. Unfortunately, the sampling of transport trucks was not permitted in the current study. This would determine the role of inter-facility transportation as a source of *C. jejuni* contamination, and sampling during transport of birds to the abattoir should be emphasized in future research examining *C. jejuni* at the subtype level of resolution. Notably, several studies have demonstrated birds become infested with *C. jejuni* during transport of broilers (Franchin et al., 2005; Ramabu et al., 2004). Collectively, the information from my study and others suggests that abattoir transportation vehicles could be important in the transmission of *C. jejuni*; although chickens infected late in the production cycle can not precluded as the source of *C. jejuni* in these birds.

4.2.2 Abattoir

Determining the presence of *C. jejuni* subtypes within the abattoir is necessary to fully understand the transmission and risk posed to human beings. The abattoir data showed that the average prevalence of *C. jejuni* was 11.6% over a 1.5 year sampling period, and this was higher than *C. jejuni* prevalence in the chicken farms. My results from abattoir carcass samples showed that *C. jejuni* was isolated from several skin samples of slaughtered birds obtained from birds from farms that were deemed to be free of *C. jejuni* as well as those from farms in which outbreaks of the bacterium occurred. These observations suggest that *C. jejuni* cross-contamination occurs in the abattoir either by *C. jejuni*-infected slaughtered birds from positive farms or likely by the presence of resident *C. jejuni* cells within the abattoir. These findings are in line with several earlier studies showing steady increase of *C. jejuni* contamination of chicken products along with poultry processing operation. Notably, there is a lack of agreement on the precise mechanisms of the transmission mode of *C. jejuni* contamination along chicken production continuum (Damjanova et al., 2011; Gruntar et al., 2015; Melero et al., 2012). For instance, a study by Grunter et al. (2015) demonstrated that the main source of *C. jejuni* contamination was from contaminated birds in the pre-

slaughtering process (i.e. during the plucking stage) within the abattoir, whereas investigations showed that abattoir environment (i.e. floor, walls, ventilation) was the major source of *C. jejuni* subtypes, independent of *Campylobacter* positive flocks entering the slaughterhouse (Allen et al., 2007; García-Sánchez et al., 2017; Melero et al., 2012)

There were few instances in which the level of *C. jejuni* contamination decreased within the slaughtering process in abattoir in the current study. As an example, some feather samples obtained from transport trucks were *C. jejuni* positive, whereas ceca and skin samples, collected from the same flock in abattoir were free of *C. jejuni*. These results suggested that the decrease in bacterial cells, was likely due to pre-slaughter processing procedures. Further longitudinal sampling are required to clarify how and the degree to which *C. jejuni* contaminates meat during the slaughter process.

4.2.3 Meat at retail

The prevalence of *C. jejuni* in retail poultry meat varied between 0% to 52.0% of meat sampled at ten different sample times during the 542 day study period. Similar rates of *C. jejuni* in retail chicken meat have reported by others (Guyard-Nicodème et al., 2015; Little et al., 2008; Pointon et al., 2008; Taremi et al., 2006; Whyte et al., 2004; Williams and Oyarzabal, 2012; Wong et al., 2007). In investigation by Guyard et al. (2015) and Taremi et al. (2006), they showed that the prevalence of *C. jejuni* in broiler meat at retail was 76% and 63%, respectively. This was in line with the current study. Considerable variation was observed in *C. jejuni* prevalence in the broiler meat at retail, which could be a result of the longitudinal design of the study and the confounding effect of season. Packaging can influence survival of *C. jejuni* likely due to the cross-contamination of the bacterium during the meat process (Burgess et al., 2005; Harrison et al., 2001; Jørgensen et al., 2002; Pointon et al., 2008), but the birds processed in the current study were not packaged before sampling. The wide variation in retail chicken meat *C. jejuni* prevalence may is influenced by the detection method applied, geographical location, and the size of the sample processed (Bohaychuk et al., 2006; Guévremont et al., 2006; Taremi et al., 2006; Whyte et al., 2004; Williams and Oyarzabal, 2012; Zhao et al., 2001). As an example, a longitudinal investigation by Williams and Oyarzabel (2012) showed that the prevalence of *C. jejuni* was influenced by season, slaughtering plant processes, the type of meat sampled, and the location of the retail store. Interestingly, data showed slightly higher *C. jejuni* prevalence in the skin samples from breast area compared to skin samples of cloaca area in several occasions. Moreover, differences were in levels of *C. jejuni* contamination between the breast and cloaca samples of skin (at the sampling time points 3 and 9).

All four outbreaks of *C. jejuni* observed in broiler farms in the current study occurred in the spring and summer seasons. The highest rates of *C. jejuni* contamination in the abattoir and retail chicken meat samples were also detected at approximately the same time period. These findings suggest that infection primarily occurs in the spring, summer and fall which is in alignment with other studies, suggesting a confined seasonal prevalence of *C. jejuni* contamination in poultry (Hermans et al., 2012; Huneau-Salaün et al., 2007; Kaakoush et al., 2015). It is possible there is a correlation between increased temperature and exposure to the environmental sources of the bacterium. Other studies however did not show a

spring-autumn seasonal prevalence of *C. jejuni* (Griekspoor et al., 2015; Williams et al., 2015). In this regard, further longitudinal large-scale investigations are needed to definitively demonstrate a relation between weather, season and prevalence of the bacterium within poultry production system.

4.3. *Campylobacter jejuni* associated with beef cattle

Beef cattle within a feedlot adjacent to Farm C were observed to frequently shed *C. jejuni* in their feces with an average prevalence of 52.1% in cattle. This observation is consistent with other studies (Besser et al., 2005; Inglis et al., 2004). *Campylobacter jejuni* was commonly detected in beef cattle feces in summer, autumn, and winter months, and less so in the spring. It is plausible that the low rate of contamination in spring was attributed to the presence of young cattle within the feedlot. Inglis et al. (2004) showed that young cattle mostly are colonized with low number of *C. jejuni* subtypes, and once the young cattle enter the feedlot, a high numbers of various *C. jejuni* subtypes colonize in their intestine likely due to the high density in feedlot cattle and horizontal transmission of *C. jejuni* within the herd (Inglis et al., 2004). The observation of frequent shedding of *C. jejuni* in beef cattle feces is consistent with observations of others (Bae et al., 2005; Besser et al., 2005; Hermans et al., 2012; Inglis et al., 2004; Stanley and Jones, 2003). Although the mechanisms of *C. jejuni* transmission in feedlot cattle is poorly understood at present, it is thought that a relatively small number of calves positive for the bacterium enter the feedlot and it is rapidly transmitted horizontally among animals due to their close proximity to one another. Since the airborne transmission of *C. jejuni* has been suggested in several studies (Wilson, 2004; Zhao et al., 2011) coupled with the high prevalence of the bacterium in feces, air samples were collected weekly adjacent to the feedlot to ascertain the degree to which *C. jejuni* liberated in aerosols could be detected. However, all the collected air samples were negative for *C. jejuni*, which may be associated with either low bacterial concentration or non-culturable *C. jejuni* within the air. The sampling strategy applied in the current study was not designed to ascertain the influence of environmental conditions on airborne dissemination of *C. jejuni*. It is noteworthy that during prolonged dry periods cattle feces rapidly dries facilitating the liberation of dry aerosols during windy periods. The degree to which precipitation, fecal moisture, and wind have on survival and airborne dissemination of *C. jejuni* are largely unknown and warrant investigation.

4.4 Recovery of *C. jejuni* isolates

Five thousand, seven hundred and fifty one *C. jejuni* isolates were recovered by direct plating and enrichment-filtration methods. These two culture-based techniques with different temperatures were employed in an attempt to minimize selection bias *C. jejuni* subtypes (Bolton et al., 1983; Bolton and Robertson, 1982; Gharst et al., 2013). Conventional enrichment culture, relies on the use of semi-selective antibiotics in the enrichment broth and subsequent agar media to prevent the growth of non-*Campylobacter* microorganisms; it is noteworthy that if selective strategies are not employed, fast-growing microorganisms overgrow *C. jejuni,* precluding isolation of the bacterium (Gharst et al., 2013; Ugarte-Ruiz et al., 2012). Importantly, the presence of selective agents and the reliance of growth of *C. jejuni* in broth are thought to bias subtype diversity (Taboada et al., unpublished). In the current study, a non-selective

enrichment broth (i.e. it did not contain selective agents) was used in combination with membrane filtration in an attempt to minimize selection bias to get a high numbers of *C. jejuni* subtypes identifying CRS. Although the prevalence of *C. jejuni* differed between the two isolation methods, a difference in subtype diversity was not observed between them. *Campylobacter jejuni* was more readily isolated from cloacal swabs, ceca digesta, and retail chicken meat samples using the enrichment-filtration method. Study results were also in consistent with previous studies demonstrating that conventional enrichment was a conspicuously effective method for low bacterial numbers or damaged bacteria such as retail chicken meat samples (Baylis et al., 2000; Speegle et al., 2009; Williams and Oyarzabal, 2012), although these studies did not examine subtype diversity. Study findings indicate that it is necessary to employ a combination of isolation methods, and that non-selective enrichment did not appreciably bias subtype recovery and therefore has value in molecular epidemiological investigations.

4.5 *Campylobacter jejuni* subtypes in the chicken production continuum

Only one *C. jejuni* outbreak was detected in Farm A (cycle 3/w4) and Farm B (cycle 1/w5), and two outbreaks were detected in Farm C (cycle 1/w6 and cycle 7/w5). Interestingly, a single predominant subtype was deemed responsible for each outbreak, supporting the hypotheses that chickens are contaminated by a limited number of *C. jejuni* subtypes, and following the establishment of a predominant subtype, it becomes widely distributed within farms and is rapidly disseminated horizontally throughout the flock. Study findings are in agreement with snapshot examinations showing a predominant *C. jejuni* subtype in farms (Perko-Mäkelä et al., 2002; Rivoal et al., 2005; Shreeve et al., 2002), and in contrast to several other studies that reported diverse strains of *C. jejuni* within poultry flocks. This may be associated with the horizontal genetic transfer within different *C. jejuni* subtypes present in birds or farm environment (Manning et al., 2001; Pokamunski et al., 1986; Rivoal et al., 2005). In some instances in the current study, *C. jejuni* subtypes not associated with outbreaks were isolated from the environment and chickens at the same time that the *C. jejuni* outbreak occurred. As discussed earlier, it is possible that these subtypes may not have been able to effectively colonize chicken intestine or that they were present in insufficient numbers to incite an outbreak. To date, the precise mechanisms involved in infection and colonization the chicken intestine by *C. jejuni* subtypes are unresolved. Moreover, the dynamics and mechanisms of transmission of *C. jejuni* subtypes within chicken farms are poorly understood. Therefore, further animal studies are needed to elucidate these mechanisms and identify the main sources of infection.

A large number of *C. jejuni* isolates were genotyped in the current study, and the CGF data demonstrated that the diversity of *C. jejuni* subtypes was low in the chicken farms, and increased as the birds continued through the slaughter and retail process. *Campylobacter jejuni* subtypes enter the abattoir during the carcass processing (Arsenault et al., 2007; Guyard-Nicodème et al., 2015; Oyarzabal et al., 2013; Pacholewicz et al., 2016; Rasschaert et al., 2007; Seliwiorstow et al., 2016; Sheppard et al., 2010). Transmission of *C. jejuni* subtypes can occur throughout the chicken production continuum (Damjanova et al., 2011; García-Sánchez et al., 2017; Gruntar et al., 2015; Melero et al., 2012). In the current study,

subtype diversity was longitudinally examined throughout the production continuum, and diversity of *C. jejuni* subtypes was observed to be substantially lower in farms, relative to the abattoir and retail. Furthermore, there was no difference in diversity of subtypes recovered from samples in the abattoir and at retail, and *C. jejuni* subtypes were often isolated on meat of birds that were free of the bacterium within the farms. It is therefore likely that the chicken, which were *C. jejuni* negative in farm, were contaminated by *C. jejuni* subtypes within the abattoir, and that bacterial cells remained viable on meat after dissemination to retail settings. It is unknown whether abattoirs are contaminated by *C. jejuni* from a small number of flocks on a continual basis, or whether a resident population of subtypes persist within the abattoir, and if so, where in the abattoir. Although the diversity of *C. jejuni* subtypes on meat in the abattoir and at retail were similar, it is also not known to what degree subtypes are transmitted at retail.

4.6 Clinically-relevant subtypes of *C. jejuni* associated with broiler chickens

Mounting evidence indicates that not all subtypes of *C. jejuni* represent an equivalent risk to human beings (Inglis et al. unpublished). In the current study only a subset of *C. jejuni* subtypes associated with chickens were found in diarrheic human beings in SWA during the study period (29 of 176 subtypes). A comparison of *C. jejuni* subtypes associated with chickens to the subtype and metadata within the C^3GFdb similarly indicated that only a subset of stains associated with chickens represent a high risk to human beings; the C^3GFdb contains subtype data for >27,000 isolates of *C. jejuni* belonging to ≈5,000 distinct *C. jejuni* subtypes across Canada . In this regard, only 45.5% of the subtypes recovered from chickens in SWA had been previously isolated from diarrheic human beings. That approximately half of the subtypes recovered from chickens were deemed CRS supports chicken as main reservoir of high risk *C. jejuni* in SWA. These findings also coincide with previous work indicating chicken meat as a primary source of human campylobacteriosis (Altekruse et al., 1999; Canadian-Food-Insepction-Agency, 2016; Guévremont et al., 2006; Ravel et al., 2017; Williams and Oyarzabal, 2012; Zhao et al., 2001). Conversely, approximately half of the subtypes recovered from chickens have not been isolated from people, supporting the hypothesis that not all subtypes represent a risk to human beings, and that it is crucial to examine risk at the subtype level of resolution. It should be noted that some subtypes found in the study were novel to the C^3GFdb, and they had not previously been detected in chickens or any other source. Detecting new subtypes and accessioning the information into the C^3GFdb will facilitate future efforts to ascertain risk and elucidate the molecular epidemiology of *C. jejuni* toward the development of effective mitigation strategies.

4.7 Beef cattle as a reservoir of CRS *C. jejuni* infecting broiler chickens

The importance of cattle as a reservoir of CRS *C. jejuni* infecting poultry has not been extensively studied. An examination of *C. jejuni* subtypes within the C^3GFdb that were isolated from chickens and beef cattle in SWA indicate that a number of subtypes occur in both species. As well, subtypes from both cattle and chickens occur in diarrheic human beings in SWA indicating that both livestock species are potential reservoirs of CRS. Examination of subtypes from broiler chickens, beef cattle (i.e. housed in a feedlot adjacent to Farm C), and diarrheic people during the study period similarly identified subtypes

infecting all three hosts. Notably, a CRS of *C. jejuni* (subtype 0735.001.002) was isolated from beef cattle, broilers within the farm, and from meat samples in the abattoir at the same time period; this corresponded to an outbreak of *C. jejuni* in Farm C. This *C. jejuni* subtype is often associated with beef cattle in Alberta. Furthermore, one of the predominant *C. jejuni* subtypes responsible for the outbreak in Farm A (0957.001.001) was also associated with chickens, cattle, and diarrheic human beings. These findings indicate that *C. jejuni* CRS transmission occurs among feedlot cattle, broiler chickens, and diarrheic human beings, supporting the possibility that cattle are important reservoir of *C. jejuni* in SWA and elsewhere. Others have suggested that cattle are a reservoir of *C. jejuni* infecting people but they did not examine *C. jejuni* at the subtype level of resolution nor did they assess the risk posed to human beings (Ridley et al., 2011a). Although *C. jejuni* readily colonizes the intestinal tract of cattle, and *C. jejuni* from cattle have been determined to be responsible for campylobacteriosis in people who consumed unpasteurized milk (Blaser et al., 1987; Kaakoush et al., 2015) and untreated drinking water (Champion et al., 2005; Clark et al., 2003; Kaakoush et al., 2015) contaminated with the bacterium, consumption of beef is not considered to be a significant risk factor. This is supported by a study that examined transmission of *C. jejuni* throughout the beef production continuum (Inglis et al. unpublished). This study showed that *C. jejuni* was readily isolated from feces of beef cattle throughout the feedlot period, from the intestinal tract, and from hides in the abattoir. A significant number of *C. jejuni*, including subtypes from the intestinal tract and hides were subsequently observed on the surface of carcasses after hide removal and storage at 4^{o}C for 24 hr. However, *C. jejuni* was not detected in ground beef made from the contaminated carcasses. Reasons for the poor survival of *C. jejuni* on and in meat from beef cattle is not well understood, but may be due to the lower pH of beef meat compared to chicken meat (Gill and Harris, 1982). Evidence from the current study supports the following scenario: *C. jejuni* subtypes readily colonize the intestinal tracts of cattle and the bacterium is frequently shed in bovine feces in large numbers from a majority of cattle housed in feedlots; a limited number of chicken-compatible *C. jejuni* from cattle are transmitted to broiler farms, and infect a small number of susceptible chickens (i.e. late in the production cycle); a single or limited number of bovine-originating *C. jejuni* subtypes is/are rapidly transmitted horizontally within the flock; the infected birds contaminate the abattoir; bovine-originating *C. jejuni* are passively transferred to meat within the abattoir, and meat infested with *C. jejuni* is transferred to retail settings; as *C. jejuni* is able to survive for prolonged periods on poultry meat, especially in the absence of freezing, the bovine-originating subtypes of the bacterium are ingested by people (i.e. of improperly handled or prepared poultry) resulting in campylobacteriosis; viable *C. jejuni* cells are released into the environment in the feces of diarrheic people, a proportion of the cells survive waste-water treatment, and *C. jejuni* cells released into surface water infect cattle on pasture or in confined feeding operations during water consumption. It is noteworthy that the mechanism by which *C. jejuni* cells are transmitted from cattle to broilers was not identified in the current study, and this warrants investigation.

Factors such as geographical region and distribution of human population are important determinants of epidemiological patterns of human campylobacteriosis and transmission routes of *C. jejuni* (Ferreira et

al., 2014; Kaakoush et al., 2015; Mullner et al., 2010; Skarp et al., 2016). For instance, Webb et al. (2018) showed that most CRS of *C. jejuni* infecting people in SWA are associated with cattle feedlots rather than chicken operations. However, SWA possesses an abnormally high density of beef cattle, coupled with the exchange of *C. jejuni* subtypes between cattle and chickens confounds conclusions on the relative importance of these two reservoirs. Other investigations have postulated that human campylobacteriosis in urban areas are primarily attributed to consumption of contaminated chicken meats, while ruminant-associated campylobacteriosis occurs more often in rural areas (E. Taboada, pers. comm.). The high prevalence of campylobacteriosis in SWA, coupled with the high density livestock production (Figure 1.4), the presence of a single and public diagnostic facility, an ≈40:60 rural: urban distribution of people, a single prominent watershed, and a spatial gradation of human activity from west to east make SWA an ideal location to identify important reservoirs and elucidate precise transmission mechanisms of CRS.

Chapter 5. General Conclusions and Future Research

5. 1 General conclusions

Campylobacter jejuni infection is one of the most common bacterial foodborne intestinal disease in the world (Kaakoush et al., 2015), and campylobacteriosis is especially prevalent in SWA (Government of Alberta, 2009; Taboada et al., 2008). Notably, the incidence of human campylobacteriosis is increasing globally (Kaakoush et al., 2015). In SWA, campylobacteriosis rates are estimated to be at least 115 cases $100K^{-1}$ (Inglis et al., 2019), and is likely underestimated by a factor of twenty (Mead et al., 1999); thus, it is possible that ≈2.3% of people living in SWA are infected by *C. jejuni* annually representing a tremendous negative influence on the well-being of people in this region, cost to the health care system, a reduction in worker productivity, and impact on the economy. The epidemiology of campylobacteriosis is poorly characterized at present (e.g. identification of important reservoirs and transmission mechanisms), and this can be attributed to a multitude of factors including a lack of standard detection methods (culture and molecular-based) (Inglis et al., 2011; Taboada et al., 2013), high genetic diversity within *C. jejuni*, many environmental and animal reservoirs of *C. jejuni* (Dingle et al., 2002), different in age and level of susceptibility in people, under-reporting of the campylobacteriosis (Havelaar et al., 2009; Young et al., 2007), different biosecurity measures to prevent disease, and the application of disease surveillance strategies. Chickens infested with *C. jejuni* is thought to be a primary risk factor for infection in human beings (i.e. via consumption and handling of chicken contaminated with the bacterium) (Ravel et al., 2017; Wagenaar et al., 2013). Factors that determine pathogenicity and virulence in *C. jejuni* are also not well understood. Importantly, *C. jejuni* is highly genetically diverse, but a degree of host specificity has been observed among subtypes (Dingle et al., 2001; Havelaar et al., 2009; McCarthy et al., 2007; Young et al., 2007) suggesting that source attribution studies can be applied to ascertain the molecular epidemiology of the bacterium. Such studies require the subtyping methods that are high-throughput and high-resolution, and a depository of fingerprint and metadata.

Although a variety of fingerprinting methods have been used to subtype *C. jejuni*, CGF40 was used in the current study. This method generates binary data (i.e. presence or absence) for 40 accessory genes within the *C. jejuni* genome that are predictive of whole genome phylogeny (Taboada et al., 2012). Importantly, the CGF method delivers high-resolution subtype data (1.1 trillion possible subtypes), is medium-throughput, is cost-effective, and there is accompanying C^3GFdb that contains >27,0000 *C. jejuni* isolates and >5,000 subtypes with metadata for each isolate. To identify important reservoirs and elucidate transmission mechanisms, *C. jejuni* associated with broiler chickens was longitudinally examined throughout the production continuum in SWA over a *ca.* 1.5 year period (i.e. three commercial broiler farms, abattoir, and retail meat). Furthermore, *C. jejuni* isolates were isolated from beef cattle adjacent to one of the poultry farms sampled, and all *C. jejuni* isolates recovered from diarrheic people in SWA over the study period were also examined. Two isolation methods were applied and *C. jejuni* isolates were identified to species using taxon-specific PCR (Inglis et al., 2018). SWA was chosen as a model agro-ecosystem because there are high rates of campylobacteriosis in the region (Inglis et al.,

2011), and there are high densities of livestock populations including beef cattle on rangeland (556,184), beef cattle in feedlots (559,807), and chickens (2,707,865) (Alberta Goverment, 2014). Furthermore, there is a single public medical diagnostic facility, which allows all *C. jejuni* isolates from diarrheic people to be characterized, and there is an ≈40:60 rural:urban distribution of people, which provides a good number of *C. jejuni* isolates from the rural cohort. Approximately 16,000 environmental and animal samples were obtained from the chicken production continuum (three commercial broiler farms, local abattoir and retail chicken), beef cattle farm adjacent to one of the chicken farms (Farm C), and stool samples from diarrheic people isolated at public diagnostic facility in SWA over the study period. Furthermore, a large subset of the *C. jejuni* isolates recovered from chicken and beef cattle, and all *C. jejuni* isolates recovered from diarrheic people were subtyped by CGF, and analyzed. Salient findings from the study include:

- Infection of chickens by *C. jejuni* in Alberta broiler farms was a rare event
- Chickens from the hatchery were free of *C. jejuni*, the bacterium was not detected in broiler feed or drinking water, and *C. jejuni* was infrequently detected in farms before population with chickens indicating that the bacterium infecting broilers entered the farm from an exogenous source(s) by an unknown mechanism
- When infection by *C. jejuni* of broilers occurred, it happened late in the production cycle and typically in the late summer and early autumn
- If a flock was exposed to *C. jejuni*, the entire flock rapidly become colonized, but by a limited number of subtypes (often one prominent subtype)
- Despite a high prevalence of infection of birds by *C. jejuni*, the bacterium was relatively rarely isolated from farm environment
- Processing at the abattoir reduced the level of contamination of meat from birds infected with *C. jejuni* on farm; however, meat from a relatively small proportion of birds was contaminated by the same subtype that the birds were infected with on farm
- Meat from a small proportion of birds infected with *C. jejuni* on farm or deemed to be free of *C. jejuni* upon entry at the processing plant was contaminated by *C. jejuni* subtypes that were not detected on farm, suggesting that the abattoir potentially becomes contaminated with *C. jejuni* from multiple farms at some point during processing in the abattoir
- Subtype diversity increased from farm to abattoir and retail, which is consistent with increasing degrees of cross-contamination moving from farm to fork
- A high prevalence of beef cattle adjacent to poultry farms shed diverse *C. jejuni* subtypes in their feces, including CRS
- *C. jejuni* subtype responsible for outbreaks in chickens were observed in feces from steers in a feedlot adjacent to the poultry farm
- Recovered isolates indicate considerable subtype diversity, and evidence of shared source origin

- Although CRS of *C. jejuni* in SWA were frequently observed in both cattle feces and chickens (abattoir samples and retail meat), only a subset of subtypes associated with poultry were CRS
- In conclusion: (i) a relatively small number of birds are able to contaminate the abattoir with C. *jejuni* subtypes, and clean birds are infested with these subtypes within the abattoir, which are subsequently transferred to retail meat; (ii) not all *C. jejuni* isolates found on chicken meat are a high risk to infect people, and mitigation strategies should target high risk subtypes; (iii) beef cattle are potentially an important reservoir of CRS infecting broilers; and (iv) Hazard Analysis and Critical Control Points (HACCP) for *C. jejuni* subtypes within abattoirs in conjunction with novel on-farm mitigation strategies are required to effectively mitigate this pathogen.

5.2 Knowledge gaps

Many studies have isolated *C. jejuni* from chicken farms, abattoirs and retail meat (Damjanova et al., 2011; Guyard-Nicodème et al., 2015; Melero et al., 2012; Pointon et al., 2008; Williams et al., 2015), but no previously conducted studies to my knowledge have longitudinally examined the transmission of *C. jejuni* at the subtype level over a prolonged period and at such a large scale, nor addressed the public health risk posed by the isolates recovered (see salient finding above). Furthermore, the importance of beef cattle as a reservoir of *C. jejuni* infecting chickens, including CRS, is a novel aspect of this research. Despite the contributions of the research reported herein to the scientific community, a number of questions remain unanswered. One salient unanswered question is how do *C. jejuni* originate from exogenous sources, such as beef cattle transmitted to and enter the farm? Some possible vectors of *C. jejuni* are ground dwelling insects (e.g. beetles), flying insects (e.g. flies), mammals (e.g. rodents), and people (e.g. broiler works) (Altekruse et al., 1999; Ekdahl et al., 2005; Hald et al., 2008; Jonsson et al., 2012; Kaakoush et al., 2015; Strother et al., 2005). In the current study, arthropods, mice, and air were sampled, but they were not identified in the transmission of *C. jejuni* from cattle to broilers. Additional unanswered questions from the study are why some subtypes of *C. jejuni* infected birds late in the production cycle and were rapidly transmitted to the entire flock, why one subtype caused outbreaks in individual farms, and why other subtypes detected in the farm did not incite an outbreak? Although it has not been examined empirically as yet, one possibility is that some subtypes of *C. jejuni* are adapted to efficiently colonizing the GIT of chickens. It is also possible that there is an inoculum threshold required to successfully colonize the intestine of chickens. In this regard, one study observed the minimum threshold of *C. jejuni* requirement to successfully colonize chickens intestine, which was between 10^2 and 10^4 bacterial cells (Shanker et al., 1990). Therefore, *C. jejuni* subtypes incited outbreaks in the current study were efficient colonizers of chickens and were present in farms in sufficient numbers initially to exceed the inoculum threshold. Coupled with the above, data obtained indicated that *C. jejuni* entrance into a broiler farm is a rare event.

As mentioned earlier, the CGF40 is a high-resolution and high-throughput subtyping method which is deployable in the context of large-scale epidemiological studies (Clark et al., 2012; Taboada et al., 2012). Despite the logistical, economic, and scientific advantages of the CGF method, a number of issues were

identified in the current study. Similarly to any PCR-based technology, consistent amplification of all 40 loci in all isolates is an unrealistic expectation, especially in fivemer multiplex reactions. In this regard, what were deemed to be false negative reactions for some amplicons, especially in multiplex seven. This necessitated clustering of isolates at ≥92.5% level of resolution (i.e. allowing two or three mismatches). Applying less than a 100% level of resolution complicated interpretation of data, including submission of CGF queries to the C^{3}GFdb, which is designed to evaluate submissions at a 100% match. Furthermore, not all of the *C. jejuni* strains identified in the current study were present in the C^{3}GFdb, even though the database contains >27,000 isolates. This illustrates the tremendous genetic diversity within the species, and it is expected that the issue of novel strain designations will be less of a obstacle as additional isolates are added to the database. Despite the issues encountered, the CGF method allowed the subtyping of a large number of *C. jejuni* isolates, the clustering of isolates based on phylogeny, the identification of important reservoirs and transmission mechanisms, and the assessment of risk posed to people. The price of sequencing the whole genome of *C. jejuni* has become much more economical in recent years, and the CGF *in silico* tool developed by Taboada and colleagues will make CGF typing reliable moving forward. The ability to link fingerprint and metadata is a crucial advantage of the CGF method.

The current study longitudinally collected animal (chickens and cattle) and environmental (outside and inside of the farms) samples from three commercial poultry farms weekly over a 542-day period. This work is considered as a large-scale sampling investigation, and >12,000 samples were obtained from broiler farms and analysed. However, the sampling strategy applied had some restrictions including number of samples per site, length of the sampling period, and some of the samples were difficult to obtain. For instance, insects (i.e. flies and beetles) and mice samples outside and inside of the chicken farms were infrequently collected during the study period. A major reason for this is that considerable sampling of farms occurred during periods when fly populations were low (e.g. winter months), and the use of insecticides in some of the farms sampled limited darkling beetle populations. Mice were not readily trapped within farms, and none of the mice trapped in adjacent rooms (e.g. feed rooms) were positive for *C. jejuni*. Airborne transmission of *C. jejuni* has been observed by other studies (Wilson, 2004; Zhao et al., 2011). Unexpectedly, only one air sample was positive for *C. jejuni* within a broiler farm during an outbreak. Dry aerosols were highly visible during sampling of farms, including during documented outbreaks of *C. jejuni*, and reasons why the bacterium was infrequently isolated from air within farms is uncertain. An inertial air sampler was used. Although this type of air sampler is the golden standard for recovery of microorganisms in air, validation of the sampler used in the current study warrants attention. Air samples were also collected outside of the chicken farms and adjacent to the beef cattle feedlot adjacent to Farm C, and no *C. jejuni* was isolated from these samples. As the air sampling strategy was designed to collect air samples that corresponded to the chicken sample acquisition schedule it was not possible to evaluate the impact of predominant weather conditions in SWA on airborne dissemination of *C. jejuni* (e.g. collection of air samples during on dry periods and on windy

days). It is expected that *C. jejuni* may be more commonly recovered from air during such period due to the liberation of dry aerosols from cattle feces. Furthermore, the implementation of a dedicated sampling strategy for arthropods and mice (e.g. as a function of distance from potential reservoirs such as feedlots) is necessary to ascertain the role of these organisms as important vectors of *C. jejuni* in SWA.

Campylobacter jejuni have been shown to be transmitted to non-infested chickens during transportation in trucks (Franchin et al., 2005; Ramabu et al., 2004) and during carcass processing in abattoir (Elvers et al., 2011; García-Sánchez et al., 2017; Rasschaert et al., 2007; Seliwiorstow et al., 2016). Due to restrictions placed on the collection of samples from broiler transport trucks and to different locations within the abattoir we were unable to ascertain key aspects of the transmission process. For instance, it is unclear whether the meat from chicken deemed free of *C. jejuni* were infested with the bacterium that was introduced into the abattoir on a daily basis or whether a resident population of *C. jejuni* subtypes exists at different locations within the abattoir. For the latter, it is possible that specific subtypes of *C. jejuni* are able to persist in the abattoir environment. For example, *C. jejuni* subtypes that readily form biofilms, are tolerant of oxygen exposure, and/or are less susceptible to sanitizers may differentially persist within the abattoir and subsequently infest poultry meat. It is also plausible that CRS differentially possess phenotypes that are able to persist extra-intestinally in the abattoir and on meat, and thus are more commonly ingested by people (i.e. rather than possessing virulence factors). This possibility is consistent with findings of recent study that compared genes present in CRS versus non-CRS of *C. jejuni* (Buchanan et al., 2017; Griekspoor et al., 2015). They determined that genes potentially linked to persistence were differentially present in CRS. Importantly, the isolates recovered and characterized in the current study are excellent candidates for subsequent experimentation to address knowledge gaps pertaining to transmission mechanisms.

The ability to assess risk posed by *C. jejuni* recovered from the poultry production continuum is a unique aspect of the current research. Given that there are no recognized pathogenicity/virulence factors present in *C. jejuni* (other than flagellar motility, which is exhibited in all isolates), a different strategy was required to ascertain human risk. In the current study, a model agroecosystem approach within a One Health framework was applied. To ascertain risk, subtypes of *C. jejuni* that readily infected human beings living in SWA were deemed to be CRS. The assumption underlying the CRS designation is that *C. jejuni* isolates recovered from diarrheic people were responsible for inciting enteritis in these individuals. Simply isolating a known pathogenic organism from a sick individual does not prove etiology. However, in a recent study conducted in SWA, it was observed that significantly more diarrheic people (9.1%) were positive for *C. jejuni* than a non-diarrheic people (1.9%) (Inglis et al., 2019). Notably, two healthy control people were culture-positive for *C. jejuni*. *Campylobacter jejuni* can be readily isolated from asymptomatic people in some developing countries, particularly in children (Figueroa et al., 1989; Lee et al., 2013; Mason et al., 2013). The bacterium has also been isolated from a low number of control individuals in Europe; for example, 0.5% (n=3) of 665 control individuals were positive for *C. jejuni* in the Netherlands (i.e. compared to 1.1% of diarrheic individuals), and 0.6% (n=14) of 2,264 control individuals were positive

for the bacterium in England. One possibility is that the *C. jejuni* strains infecting asymptomatic people do not incite enteritis. Another possibility is that such people possess adaptive immunity to the strain they are infected with, and by neglecting to account for this may lead to under estimation of exposure and infection (Havelaar et al., 2009). Regardless of the limitations of the CRS strategy, it is an effective way to ascertain risk in an epidemiological context. Furthermore, SWA is an ideal locale to apply this strategy given the high rates of campylobacteriosis coupled with intensive livestock agriculture and the accession of a very large number of *C. jejuni* strains from diarrheic people within the C^3GFdb.

5.3. Future research

Campylobacter jejuni subtypes are readily isolated from farm animals, wild animals and environment in Alberta and elsewhere in Canada (Griekspoor et al., 2015; Kaakoush et al., 2015). However, only a subset of subtype represent a significant public health risk. Results of the current study showed that CRS of *C. jejuni* are associated with livestock species primarily cattle and chicken in SWA. Campylobacteriosis in SWA that is linked to *C. jejuni* subtypes primarily associated with cattle reservoirs is thought to be the result of occupational contact, whereas CRS of *C. jejuni* infecting people that are primarily associated with poultry are predominantly thought to be linked to consumption of poultry meat (Inglis et al., 2011). However, a salient finding of the current study is that CRS present in both reservoirs are involved in the complex epidemiology of campylobacteriosis. Future research to explore the relationship between *C. jejuni* associated with cattle, chickens, and people is warranted. Such research should take a multipronged approach. This could include an epidemiological approach in which *C. jejuni* subtypes associated with cattle, chickens, and humans is explored on a regional and national basis (see below). This should be coupled with comparative genomic approaches (e.g. Genome-Wide Association Studies; GWAS), as well as empirical approaches to elucidate the mechanisms by which *C. jejuni* are transmitted from cattle to chickens, and ascertaining the rates and mechanisms of GIT colonization by relevant subtypes in broilers. It is noteworthy, that conducting such studies would deliver valuable information in support of the source attribution hypothesis for *C. jejuni.*

The recently completed Microbiological Baseline Study (MBS) conducted by the Canadian Food Inspection Agency in partnership with the Public Health Agency of Canada involved the snapshot sampling of poultry across Canada (all provinces), the isolation and quantification of *C. jejuni*, and subtyping of recovered *C. jejuni* isolates by CGF (E. Taboada pers. comm.). The MBS is the most comprehensive surveillance project on the Canadian chicken supply chain conducted to date. The collection of characterized *C. jejuni* isolates (n=2,820) obtained by the MBS provides a valuable strain resource that will enhance the C^3GFdb (currently contains >9,000 *C. jejuni* isolates from chickens). Furthermore, analyzing the MBS strain information with the strain data obtained in the current longitudinal study in SWA would be expected to provide additional insight on important reservoirs, transmission mechanisms, and risk at a national level of resolution. Such an analysis would allow the selection of relevant CRS and non-CRS of *C. jejuni* to be whole genome sequenced and GWAS conducted to identify genetic markers predictive of CRS for diagnostics and effective vaccine development. Furthermore, the

data would be instrumental in ascertaining the factors contributing to resistance to antibiotics in *C. jejuni*. It in noteworthy that resistance to fluoroquinolone antibiotics in *C. jejuni* has been identified as a major risk to human health by the World Health Organization, (World Health Organization, 2017) and resistance to fluoroquinolones (e.g. ciprofloxacin and enrofloxacin) in clinical *C. jejuni* is increasing significantly in SWA, but the reasons for this increase are currently enigmatic (D. Inglis, pers. comm.). Interestingly, resistance to antibiotics in *C. jejuni* associated with cattle in feedlots using comparative analysis of population structures reported that increasing resistance rates to ciprofloxacin but not to tetracycline were subtype-specific (Webb et al., 2018). It would be beneficial to examine mechanisms of resistance develop in *C. jejuni* (e.g. to fluoroquinolones) as component of molecular epidemiological, genomic, and empirical approaches.

Applying an experimental poultry infection model would be exceptionally valuable to answer many questions regarding GIT colonization by CRS of *C. jejuni* in broiler chickens. Broilers could be inoculated with relevant *C. jejuni* subtypes alone and in combination (e.g. CRS exclusively associated with cattle versus those found in both cattle and chickens). Whole genome sequence information could be used to design strain-specific primers and probes to temporally characterize the GIT colonization process. The experimental poultry model could be used to clarify whether the GIT colonization process is a strain specific phenomenon, and the degree to which and why there is a dose threshold requirement for colonization success (e.g. by different *C. jejuni* subtypes). Furthermore, these studies could elucidate key phenotypic characteristics that allow CRS to colonize chicken intestine, persist extra-intestinally, and subsequent disseminate through flocks. It would be also promising to investigate the ability of selected *C. jejuni* subtypes to competitively colonize chicken GIT and to identify if there is specific intestinal niches for *C. jejuni* subtypes. Subsequently, populating chicken intestine with an optimal microbiota that persistently occupy niches colonized by CRS of *C. jejuni* may prove to competitively exclude the bacterium from the chicken GIT. Finally, the experimental chicken infection model could be used to ascertain why young birds are not observed to be colonized in production farms, and whether this is a strain-specific phenomenon.

Empirical chicken models could also be used to study transmission mechanisms. Inter-bird transmission of *C. jejuni* (i.e. horizontal transmission) could be examined by inoculating relevant *C. jejuni* subtypes in an experimental mini-chicken flock. For example, a single marked bird could be inoculated with a few specific *C. jejuni* subtypes and introduced to flock, and then the transmission between and within flock could be monitored. It also would be interesting to see how these subtypes are transmitted within farm environment by experimentally introducing *C. jejuni* subtypes in samples such as insects, food, and water. For example, flies infested with specific *C. jejuni* subtypes could be introduced into the mini-flock (i.e. in cages within netting that preclude entry and exit of flies), and transmission of the subtypes temporally measured within birds using strain-specific primers. Moreover, the mechanisms by which beef cattle might serve as a reservoir of *C. jejuni* subtypes infecting poultry could be examined using an experimental feedlot, such as the feedlot infrastructure located at the AAFC Lethbridge

Research and Development Centre. For such a study, the presence of *C. jejuni* subtypes associated with cattle, arthropods and rodents in proximity to the feedlot, and *C. jejuni* liberated in air could be examined as a function of a variety of parameters (e.g. arthropod and rodent densities, temperature, precipitation, fecal and soil moisture, wind speed and direction). The situation of mini-flocks of broilers in proximity to the feedlot, and monitoring their colonization in conjunction with the previous metrics could be included as a component of this experiment. Importantly, the above experimentation would be designed to obtain evidence that cattle are primary source of *C. jejuni* infection contaminating chickens. Such information would be instrumental in designing effective biosecurity programs.

A primary finding of the current study is that the abattoir is the primary step at which CRS of *C. jejuni* infest carcasses and meat. Currently there are no quality control programs for *C. jejuni* in slaughter houses in Alberta. Future studies should obtain samples throughout the slaughtering process, and apply comprehensive isolation strategies with subtyping of *C. jejuni* isolates. Importantly, samples should be obtained from each step of continuum starting with birds entering the abattoir (transport trucks, transport crates, worker gloves) and ending with the packaging room. As observed in the current study, determining if the birds are free of *C. jejuni* subtypes (or not) before transport to the abattoir provides a key opportunity to ascertain contamination during transport and/or processing. Thus, the sampling of broiler farms late in the production cycle would be an invaluable addition to the experiment. Applying this approach is a prerequisite to achieve effective HACCP analysis against *C. jejuni*. Importantly, the information gained in the current study will allow the design of effective sampling strategies (e.g. to complete power analysis to ascertain effective sample sizes). Despite the crucial information that this experimentation would deliver, conducting such a study is expensive, and both logistically and scientific challenging. It requires the participation of multiple producers who are willing to allow sampling of birds at or near the end of the cycle, bird catchers, and transporters, and abattoir management and workers. Sampling birds, carcasses, and meat all represent potential losses to the individuals and companies involved (e.g. due to delays in processing), and achieving this comprehensive sampling regimen necessitates buy-in from all parties, which is challenging to achieve as was experienced in the current study. Crucially, effective mitigation of *C. jejuni* must be promoted as a positive for the sector, and that the research will deliver key and tangible knowledge to stakeholders to allow them to cost-effectively reduce risk and promulgate the sector as a producer of safe products. That only a subset of *C. jejuni* subtypes represent a risk to human beings (i.e. risk cannot be applied at the species level of resolution) is a major deliverable of the current study, and this will be instrumental in gaining support from the sector to conduct ancillary experimentation to achieve effective mitigation of *C. jejuni* moving forward.

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