A Canadian farm-to-fork quantitative microbial risk assessment of ciprofloxacin-resistant *Campylobacter* spp.

by

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Abstract

Antimicrobial resistance (AMR) is one of the largest public health threats of the 21st century with 4.95 million global deaths associated with bacterial AMR in 2019. Continued spread of bacterial AMR threatens the possibility of a post-antimicrobial era, where humans and animals may succumb from previously treatable illnesses. Widespread and persistent ciprofloxacin-resistant (CIPR) *Campylobacter* has been noted in humans and food-producing animals, especially broiler chickens (chickens raised for meat). *Campylobacter* itself is an extremely prevalent foodborne bacteria causing over 95 million global cases of gastroenteritis annually. Ciprofloxacin-resistant *Campylobacter* illnesses may have an elevated probability of more intense symptomology with increased risk of treatment failure in immunocompromised or vulnerable patients.

The objective of this thesis was to model transmission of CIPR *Campylobacter* along the farm-to-fork pathway of broiler chicken and estimate incidence of consequent CIPR illness in Canada. The quantitative microbial risk assessment (QMRA) framework was adopted, and four principal tasks were accomplished: an exposure assessment, a dose-response model (DRM), a risk characterization, and model analysis.

The exposure assessment was constructed with nine nodes tracing the broiler farm-to-fork pathway and modeled using stochastic processes and Monte Carlo simulations. At each node, the prevalence among all chickens with external *Campylobacter* and CIPR *Campylobacter* was estimated, and the corresponding concentrations per bird. Changes to prevalence and concentration at each node were modeled from peer-reviewed literature and federal surveillance data. The final estimate was the probability distribution of consuming a serving of broiler meat

with *Campylobacter* or CIPR *Campylobacter* and how much *Campylobacter* and CIPR *Campylobacter* was ingested.

A novel AMR dose-response model was constructed, adapted from a recently proposed framework. In cases of no prior ciprofloxacin use or in cases of CIPR *Campylobacter* exposure in the presence of ciprofloxacin, the traditional beta-Poisson DRM was used with parameters determined by a previous QMRA. The novel model was used in cases of prior ciprofloxacin use and exposure to ciprofloxacin-susceptible *Campylobacter*, for which new shape parameters were determined using pharmacodynamic data. An inequality comparing survival probabilities of CIPS *Campylobacter* versus CIPR *Campylobacter* was used to predict if an infection would be resistant to ciprofloxacin. A conditional probability was used to further estimate the probability of symptomatic illness given an infection.

The risk characterization had two major metrics, estimated for both overall *Campylobacter* and CIPR *Campylobacter*: probability of illness from one random serving of chicken and the incidence of illness in Canada per 100,000 population. The former was completed through pairing the exposure assessment outputs with the DRM to determine the probability of illness from a serving. Multiplying this probability of illness per serving by the total number of servings consumed annually and scaling by Canadian population yielded the estimated incidence illnesses.

Lastly, sensitivity and scenario analyses were performed on the model described above. The sensitivity analysis used conditional medians to determine which data inputs were most influential in determining the final probabilities of overall and CIPR *Campylobacter* illness per one serving. Additionally, seven hypothetical scenarios were independently applied to the

baseline model to simulate potential procedure changes and resulting incidences were compared to the baseline to determine intervention efficacy.

The estimated probabilities of total and CIPR *Campylobacter* illness from any serving of chicken were 0.015% (90% CrI: 0.000082-3.1%) and 0.002% (90% CrI: 0.000012-0.44%), respectively. The estimated incidence per 100,000 population for total and CIPR illness were 1,101 (90% CrI: 6-223,000) and 143 (90% CrI: 1-31,500), respectively.

The sensitivity analysis revealed that the temperature during cooking, within-flock spread during transportation to the abattoir, and within-flock contamination during evisceration were most influential in determining probability of overall illness per serving. The fraction of flocks colonized with CIPR *Campylobacter* was most critical in determining if an illness from a serving would be CIPR. Reducing the prevalence of contaminated birds leaving the abattoir, limiting flock colonization, and reducing consumer mishandling were most effective at reducing overall incidence. No scenario significantly altered CIPR illness incidence.

Additionally, this thesis compares our findings with published literature to assess the validity of the model. Knowledge gaps in the field are identified and directions of future work are discussed. Ultimately, this model provides unparalleled insight on transmission of CIPR *Campylobacter* to the Canadian public and guidance for risk managers and policy makers.

Preface

This thesis is an original work by Dana Tschritter. Parts of this work have been previously presented at various conferences (below). No part of this work has been previously published in peer-reviewed journals. A manuscript on the novel dose-response model was submitted for review to Epidemiology and Infection on February 28, 2022.

1. Tschritter D, Carson CA, Murphy CP, Smith BA, Otten A, Reid-Smith RJ, Li Q, Ashbolt NJ, Otto SJG. Modeling human exposure to fluoroquinolone-resistant *Campylobacter*. Poster presented at: This is Public Health Week; November 4-8, 2019; Edmonton, Canada.
2. Tschritter D, Smith BA, Reid-Smith RJ, Li Q, Ashbolt NJ, Carson CA, Murphy CP, Otten A, Otto SJG. Modeling human exposure to and risk from fluoroquinolone-resistant *Campylobacter* from retail chicken in Canada. Oral presentation at: Conference of Research Workers in Animal Diseases; December 4-8, 2020; virtual.
3. Tschritter D, Li Q, Smith BA, Reid-Smith RJ, Carson CA, Murphy CP, Ashbolt NJ, Otto SJG. Quantitative risk assessment of ciprofloxacin-resistant *Campylobacter* in Canadian chicken meat. Oral presentation at: One Health Antimicrobial Stewardship Conference; March 10-12, 2021; virtual.

“All models are wrong, but some are useful”

- George E. P. Box

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Lastly, this work exists because of my father's tireless technical support, my mother's thoughtful care packages, and my sister's begrudging mathematics knowledge, and ultimately, because of their love. Thank you.

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List of Acronyms and Abbreviations

AC	Air chilling
ADI	Acceptable daily intake
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ANSES	The French Agency for Food, Environmental and Occupational Health & Safety
AST	Antimicrobial susceptibility testing
BFP	Between-flock prevalence
CAC	Codex Alimentarius Commission
CC	Immersion chilling with chlorinated water
CDC	Centers for Disease Control and Prevention
CFIA	Canadian Food Inspection Agency
CFU	Colony forming unit
CH	Chilling
CK	Cooking
CS	Cold storage
CIPR	Ciprofloxacin-resistant
CIPS	Ciprofloxacin-susceptible
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
DDD	Defined daily doses
DF	Defeathering
DP	Depopulation

DRM	Dose-response model
EV	Evisceration
FAO	Food and Agriculture Organization
FDA	United States Food and Drug Administration
FLQR	Fluoroquinolone-resistant
GI	Gastrointestinal
GBS	Guillain-Barré Syndrome
HS	Hard scald
IE	Intervention efficacy
LC	Log change
MIC	Minimum inhibitory concentration
MRA	Microbial risk assessment
N flock	Flocks with no birds colonized by *Campylobacter* at the time of depopulation
OR	Odds ratio
P flock	Flocks colonized with *Campylobacter*, regardless of antimicrobial resistance
PCU	Population correction unit
PD	Pharmacodynamic
PHAC	Public Health Agency of Canada
QMRA	Quantitative microbial risk assessment
QRDR	Quinolone resistance determining region
R flock	Flocks with at least one bird colonized with CIPR *Campylobacter*
S flock	Flocks colonized with CIPS *Campylobacter*
SC	Scalding

SNP	Single nucleotide polymorphism
SS	Soft scald
TR	Transportation
TxR	Treatment resistant
TxS	Treatment susceptible
WA	Washing
WC	Immersion chilling with water only
WFP	Within-flock prevalence
WHO	World Health Organization
XC	Cross-contamination in the kitchen

Chapter 1: Literature Review and Objectives

1.1. Campylobacter

Campylobacter was first isolated in 1906 from sheep but was not taxonomically classified until 1927, when it was filed under the genus *Vibrio*.[1,2] Sebald and Véron proposed a new genus in 1963 named *Campylobacter*.[3,4] However, it would take another ten years and the work of Véron and Chatelaine in 1973 for the name to be widely accepted within the scientific community.[3,5] In the same year, Butzler *et al.* proposed *Campylobacter* as being a cause of human gastroenteritis, piquing the interest of the human medicine community and inspiring further research.[3,6] Today, the genus *Campylobacter* has 24 known species, and an additional ten subspecies, and is widely understood as one the major culprits of foodborne illness in the world.[7–9]

Campylobacter species are curved or spiral-shaped, motile, Gram-negative bacteria and can be separated into four broad groups: thermotolerant *Campylobacters*; those that infrequently cause human disease and are associated with livestock; those implicated in periodontal disease; and those not associated with human illness.[8] The former grouping, thermotolerant *Campylobacters*, are the most relevant to human medicine and consists of *Campylobacter jejuni* (including the subspecies *C. jejuni jejuni* and *C. jejuni doylei*), *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis*.[8] These *Campylobacters* proliferate easily between 37-42°C but have been shown to survive for long periods of time in temperatures as low as 4°C.[2,8,10] Additionally, these bacteria grow best between pH levels of 6.5-7.5 and in microaerobic conditions (5% O_2, 10% CO_2, 85% N_2), although recent reports have noted an emerging tolerance of aerobic conditions.[11,12]

Two species predominate in initiating animal colonization and subsequent human infection: *C. coli* and *C. jejuni*, hereafter collectively referred to as *Campylobacter* spp.[7] *C. jejuni* is more frequently the culprit of clinical human infection but the frequencies of *C. coli* and *C. jejuni* colonization among animal populations vary by species. In chickens, one of the largest exposure sources of *Campylobacter* spp. to humans, *C. jejuni* also predominates. Recent speciation studies have reported about three quarters of *Campylobacter* found in broiler caeca and on carcasses during production are *C. jejuni*, with the remaining one quarter predominately identified as *C. coli*.[13–15] The same pattern is found in cattle, however research with swine samples has reported *C. coli* is the primary species of *Campylobacter*.[16]

The ability of *Campylobacter* spp. to colonize the intestinal tract of agriculturally important animals and exist within the natural environment, particularly surface water, is a critical feature in its pervasiveness and resilience.[7] Some researchers have indicated that *Campylobacter* spp. is essentially ubiquitous among broiler flocks worldwide.[17–19] This dominance is reflected by source attribution and sequence typing of human campylobacteriosis infections. Two-thirds of human cases can be genetically identified as being from poultry.[20–22] These studies also indicate approximately one-fifth of human *Campylobacter* illness may have originated from cattle sources.[20,21] This corroborates exposure studies, which conclude that the human exposure for *Campylobacter* spp. is in the form of undercooked poultry meat and unpasteurized milk.[7]

Campylobacter spp. has consistently been ranked as one of the most pervasive foodborne pathogens globally, initiating the gastrointestinal illness campylobacteriosis. In 2015, the World Health Organization (WHO) reported over 95 million cases of campylobacteriosis occur yearly around the world, or 1,390 per 100,000 population.[23] While considered common in both

developing and developed nations, the epidemiology of campylobacteriosis in these environments are quite different.[23] This thesis focuses on scenarios that are similar to the Canadian context and therefore will not discuss exposure and illness in developing regions. Campylobacteriosis has consistently ranked the most common bacterial foodborne illness in the European Union since 2005, with 64.1 cases per 100,000 population in 2018.[24] In the case of Germany in 2014, this represented 71% of all reported intestinal bacterial infections.[7] Similar reports are found in North America, with 19.5 laboratory-confirmed cases per 100,000 in the United Stated in 2019 and 27.6 cases per 100,000 population in 2018 in Canada.[25,26] These reported incidences are likely significantly under-estimated as the majority of infected people will not seek medical treatment.[27] In 2013, Thomas *et al.* estimated the Canadian incidence of campylobacteriosis to be approximately 447 cases per 100,000 population.[27]

Campylobacteriosis typically presents as mild to moderate diarrhea, fever, and abdominal cramping.[7,28] An incubation period of 24-72 hours is common, but it may take up to 7 days for illness to appear, with lower magnitudes of exposure possibly extending this incubation period.[28,29] Interestingly, a clear association between magnitude of exposure (i.e., ingested dose) and likelihood of subclinical illness has been documented, however the same relationship has not been found for exposure and likelihood of clinical illness.[28,30] Illness is usually self-limiting, with symptomology peaking between 24-48 hours following onset. [28,29] Approximately 1% of cases in the United States between 1998 and 2009 required hospitalization with <1% of these hospitalizations resulting in death.[28] Rarely, other serious health conditions can be directly linked to *Campylobacter* spp. infection. Most importantly, this includes Guillain-Barré Syndrome (GBS), a neurologic disease that causes ascending paralysis and can lead respiratory immobilization and death. [31,32] While only approximately <1 GBS case develops per 1,000

campylobacteriosis cases, this represents 30% of all GBS cases. [31] Additionally, GBS cases that develop from *Campylobacter* spp. infections are often more intense and the patient has an increased likelihood of requiring mechanical ventilation and acquiring permanent neurologic damage.[32]

Looking toward the future, it is essential we understand the nature of *Campylobacter* spp. transmission and associated emerging threats. While research connecting the climate crisis and foodborne illness is limited, a recent model predicting future changes to campylobacteriosis incidence in Nordic countries reported a possible 1,484 excess cases due to climate change per 100,000 population annually by the year 2089.[33] When taken in conjunction to the growing frequency of antimicrobial resistance in *Campylobacter* spp. infections (discussed below), there are major concerns and huge areas of uncertainty regarding what the rest of the century may look like in terms of the epidemiology of *Campylobacter* spp.

1.2. Fluoroquinolones

Fluoroquinolones are a class of antimicrobials that developed from their parent class, quinolones, in 1976, with the discovery of flumequine.[34] In the 1970s and 1980s, researchers discovered that the addition of a fluorine atom at position 6 of the 4-quinolone skeleton significantly increased the drug's antimicrobial properties (Figure 1.1).[35–37] Quickly, additional fluoroquinolones were developed and deployed into human and veterinary medicine, particularly ciprofloxacin and enrofloxacin, respectively.[34] These emerging antimicrobials were enthusiastically adopted for therapeutic use due to their impressive broad-spectrum of activity against Gram-positive and Gram-negative bacteria, and improved pharmacokinetics, like increased oral bioavailability.[34,38]

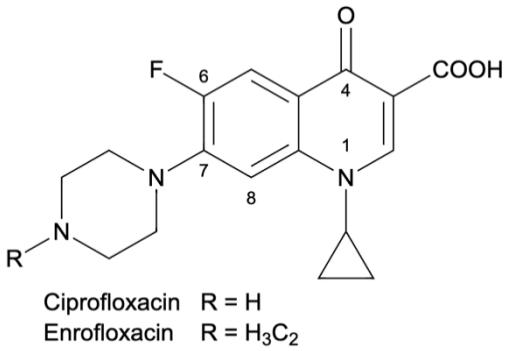

Figure 1.1. The general second-generation fluoroquinolone chemical structure with R groups listed for ciprofloxacin and enrofloxacin.
Fluoroquinolones are distinguished from quinolones by the addition of a fluorine atom at position 6. Second-generation fluoroquinolones are distinguished by a piperazine ring at position 7.[37]

Fluoroquinolones selectively target bacterial enzymes that human cells lack: DNA gyrase and topoisomerase IV.[36] It is believed that this dual targeting is partially what allows fluoroquinolones to have a broad-spectrum of activity, with anti-DNA gyrase activity primarily targeting Gram-negative species, like *Campylobacter*, and anti-topoisomerase IV activity working mostly against Gram-positive bacteria.[36,38] DNA gyrase is an essential enzyme in the negative supercoiling of DNA that occurs during bacterial cell replication.[35,36] DNA gyrase works to induce negative twists ahead of the replication fork, or in lay terms allows for DNA to unwind, so that genetic replication processes may continue along the chromosome.[35] Meanwhile, topoisomerase IV is responsible for delinking the newly replicated daughter chromosomes, a process called decatenation, so that cell division may conclude.[36] Without either of these essential functions, the bacterial cell would fail to replicate and rapidly die thus creating the intended effect of fluoroquinolone drugs.

Quickly after their discovery, fluoroquinolones, especially ciprofloxacin and norfloxacin, displayed a broad array of bacterial species against which they were effective. These include, but

are not limited to, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Hemophilus influenza*, *Neisseria* spp. (including *Neisseria gonorrhoeae*), *Staphylococcus aureus*, and *Escherichia coli* and *Campylobacter* spp.[34,38] Oral administration of fluoroquinolones also showed improved bioavailability and wide distribution in mammalian tissues and body water.[34] This widespread distribution throughout the body and a vast array of target bacteria made fluoroquinolones a favoured class of antimicrobial agents in human medicine. In the late 20th century, they were often prescribed for urinary tract infections, sexually transmitted infections, respiratory infections, and bacterial gastroenteritis.[34,38]

Today, these trends of fluoroquinolone use have changed drastically due to the emergence of resistant strains in many species of bacteria. Despite early warnings of growing resistance to fluoroquinolones, their prescriptions in Canada rose between 1995 and 2010.[39] Increased ciprofloxacin prescribing contributed considerably to this trend, with a rise from approximately 0.11 to 0.18 prescriptions per 1,000 inhabitant-days during this time period.[39] This reported increase in use seems to be driven by fluoroquinolone prescription dispensing at community or retail pharmacies, as opposed to in-hospital use, where use is reported to have significantly dropped by 42% from 2009 and 2016.[40,41] Meanwhile, prescriptions from Canadian community pharmacies have increased from 2007 to 2018.[41,42] Fortunately, ciprofloxacin consumption in more recent years showed a slight downward trend with approximately 1.17 defined daily doses (DDD) per 1,000 inhabitant-days in 2014 and steadily declining to 0.9 in 2017.[43] However, overall, it remains the fifth most prescribed antimicrobial in Canada, and the second most common in those over the age of 60.[42,43]

Naturally, fluoroquinolone use in veterinary medicine paralleled the quick uptake in humans in the 1980s and 1990s, with the majority of animal use in food-producing animals.[41]

However, national Canadian surveillance of use in animals only began in 2006 and assessing the frequency of antimicrobial use, particularly fluoroquinolone use, before this time can be quite difficult.[41] Use of fluoroquinolones in animals rose 40% between 2010 and 2014, with use peaking in 2015 at 860 kg.[41,42] Since then there has been a 21% decrease to 677 kg in 2018.[42] While specific numbers are not reported, the Public Health Agency of Canada reports that these trends remain consistent with using either the European weights or Canadian weights for scaling by population correction units (PCU).[42]

When discussing antimicrobial use in broiler flocks, particularly fluoroquinolones, it is essential to mention the recent history of regulations and policies restricting veterinary use of these drugs. In 2005, the United States Food and Drug Administration (FDA) made a landmark ruling prohibiting use of all fluoroquinolones in American poultry production, effectively eliminating fluoroquinolone use in flocks.[44] Canada followed suit in 2014 when an industry-led decision from the Chicken Farmers of Canada restricted all 'Category I' antimicrobials (those listed as very high importance and critical to human health, including fluoroquinolones) from prophylactic use in broiler production.[45,46] In 2018, fluoroquinolones represented only 0.07% of all veterinary antimicrobial use in Canada, a proportion which has remained relatively constant for the past decade.[41,42] This sweeping restriction and the fact that over 80% of antimicrobial use in broiler production was specifically used preventatively helps explain why reported use of fluoroquinolones in broiler production has become quite rare.

Therefore, the most predominant classes of antimicrobials used in broilers flocks are bacitracins, streptogramins, trimethoprim-sulfas, and orthosomycins.[43] In Canada, approximately 150 mg per PCU of antimicrobials were used in 2014 with a decline to 130 mg per PCU in 2017.[43] Antimicrobial use in broiler flocks continue to be largely for disease prevention,

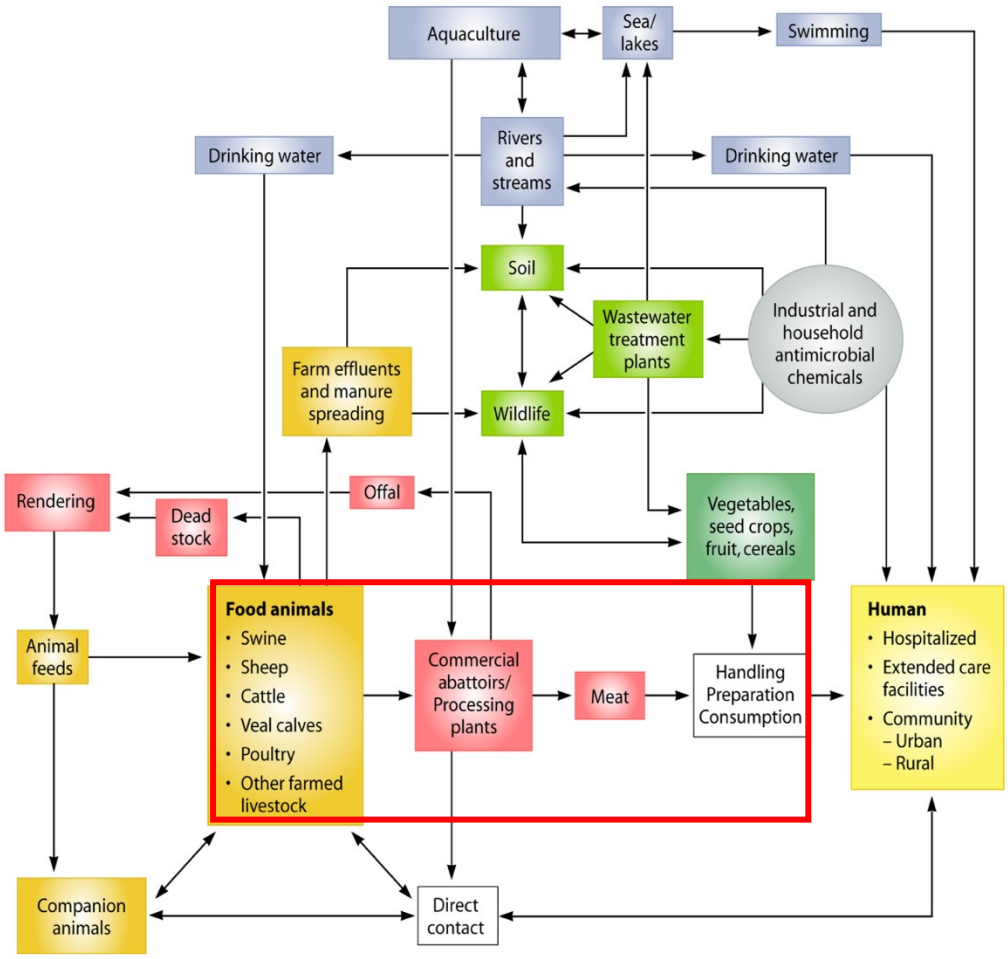

Figure 1.2. A schematic exemplifying the pathways through which bacteria, and therefore their antimicrobial resistant genes, travel.
Captured in the red box is the pathway of interest in this thesis.
Adopted from Davies & Davies.[47]

particularly necrotic enteritis caused by *Clostridium perfringens* and is primarily administered as a feed additive.[41]

While still widespread in human medicine, the drastic decrease of fluoroquinolones to merely 0.07% of all animal antimicrobial use is astonishing. Despite their miraculous achievements in human and veterinary medicine, many now consider the possibility of a time when antimicrobials will no longer be usable. To understand the drivers of this decrease and the continued essential use in human medicine one must turn attention to the rising tide of

antimicrobial resistance, which has had sweeping and permanent changes on how clinicians and researchers approach infectious disease treatment, prevention, and food safety.

1.3. Antimicrobial Resistance

The first major event of antimicrobial resistance (AMR) related disease occurred during the Second World War. The first class of commercially-available antimicrobials, sulfonamides, had been launched into therapeutic use in 1937 and clinicians in military hospitals noted sulfonamide-resistant *Streptococcus pyogenes* emerging only a few years later during the war, a time of intensive antimicrobial use.[47,48] Even Sir Alexander Fleming, the physician credited with discovering penicillin in 1928, warned of the possibility of antimicrobial resistance in cases of improper administration in his Nobel Prize lecture in 1945.[49] Antimicrobial resistance is not a new concept. However, for many decades the appropriate level of funding, surveillance, and government action did not meet what experts were calling for.[50] Humanity has now reached a time where AMR is recognized as a major threat to human health and one whose solutions must be international and multidisciplinary.[50]

It is difficult to characterize a historic global trend of AMR. Not only were formal surveillance programs of these bacteria extremely scarce in the first several decades of antimicrobial use, but surveillance continues to lack coordination and completeness.[50,51] Additionally, different economic and pharmacologic policies and practices in various regions have created vastly different landscapes of AMR and reported metrics.[52] To briefly summarize the consequences in Canada specifically, it has been estimated that 1 in every 180 hospital admissions suffers from an infection with AMR, with 20% of these cases being fatal.[42] The

Council of Canadian Academies estimates this to total 14,000 annual deaths in Canada due to AMR.[53]

Despite historically weak surveillance frameworks, it is well understood that shortly after the introduction of an antimicrobial into routine clinical use, resistant species will arise.[54] Such was the case with fluoroquinolone resistant (FLQR) bacteria; fluoroquinolones entered clinical use in the early 1980s and resistant species were detected before the end of the decade, particularly in *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[35,55,56] In particular, FLQR *Campylobacter* began to be isolated in mainland Europe in 1989, and was first detected in the UK in 1991.[57,58] Unlike in Europe in 2018, where approximately 59.3% of *Campylobacter* spp. samples were resistantly to fluoroquinolones, in Canada 23.9% of *Campylobacter* spp. isolates tested as FLQR, although this has dramatically risen from 2.4% in 2004.[59,60] Interestingly, antimicrobial resistant appears to be more common in *C. coli* rather than *C. jejuni*.[61,62] Otto *et al*.'s 1999-2006 data from Saskatchewan, Canada, exemplifies this trend well: 9.4% of *C. jejuni* samples were classified as ciprofloxacin resistant, while 15.5% of *C. coli* samples were ciprofloxacin resistant.[61]

The greatest predictor of the development of AMR is prior antimicrobial use (AMU). Often, we see resistant genes and bacterial strains emerge shortly after widespread use of an antimicrobial due to the natural evolutionary process of selection pressure.[47,63] While historically, the bacterial genome has evolved to favour those with survival advantages overtime, in the presence of antimicrobials, those cells showing any tolerance to the antimicrobial are rapidly selected.[47,63] Those with the structural defenses to survive amidst an antimicrobial, either through spontaneous mutations, acquired genes, or intrinsic properties, are able to vertically or horizontally pass on these genes.[63,64] Mobile genetic elements carrying genetic material with

bacterial AMR plays a recently understood but critical role in horizontal transmission of resistance.[47,63] In these cases, the bacteria are able to acquire new resistance genes from plasmids (conjugation), bacteriophages (transduction), or simply encountering these resistant genes in their environment (transformation).[63] Horizontal transmission of FLQR remains rare and often does not impart full resistance when shared, however it may contribute to some increased tolerance to fluoroquinolones and strengthen the bacteria for future selective pressures.[47]

On a cellular level, there are many mechanisms by which a bacterium may enact resistance to an antimicrobial agent. Innate immunity (otherwise known as intrinsic resistance) is the result of a natural structure or process belonging to a bacterium that precludes antimicrobial activity. Acquired resistance typically refers to gained resistance mechanisms by a once susceptible species and is largely the focus of AMR research.[64,65] Due to the multiplicity of possible mechanisms, they can be categorized broadly as such: limiting uptake of the antimicrobial; modifying the antimicrobial's target; inactivating an antimicrobial; antimicrobial efflux[65].

Species resistant to fluoroquinolones particularly rely on target modification, specifically mutations to the bacterial *gyrA* and *gyrB* genes which encode DNA gyrase, and *grlA* and *grlB* which encode topoisomerase IV.[65,66] These change the cells chromosome and mutate DNA gyrase and/or topoisomerase IV enough to prohibit fluoroquinolone binding while allowing the bacterium to survive.[64] Less commonly, fluoroquinolone resistance can be achieved through drug inactivation and increased efflux activity.[65,66] *Campylobacter* resistance to fluoroquinolones relies heavily on mutations to *gyrA*, particularly the single neucleotide polymorphism (SNP) of threonine to isoleucine at base pair 86 (i.e., T86I). Although other SNPs within the quinolone resistance-determining region (QRDR) of the *Campylobacter* chromosome which mutate DNA

gyrase have been reported, none are as globally pervasive at the T86I mutation.[59,67,68] Interestingly, recent research has demonstrated an antithetical gain in fitness due to *gyrA* mutations in *Campylobacter* and *Salmonella enterica*.[69–72] In the case of the T86I *gryA* mutation in *Campylobacter*, this gain of fitness has been shown consistent in *in vivo* scenarios as well as having the ability to entirely outcompete susceptible counterparts in the absence of fluoroquinolone selection pressure, theoretically due to changes in DNA supercoiling.[71,72]

In addition to the misuse and overuse of antimicrobials for human medicine being culpable for the development and spread of these AMR species, the role of animal health and the environment must also be considered. Antimicrobials are commonly used as growth promoters, prophylactics, and therapeutics in agriculture, aquaculture, plant pest control, and in household cleaning products.[47] The large and often uninhibited entry of antimicrobials into our environment has created the existence of environmental reservoirs of resistant species and their DNA (Figure 1.2).[47] In particular to broiler production, antimicrobials are less commonly used as growth promoting drugs but rather for prophylactic and treatment purposes.[73] In Canada, fluoroquinolones are not approved for use in broilers and their extralabel administration creates challenges in regulating and reporting its use.[61,73]

It has been demonstrated that a significant reason for the emergence of AMR is due to the heavy use of antimicrobials in veterinary practice, agriculture, and aquaculture, particularly in Europe.[63] Studies which examined the prevalence of ciprofloxacin resistant *Campylobacter* in poultry flocks before, during, and after treatment with fluoroquinolones demonstrably show a large amplification of the FLQR *Campylobacter* populations to nearly 100%, which then persists after treatment withdrawal, and ostensibly to continue to the consumer and enter the environment.[67,74] While improving surveillance and practices in the realm of human medicine is

critical in curbing the spread of AMR, due focus must also be placed on how these drugs are utilized within the animal sphere.

1.4. Quantitative Microbial Risk Assessment

A comprehensive risk analysis has three components: risk assessment, the most computationally complex piece describing the occurrence and severity of an adverse health affect due to a certain exposure; risk management, where risk assessment outcomes and policy alternatives are considered in the context of health protection and fair trade policy; and lastly risk communication, where stakeholders are consulted and the risk assessment outcomes and risk management decisions are interpreted and shared.[75–77] In 1999, the Codex Alimentarius Commission (CAC) published a guide codifying a structured framework for microbial risk assessment in an attempt to unify methodology and increase transparency of research.[77] The CAC acknowledges that ideally microbial risk assessments (MRA) should have a quantitative basis but in some instances data may be too limited to execute viable estimations of risk, and can therefore be further classified as either quantitative, semi-quantitative, or qualitative.[75–77] A quantitative microbial risk assessment (QMRA) can be defined as one that provides "numerical expressions of risk and … attendant uncertainties", while qualitative microbial risk assessments use risk rankings or descriptive categorizations created through expert opinions of limited numerical data.[75,76] The science behind microbiological risk analysis has evolved in the 20 years since original publication, but the basic framework behind the risk assessment components remains the same: hazard identification, exposure assessment, hazard characterization, and risk characterization (Figure 1.3).[77]

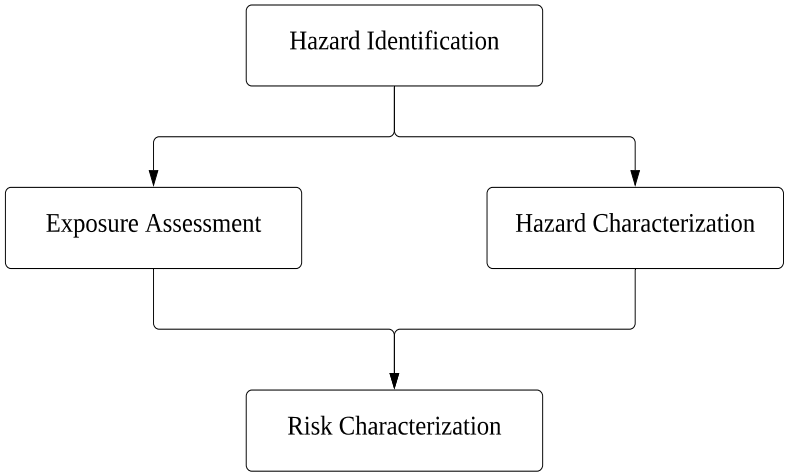

Figure 1.3. Risk assessment framework as proposed by the Codex Alimentarius Commission (CAC) in 1999.
Adopted from the Food an Agriculture Organization (FAO) & World Health Organization (WHO).[77]

Hazard identification serves as the qualitative stage where literature is collected on a certain microbe of interest and its consequences to a population in order to describe transmission routes and other pertinent characteristics.[75,78] Based on findings from the hazard identification and available data, an exposure assessment and hazard characterization can be conducted simultaneously. The main objective of an exposure assessment is to quantify the intensity and duration of exposure to the microbe of interest that a pre-defined population may experience.[78] The tools and metrics used to assess exposure quantities and pathways vary greatly and are highly dependent on the organism and context in question. However, the CAC specifies that frequency of contamination (i.e., prevalence or incidence over a time period or within a population) and level of contamination (i.e., magnitude of the exposure dose) are essential outputs in an exposure assessment.[75] In cases of a foodborne pathogens, a farm-to-fork exposure assessment may be used.[77] These models quantitatively track the path of a food product from its

lifespan on the farm through the chronological stages the product goes through before being prepared and eaten at a consumer's home.[77,79,80]

Completed in parallel to an exposure assessment, a hazard characterization aims to assess the consequences of exposure to the microbe of interest, generally in terms of severity and duration of consequences.[80] Dose-response models (DRMs) are the quantitative best-practice tool used in the hazard characterization, whereby some exposure amount (i.e., dose) is mathematically related to the probability of infection or illness (i.e., response) in an individual.[77,78] These can be extrapolated to estimate the likelihood of consequences on a population level over a time-period. In addition, pathogen infectivity and virulence, host susceptibility, population characteristics, and other infectivity characteristics can be taken into consideration.[30,77] As with the exposure assessment, many modeling decisions in the hazard characterization depend on the microbe and context being studied, as well as data availability.

The final component of microbial risk assessments as laid out by the CAC is risk characterization, or the pairing of the exposure assessment and hazard characterization models.[75] The magnitude of consequences caused by the organism given the specific exposure pathway studied are estimated.[75,78] Additionally, the uncertainty around these estimates can create a fuller picture of the likelihood and severity of illness from the microbe.[75,78]

In acknowledgement of the growing threat of foodborne antimicrobial resistant pathogens globally, the CAC published an update of microbial risk assessment guidelines specifically for consideration of such species with AMR.[81,82] Fundamentally, the risk assessment framework and guidelines are unchanged, but attention is called to the unique challenges and considerations regarding antimicrobial resistant organisms transmitted to humans through the foodchain.[82] In particular, the CAC stresses attention to the specifics of antimicrobial susceptibility of the

organism, the spread of resistance throughout an animal or crop population, selective pressure effects if these resistant organisms are exposed to antimicrobial treatments during production, probability and severity of treatment failures in clinically ill humans, and so on.[81,82]

According to Chapman *et al.* 10 QMRAs regarding risk from *Campylobacter* via broiler meat with a farm-to-fork lens have been conducted, as of 2016.[83] When not limited to a full farm-to-fork scope and updated to 2020, this number increases to 24 publications.[84] Haas *et al.* estimated that were 157 QMRA-related publications between 1998 and 2013.[78] Using Chapman *et al.*'s estimate, farm-to-fork *Campylobacter*-broiler QMRAs make-up approximately 6% of the total field of literature. Each of these analyses tackles a slightly different objective, scope, and/or geographic location or population, contributing to a broad understanding of how *Campylobacter* is transmitted to humans from broiler flocks via 'traditional' processing procedures.[85] Generally, the largest contributing sources of *Campylobacter* risk from the broiler farm-to-fork pathway comes from the level of contamination on the birds leaving the farm, the amount spread within the abattoir during defeathering and evisceration of the bird, and at-home preparation factors (e.g., failing to washing hands, cooking temperature etc.).[84–86] However, knowledge gaps remain. In particular, this includes sources of colonization of broilers with *Campylobacter* in the first place, cross-contamination transmission dynamics between birds and flocks during transportation and within the abattoir, as well as variations in at-home consumer behaviour when preparing the poultry meat.[83,85] (Discussed further in Chapter 4.)

These deficits in our understanding grow substantially when considering antimicrobial resistant foodborne pathogens. While several risk assessments of foodborne antimicrobial resistant pathogens exist, not all have a substantial quantitative basis and only some follow the entire farm-to-fork scope.[82,87] While this represents considerable progress in the field of risk

assessment, many assumptions and expert opinions remain necessary in modeling transmission and risk of organisms with AMR along the farm-to-fork pathway.[87] Given the urgency called for in AMR research, filling the knowledge gaps that exist in foodborne AMR risk is integral in stemming the spread of resistant infectious disease.[88–90]

1.5. Objectives

The purpose of this thesis is to develop and interpret a novel QMRA centred around ciprofloxacin-resistant *Campylobacter* spp. using a Canadian-based farm-to-fork framework of commercial broiler chicken production. This project aims to fill knowledge gaps that exist in the farm-to-form transmission of AMR *Campylobacter* spp., as well as clearly identify others that still exist. Addressing these data gaps will be a critical task for the food microbiology and veterinary epidemiology communities in the future, especially as genetically diverse and environmentally tolerant species of *Campylobacter* continue to emerge. Additionally, results from this QMRA will create evidence-based policy recommendations for government and industry, specific to the contemporary Canadian context.

This thesis will follow the QMRA framework proposed by the Codex Alimentarius Commission (Figure 1.3) and consist of four principal tasks, listed below.[75]

I. Estimate magnitude and prevalence of exposure to ciprofloxacin-resistant *Campylobacter* spp. from Canadian broiler chicken meat

A quantitative farm-to-fork exposure assessment will be constructed following the pathway of Canadian broiler meat. In the interest of scope, the pathway will begin at depopulation (the time at which birds are fully grown and ready to leave the farm) and will not

consider on-farm or hatching policies or practices. This exposure assessment will include the five abattoir stages most common in farm-to-fork exposure assessments (scalding, defeathering, evisceration, washing, chilling) and will conclude with a consumer-based model to represent at-home preparation and cooking. Stochastic processes and Markov chains will be used (discussed further in Chapter 2).

II. Modify an existing *Campylobacter* spp. dose-response model to account for ciprofloxacin resistance

For scope, this QMRA's hazard characterization has been pared down to the most integral quantitative component, the dose-response model (DRM). This DRM will utilize previous data collected from human feeding studies as well as incorporate a novel approach for considering antimicrobial resistance in dose-response modeling. Additional considerations made include an individual's likelihood of recent fluoroquinolone use, probability of a response being treatment resistant to fluoroquinolones, and likelihood of developing illness given a subclinical infection has been initiated.

III. Estimate probability of ciprofloxacin-resistant campylobacteriosis from one serving of Canadian-raised broiler meat and the incidence of resistant campylobacteriosis among the national population

This task will pair exposure results from the exposure assessment with the dose-response model to estimate risk of ciprofloxacin-resistant illness in the Canadian population. The final estimates of risk produced here will include the probability of illness from one serving of broiler meat and the number of illnesses per 100,000 inhabitants in Canada annually. Both measures of

risk will be estimated for total campylobacteriosis and specifically for ciprofloxacin-resistant campylobacteriosis.

IV. Analysis of the baseline model using a sensitivity analysis and scenario analysis

These analyses will help inform future surveillance and policy directions. The sensitivity analysis will reveal which data inputs are most impactful to the final risk estimate while a scenario analysis is able to evaluate the efficacy of potential interventions in reducing risk. In addition to these analyses, a general evaluation and summary of knowledge gaps in the realm of ciprofloxacin-resistant *Campylobacter* and broiler meat will be collected and presented.

Chapter 2: Methods

2.1. Exposure Assessment

A module-based exposure assessment was constructed that chronologically follows the broiler chicken farm-to-fork pathway in Canada. Two farm-to-fork quantitative microbial risk assessments (QMRAs) of *Campylobacter* on broiler meat were used as foundational models in the construction of the present model. The first was published by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) in 2009 and serves as a literature review and comprehensive assembly of the best-practice farm-to-fork QMRA techniques up to 2009.[30] The QMRA and associated report was published with the intention of being adaptable for use and analysis in other countries or with more tailored data points.[30] The second foundational QMRA is also a farm-to-fork QMRA for *Campylobacter* in broiler chicken, specifically representing the scenario in the United States, published in 2019 from Dogan *et al*.[91] With data gathered through a systematic literature review and compiled through meta-analysis, this currently represents the best available data for this QMRA.[91] The 2019 USA QMRA follows the same farm-to-fork exposure pathway as the 2009 WHO/FAO QMRA, and consequently is the pathway adopted here (Figure 2.1).

It should also be noted that neither of these QMRAs distinguish between *Campylobacter* species, grouping *Campylobacter jejuni* and *Campylobacter coli* under the term *Campylobacter* spp.[30,91] While there are some distinguishing epidemiologic features between these two species, biologically it is believed that *C. jejuni* and *C. coli* act nearly identically and can be studied together, as is frequently done. Additionally, QMRAs are very data-intensive, a feature that can be prohibitive. Often when studying *Campylobacter* spp., it is analytically efficient to group both

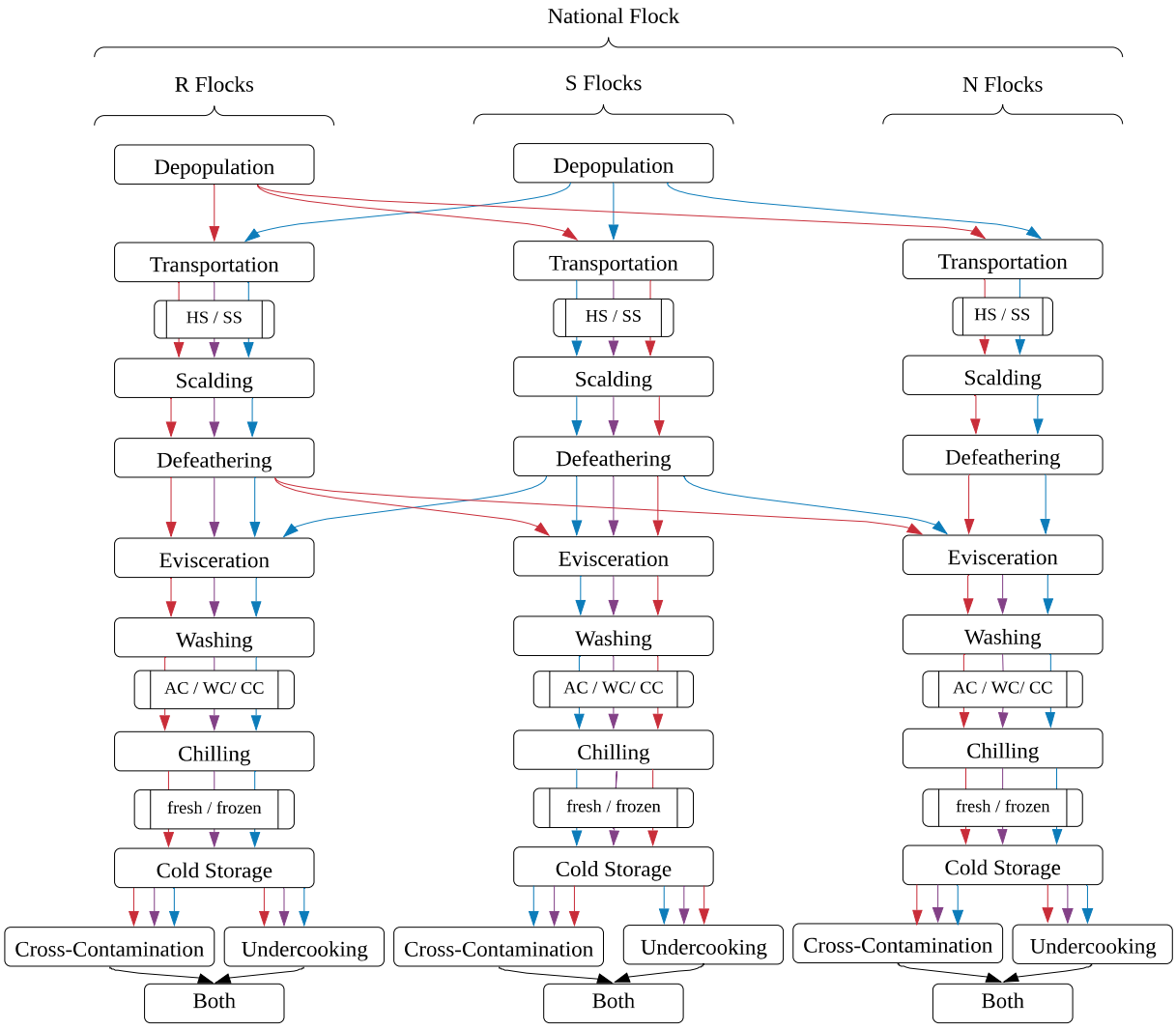

Figure 2.1. Schematic of the farm-to-fork exposure pathway, vertically separated by flock type. R flocks are those colonized by ciprofloxacin-resistant *Campylobacter* (CIPR); S flocks are those colonized by ciprofloxacin-susceptible (CIPS) *Campylobacter*; N flocks are those with no colonized birds

Red arrows denote transmission of CIPR only birds, blue arrows represent transmission of CIPS only birds, and purple arrows indicate 'mixed' birds, those carrying both CIPR and CIPS *Campylobacter*.

Note that by definition, N flocks do not have on-farm *Campylobacter* contamination, hence the omission of Depopulation for N flocks.

All cross-contamination, undercooking, and both nodes represent doses that are ingested by humans.

HS: hard scald; SS: soft scald; AC: air chill; WC: immersion chill with water; CC: immersion chill with chlorinated water.

C. jejuni and *C. coli* together. Knowledge gaps are further discussed in Chapter 4. Following suit with previous QMRAs, here *C. coli* and *C. jejuni* are pooled as *Campylobacter* spp., henceforth referred to as *Campylobacter*. Based on the aforementioned QMRAs, the current exposure assessment consists of three stages along the exposure pathway and nine nodes within these (Figure 2.1).

Broiler flocks are categorized among three classifications based upon their status on the farm: type S, R, or N flocks. Type S flocks are those that have at least one bird colonized on the farm with *Campylobacter* but do not include any ciprofloxacin-resistant *Campylobacter* (therefore, assumed that only ciprofloxacin-susceptible *Campylobacter* [CIPS] is present); R flocks have at least one bird colonized with ciprofloxacin-resistant *Campylobacter* (CIPR); N flocks are those that have no birds colonized with *Campylobacter*. These labels are static and do not change along the pathway regardless of any cross-contamination events that may occur downstream. The within-flock prevalences ($WFP_{[node],[flock\ type],[bird\ type]}$) and counts of external contamination ($C_{[node],[flock\ type],[bird\ type]}$) of both overall *Campylobacter* and CIPR *Campylobacter* are estimated at each node for the three flock types. In cases of between-flock cross contamination where the bacteria from one flock-type contaminates a different type of flock, this is modeled and estimated.

Broadly, the farm-to-fork path assessed in this model begins on the farm at the time of depopulation (specific on-farm interventions prior to depopulation are not modeled) and includes transportation from the farm to the abattoir and five distinct processing stages within the abattoir. It then considers chilled storage at retail and at home and consumer behaviour during preparation of the meat. Between-flock contamination of CIPR and CIPS species is modeled during the transportation and the evisceration nodes.

Computationally, stochastic models allow for randomness and variability in simulation models, with output distributions dependent on the randomness of one or several input parameters. More specifically this model uses Markov chain Monte Carlo simulation (a subset of stochastic modeling), where the input value will be selected from within a predefined range (i.e., index) that has some defined chance of being sampled.[92] This randomly selected input value will then generate a unique output value when following the equation in question. After many random samplings, or iterations, of this process a similar probability distribution for the likelihood of output values is created, thereby creating an estimation that has uncertainty around it, carried forward from the uncertainty defined in the input(s).[78]

The number of iterations required to find a converged model was determined to be the number at which a sentinel node (probability of illness for one serving, discussed in section 2.3.1.) produced the same 5^{th}, 50^{th}, and 95^{th} distribution percentiles to two significant digits using a random seed. The final model was run for 400,000 iterations and a fixed seed of 1004. Modeling was completed using BayesFusion (GeNIe Version 3.0).

2.1.1. Pre-Processing Module

The Canadian Integrated Program from Antimicrobial Resistance Surveillance (CIPARS) performs Canada-wide surveillance for antimicrobial use and resistance on farms, in processing plants, and in retail meat products. The prevalence of on-farm broiler flock antimicrobial resistance data is determined using pooled fecal samples from four quadrants of barn floors of flocks representatively selected across the country.[93] From this CIPARS data, the overall prevalence of broiler flocks in Canada that show any amount of *Campylobacter* at the time of depopulation (P flocks) is given by BFP_P (Table 2.1).[94] This prevalence is derived from

Table 2.1. Parameters used for pre-processing module in farm-to-fork exposure assessment.

Variable	Description	Value or Distribution	Unit	Source
BFP_P	Between-flock prevalence of all positive flocks in the national flock, regardless CIP susceptibility	Beta(122+1, 560-122+1)	Prevalence	[94]
BFP_Z	CIPR between-flock prevalence, among positive flocks	Beta(16+1, 122-16+1)	Prevalence	[94]
N_{flock}	Flock size	Normal(23735, 13477)	Birds	[95]
r_t	Biologic transmission rate	Normal(2.37, 0.295)	Birds/day	[96]
t_e	Time of exposure	Uniform(14, 21)	Days	[97]
t_s	Time of slaughter (i.e., depopulation)	Normal(34.5, 4.4)	Days	[95]
$Conc_{depop}$	External concentration at time of depopulation	Custom probability density function*	Log_{10} CFU/bird	[98]
N_{trans}	Flocks transported in 1 day	Uniform(0.5,5.4)	Flock	[99]
P_{DCT}	Probability of direct contamination during transport	Custom probability density function*	Probability	[99]
$Conc_{trans}$	External concentration after transport	Custom probability density function*	CFU/bird	[98]

*For custom functions see Appendix A.1.

representative national sampling of broiler flocks from across Canada in one year (i.e., the national flock). Additionally, these positive samples undergo antimicrobial susceptibility testing (AST) by CIPARS and can become classified as ciprofloxacin-resistant (CIPR) or ciprofloxacin-susceptible (CIPS) *Campylobacter*.[93]

The prevalence of CIPR flocks within all positive flocks is defined here as BFP_Z. Therefore, among all Canadian flocks in one year, R flocks (those with only CIPR *Campylobacter*) occur with a prevalence of $BFP_R = BFP_P \times BFP_Z$; S flocks (those with only CIPS *Campylobacter*) occur at $BFP_S = BFP_P – BFP_R$; and N flocks (those with no *Campylobacter*) occur at the frequency of $BFP_N = 1 – BFP_P$; such that $BFP_R + BFP_S + BFP_N = 1$. To account for some variability from the point estimates made by the surveillance program, a

beta distribution was used (Eq. 1), where x is the number of positive flocks and n is the total number of flocks tested.[99]

$$BFP = Beta(x + 1, n - x + 1) \quad (1)$$

Logically, all flocks that have at least one *Campylobacter* positive bird (S and R flocks) have a within-flock prevalence (WFP), or the fraction of birds within the flock colonized with either CIPR or CIPS *Campylobacter* at the time of depopulation. By definition N flocks do not have a within-flock prevalence or external contamination count (C) at the time of depopulation (DP) ($WFP_{DP,N} = 0$, $C_{DP,N} = 0$). Based on *in vivo* experimental evidence, we assumed that CIPR *Campylobacter* will entirely outcompete CIPS *Campylobacter* in colonizing the intestine of a broiler chicken.[71,72] Therefore, at the time of depopulation R flocks have only CIPR and S flocks have only CIPS. Katsma *et al*. developed a model (Eq. 2) that represents the within-flock transmission dynamics of *Campylobacter* within a large, commercial broiler flock. [18] N_{inf} represents the number of infected birds in a flock at the time of depopulation (i.e., end of time on the farm).

$$N_{inf} = \frac{N_{flock}}{1 + (N_{flock} - 1)e^{-r_t(t_s - t_e)}} \quad (2)$$

N_{flock} represents the total flock size, r_t the biologic transmission rate, and t_s–t_e indicates the duration of exposure in days. Dividing this by the total flock size yields the within-flock prevalence, such that $WFP_{DP} = N_{inf} / N_{flock}$. Stern *et al.* collected data on the external contamination of *Campylobacter* on broilers at the time of depopulation ($Conc_{depop}$) which is applied to the fraction of birds contaminated at depopulation.[98]

During transportation (TR), the live birds are loaded into transport crates on trucks and delivered to abattoirs. Transportation presents two opportunities for additional external

contamination to a bird: direct contamination by fecal matter from a colonized and shedding bird in the truck of the same flock; and indirect contamination from materials on the truck contaminated by a previous flock. The model describing the route of direct contamination is described in detail elsewhere.[30,99] Briefly, the vertical and horizontal distance between a bird of interest and a potentially colonized bird is used to calculate all possible probabilities of within-flock external contamination during transportation.[99] Hartnett derived a custom distribution to describe this range of probabilities (P_{DCT}), which was adopted here.[99]

The data regarding indirect contamination from transportation equipment between flocks remains scarce. Indirect contamination is contingent on a positive flock being transported with the same materials earlier that day and the assumptions that there was insufficient cleaning between flocks during the day and entirely effective cleaning between days.[86] The probability of a prior positive flock within that day (P_{ppf}) is given by Equation 3.

$$P_{ppf} = \begin{cases} 1 - (1 - BFP_P)^{N_{trans}-1}, & N_{trans} - 1 \neq 0 \\ 0, & N_{trans} - 1 = 0 \end{cases} \quad (3)$$

Here, N_{trans} is the number of flocks transported that day so far, including the flock of interest. P_{ppf} can be modified to account for the specific probability of a prior flock type by substituting BFP_P with BFP_R or BFP_S, calculating $P_{ppf,R}$ and $P_{ppf,S}$, respectively (Eq. 3). Due to a lack of quantitative information about the contamination of transport equipment with *Campylobacter* from broilers, we assume that any prior positive flock has an unknown chance of contaminating equipment.[99] Therefore, the probability of indirect contamination (P_{ICT}) is given in Equation 4.

$$P_{ICT} = Uniform(0,1) \times P_{ppf} \quad (4)$$

This is the first occurrence of between-flock contamination in the exposure pathway, where R and S flocks may henceforth be contaminated with both CIPS and CIPR *Campylobacter*. Equation 4 may be modified to estimate the probability of indirect

contamination specifically from a prior R ($P_{ICT,R}$) or S ($P_{ICT,S}$) flock by using $P_{ppf,R}$ or $P_{ppf,S}$, respectively.

The overall probability of contamination (P_{cont}) during transportation is the combination of indirect contamination and direct contamination (Eq. 5).[30] By definition, negative flocks have a P_{DCT} of 0, therefore $P_{cont,N} = P_{ICT,N}$.

$$P_{cont} = P_{DCT} + P_{ICT} - (P_{DCT} \times P_{ICT}) \quad (5)$$

Equation 6 represents the post-transportation within-flock prevalence (WFP_{TR}) which accounts for the probability of being contaminated before transportation (WFP_{DP}) and the probability of being contaminated during transportation (P_{cont}).

$$WFP_{TR} = WFP_{DP} + P_{cont} \times (1 - WFP_{DP}) \quad (6)$$

Stern *et al.* additionally recorded the external *Campylobacter* contamination on broilers after transportation to the abattoir from the farm ($Conc_{trans}$).[98] This distribution is used here to estimate C_{TR} for those birds whose $P_{cont} \neq 0$. For birds whose $P_{cont} = 0$, their C_{TR} remains estimated by Stern *et al.*'s $Conc_{depop}$ (i.e., remains as it was before transportation).

In cases where a positive flock is preceded by a positive flock with the opposite CIP status and indirect contamination occurs, the flock of interest is now contaminated in some capacity with both strains of *Campylobacter*. For example, an S flock may become contaminated with some amount of CIPR *Campylobacter* (the magnitude of this value is described below) with a probability of $P_{ICT,R}$. In this case, a within-flock prevalence of "mixed" birds in the S flock now exists ($WFP_{TR,S,mixed}$) as well as a fraction of birds who were previously not externally contaminated and now only have CIPR *Campylobacter* ($WFP_{TR,S,CIPR}$). These prevalences are functions of the probability of indirect CIPR contamination and the within-flock prevalence of either birds with CIPS from depopulation or from direct contamination (for mixed birds) or

negative birds (for CIPR only birds) (Eqs. 7 & 8). The reverse is true for R flocks with indirection contamination from prior S flocks (Eqs. 9 & 10).

$$WFP_{TR,S,mixed} = P_{ict,R} \times \left(WFP_{DP,S} + P_{dct} \times \left(1 - WFP_{DP,S}\right)\right) \quad (7)$$

$$WFP_{TR,S,CIPR} = P_{ict,R} \times \left(1 - WFP_{DP,S}\right) \times (1 - P_{dct}) \quad (8)$$

$$WFP_{TR,R,mixed} = P_{ict,S} \times \left(WFP_{DP,R} + P_{dct} \times \left(1 - WFP_{DP,R}\right)\right) \quad (9)$$

$$WFP_{TR,R,CIPS} = P_{ict,S} \times \left(1 - WFP_{DP,R}\right) \times (1 - P_{dct}) \quad (10)$$

At the end of transportation S flocks may have four types of birds: CIPS only birds ($WFP_{TR,S,CIPS}$), mixed birds ($WFP_{TR,S,mixed}$), those with only CIPR ($WFP_{TR,S,CIPR}$), and those without any external contamination ($WFP_{TR,S,NEG} = 1 - WFP_{TR,S,CIPS} - WFP_{TR,S,mixed} - WFP_{TR,S,CIPR}$). R flocks also have four bird types ($WFP_{TR,R,CIPR}$, $WFP_{TR,R,mixed}$, $WFP_{TR,R,CIPS}$, and $WFP_{TR,R,NEG}$). N flocks whose $P_{cont} \neq 0$ will now have a within-flock prevalence of externally contaminated birds post-transportation due entirely to indirect contamination from either CIPR or CIPS flocks. It is assumed that there will be no mixed birds post transportation for N flock since we assume that prior flocks can only transmit one *Campylobacter* strain through indirect contamination. Recall that $WFP_{DP,N}$ and P_{DCT} both equal 0, therefore the $WFP_{TR,N,CIPS} = P_{ICT,S}$ and $WFP_{TR,N,CIPR} = P_{ICT,R}$.

Regarding the amount of *Campylobacter* transferred from indirect contamination, this model adopts the assumption made by Hartnett regarding the microbial transfer rate when surfaces come in contact, based on the experimental work by Zhao *et al*.[99,100] Two contact events are required for the contamination from a prior flock to reach a future flock: a contaminated bird must come into contact with an intermediary surface (e.g., worker's hands, transport crates) and the intermediary surface must come into contact with the bird of interest. With an equal rate of transfer of 10% of the total source *Campylobacter* population at both, the overall amount

transmitted from indirect contamination is 1% of the average external contamination present on the prior flock (e.g., C_{DP}).[99,100] We assumed that both CIPR and CIPS *Campylobacter* have equal transfer rates. Therefore, in cases of indirect contamination causing cross-contamination from a flock with differing CIP status, the amount cross-contaminated ($C_{TR,S,CIPR}$ for S flocks, $C_{TR,R,CIPS}$ for R flocks) will equal 1% of C_{DP}, which is equivalent in both S and R flocks. The amount of opposite status *Campylobacter* transferred divided by the total amount of external *Campylobacter* post-transport (C_{TR}) gives the fraction of external contamination on a mixed bird that is of the opposite status at that time ($Fr_{TR,S,CIPR}$ or $Fr_{TR,R,CIPS}$, respectively).

2.1.2. Processing Module

As mentioned above, Dogan *et al.* conducted a systematic review and meta-analysis of the changes in *Campylobacter* prevalence and external contamination on broilers within the abattoir and reported these estimates.[91] We adopted these values for the processing module (Table 2.2) and assumed that prevalence and concentration changes affect CIPR and CIPS *Campylobacter* populations at the same rates through the processing nodes.

Changes in WFP are reported as odds ratios, or the odds of a fraction increasing (when greater than 1) or decreasing (when less than 1).[91] Equation 11 allows for such an odds ratio to be applied to an initial WFP_i from a previous stage to calculate WFP_{new}. For example, OR_{DF} and WFP_{SC} can be used to calculate WFP_{DF}.

$$WFP_{new} = \frac{WFP_i \times OR}{1 - WFP_i + WFP_i \times OR} \quad (11)$$

Changes in external contamination are reported as log changes.[91] The contamination levels from the post-transport stage are converted from the normal scale to the log scale and the log change is simply added (Eq. 12).

Table 2.2. Parameters used for processing module in farm-to-fork exposure assessment.

Variable	Description	Value/Distribution	Unit	Source
OR_{hs}	Within-flock prevalence (WFP) change due to hard scald	Exp(Normal(-1.71, 0.62))	Odds ratio	[91]
LC_{hs}	Log change of contamination due to hard scald	Normal(-1.85,0.13)	Log_{10} CFU	[91]
P_{hs}	Probability of a flock being hard scalded	0.82	Probability	[87]
OR_{ss}	WFP change due to soft scald	Exp(Normal(-1.74, 0.3))	Odds ratio	[91]1
LC_{ss}	Log change of contamination due to soft scale	Triangular(-2.62, -1.22, 0.29)	Log_{10} CFU	[91]
P_{ss}	Probability of a flock being soft scalded	0.18	Probability	[87]
OR_{df}	WFP change due to defeathering	Exp(Normal(0.24, 0.34)	Odds ratio	[91]
LC_{df}	Log change of concentration due to defeathering	Triangular(-2.4, 0.41, 2.17)	Log_{10} CFU	[91]
OR_{ev}	WFP change due to evisceration	Exp(Normal(0.12,0.13))	Odds ratio	[91]
LC_{ev}	Log change of concentration due to evisceration from within-flock effects	Triangular(-2.36, 0.45, 3.01)	Log_{10} CFU	[91]
P_{damage}	Probability of damage to a bird's viscera	Uniform(0,1)	Probability	[30]
$Conc_{caec}$	*Campylobacter* concentrations in a broiler chicken's caeca	Custom probability density function*	Log_{10} CFU per 1 gram	[30]
OR_{wa}	WFP change due to washing	Exp(Normal(-0.25, 0.36))	Odds ratio	[91]
LC_{wa}	Log change of concentration due to washing	Triangular(-0.72, -0.52, -0.34)	Log_{10} CFU	[91]
OR_{ac}	WFP change due to air chilling	Exp(Normal(-0.13, 0.19))	Odds ratio	[91]
LC_{ac}	Log change of concentration due to air chill	Normal(-0.51, 0.86)	Log_{10} CFU	[91]
P_{ac}	Probability of a flock being air chilled	0.74	Probability	[87]
OR_{wc}	WFP change due to immersion chilling with water	Exp(Normal(0.44, 0.33))	Odds ratio	[91]
LC_{wc}	Log change of concentration due to immersion chilling with water	Triangular(-3.13, -1.91, -0.89)	Log_{10} CFU	[91]

P_{wc}	Probability of a flock being immersion chilled with water only	0.24	Probability	[87]
OR_{cc}	WFP change due to immersion chilling with chlorinated water	Triangular(0, 1, 2.55)	Odds ratio	[91]
LC_{cc}	Log change of concentration due to immersion chilling with chlorinated water	Triangular(-3.68, -0.77, -0.11)	Log_{10} CFU	[91]
P_{cc}	Probability of a flock being immersion chilled with chlorinated water	0.01	Probability	[87]

* For probability density functions see Appendix A.1.

$$C_{new} = C_i + LC \tag{12}$$

As illustrated in Figure 2.1, the processing module is a linear pathway through the abattoir with five distinct nodes: scalding, defeathering, evisceration, washing, and chilling. Generally, there are two methods of scalding: a hard scald at 58-60°C for two minutes; and soft scald at 50-52°C for 3.5 minutes.[87] According to unpublished data collected by the Canadian Food Inspection Agency (CFIA), soft scalding is used on 18% of the incoming broiler flocks in Canada, while hard scald is used with the remaining 82% of flocks.[87] To account for this, these frequencies are used to weight results for hard and soft scalding, before combining to find an overall post-scald with-flock prevalence (WFP_{SC}) and external contamination count (C_{SC}) for all eight bird types across the three flock types (CIPS only, mixed, and CIPR only birds in S flocks; CIPR only, mixed, and CIPS only birds in R flocks; and CIPS only and CIPR only birds in N flocks).

OR_{DF} and LC_{DF} are used to calculate post-defeathering WFPs and Cs and applied uniformly to all bird types. Due to our previously stated assumption that CIPS and CIPR *Campylobacter* have the same survival rates within the abattoir environment, the fraction of

CIPS and CIPR *Campylobacter* on mixed birds in S and R flocks does not change. Similarly, newly calculated WFPs are the overall WFP for any positive bird within that flock, the proportion of these positive birds that belong to each bird type remains constant. Hypothetically, if 80% of all positive birds in an S flock post-scalding were CIPS only, then 80% of all positive birds in the flock post-defeathering are still CIPS only, even if the overall WFP of all positive birds has changed.

The evisceration node diverges from this straightforward application OR and LC parameters.[91] In addition to the changes in a flock due to evisceration proposed by Dogan *et al*., a model for between-flock cross-contamination during evisceration proposed by Hartnett was also adopted.[91,99] Evisceration is often recognized as the stage in the abattoir where bacterial contamination of the broiler carcass is most likely to increase due to the hazard of removing and potentially damaging internal organs, thus contaminating the evisceration equipment and affecting downstream flocks.[11,86] Using Equation 3, the probability of a prior positive flock (P_{ppf}) in addition to a random probability of damage to a bird's viscera (P_{damage}) and the probability that that bird is colonized with *Campylobacter* (WFP_{DP}), the probability of between-flock cross-contamination at evisceration ($P_{EV,BFXC}$) is determined (Eq. 13). If between-flock cross-contamination occurs, then C_{EV} follows Equation 10 plus some random amount of contamination from a prior flock (Eq. 14).

$$P_{EV,BFXC} = P_{ppf} \times WFP_{DP} \times P_{damage} \tag{13}$$

$$C_{EV} = C_{DF} + LC_{EV} + \begin{cases} Uniform(0,1) \times Conc_{caec}, & P_{EV,BFXC} \neq 0 \\ 0, & P_{EV,BFXC} = 0 \end{cases} \tag{14}$$

The probability of between-flock cross-contamination by a specific flock type is calculated by simply substituting $P_{ppf,R}$ or $P_{ppf,S}$ for P_{ppf} in Equation 13 and using the

corresponding $WFP_{DP,R}$ or $WFP_{DP,S}$ rather than WFP_{DP}. The colonization status of a bird is a reflection of the flock's on-farm within-flock prevalence (WFP_{DP}) and carries the assumption that all birds with external contamination and only those birds are colonized. Therefore, N flocks do not contribute to between-flock cross-contamination during evisceration as they were not colonized on the farm.

In cases where an S or R flock is cross-contaminated with the opposite strain of *Campylobacter* (i.e., S flocks get CIPR contamination or R flocks get CIPS contamination), the total amount of this "opposite" type is the 1% that was transmitted during transportation (discussed above) plus some random amount from that bird's caecal concentration ($Conc_{caec}$). $Conc_{caec}$ is equivalent in both R and S flocks because of the assumption that within-flock transmission dynamics on the farm are not different between the strains. Equation 15 shows this case for an S flock and Equation 16 for an R flock.

$$C_{EV,S,CIPR} = \left(C_{DF,S} + LC_{EV} - 2\right) + \begin{cases} Uniform(0,1) \times Conc_{caec,}, & P_{EV,R} \neq 0 \\ 0, & P_{EV,R} = 0 \end{cases} \quad (15)$$

$$C_{EV,R,CIPS} = \left(C_{DF,R} + LC_{EV} - 2\right) + \begin{cases} Uniform(0,1) \times Conc_{caec}, & P_{EV,S} \neq 0 \\ 0, & P_{EV,S} = 0 \end{cases} \quad (16)$$

Post-evisceration within-flock prevalence (WFP_{EV}) is the total probability of being contaminated before and/or during evisceration from within-flock sources and/or being contaminated from between-flock sources (Eq. 17).

$$WFP_{EV} = P_{EV,BFXC} + \left(\frac{WFP_{DF} \times OR_{EV}}{1 - WFP_{DF} + WFP_{DF} \times OR_{EV}}\right) \times \left(1 - P_{EV,BFXC}\right) \quad (17)$$

The within-flock prevalence of mixed birds in an S flock post-evisceration ($WFP_{EV,S,mixed}$) is the sum of the mixed birds before evisceration, CIPR only birds that get between-flock CIPS contamination, and CIPS only birds that get between flock CIPR contamination (Eq. 18). The reverse is true for the within-flock prevalence of mixed birds in an R flock (Eq. 19).

$$WFP_{EV,S,mixed} = WFP_{DF,S,mixed} + WFP_{EV,S,CIPS} \times P_{EV,BFXC,R} + WFP_{EV,S,CIPR} \times P_{EV,BFXC,S} \quad (18)$$

$$WFP_{EV,R,mixed} = WFP_{DF,R,mixed} + WFP_{EV,R,CIPR} \times P_{EV,BFXC,S} + WFP_{EV,R,CIPS} \times P_{EV,BFXC,R} \quad (19)$$

During evisceration, N flocks may also now develop mixed birds in cases where a CIPS only bird receives contamination from a previous CIPR flock, or vice versa. The prevalence of mixed birds in N flocks after evisceration ($WFP_{EV,N,mixed}$) is the prevalence of CIPS only birds in N flocks multiplied by the probabilities of between-flock cross-contamination from a prior CIPR bird plus the reverse scenario (Eq. 20).

$$WFP_{EV,N,mixed} = WFP_{EV,N,CIPS} \times P_{EV,BFXC,R} + WFP_{EV,N,CIPR} \times P_{EV,BFXC,S} \quad (20)$$

Additionally, since all of the mixed birds became mixed because of cross-contamination as evisceration, a random probability term of Uniform(0,1) is not required (Eq. 21).

$$C_{EV,N,mixed} = C_{DF,N} + LC_{EV} + Conc_{caec} \quad (21)$$

The final modification to the processing module is similar to that made at the scalding node. Three forms of chilling are performed in Canada according to the CFIA, each occurring at different frequencies and with different effects on *Campylobacter*.[87,91] Air chilling reportedly occurs 74% of the time, immersion chilling with water 24%, and immersion chilling with chlorinated water only 1% of the time.[101] Specific data on temperatures and duration of each chilling method in Canada is currently unavailable. The WFPs and Cs from each chilling method are weighted according to these frequencies and combined to create an overall post-chill WFP_{CH} and C_{CH}.

2.1.3. Post-Processing Module

Following processing at the abattoir, the poultry meat remains in cold storage until preparation at home by the consumer. This cold storage is modeled here in two phases, retail and home, as proposed by Dogan *et al*.[91] At either location, the meat may be refrigerated (considered fresh) or frozen. Dogan *et al*. proposed three main possibilities in terms of external contamination changes and two possibilities for within-flock prevalence changes.[91] If the chicken is ever frozen (either at retail or by the consumer at home) then the OR_{frozen} is used, otherwise the new prevalence is calculated using OR_{fresh}, using Equation 9 (Table 2.3). These effects on prevalence are weighted using the probability of occurring (P_{fresh} [Eq. 22] and P_{frozen} [Eq. 23]) and combined to create an overall WFP_{CS}, as was done above with scalding and chilling.

$$P_{fresh} = 0.85 \times (1 - P_{frozen,home}) \tag{22}$$

$$P_{frozen} = 1 - P_{fresh} \tag{23}$$

These probability weights are also used in calculating the overall post-cold storage external contamination (C_{CS}) with added differentiation regarding where the product was frozen.

As with the within-flock prevalence change if the poultry is never frozen the time-dependent LC_{fresh} (Eq. 24) with a weight of P_{fresh} is used. If the poultry is sold fresh but frozen at home a separate $LC_{frozen,home}$ (Eq. 26) is used and weighted with $P_{frozen,home}$, whereas if the chicken is sold frozen (regardless of status at home) a third log change is used (LC_{frozen}; Eq. 25) with the weight of $P_{frozen,retail} = 0.15$ used. The equations calculating log changes from refrigeration and freezing poultry (Eq. 24 & Eq. 25) were adopted by Bhaduri & Cottrell.[102]

$$LC_{fresh} = 0.03 - 0.14t_{fresh} + 0.007t_{fresh}^2 \tag{24}$$

$$LC_{frozen} = -0.23 \ln(t_{freeze}) - 1.58 \tag{25}$$

$$LC_{frozen,home} = LC_{fresh} + LC_{frozen} \tag{26}$$

Table 2.3. Parameters used for the post-processing module.

Variable	Description	Value/Distribution	Unit	Source
OR_{fresh}	WFP change due to cold storage in at refrigeration temperatures only	Exp(Normal(-1.76, 0.51))	Odds ratio	[91]
OR_{frozen}	WFP change due to cold storage if ever frozen	Triangular(0, 0.00825, 0.38)	Odds ratio	[91]
$P_{frozen,home}$	Probability of being sold fresh at retail and frozen at home	Custom probability density function*	Probability	[103]
t_{retail}	Time the product is refrigerated for at retail if sold fresh	Triangular(0, 1, 2)	Days	[91]
t_{home}	Time the product is refrigerated for at home	Custom probability density function*	Days	[103]
t_{frozen}	Time the product is frozen for	Uniform(0, 90)	Days	[91]
Fr_{loose}	Fraction of the product with loose cells	Uniform(0.01, 0.1)	Proportion	[30]
V_{dilute}	Volume of fluid from the product diluting the loose cells	Uniform(150, 250)	mL	[30]
V_{ingest}	Volume of the contaminated fluid ingested	Uniform(0.5, 1.5)	mL	[30]
D	D-value	$10^{-0.96}$	Minutes	[104]
z	z-value	12.3	°C	[104]
t_{cook}	Time the product is heated for	Uniform(15, 60)	Minutes	[91]
T_{cook}	Temperatures the product is heated at	Custom probability density function*	°C	[91]
$Fr_{portion}$	Fraction of one bird ingested as one portion	$1/(w_{bird}/w_{serving})$	Proportion	[30,91]
w_{bird}	Weight of one bird after processing	Gamma($(1495.3/303.4)^2$, $303.4^2/1495.3$)**	Grams	[105]
$w_{serving}$	Weight of one serving of chicken meat ingested	Custom probability density function*	Grams	[106]

* For custom functions see Appendix A.1.
** Where mean is 1495.3 and standard deviation is 303.4.

Lastly, the time total time refrigerated (t_{fresh}) for poultry sold fresh is modified to reflect the length of time before either preparation or freezing (Eq. 27), depending on if the chicken is frozen at the consumer's home, as described above.

$$t_{fresh} = \begin{cases} t_{retail} + t_{home}, & P_{fresh} \\ t_{retail}, & P_{frozen,home} \end{cases} \quad (27)$$

This exposure assessment model evaluates two mechanisms of ingesting some amount (i.e., dose) of *Campylobacter* from broiler meat during the at-home preparation process: cross-contamination of uncooked fluid from the meat; and remaining *Campylobacter* on the cooked poultry. Here, the drip-fluid model as first described by the WHO/FAO is used to estimate doses of ingested *Campylobacter* from cross-contaminated fluid.[30] The fraction of loosely attached cells on the poultry product (Fr_{loose}) is estimated as well as the volume of diluting fluid (V_{dilute}) that may come from the chicken (i.e., a representation of the concentration and volume with the potential to become cross-contaminated). This is then scaled by the volume of fluid that becomes ingested (V_{ingest}) and the total contamination on the product post-cold storage (C_{CS}), leaving a contamination dose (D_{XC}) ingested through this fluid (Eq. 28).[30]

$$D_{XC} = C_{CS} \times \left(\frac{Fr_{loose}}{V_{dilute}} \times V_{ingest}\right) \quad (28)$$

While ingestion through cross-contamination is an import exposure pathway for foodborne organisms on poultry, exposure from remaining bacteria on a cooked product remains a significant exposure possibility. Dogan *et al*. modeled thermal inactivation of *Campylobacter* on broiler meat at cooking temperatures using the decimal reduction time (D-value; time required for a 1 log reduction in organisms) and z-value (degrees Celsius to change the D-value by 1 log) published by Adams & Moss.[91,104] Equation 29 outlines the log change in contamination produced after a certain cooking duration (t_{cook}) at a certain temperature (T_{cook}), given these *Campylobacter*-specific D- and z-values.

$$LC_{cook} = \frac{t_{cook}}{D \times (10^{70 - T_{cook}/z})} \quad (29)$$

Ingested doses from a cooked product (D_{CK}) can be calculated using Equation 10 and C_{CS} and multiplying by the fraction of the bird being consumed in one portion ($Fr_{portion}$), estimated to be somewhere between 1/4 to 1/3 of the full broiler.[30,91] Logically, given these two exposure pathways, there are three possibilities for an ingested dose: D_{XC}, D_{CK}, or $D_{both} = D_{XC} + D_{CK}$.

2.2 Hazard Characterization

2.2.1. Traditional Dose-Response Model

The hazard characterization of this QMRA consists of dose-response modeling, the quantitative process by which a magnitude of exposure is related to a probability of an adverse outcome. Historically, these relationships were devised using experimental data from human feeding trials. Due to developments in research ethics such studies are rarely conducted, and dose-response data for emerging pathogens (such as antimicrobial resistant infections) is sparse.[107] In 1988, Black *et al.* published the data set that has formed the basis for the standard *Campylobacter* dose-response model, derived from orally administering *Campylobacter jejuni* (strains A3249 and 81-176) exposures to human volunteers and monitoring both microbiologic infection status and symptomology.[108] Later, Medema *et al.* fit Black *et al*.'s strain A3249 infection data to the approximate beta-Poisson model (Eq. 30) and produced the first dose-response model (DRM) for campylobacteriosis.[109]

$$DRM_{traditional} = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha} \quad (30)$$

In 2009, the Food and Agriculture Organization (FAO) & World Health Organization (WHO) pooled the infection data from both of Black's strains (A3249 and 81-176) and produced more comprehensive parameters for a *Campylobacter* DRM (α=0.21; β=59.59).[30] In addition to creating new pooled parameters, the FAO & WHO work also deduced that a rearranged form of

the beta-Poisson DRM that conserved variability in the probability of response was appropriate.[30] Given these developments, the best available DRM for a *Campylobacter* exposure follows Equation 31, where $P_{inf,1}$ represents the probability of infection from one colony forming unit (CFU) of exposure and follows a Beta(0.21, 59.59) distribution. The dose is the total ingested amount of *Campylobacter* in CFUs.

$$DRM_{simplified} = 1 - (1 - P_{inf,1})^{dose} \quad (31)$$

2.2.2. Antimicrobial Resistant Dose-Response Model

A novel DRM proposed by Chandrasekaran & Jiang in 2019 was adopted for this study to quantify the relationship between exposure and outcome when ciprofloxacin-resistant (CIPR) *Campylobacter* was ingested.[110] These authors developed a model for an exposure that contains both antibiotic-susceptible and -resistant bacteria in the presence of an ambient antibiotic concentration.[110] Equation 32 models the overall probability of response from a mixed dose given the independent extinction probabilities of the susceptible portion of the dose ($P_{ext,S}$) and the resistant portion of the dose ($P_{ext,R}$).

$$P_{response} = 1 - P_{ext,S} \times P_{ext,R} \quad (32)$$

$$P_{ext,S} = \left(1 + \left(\frac{d \times (1 - f_R)}{\beta_S}\right)\right)^{-\alpha_S} \quad (33)$$

$$P_{ext,R} = \left(1 + \left(\frac{d \times f_R}{\beta_R}\right)\right)^{-\alpha_R} \quad (34)$$

In Equations 33 and 34, d is the total magnitude of exposure in CFU and f_R is the fraction of the ingestion dose that is antimicrobial-resistant (Table 2.4). Most critically, for a 'mixed' exposure this model requires two sets of alpha-beta parameters (denoted by S and R subscripts), as the antimicrobial susceptible and resistant populations will die at different rates when in the

Table 2.4. Parameters used for dose-response modeling.

Variable	Description	Value	Unit	Source
d	Ingested dose of *Campylobacter*	D_{CK}, D_{XC}, D_{both}	CFU	Exposure assessment
α_R	Alpha parameter for a "traditional" *Campylobacter* DRM	0.21	-	[30]
β_R	Beta parameter for a "traditional" *Campylobacter* DRM	59.59	-	[30]
f_r	Fraction of total ingested *Campylobacter* dose that is CIPR	$Fr_{EV,S,CIPR}$, $Fr_{EV,R,CIPR}$, $Fr_{EV,N,CIPR}$	Fraction	Exposure assessment
t_{inc}	The last day of *Campylobacter*'s incubation period	4	Days	[111]
k_{max}	The maximum rate that ciprofloxacin can kill *E coli*	8.4	h^{-1}	[112]
EC_{50}	The concentration of ciprofloxacin at which half (or 50%) of the maximal effect is reached	0.0035	mg/l	[112]
C_{body}	The ambient concentration of ciprofloxacin in the body at the time of *Campylobacter* exposure	0.025 * 0.013	mg/l	[110,112]
α_S	Alpha parameter for CIPS *Campylobacter* in the presence of ciprofloxacin	0.2182	-	[110]
β_S	Beta parameter for CIPS *Campylobacter* in the presence of ciprofloxacin	1088.37	-	[110]
DDD	Defined daily doses of fluoroquinolones per 1000 inhabitant-days	1.5074	DDD	[42]
t_{AMU}	The average length of treatment with ciprofloxacin	Uniform(7,14)	Days	[113]
t_{EXP}	Length of time residual effects of taking antibiotics may remain present in the body	45	Days	[114]
$P_{undercook}$	Probability of undercooking occurring	Appendix A.2.	Probability	[87,115]
$P_{handwashing}$	Probability of no handwashing occurring	Beta(38+1, 100-38+1)	Probability	1

presence of any amount of the antimicrobial.[110] The parameters used for the extinction probability for the resistant fraction of the dose (i.e., the CIPR cells) in the presence of the antimicrobial ciprofloxacin (α_R and β_R) will be equal to the traditionally accepted Beta-Poisson DRM parameters (α_R=0.21, β_R=59.59). Chandrasekaran & Jiang assume that resistant bacteria in the presence of the antibiotic will behave the same as susceptible bacteria in the absence of the antibiotic.[110] The authors propose a methodology, detailed elsewhere, of calculating the survival of the susceptible portion of the ingested dose (i.e., the CIPS cells) given some concentration of the antimicrobial is present in the body (C_{body}), as well as the bacteria's maximum incubation period (t) and the pharmacodynamic relationship specific to the "bug-drug" combination (E_{max}, EC_{50}).[110] Equation 35 describes this process of converting the traditional (now α_R and β_R) parameters into r_S, which is then fit to a beta distribution to calculate the susceptible parameters such that $r_S \sim Beta(\alpha_S, \beta_S)$.[110] The results from this process are presented in Table 4.

$$r_S = -ln\left(1 - exp\left(-t_{inc} \times \left(\frac{ln(1 - \exp(-Beta(\alpha_R, \beta_R)))}{-t_{inc}} + \frac{k_{max} \times C}{EC_{50} + C}\right)\right)\right) \quad (35)$$

For the estimation of k_{max}, EC_{50}, and C_{body}, data regarding ciprofloxacin's relationship with *E. coli* was used as a substitute due to a lack of pharmacodynamic data regarding ciprofloxacin and *Campylobacter*. *E. coli* was chosen as the closest available substitute due their similar exposure mechanisms, abilities to initiate gastroenteritis, Gram-negative status, and relationship to fluoroquinolones.[116] Chandrasekaran & Jiang suggest using a small percentage (0.5%-2.5%) of the bacteria'ees minimum inhibitory concentration (MIC) as the C_{body} value (Table 2.4), therefore, 2.5% of the *E. coli* MIC (0.013 mg/l) from the same pharmacodynamic study was used to ensure consistency in the pharmacodynamic variables.[110,112]

2.2.3. Exposure Mechanisms and Scenarios

The utilization of Chandrasekaran & Jiang's novel DRM is predicated on the probability of both prior ciprofloxacin exposure and the probability of ingesting a 'mixed' dose (containing both CIPR and CIPS *Campylobacter*) through chicken meat. Thus, this model is only utilized in a fraction of all possible exposure scenarios. In cases of a 'single' exposure (entirely CIPR *Campylobacter* or CIPS *Campylobacter*) the traditional beta-Poisson form is used to estimate probability of infection (Eq. 30). Table 2.5 outlines the various equation and parameter combinations. Table 2.6 highlights which scenarios are used with modeling schemes, depending on exposure mechanism, within- and between-flock prevalences, and the presence of prior antimicrobial use (AMU).

The probability of prior antimicrobial use creating some ambient concentration of the drug remaining in the body is modeled by P_{AMU} (Eq. 36) and was adopted from Collineau *et al*.[87]

$$P_{AMU} = 1 - \left(1 - \frac{DDD/1000}{t_{AMU}}\right)^{t_{EXP}} \tag{36}$$

Where DDD represents the defined daily doses of ciprofloxacin per 1000 inhabitant-days, t_{AMU} is the average length of treatment with ciprofloxacin, and t_{EXP} is the length of time that residual effects of taking antibiotics may remain in the body. National defined daily doses of fluoroquinolones were used as a substitute for a measure specific to ciprofloxacin due to data availability.

As described in Section 2.1.3, contamination from a bird can be ingested through three exposure mechanism: undercooked meat (D_{CK}), cross-contamination through fluid (D_{XC}), and both (D_{both}).[42] The exposure assessment model also creates the possibility for nine types of birds across three flock types. Additionally, we must consider whether there was prior ciprofloxacin

Table 2.5. Dose-response equations and parameters used for various dose compositions and ciprofloxacin use scenarios.

Model Scenario	Model Equation	Parameters
I	Traditional DRM (Eq. 26)	α_R, β_R
II	Traditional DRM (Eq. 26)	α_S, β_S
III*	Novel DRM (Eq. 28)	α_R, β_R
IV	Novel DRM (Eq. 28)	α_S, β_S, α_R, β_R

*In the case of scenario III, the same parameters (α_R and β_R) are used for both the susceptible (Eq. 29) and resistant (Eq. 30) extinction probabilities.

use. Therefore, 54 distinct dose-response scenarios must be constructed for each dose produced through the various combinations of bird type, exposure mechanism, and antimicrobial use history. Table 2.6 outlines which model scenario is used in which instance. These 54 scenarios can be consolidated to 27 by weighting by P_{AMU} to create and overall model for that dose-exposure mechanism combination, regardless of AMU status.

Each of these 27 dose-response scenarios must then be paired with a probability of exposure which accounts for that bird type frequency within the national flock as well as the probability of the exposure mechanism occurring. The probability of exposure occurring through undercooking alone is given in Equation 37.

$$P_{EXP,CK} = P_{undercook} \times \sum \left(WFP_{bird\ type} \times BFP_{flock\ type} \right) - P_{EXP,both} \tag{37}$$

These probabilities of exposure are dependent on their overall prevalence among the national flock, therefore $P_{EXP,CK}$ can be further specified to $P_{EXP,CK,CIPS}$ by substituting WFP_{CS} and $P_{EXP,both}$ for $WFP_{CS,CIPS}$ and $P_{EXP,both,CIPS}$. These same changes are made for CIPR only doses and mixed doses. Similarly, the probability of exposure through cross-contamination in the kitchen alone is described by Equation 38. Also being a function of overall prevalence and therefore specific to

Table 2.6. The dose-response scenarios, differentiated by flock status, bird statuses, exposure mechanism, and antimicrobial use history.

		S flock			R flock			N flock		
		CIPS only	Mixed	CIPR only	CIPS only	Mixed	CIPR only	CIPS only	Mixed	CIPR only
No prior AMU	D_{CK}	I	III	I	I	III	I	I	III	I
	D_{XC}	I	III	I	I	III	I	I	III	I
	D_{both}	I	III	I	I	III	I	I	III	I
Prior AMU	D_{CK}	II	IV	I	II	IV	I	II	IV	I
	D_{XC}	II	IV	I	II	IV	I	II	IV	I
	D_{both}	II	IV	I	II	IV	I	II	IV	I

bird type, the same substitutions described above must be made here.

$$P_{EXP,XC} = P_{handwashing} \times \sum \left(WFP_{bird\ type} \times BFP_{flock\ type} \right) - P_{EXP,both} \tag{38}$$

Lastly, the probability of exposure through both undercooking and cross-contamination is the combined probabilities of undercooking occurring, cross-contamination occurring, and the overall within-flock prevalence of that bird type (Eq. 39).

$$P_{EXP,both} = P_{undercook} \times P_{handwashing} \times \sum \left(WFP_{bird\ type} \times BFP_{flock\ type} \right) \tag{39}$$

$P_{undercook}$ is based on American survey data collected by Bruhn and is based on the frequencies of five cooking methods used to cook chicken (grilling, frying, oven roasting, boiling, and pressure cooking) and the individual probabilities of undercooking the meat by each of these methods (Appendix A.2).[115] $P_{handwashing}$ is also adopted from the same Bruhn survey and is estimated by placing a beta distribution around the reported fraction of people who do not wash their hands after handling raw chicken during preparation.[115]

Multiplying the results of a specific DRM scenario (Table 2.6) with its corresponding probability of exposure will generate the specific probability of infection (P_{inf}) in the general

population for that bird type-exposure mechanism combination (Eq. 40). This discussion is continued in Section 2.3.

$$P_{inf} = DRM \times P_{EXP} \tag{40}$$

2.2.4. Infection Susceptibility to Ciprofloxacin

This model assumes that in cases of exposure to a single *Campylobacter* type, the resulting ciprofloxacin susceptibility status of the infection will be aligned with the status of the *Campylobacter* population ingested. That is, if only CIPS *Campylobacter* is consumed, any resulting campylobacteriosis is assumed to be treatment-susceptible (TxS) to ciprofloxacin, and CIPR *Campylobacter* only exposures can only initiate treatment-resistant (TxR) infection. Additional considerations are needed when predicting infection status in cases of a mixed exposure.

Chandrasekaran & Jiang provide a conditional inequality for predicting whether any resulting infection from a mixed dose will be TxR (Eq. 41).[110] In our case, whether any resulting *Campylobacter* infection will be ciprofloxacin resistant. $P_{ext,S}$ and $P_{ext,R}$, the extinction probabilities, are defined in Equations 29 and 30, respectively.

$$P_{TxR} = \begin{cases} 0, & (1 - P_{ext,S}) \times P_{ext,R} > 1 - P_{ext,R} \\ 1, & otherwise \end{cases} \tag{41}$$

Therefore, P_{TxR} is dependent on dose, fraction of the dose that is CIPR, and AMU status. Two P_{TxR} inequalities are created for each exposure mechanism and bird type combination, differing by AMU status. Similar to how mixed dose DRM scenarios are weighted and combined by P_{AMU}, these two P_{TxR} are also scaled by P_{AMU}, defined by Equation 32, and summed to create an overall P_{TxR} for a mixed dose for that exposure mechanism, regardless of AMU status.

This process occurs for the nine possibilities for mixed doses (three types of mixed birds, one per flock type, each with three possible exposure mechanisms). Similar to what is described in section 2.2.3, the three P_{TxR} for a bird type (one for each exposure mechanism) are then weighted by probability of exposure mechanism occurring for that bird type ($P_{EXP,XC,mixed}$, $P_{EXP,CK,mixed}$, $P_{EXP,both,mixed}$), described in Equations 37, 38, and 39. When weighted and summed this produces a total probability that *Campylobacter* infection from a mixed dose will be ciprofloxacin-resistant.

For mixed doses, multiplying the P_{inf} for the specific bird type-exposure mechanism combination (Eq. 40) by its P_{TxR} generates the probability of a ciprofloxacin-resistant infection for that exposure. Logically, this is a subset of the total P_{inf} for that mixed dose-exposure mechanism.

2.3. Risk Characterization

2.3.1. Risk from One Serving

The first major outputs from this QMRA are the estimations of the probability of *Campylobacter* illness from one random serving of chicken meat and the probability of ciprofloxacin-resistant *Campylobacter* illness from a random serving. The total probability of general infection from any random serving ($P_{inf,overall}$) is simply the sum of the individual probabilities of infection (P_{inf}), as described in section 2.2.3 (Eq. 42).

$$P_{inf,overall} = \sum_{i=1}^{27} P_{inf,[bird\ type],[exposure\ mechanism]} \tag{42}$$

When human feeding trials are completed to measure the biologic relationship between *Campylobacter* exposure and consequent effect, a clear relationship was documented between

microbiologic infection status and exposure magnitude. However, such was not the case with the relationship between exposure and symptomatic illness, as exemplified by the data published by Black *et al*. [108,109] Therefore, an additional probability factor is applied for estimating the conditional probability of illness given infection. The FAO & WHO used a dose-independent probability for this, using the pooled Black *et al.* data (both A3249 and 81-176 *C. jejuni* strains) where 89 participants were microbiologically infected and 29 of these became symptomatic.[30,108] The distribution for the probability of illness given infection ($P_{ill|inf}$) is shown in Table 2.7. Therefore, the overall probability of illness given infection for one serving of broiler meat follows Equation 43.

$$P_{ill,overall} = P_{inf,overall} \times P_{ill\_inf} \tag{43}$$

When considering the probability of developing ciprofloxacin-resistant campylobacteriosis, all CIPR *Campylobacter* only probabilities of infection (across all exposure mechanisms) are summed with the TxR fraction of all mixed dose probabilities of infection, as described in section 2.2.4. (Eq. 44).

$$P_{inf,CIPR} = \sum_{i=1}^{9} P_{inf,CIPR,[flock\ type],[exposure\ mechanism]} + \left(\sum_{i=1}^{9} P_{inf,mixed,[flock\ type],[exposure\ mechanism]} \times P_{TxR,[flock\ type],[exposure\ mechanism]} \right) \tag{44}$$

To estimate the total probability of ciprofloxacin-resistant campylobacteriosis from any random serving on chicken ($P_{ill,CIPR}$), Equation 39 is used, substituting $P_{inf,overall}$ for $P_{inf,CIPR}$. To estimate the total probability of ciprofloxacin-resistant campylobacteriosis from any random serving on chicken ($P_{ill,CIPR}$), Equation 39 is used, substituting $P_{inf,overall}$ for $P_{inf,CIPR}$.

Table 2.7. Parameters used for estimating illnesses.

Variable	Description	Value	Unit	Source
P_{ill_inf}	The probability of being symptomatic given a positive infection status	Beta(29+1, 89-29+1)	Probability	[30]
w_{total}	The total eviscerated weight of broiler carcasses produced in Canada in one year	1,298,000,000	kg	[117]
$w_{serving}$	The average weight of one serving	Custom distribution function *	g	[106]
N_{CANpop}	The mid-year Canadian population in 2019	37,593,384	Persons	[118]

* For custom functions see Appendix A.1.

2.3.2. Estimating Illness in the Population

On a population level, multiplying the likelihood of an outcome from one serving by the total number of servings consumed in one year by all people living in Canada ($N_{serving,total}$) will yield the total number of outcomes expected in Canada for that year ($N_{ill,total}$), as shown in Equation 45.

$$N_{ill,total} = P_{ill} \times N_{servings,total} \quad (45)$$

Logically, subbing P_{ill} in Equation 41 for $P_{ill,CIPR}$ will calculate the total number of ciprofloxacin-resistant illnesses ($N_{ill,CIPR,total}$).

The total number of servings prepared and consumed in private Canadian homes annually ($N_{servings,total}$) is estimated by dividing the total weight of viable broiler meat produced and eaten in one year by the average weight of one serving of broiler meat eaten ($w_{serving}$) (Eq. 46). The total eviscerated weight of broilers produced (w_{total}) in Canada in 2019 was approximately 1.3 billion kg.[117] Statistics Canada estimates that only 49.12% of a broiler's eviscerated weight is eaten as lean meat, after discounting the weight of bones in the carcass and estimating the average amount of meat lost due to food waste at retail and home.[119] Agriculture

and Agri-Food Canada estimates that of all the broiler carcasses produced in Canada, 59% are sold through retail to Canadian households.[117] These factors are used to estimate the total weight of lean broiler meat eaten by those living in Canada in 2019. Australian survey data from 2016 that presents the median weights and interquartile ranges of serving sizes of chicken was used.[106] Due to the lack of similar Canadian data, the assumption was made that Australians and Canadians consume similar amounts of broiler chicken in one sitting. Interestingly, these data are stratified by gender. [106] To find the overall average serving size, the gender averages were weighted by 50% each and combined.

$$N_{servings,total} = \frac{w_{total} \times 0.4912 \times 0.59}{w_{serving}/1000} \quad (46)$$

An incidence of illness per 100,000 people is calculated by dividing the total number of illnesses by that fraction of the Canadian population (Eq. 47). As with other determinations, subbing $N_{ill,total}$ for $N_{ill,CIPR,total}$ will give the incidence of ciprofloxacin-resistant illnesses per 100,000 Canadian inhabitants.

$$N_{ill,100k} = \frac{N_{ill,total}}{N_{CANpop}/100,000} \quad (47)$$

2.3.3 Sensitivity Analysis

Following construction of the baseline model and estimation of the expected probability of illness per one serving and number of illnesses per 100,000 inhabitants for both total *Campylobacter* exposure and CIPR *Campylobacter*, the model can be analyzed. The first of these analyses is a sensitivity analysis with attempts to identify which data inputs within the full model are most influential in determining the median value of the final estimated results.

While there are many methodologies for performing sensitivity analyses, this thesis employs a conditional sensitivity analysis that examines the change in median across pre-specified domains. Using input and output distributions at 400,000 iterations with an initial seed of 1004, input values are sorted in ascended order while maintaining its pairing with the corresponding output value for each iteration. All iterations are then segregated into 10 bins with cutoffs at every 10^{th} percentile of the input distribution. In our case, each of these bins would hold 40,000 iterations. Medians of the output within each of these bins are found to assess how the output varies while being conditioned by different ranges of inputs. This process is repeated for each of the input variables of interest. Those variables that exhibit the largest 'swing,' or largest difference between the smallest output median and largest output median among across all 10 bins, are considered to show the greatest influence on that output variable.

$P_{ill,overall}$ (probability of overall illness from one serving), $P_{ill,CIPR}$ (probability of CIPR illness from one serving), and $Fr_{ill,CIPR}$ were used as outputs of interest and their iteration data were placed into 10 bins according to the input of interest's distribution. A total collection of data inputs across the entire model can be found within Tables 2.1-2.4. and 2.7. To shorten this list to a feasible amount for conditional analysis, a Spearman rank correlation coefficient was found for each of the input distributions and the three output distributions of interest. Ten variables were identified as having a significant correlation ($p < 0.001$) and a coefficient larger than 0.1 ($\rho > 0.1$) for the outputs $P_{ill,overall}$ and $P_{ill,CIPR}$ and were consequently selected for the conditional sensitivity analysis. Then 10 inputs with the highest correlation coefficients with $Fr_{ill,CIPR}$ were chosen for conditional analysis with that output, although not all of these correlation coefficients had a $p < 0.001$.

The 10 data input distributions identified for further conditional analysis for $P_{ill,overall}$ and $P_{ill,CIPR}$ were: $Conc_{depop}$; LC_{DF} ; LC_{EV}; LC_{HS}; N_{inf}; N_{proc}; P_{damage}; P_{DCT}; $P_{undercook}$; T_{cook}. A different set of ten input variables were chosen through Spearman coefficients to be correlated with $Fr_{ill,CIPR}$ and went on for further analysis: BFP_Z; $Conc_{caec}$; $Conc_{depop}$; N_{inf}; N_{proc}; N_{trans}; OR_{HS}; P_{AMU}; P_{damage}; P_{ICT}.

2.3.4. Scenario Analysis

The second major analysis performed on the baseline model is a scenario analysis where some data within the baseline model are changed to simulate a potential intervention and the results from the baseline and the altered model are compared. Outlined in Table 2.8. are the seven independent scenarios assessed. These scenarios were selected based on findings from the literature and expert opinion (Drs. Agnes Agunos, Carolee Carson, Anne Deckert, Qiaozhi Li, Colleen Murphy, Simon Otto, Richard Reid-Smith, and Mr. Ben Smith, email communication, September 2021).

Scenario A was achieved by multiplying BFP_P by 0.5, which effectively reduces the prevalence of S and R flocks (BFP_S and BFP_R) by half. The proportion of all P flocks that are CIPR is maintained while prevalence of N flocks increases to absorb the number of flocks no longer contaminated at depopulation due to the relationship between these parameters described in Section 2.1.1.

Scenario B was achieved by multiplying P_{ICT} by 0.1, causing downstream changes to the probability of indirect contamination from both preceding R and S flocks. Similarly, $P_{EV,BFXC}$ was multiplied by 0.1 in Scenario C to create a 90% reduction of the probability of between-flock cross-contamination during evisceration.

Table 2.8. Description of the scenarios compared against the baselined model for scenario analysis.

Scenario	Node	Description	Baseline Value	Intervention Value
A	Depopulation	Reduce on-farm between-flock prevalence by 50%	BFP_P	$0.5*BFP_P$
B	Transportation	Reduce indirect contamination (i.e., between flock) during transportation by 90%	P_{ICT}	$0.1*P_{ICT}$
C	Evisceration	Reduce between-flock cross-contamination at evisceration by 90%	$P_{EV,BFXC}$	$0.1*P_{EV,BFXC}$
D	Chilling	Reduce within-flock prevalence entering retail by 90%	$WFP_{CH,P}$; $WFP_{CH,N}$	$0.1*$ $WFP_{CH,P}$; $0.1*WFP_{CH,N}$
E	Cold Storage	Reduce overall contamination per bird by 1 log entering retail	$C_{CH,P}$; $C_{CH,N}$	$C_{CH,P} - 1$; $C_{CH,N} - 1$
F	Preparation	Reduce probabilities of undercooking and kitchen cross-contamination by 90%	$P_{handwashing}$; $P_{undercook}$	$0.1*P_{handwashing}$; $0.1*P_{undercook}$
G	Dose-Response Model	Reduce human fluoroquinolone use by 90%	P_{AMU}	$0.1*P_{AMU}$

Scenario D involves changing within-flock prevalences between two nodes. To accomplish this, the post-chilling within flock prevalence was multiplied by 0.1 within four relevant versions of Equation 11: when OR_{fresh} and OR_{frozen} are used for with either $WFP_{CH,N}$ or $WFP_{CH,N}$. Scenario E is complimentary to Scenario D in that changes are made in the same node in the exposure assessment, but rather than manipulating within flock prevalence, contamination from cold storage is reduced by 1 log. Six versions of Equation 12 exist at cold storage (LC_{fresh}, LC_{froxen}, $LC_{frozen,home}$ for both P and N flocks), for each of which C_i is $C_{CH,P} - 1$ or $C_{CH,N} - 1$, respectively, in Scenario E.

Similar to Scenarios B and C, the probability of adverse events during home preparation are reduced by 90% by multiplying both the probability of ingesting raw fluid because of no

handwashing ($P_{handwashing}$) and the probability of undercooking the meat ($P_{undercook}$) by 0.1, simultaneously. Lastly, in Scenario G the probability of ambient fluoroquinolones in the body prior to any *Campylobacter* ingestion is reduced by 90%.

To compare risk between these alternate scenarios and the baseline modeled, the number of overall *Campylobacter* and ciprofloxacin-resistant *Campylobacter* illnesses per 100,000 inhabitants of Canada are compared following Equation 48, where $N_{ill,100k}$ is the estimated number of illnesses per 100,000 inhabitants, as described above. The intervention efficacy regarding CIPR illness can be assessed using Equation 48 and substituting $N_{ill,100k}$ with $N_{ill,CIPR,100k}$.

$$IE_{[scenario],all} = \frac{N_{ill,100k} - N_{ill,100k,[scenario]}}{N_{ill,100k}} \quad (48)$$

The percent change for intervention efficacy (IE) estimation is calculated individually for each iteration comparison, allowing for a distribution of results to be created. That is to say, the first iteration in the baseline model is directly compared to the first iteration of the Scenario A model, and so on for all 400,000 iterations, and across all seven scenarios. This allows for a more detailed and nuanced assessment of results, as opposed to simply using point estimates.

The altered scenario models were run for 400,000 iterations with the seed set to 1004 in accordance with the baseline model to allow direct comparison between iterations.

Chapter 3: Results

3.1. Exposure Assessment

3.1.1. Farm-to-Fork Exposure Pathway

The first task outlined in Section 1.5 of this thesis was to estimate the prevalence and concentration of all *Campylobacter* (both *C. jejuni* and *C. coli*) on a bird along the farm-to-fork production chain, as well as that specifically for ciprofloxacin-resistant (CIPR) *Campylobacter*. Table 3.1 presents the median estimates and accompanying 5th and 95th percentiles of estimated prevalence and concentration at each stage of the farm-to-fork pathway. Figure 3.1 displays these distributions graphically.

Transportation and evisceration cause the greatest increases in prevalence of birds externally contaminated by *Campylobacter* with the latter associated with the highest median along the pathway of 32.7%. A similar pattern is shown for the overall prevalence of birds with any CIPR *Campylobacter* contamination, with this frequency peaking at 4.9% at evisceration. It should be made clear that the prevalence of birds with CIPR *Campylobacter* is not among positive birds but among all total birds. Therefore, it could be considered that among all positive birds at evisceration, approximately 15.0% have some amount of CIPR *Campylobacter*. The same interpretations can be made for all other stages along the farm-to-fork pathway.

Regarding the magnitude of contamination with either total or CIPR *Campylobacter*, Figure 3.1 illustrates the generally decreasing trend of average contamination. Two things should be kept in mind: 1) as discussed above, the general trend for the overall prevalence among birds is increasing, meaning more birds are being contaminated with some small amount that plays a part in driving the average down; 2) the breadth of uncertainty around the median estimation of concentrations increases noticeably as the farm-to-fork pathway progresses, indicating that while

Table 3.1. The 5th, 50th, and 95th percentiles from the generated prevalence (percent of national flock) and concentration (log_{10} CFU/bird) probability distributions of external *Campylobacter* and CIPR *Campylobacter* contamination.
DP: Depopulation; TR: Transportation; SC: Scalding; DF: Defeathering; EV: Evisceration; WA: Washing; CH: Chilling; CS: Cold storage

Node	**Prevalence of birds with any *Campylobacter* contamination, % (median [5th, 95th])**	**Concentration of any *Campylobacter* contamination on one bird, log_{10} CFU/bird (median [5th, 95th])**	**Prevalence of birds with CIPR *Campylobacter* contamination, % (median [5th, 95th])**	**Concentration of CIPR *Campylobacter* contamination on one bird, log_{10} CFU/bird (median [5th, 95th])**
DP	21.0 [0, 24.5]	6.97 [3.12, 7.38]	2.6 [0, 4.1]	6.97 [3.12, 7.38]
TR	30.6 [8.3, 60.0]	5.94 [1.76, 7.76]	4.3 [1.1, 10.0]	5.83 [1.66, 7.68]
SC	23.0 [1.6, 35.5]	4.42 [0.19, 7.17]	3.3 [0.2, 6.5]	4.26 [0.12, 7.07]
DF	23.4 [1.9, 39.5]	4.32 [0.36, 7.61]	3.4 [0.2, 7.0]	4.18 [0.32, 7.51]
EV	32.7 [2.7, 63.2]	4.84 [0.71, 9.82]	4.9 [0.4, 11.5]	4.59 [0.64, 9.14]
WA	30.2 [2.0, 60.2]	4.38 [0.30, 9.42]	4.7 [0.3, 10.9]	4.10 [0.27, 8.68]
CH	30.1 [2.0, 62.1]	3.53 [0.00, 8.75]	4.7 [0.3, 11.1]	3.27 [0.01, 8.05]
CS	22.5 [0.2, 33.1]	2.13 [0.00, 8.15]	3.5 [0.0, 6.8]	1.76 [0.00, 6.92]

some newly contaminated birds will have little external contamination, there are also many birds whose magnitude of contamination is increasing. Another interesting trend in total external contamination versus resistant contamination is that the magnitudes are generally very close to each other along the pathway, unlike the comparison for prevalence. This is representative of the fact that most birds contaminated with CIPR *Campylobacter* in our model have only CIPR *Campylobacter* contamination.

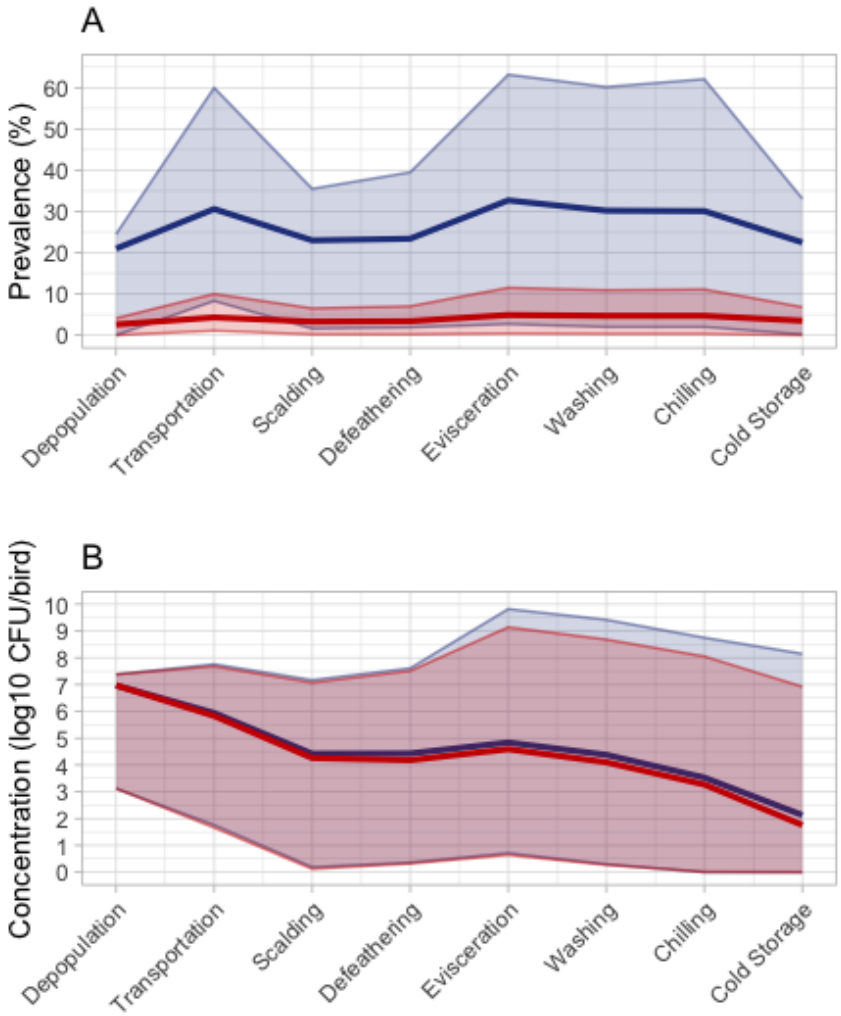

Figure 3.1. The prevalence of contaminated birds among the national flock (A) and the concentration per bird (B) along the farm-to-fork exposure pathway.
Median total *Campylobacter* contamination is represented in blue while median ciprofloxacin-resistant *Campylobacter* is represented in red.
Shaded regions represent 95% credible intervals from the probability distributions surrounding the respective metric.

Even though the average contamination of CIPR *Campylobacter* is very similar to that of total *Campylobacter*, the frequencies at which these magnitudes are occurring is drastically different. For example, at defeathering it can be interpreted that the model estimates 23.4% of all birds have an average 4.32 $\log_{10}$ CFU per bird of total *Campylobacter*, meanwhile 3.4% of all

birds (or 14.5% of all positive birds) at defeathering have on average 4.18 $\log_{10}$ CFU of CIPR *Campylobacter*.

3.1.2. Estimated Doses

Following cold storage (CS) along the farm-to-fork pathway is at-home preparation, which includes cross-contamination (XC) and cooking (CK). At this point, magnitudes of ingested *Campylobacter* and CIPR *Campylobacter* per serving of broiler chicken meat were estimated. Following cold storage, contamination units are presented in CFU/serving (as opposed to $\log_{10}$ CFU/bird as in the exposure assessment). Associated probabilities of exposure mechanisms (i.e., CK, XC, both) occurring were also estimated.

As described in Section 2.2.3, estimated magnitudes and frequencies of exposure are kept separate throughout the dose-response modeling for each exposure pathway, with 27 scenarios created, presented in Table 3.2. These values serve as the direct input for the 54 dose-response scenarios (each dose has a scenario with and without prior antimicrobial use). Readers should bear in mind that the results presented in Table 3.2 are not directly interpretable as estimates of risk since they do not yet incorporate frequencies of exposure. For example, the median doses from contaminated mixed birds in N flocks are much higher than any other type of bird, however the probability of exposure to this bird type is very small. Similarly, among all bird types, exposure through both mechanisms (undercooking and cross-contamination) is associated with the highest median dose, but this is also the least likely mechanism of exposure since it requires both undercooking and cross-contamination to occur (Eq. 39).

Overall, the exposure assessment estimates that 12.2% (90% CrI: 0.1-19.7) of all ingested servings in Canada annually may have *Campylobacter* contamination of any type. Similarly, of

Table 3.2. Estimated magnitudes and frequencies of doses used as inputs for the dose-response scenarios (corresponding to Table 2.6).
All estimations are displayed as median [5th percentile, 95th percentile].
Flock types (S, R, N) are distinguished by their original contamination status at depopulation.
Exposure mechanisms include undercooking (CK), cross-contamination (XC), and both.
S flocks are those colonized by ciprofloxacin-susceptible (CIPS) *Campylobacter* at Depopulation (DP); R flocks are those colonized by ciprofloxacin-resistant (CIPR) *Campylobacter* at DP; N flocks are those without colonized birds at DP.

		CK		**XC**		**Both**	
		Dose (CFU)	**Freq (%)**	**Dose (CFU)**	**Freq (%)**	**Dose (CFU)**	**Freq (%)**
S flocks	**CIPS only**	1 [1, 4.77e+04]	3.48 [0, 6.55]	1 [1, 4.52e+05]	3.56 [0.01, 5.95]	2 [2, 2.92e+06]	2.12 [0, 4.13]
	Mixed	1 [1, 4.77e+04]	0.11 [0, 0.5]	1 [1, 4.52e+05]	0.11 [0, 0.47]	2 [2, 2.92e+06]	0.07 [0, 0.31]
	CIPR only	1 [1, 14]	0 [0, 0.01]	1 [1, 6]	0 [0, 0.01]	2 [2, 59]	0 [0, 0.01]
R flocks	**CIPS only**	1 [1, 49]	0 [0, 0.01]	1 [1, 46]	0 [0, 0.01]	2 [2, 454]	0 [0, 0.01]
	Mixed	1 [1, 4.77e+04]	0.1 [0, 0.45]	1 [1, 4.52e+05]	0.11 [0, 0.43]	2 [2, 2.92e+06]	0.06 [0, 0.28]
	CIPR only	1 [1, 4.77e+04]	0.37 [0, 0.92]	1 [1, 4.52e+05]	0.38 [0, 0.86]	2 [2, 2.92e+06]	0.23 [0, 0.58]
N flocks	**CIPS only**	1 [1, 66]	0.25 [0, 2.35]	1 [1, 322]	0.26 [0, 2.29]	2 [2, 2.03e+03]	0.16 [0, 1.46]
	Mixed	1 [1, 1.78e+06]	0 [0, 0.04]	39 [1, 4.10e+05]	0 [0, 0.04]	156 [2, 4.79e+06]	0 [0, 0.03]
	CIPR only	1 [1, 66]	0.03 [0, 0.36]	1 [1, 322]	0.04 [0, 0.35]	2 [2, 2.03e+03]	0.02 [0, 0.22]

all ingested broiler chicken servings annually, an estimated 2.1% (90% CrI: 0.02-4.4) may have CIPR *Campylobacter*. This model estimates the median exposure of any *Campylobacter* through chicken prepared in a Canadian home is 1 CFU (90% CrI: 0-9.38E+05) while the median exposure to CIPR *Campylobacter* on those prepared servings with the resistance species is also estimated at 1 CFU (90% CrI: 0-1.38E+06).

3.2. Antimicrobial Resistance Modeling

A key novel component of this QMRA is the modeling developed to account for antimicrobial resistance (AMR) in the hazard characterization. This was done using methods proposed by Chandrasekaran & Jiang, described in Section 2.2.2.[110] This novel AMR dose-response model (DRM) is only suitable for cases where the subject has experienced prior antimicrobial use (AMU) or exposure, necessitating an estimation of the probability that such prior AMU occurred. Using Equation 36 and the parameters described in Table 2.4 the median probability of prior fluoroquinolone use in the past 45 days was found to be 0.64% (5^{th} percentile: 0.50%; 95^{th} percentile: 0.92%).

The other additional calculation required for Chandrasekaran & Jiang's AMR DRM was novel alpha and beta parameters describing the probability of a single CFU of the resistant bacteria initiating a response in the presence of the antimicrobial. A beta distribution was fit to the simulated data set produced by Equation 35. The parameters that best fit this data were α_S = 0.2182 and β_S = 1088.37. Figure 3.2 shows a comparison of the simplified model (Eq. 30) with the resistant parameters (α_R, β_R), analogous to the traditional model, and the simplified model with novel parameters (α_S, β_S). The latter is analogous to a scenario of ingested dose comprised entirely of CIPS *Campylobacter* in the presence of 0.325 μg/L ciprofloxacin (i.e., 2.5% of *E. coli*'s MIC, 13 μg/L).[112] For the traditional model, the infectious dose that yields a 50% chance of infection (ID_{50}) is 3.19 $\log_{10}$ CFU of *Campylobacter*, while that for the novel parameters in the simplified model form is 4.40 $\log_{10}$ CFU.

With the understanding that a segment of this novel DRM is modeling the probability of infection by ciprofloxacin-susceptible (CIPS) *Campylobacter* in the presence of ciprofloxacin, it makes sense that almost all such scenarios show a lower probability of infection, due to the

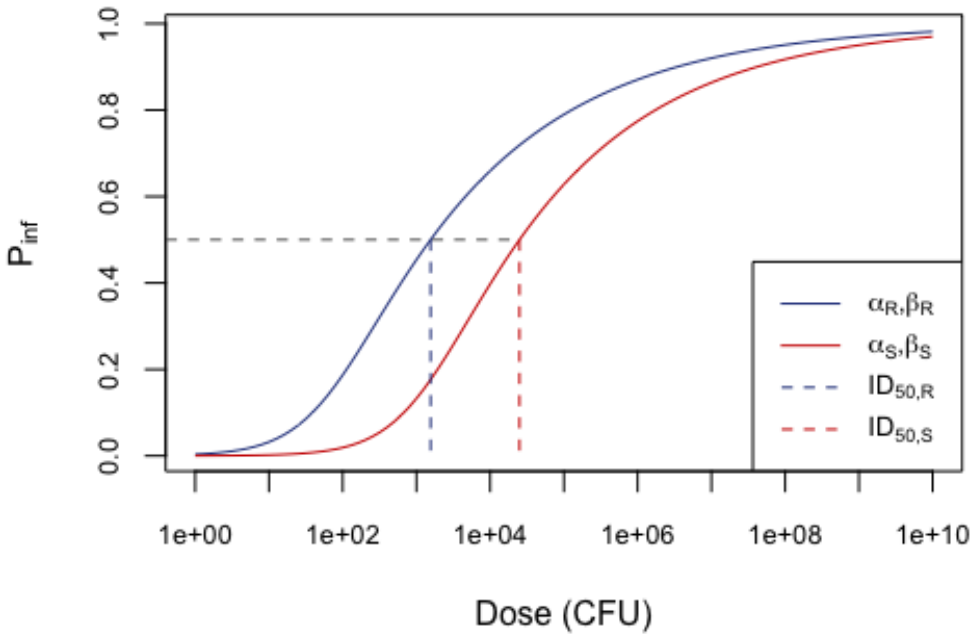

Figure 3.2. The traditional *Campylobacter* dose-response model (blue) and the novel ciprofloxacin-susceptible *Campylobacter* shape parameters in the simplified beta-Poisson model (red).
In our model, the traditional curve represents scenarios of no prior ciprofloxacin use or resistant *Campylobacter* in the presence of ciprofloxacin, and uses parameters from the FAO & WHO.[30] The novel model here represents a scenario if 100% of an exposure was susceptible *Campylobacter* in the presence of ciprofloxacin.
The ID_{50} of the traditional model ($ID_{50,R}$) is 3.19 $\log_{10}$ CFU, while the ID_{50} for the simplified model with novel parameters ($ID_{50,S}$) is 4.40 $\log_{10}$ CFU.

action of ciprofloxacin on the susceptible bacteria. This modeling framework was indeed developed because its ability to estimate risk from CIPR *Campylobacter*, however it intrinsically does this through comparison of risk from CIPS *Campylobacter* after altering the dose-response relationship with the latter. So, while CIPR *Campylobacter* remains of principle interest in this QMRA our estimation of its dose-response effects are dependent on its susceptible counterpart's relationship to the drug of interest.

The results of this novel DRM are specific to the parameter inputs used to create it (Table 2.4), such as ambient concentration of ciprofloxacin and the pharmacodynamic variables. The choice and implication of these parameters is discussed further in Chapter 4.

3.3. Estimated Illnesses

Finally, pairing the estimated dose distributions and their associated frequencies and the dose-response model framework described in Section 2.2 yields estimates of risk to the public from *Campylobacter* and CIPR *Campylobacter* on the national flock. Two forms of risk estimates from exposure to broiler meat were determined: probability of infection or illness per serving and number of infections or illnesses per 100,000 inhabitants annually. Further, due to the nature of this QMRA these estimates can be extended for both overall *Campylobacter* infection or illness, or specifically for CIPR infection or illness. These estimates, presented in Table 3.3 represent the final outputs of the baseline model of this QMRA.

From any one random serving of domestically produced, purchased, and prepared broiler meat the median probability of a campylobacteriosis was 0.015% (90% CrI: 0.000082-3.1%) and

Table 3.3. Risk estimates per one serving of broiler meat and per 100,000 inhabitants for both all *Campylobacter* exposures and specifically for ciprofloxacin-resistant (CIPR) *Campylobacter*. All measures are presented as median [5th percentile, 95th percentile].

	Probability of infection from one serving (P_{inf})	**Probability of illness from one serving (P_{ill})**	**Number of infections per 100,000 inhabitants ($N_{inf,100k}$)**	**Number of illnesses per 100,000 inhabitants ($N_{ill,100k}$)**
All *Campylobacter*	4.5e-04 [2.5e-06, 0.093]	1.5e-04 [8.2e-07, 0.031]	3,321 [18, 6.75e+05]	1,101 [6, 2.23e+05]
CIPR *Campylobacter*	6.0e-05 [3.7e-07, 0.013]	2.0e-05 [1.2e-07, 4.4e-03]	432 [3, 9.57e+04]	143 [1, 3.15e+04]

the probability of a ciprofloxacin resistant illness was 0.002% (90% CrI: 0.000012-0.44%). When multiplied by the total amount of chicken consumed in Canada annually, these estimates per serving can be used to estimate the total number of illnesses in the population. This QMRA estimated 1,101 total campylobacteriosis cases per 100,000 inhabitants annually in Canada, and 143 CIPR campylobacteriosis cases per 100,000 inhabitants annually. Therefore, among total illnesses approximately 13.0% will be ciprofloxacin resistant.

3.4. Sensitivity Analysis

The sensitivity analysis examines which data inputs are most influential in the final estimates of risk (Section 2.3.3). This analysis compared conditional medians of the probability of overall illness and CIPR illness from one serving ($P_{ill,overall}$ and $P_{ill,CIPR}$) across 10 different data inputs, chosen from Spearman rank correlation coefficients (not shown). Additionally, the ten inputs with highest Spearman rank correlation coefficient with the fraction of overall risk that is CIPR ($Fr_{ill,CIPR}$) were also tested with conditional median analysis. The smallest and largest conditional medians for each output when the various inputs are conditioned can be found in Table 3.4, along with the consequent swing.

Tornado plots visualizing the fluctuation among conditional medians of $P_{ill,overall}$, $P_{ill,CIPR}$, and $Fr_{ill,CIPR}$ for each input are shown in Figure 3.3. The distance from smallest conditional median to the largest conditional median are shown, demonstrating the change in output values that were influenced by different values of each input distribution. The baseline estimates from all iterations are shown for comparison. Those bars which extend to the left of the baseline estimation show an increased probability of illness for some segments of the input distribution. Inversely, extension of bars to the right indicates conditional medians that reduce the probability

Table 3.4. Sensitivity analysis results for the overall probability of illness from one serving ($P_{ill,overall}$), the probability of CIPR illness from one serving ($P_{ill,CIPR}$), and the fraction of total risk that is CIPR ($Fr_{ill,CIPR}$).
Swing represents the difference between the output's smallest and largest conditional medians when the input is varied within its own probability distribution.

Variable	Description	Smallest conditional median	Largest conditional median	Swing
$P_{ill,overall}$				
$Conc_{depop}$	Concentration of total *Campylobacter* on exterior of a bird at depopulation	1.14e-04	2.31e-04	1.17e-04
LC_{DF}	Change in external contamination on a bird due to defeathering	1.11e-04	5.84e-04	4.72e-04
LC_{EV}	Change in external contamination on a bird due to within-flock sources during evisceration	1.08e-04	1.08e-03	9.71e-04
LC_{HS}	Change in external contamination on a bird due to hard scalding	1.16e-04	5.56e-04	4.40e-04
N_{inf}	Number of birds in a flock colonized with *Campylobacter* at time of depopulation	4.04e-06	2.65e-04	2.61e-04
N_{proc}	Sequential order a flock was processed in throughout one day within the abattoir (i.e., first, second, etc.,)	1.09e-04	3.04e-04	1.57e-04
P_{damage}	Probability of a bird's viscera being damaged during evisceration	1.12e-04	1.06e-03	1.92e-04
P_{DCT}	Probability of direct (or within-flock) contamination during transportation	1.22e-04	1.06e-03	9.36e-04
$P_{undercook}$	Probability of chicken being undercooked during at-home meat preparation	6.61e-05	2.02e-04	1.41e-04
T_{cook}	Final internal temperature a chicken reaches during at-home cooking	1.32e-04	1.77e-03	1.63e-03
$P_{ill,CIPR}$				
$Conc_{depop}$	Concentration of total *Campylobacter* on exterior of a bird at depopulation	1.46e-05	2.88e-05	1.42e-05
LC_{DF}	Change in external contamination on a bird due to defeathering	1.42e-05	7.24e-05	5.82e-05
LC_{EV}	Change in external contamination on a bird due to within-flock sources during evisceration	1.36e-05	1.35e-04	1.21e-04
LC_{HS}	Change in external contamination on a bird due to hard scalding	1.47e-05	6.83e-05	5.36e-05
N_{inf}	Number of birds in a flock colonized with *Campylobacter* at time of depopulation	6.7e-0.7	3.36e-05	3.29e-05
N_{proc}	Sequential order a flock was processed in throughout one day within the abattoir (i.e., first, second, etc.,)	1.47e-05	3.16e-05	1.69e-05
P_{damage}	Probability of a bird's viscera being damaged during evisceration	1.5e-05	3.57e-05	2.07e-05
P_{DCT}	Probability of direct (or within-flock) contamination during transportation	1.57e-05	1.39e-04	1.23e-04
$P_{undercook}$	Probability of chicken being undercooked during at-home meat preparation	8.62e-06	2.83e-05	1.97e-05

T_{cook}	Final internal temperature a chicken reaches during at-home cooking	1.7e-05	2.18e-04	2.01e-04
$Fr_{ill,CIPR}$				
BFP_Z	Proportion of all colonized flocks colonized with CIPR *Campylobacter*	0.089	0.190	0.101
$Conc_{caec}$	Concentration of total *Campylobacter* in 1 gram of caecal contents	0.132	0.135	0.003
$Conc_{depop}$	Concentration of total *Campylobacter* on exterior of a bird at depopulation	0.133	0.135	0.002
N_{inf}	Number of birds in a flock colonized with *Campylobacter* at time of depopulation	0.131	0.148	0.018
N_{proc}	Sequential order a flock was processed in throughout one day within the abattoir (i.e., first, second, etc.,)	0.131	0.138	0.007
N_{trans}	Similar to N_{proc}, sequential order a flock was transported in throughout one day on one truck	0.131	0.137	0.006
OR_{HS}	Odds of a bird becoming newly externally contaminated during hard scalding	0.132	0.135	0.003
P_{AMU}	Probability of a human subject having taken fluoroquinolones within 45 days prior to *Campylobacter* exposure	0.133	0.136	0.003
P_{damage}	Probability of a bird's viscera being damaged during evisceration	0.132	0.137	0.005
P_{ICT}	Probability of indirect (or between-flock) contamination during transportation	0.129	0.136	0.007

of illness. Swing is simply the difference between the largest conditional median and the smallest conditional median.

The purpose of sensitivity analysis is not to identify areas in the model that most drastically reduce the probability of illnesses. Rather, this purpose is to identify those inputs that are responsible for a large amount of variability in the final outcome. The swing, or length of the bars in Figure 3.3 is more critical for interpretation than how far to the right that bar might go. Bars that are closely aligned with the baseline indicate that the ten conditional medians for the output of interest do not greatly vary as the input varies; values at an input's distribution extremes can lead to a similar output value.

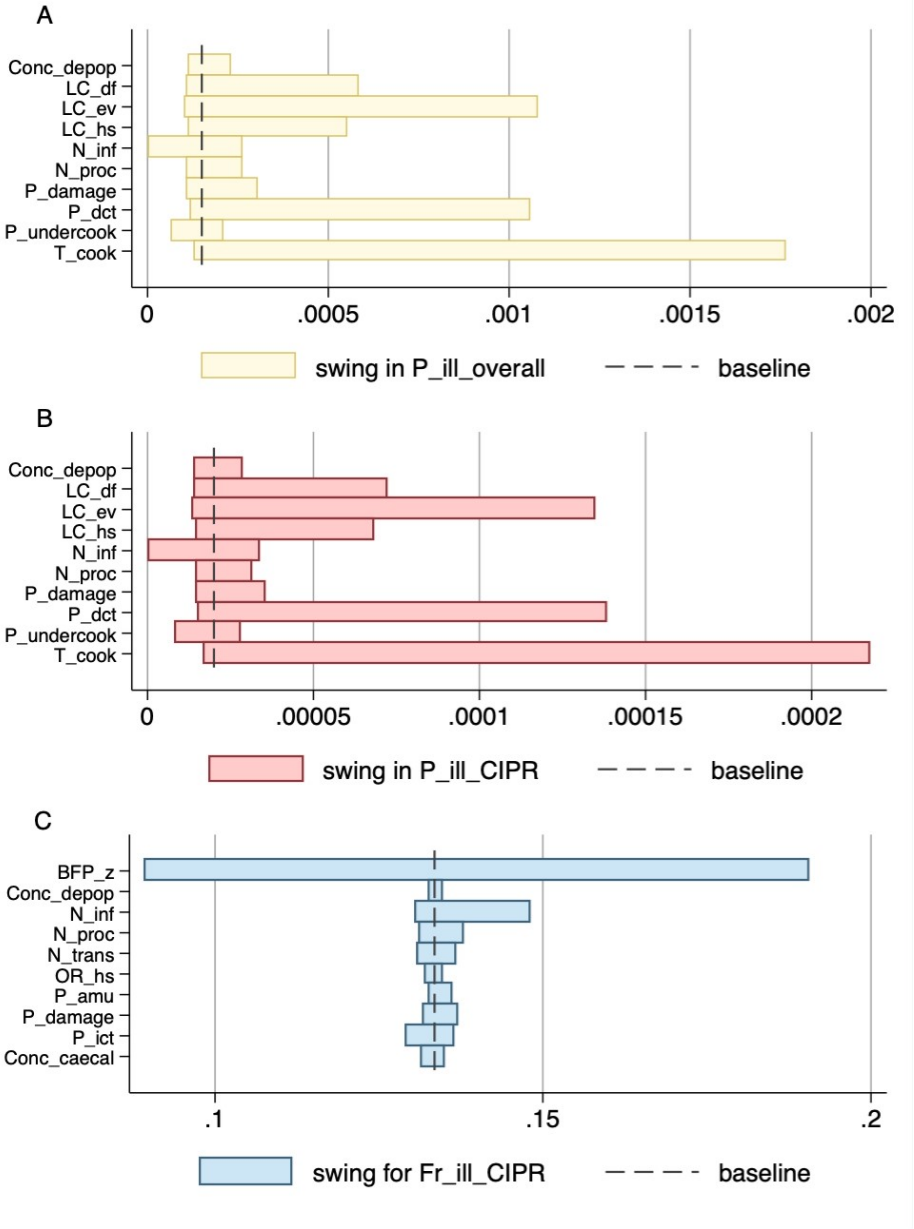

Figure 3.3. Tornado plots visualizing the change in output median for probability of overall illness per one serving (A), probability of CIPR illness per one serving (B), and the fraction of overall risk that is CIPR (C).
Displayed is the baseline of all outputs for comparison.
Bars extending to the left of the baseline show an increased magnitude of the output for some conditional medians, while extension to the right indicates lowered magnitudes of conditional medians.
Definitions for all variables can be found in Table 3.4.

For both $P_{ill,overall}$ and $P_{ill,CIPR}$, the internal temperature at cooking (T_{cook}) input has the largest effect on the probabilities of illness. Two additional inputs that show notable influence on $P_{ill,overall}$ and $P_{ill,CIPR}$ are the probability of direct (or within-flock) contamination during transportation (P_{DCT}) and the amount of cross-contamination spread within a flock during evisceration (LC_{EV}). Interestingly, of the 10 data inputs considered, the one that shows the least influence on these risk estimates is $Conc_{depop}$, or the average external contamination per bird at the time of depopulation. This can also be considered as the amount of contamination entering the system at the beginning of the exposure pathway.

Overall, the pattern of data inputs' influence of $P_{ill,overall}$ and $P_{ill,CIPR}$ is largely the same, with one small difference. While P_{DCT} and LC_{EV} remain the second and third most influential input distributions on $P_{ill,overall}$ and $P_{ill,CIPR}$, the former causes slightly more variation in $P_{ill,overall}$ while the latter is slightly more influential of the two for $P_{ill,CIPR}$. Although the differences between their effects on these two outputs are very small.

The relationships between the conditional median of the outcome variable at each bin of the top influential data inputs are further explored in Figure 3.4. Figure 3.4.A shows the conditional median for $P_{ill,overall}$ decreasing drastically as T_{cook} increases. The same trend can be found in Figure 3.4.B for the effect of T_{cook} effect on $P_{ill,CIPR}$. It appears that once T_{cook} enters its third bin, which corresponds to the 21^{st}-30^{th} percentiles (approximately 66.7°C), the condition medians of $P_{ill,overall}$ and $P_{ill,CIPR}$ stabilize at the overall baseline median of these outputs. The other two data inputs that cause noticeable variation in the overall and CIPR-specific risk of illness per one random serving were LC_{EV} and P_{DCT}. The changing values of LC_{EV} do not impact $P_{ill,overall}$ and $P_{ill,CIPR}$ until approximately bin 8 (71^{st}-80^{th} percentiles), at which point LC_{EV} has

values of 1.16 $\log_{10}$ CFU/bird and larger. Similarly, the medians of $P_{ill,overall}$ and $P_{ill,CIPR}$ begin to rise at bin 8 of

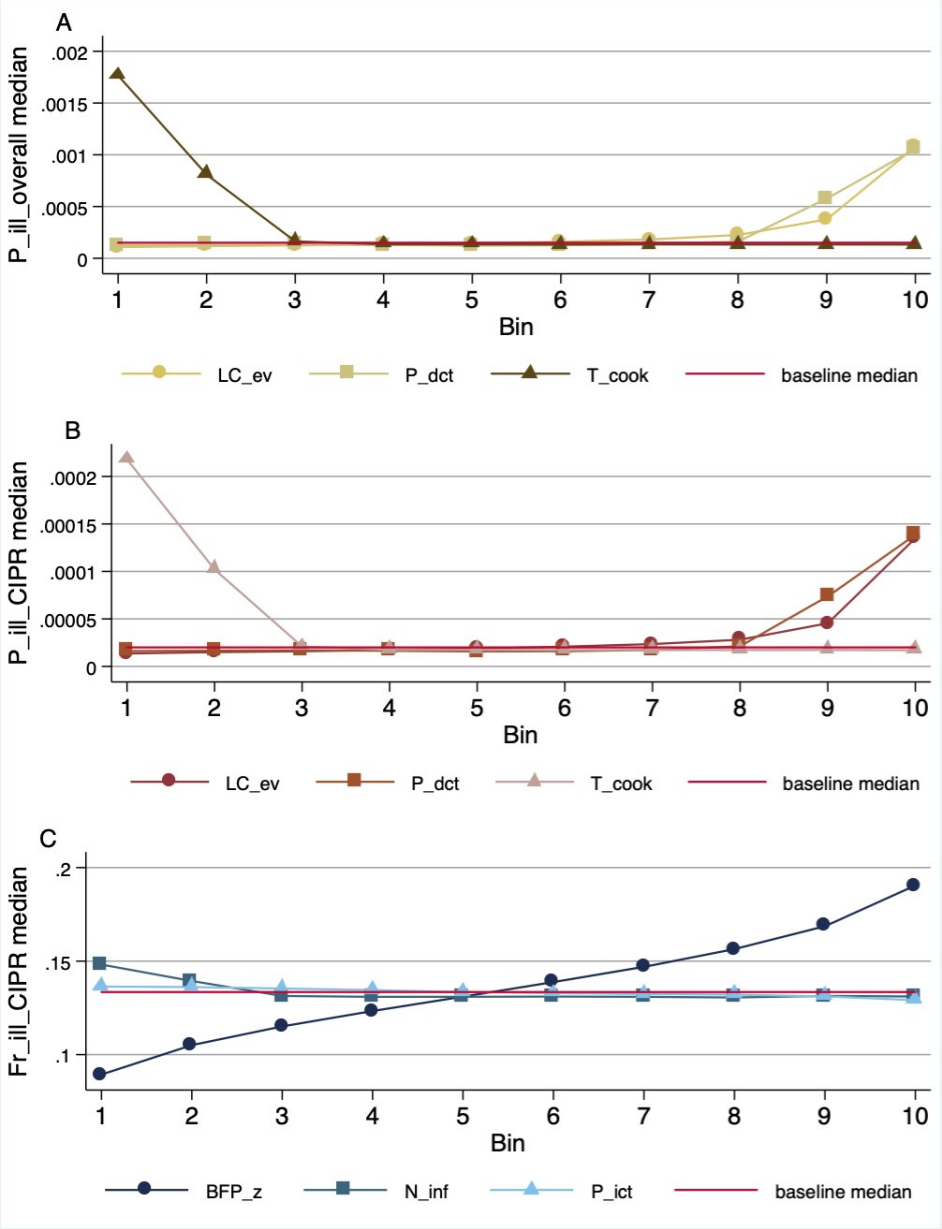

Figure 3.4. Spider plots for the top three most influential input variables on median of $P_{ill,overall}$ (A), $P_{ill,CIPR}$ (B), and $Fr_{ill,CIPR}$ (C).
Each bin represents 10 percentiles of inputs distribution, with the consequential conditional median for the output of interest.
The most influential input variables for $P_{ill,overall}$ and $P_{ill,CIPR}$ were the meat temperature at the end of cooking (T_{cook}), the probability of within-flock contamination occurring during transportation (P_{DCT}), and the magnitude of contamination from within-flock sources at evisceration (LC_{EV}).
The three most influential data inputs for $Fr_{ill,CIPR}$ were the fraction of positive flocks colonized with CIPR *Campylobacter* (BFP_Z), the number of birds in a flock colonized at the time of

depopulation (N_{inf}), and the probability of contamination from contaminated equipment occurring during transportation (P_{ICT}).

the P_{DCT} conditioned values, when P_{DCT} takes values of 0.4995 and higher. Figure 3.4.C illustrates the strong influence BFP_Z has on the median value of $Fr_{ill,CIPR}$. When the number of positive flocks colonized by CIPR *Campylobacter* (BFP_Z) is at its lowest range, the fraction of total illnesses that are CIPR is lowered, while when BFP_Z is set to its higher values this fraction drastically increases.

The input variables included for conditional median analysis of $Fr_{ill,CIPR}$ were quite different from those included for analysis of $P_{ill,overall}$ and $P_{ill,CIPR}$. There is one standout data input that had massive impact on the conditional medians of $Fr_{ill,CIPR}$: the fraction of all colonized flocks that are colonized by CIPR *Campylobacter* (BFP_Z). Given the lack of CIPR *Campylobacter* specific relationships and data inputs, necessitating the assumption that the resistant bug has the same survival and tolerance characteristics as its susceptible counterparts, it is understandable that the data input driving the fraction of all risk that is CIPR is the fraction of all contaminated flocks that are CIPR. In this model, the proportion CIPR at the outset is carried through the farm-to-fork exposure pathway and directly determines the proportion of risk that is CIPR. All other data inputs included in this analysis only had marginal impact on $Fr_{ill,CIPR}$ by comparison.

3.5. Scenario Analysis

The purpose of scenario analysis is to evaluate changes to the farm-to-fork pathway that may cause the greatest reductions in risk and help guide future policy. Seven different hypothetical interventions were evaluated against the baseline model and compared by percent

change (i.e., intervention efficacy [IE]) in the number of illnesses (total and CIPR) per 100,000 population and the fraction of illnesses that are CIPR (Table 3.5). Figure 3.5 shows box plots

Table 3.5. Results of the scenario analysis for the incidence of all campylobacteriosis per 100,000 population ($N_{ill,100k}$), the incidence of CIPR campylobacteriosis per 100,000 population ($N_{ill,CIPR,100k}$), and the fraction of illnesses that are CIPR ($Fr_{ill,CIPR}$).
All estimates are presented as median, and their associated 5th and 95th percentile values.
Intervention efficacy is calculated as per Equation 48.
Scenario A: on-farm flock prevalence (BFP_P) reduced; Scenario B: probability of between-flock contamination during transportation reduced; Scenario C: probability of between-flock contamination during evisceration reduced; Scenario D: total prevalence of birds contaminated leaving abattoir reduced; Scenario E: average contamination of birds leaving abattoir reduced; Scenario F: probabilities of kitchen cross-contamination and undercooking simultaneously reduced; Scenario G: probability of prior ciprofloxacin use reduced.
** 95% credible interval does not include the baseline.

Scenario	**$N_{ill,100k}$ (cases per 100,000)**	**Intervention Efficacy (percent change)**	**$N_{ill,CIPR,100k}$ (cases per 100,000)**	**Intervention Efficacy (percent change)**	**$Fr_{ill,CIPR}$ (percent)**	**Intervention Efficacy (percent change)**
Baseline	1101 [6, 2.23e+05]	-	143 [1, 3.15e+04]	-	13.3% [7.8%, 20.0%]	-
A	483 [3, 8.92e+04]	50.7% [47.4%, 95.4%] **	63 [0, 1.23e+04]	50.4% [42.0%, 95.6%] **	13.4% [8.4%, 19.7%]	0 [-23.2%, 76.2%]
B	966 [1, 2.14e+05]	4.5% [0%, 90.6%]	130 [0, 2.98e+04]	3.1% [-1.7%, 90.5%]	13.4% [8.7%, 20.0%]	0 [-25.3%, 2.6%]
C	894 [5, 1.27e+05]	0.6% [0%, 98.9%]	111 [1, 1.69e+04]	0.5% [0%, 99.2%]	13.2% [6.9%, 20.3%]	0 [-3.1%, 21.3%]
D	29 [1, 4624]	97.4% [90.2%, 99.3%] **	3 [0, 646]	97.5% [90.2%, 99.3%] **	13.2% [7.4%, 19.9%]	0 [-3.9%, 15.4%]
E	878 [5, 1.86e+05]	10.8% [0%, 83.3%]	[1, 2.57e+04]	8.1% [0%, 83.6%]	13.4% [7.9%, 20.0%	0 [-8.1%, 3.1%]
F	110 [1, 2.41e+04]	90.0% [88.9%, 90.0%] **	14 [0, 3402]	90.0% [88.9%, 90%] **	13.3% [7.8%, 20.0%]	0 [-0.1%, 0.2%]
G	1104 [6, 2.23e+05]	0% [0%, 0%]	143 [1, 3.15e+04]	0% [0%, 0%]	13.3% [7.9%, 19.8%]	0 [-0.3%, 0]

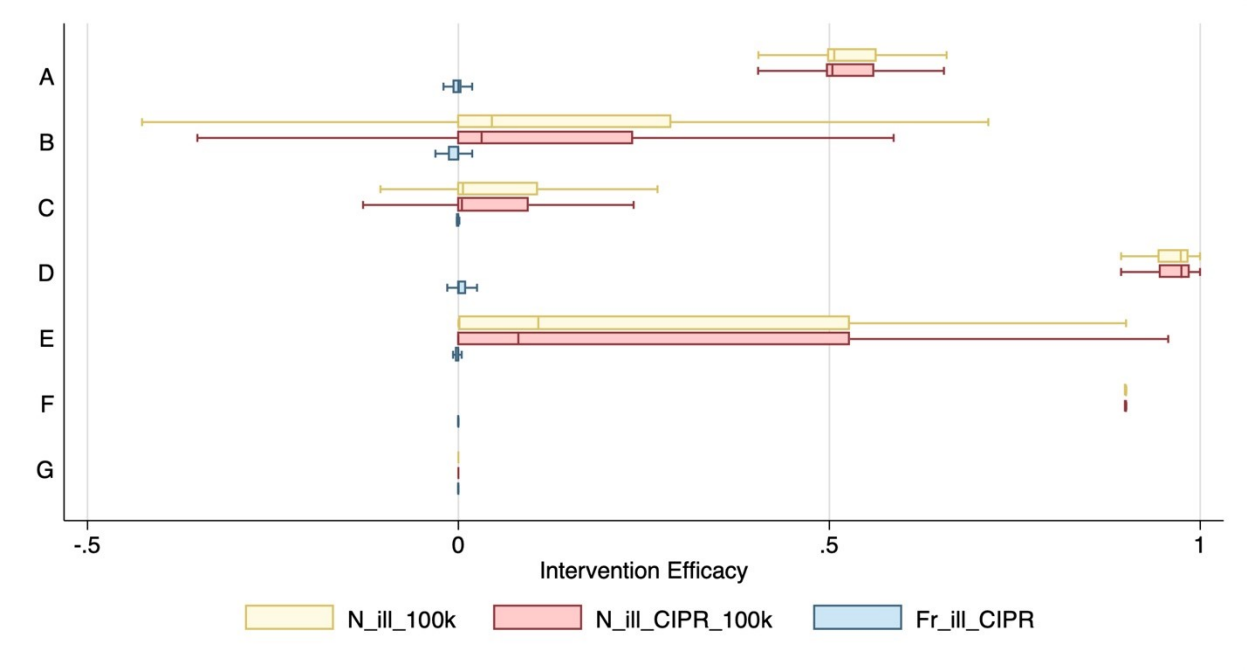

Figure 3.5. Intervention efficacy of seven interventions on incidence of overall campylobacteriosis per 100,000 population ($N_{ill,100k}$), incidence of CIPR campylobacteriosis per 100,000 population ($N_{ill,CIPR,100k}$), and the fraction of illnesses that are CIPR ($Fr_{ill,CIPR}$). Boxes are marked by the distribution's median in the centre while the left and right edges of the box are the distributions 25th and 75th percentiles, respectively. Whiskers denote the distributions adjacent values.
Scenario A: on-farm flock prevalence (BFP_P) reduced; Scenario B: probability of between-flock contamination during transportation reduced; Scenario C: probability of between-flock contamination during evisceration reduced; Scenario D: total prevalence of birds contaminated leaving abattoir reduced; Scenario E: average contamination of birds leaving abattoir reduced; Scenario F: probabilities of kitchen cross-contamination and undercooking simultaneously reduced; Scenario G: probability of prior ciprofloxacin use reduced.

comparing these findings side-by-side. (Readers are advised that the box plot whiskers in Figure 3.4 are Tukey's adjacent values, not 5th and 9th percentiles of the distribution, therefore, values are not reflected in Table 3.5.[120]) Additionally, the boxes themselves represent the interquartile range and the distribution's median.) An IE of zero indicates no change from the baseline model. An IE larger than zero indicates the percent reduction in total risk compared to the baseline model, while an IE less than zero indicates the possibility of increasing risk.

The largest percent reductions in median number of total illnesses per 100,000 ($N_{ill,100k}$) occurred in Scenarios D (reduced prevalence leaving the abattoir) and F (reduced probabilities of kitchen cross-contamination and undercooking), with approximately 97.4% and 90.0% less total cases, respectively. Conversely, Scenarios C and G showed the least intervention efficacy towards $N_{ill,100k}$ with only 0.5% reduction from Scenario C (reduced probability of between-flock contamination at evisceration) and no change from Scenario G (reduced probability of prior ciprofloxacin use). These trends were mirrors for scenario IE towards CIPR campylobacteriosis incidence ($N_{ill,CIPR,100k}$).

Unfortunately, no scenario caused any notable changes in the fraction of risk that is CIPR ($Fr_{ill,CIPR}$). This is most surprising in the case of Scenario G, where probability of prior ciprofloxacin use is decreased compared to the baseline, where we may have expected less prior AMU to lower $Fr_{ill,CIPR}$ since more CIPS *Campylobacter* would be able to survive and initiate illness. However, given that P_{AMU} was estimated to be 0.64% (90% CrI: 0.50-0.92%) this finding is likely because of the very small probability of occurrence in the baseline initially.

Chapter 4: Discussion

This thesis used a QMRA framework to meet four objectives: I) estimate magnitude and frequency of exposure to ciprofloxacin-resistant *Campylobacter* through broiler meat; II) construct and evaluate a novel dose-response model that accounts for antimicrobial resistance; III) estimate the risk of ciprofloxacin-resistant campylobacteriosis through exposure to broiler meat; and IV) analyze the baseline model using sensitivity and scenario analyses and assess knowledge gaps to guide future research, surveillance, and policy.

The exposure assessment tracked total *Campylobacter* and ciprofloxacin-resistant *Campylobacter* through the farm-to-fork food chain. The final estimation of median magnitude of ingested doses of total and CIPR *Campylobacter* were almost always 1 CFU per serving. This pattern changes when the exposure mechanism was through both raw cross-contaminated fluids and undercooked meat, where the estimated median dose was 2 CFU per serving (1 CFU from each mechanism). The 95th percentiles of these estimation probability distributions reveal the upper, yet unlikely, possibilities of these doses range from 47,700 CFU per serving to 2,920,000 CFU per serving. In terms of sheer magnitude, the size of doses does not meaningfully change between doses composed of CIPS *Campylobacter* versus CIPR *Campylobacter*.

A novel dose-response model was applied in cases of prior fluoroquinolone use by a human within 45 days prior to any CIPS *Campylobacter* exposure. New parameters were calculated for this beta distributions of α=0.22 and β=1088.37. In cases of no prior antimicrobial use or exposure to CIPR *Campylobacter*, a traditional dose-response model was used with α and β parameters of 0.21 and 59.59, respectively. By pairing our dose-response model with the magnitudes and frequencies of exposure, risk estimates were generated for both overall risk from any *Campylobacter* species and that specifically for CIPR *Campylobacter*. The total probability

of developing campylobacteriosis from one serving of broiler meat was estimated to have a median 0.015%. The median probability of developing a ciprofloxacin-resistant case of campylobacteriosis was estimated to be 0.002%. The median estimated incidence of total campylobacteriosis cases in Canada annually was 1,101 per 100,000 inhabitants, while the median estimated incidence of CIPR campylobacteriosis cases was 143 per 100,000 inhabitants.

Lastly, analysis was performed on this baseline model. Sensitivity analysis was conducted with 11 independent data inputs and analyzed conditional changes in the median of the probability of total *Campylobacter* illness from one broiler meat serving and the fraction of total risk that is CIPR. This analysis indicated the top three probabilities driving variability in the risk per serving were the temperature of cooking, the probability of within-flock contamination at transportation, and the magnitude of within-flock contamination at evisceration. The top data inputs driving variability in the fraction of risk were the percent of flocks colonized by CIPR *Campylobacter* on-farm and the within-flock prevalence of birds externally contaminated at the time of depopulation. Scenario analysis assessed changes in estimated incidence of illness in the population and changes in the fraction of risk that is CIPR if hypothetical changes were made along the farm-to-fork pathway. The top three scenarios that reduced the total incidence of all *Campylobacter* and CIPR illness were reducing the number of flocks colonized on-farm, reducing the number of birds externally contaminated at retail, and improving at-home chicken preparation practices. Surprisingly, no scenario meaningfully changes the fraction of illnesses in the population that were CIPR.

4.1. Model Validation

As described by Cox, quantitative risk models must be validated against real-world observational data before being accepted for risk management decision making, either through

comparison with microbiological enumeration for exposure assessments, or in rates of illness for risk characterization.[76] While formal statistics tests do exist for comparing a predictive model with laboratory-confirmed data, such tests were not employed here due to the scope of the project and limited viable data for such rigorous methods.[78] Often, acceptable model validation can be conducted simply by visually comparing the model estimates with a critical eye to published findings in the literature.[76,121]

In the context of contamination of broiler carcasses with *Campylobacter*, and especially ciprofloxacin-resistant *Campylobacter*, data specific to the current Canadian farm-to-fork context were difficult to identify. Studies may have been completed in different countries with different processing procedures, different policies regarding antimicrobial use in production, or the data were simply not gathered with the intent of being more broadly representative. Additionally, the *Campylobacter* found on birds in the abattoir, farm, or retail environments rarely undergo extensive antimicrobial susceptibility testing (AST) required to make reasonable conclusions about prevalences and concentrations for CIPR *Campylobacter*.[80] The field of data to reasonably validate the model was further diminished due to concerns around autocorrelation, since data that was used to construct the model cannot be used to subsequently validate it.[76]

4.1.1. Depopulation

At the time of depopulation, our model estimated 21.0% of the national flock (all birds produced across the country in a year) had external *Campylobacter* contamination, while 2.6% of the national flock had external CIPR *Campylobacter* contamination (Table 3.1). When validating these prevalence estimates against data in the literature two important considerations must be

noted: the *between*-flock prevalence in other settings, and bird colonization versus external contamination.

It is well documented that rapid horizontal transmission of *Campylobacter* occurs in broiler flocks while on the farm, and very frequently 100% of caecal samples or fecal droppings from commercial broiler flocks contain *Campylobacter*.[18,62,96,122] Ultimately, this indicates that thousands of birds housed closely together (as in commercial farms) can be colonized inside of a week and a prevalence of 100% at depopulation should be expected.[17,96] As reported by the federal surveillance program, CIPARS, the between flock prevalence (or the percent of all flocks that have at least one *Campylobacter*-positive sample, regardless of the frequency within that flock) is 21.8% (122 positive flocks of 560 sampled in 2018). Our pre-processing module makes the logical assumption that flocks that show no *Campylobacter* in any of the fecal between-flock samples (i.e., the other 438 flocks) cannot develop a within-flock prevalence and do not exhibit horizontal transmission. Therefore, in Canada, the theoretical maximum overall prevalence of birds that could show *Campylobacter* contamination at depopulation is 21.8%. In other words, within these positive flocks, our estimation of 21.0% of birds in the national flock having *Campylobacter* contamination is approximately 96.3% of the potential maximum. This is directly on target with data in the literature.

The other caveat when validating our model estimates is the source of these data: fecal samples or caecal colonization versus external contamination. The latter was chosen as our estimate in depopulation to allow for enable easier comparison of prevalence and concentration trends throughout the rest of the exposure pathway. This required the assumption that all birds colonized by *Campylobacter* also have external contamination on their skin or feathers. Published data on external *Campylobacter* prevalence and magnitude while on the farm are

extremely sparse. In 1995, Stern *et al*. measured the caecal and external contamination on randomly selected broilers from ten different farms in Georgia, USA.[98] This study shows that six out of 10 farms showed no external *Campylobacter* contamination while this fraction grew to nine out of 10 when examining the same birds' caeca. More recently, Mendes *et al*. published similar data from Portugal in 2020.[123] Forty-six fecal samples were positive for *Campylobacter*, while only 37 skin swabs showed *Campylobacter*. Lastly, Seliwiorstow *et al*. used a robust binomial regression model to find a statistically significant relationship ($p < 0.01$) between colonization of a birds' caeca and the external contamination of the bird, concluding that a reduction of colonization frequency can reduce external contamination frequency.[124] Overall, this indicates that prevalence of birds colonized versus that with external contamination may be slightly different (with the latter measure appearing to be slightly lower) but fluctuate in tandem and are closely linked.

As mentioned above, the Stern *et al*. data above were used to estimate magnitude of external contamination on a bird at depopulation. Using whole carcass rinses, Stern *et al*. estimated a median of 6.97 $\log_{10}$ CFU/bird at depopulation. Mendes *et al*. reported skin contamination of broilers at depopulation in Portugal with a mean of 1.43 $\log_{10}$ CFU/cm^2. Unfortunately, no conversion factors have been established to translate between contamination per whole bird and per cm^2.[125] Gill & Baduri reported that on average birds weighting 1.46 kg has an external surface area of 845 cm^2.[126] Mendes *et al*. reported that the birds in their study had a median of approximated 1.7 kg, 16% larger than those measured by Gill & Baduri.[123,126] Assuming weight and surface area increase proportionally, a 16% increase in 845 cm^2 is 980 cm^2. If the birds in Mendes *et al*. had a total surface area of 980 cm^2 and 1.43 $\log_{10}$ CFU/cm^2 as reported, these birds would have approximately 4.42 $\log_{10}$ CFU/bird at depopulation.

Alternatively, Zhao *et al*. estimated that birds have an approximate average surface area of 1,200 cm^2 for birds weighing on average 1.6 kg.[127] In this case, the Mendes *et al*. estimation of 1.43 log_{10} CFU/cm^2 would lead to 4.51 log_{10} CFU/bird. A final approximation uses a linear relationship published by Thomas in 1978: y=0.67x+536, where y is external surface area in cm^2 and x is broiler weight in g. Using the Thomas relationship, the Mendes birds would have had an average of 4.65 log_{10} CFU of *Campylobacter* per bird. This rough approximation appears to indicate that the Stern *et al.* estimate used by our model may be overestimating the magnitude of *Campylobacter* contamination on a broiler chicken at depopulation, although no further data exists to corroborate this.

When considering the role of CIPR *Campylobacter* in external contamination prevalence and magnitude, some on-farm flock prevalence estimates have been published but no magnitude data exist. The prevalence of ciprofloxacin-resistant samples among all *Campylobacter*-positive samples taken from mature flocks appears to vary from approximately 10% to 35% in studies in the last four years.[128–132] Our model predicted 2.6% of the national flock, or 12.4% of all positive birds will be externally contaminated with CIPR *Campylobacter* at the time of depopulation. The closest estimate to this in North America is from Beier *et al*. in Texas, where 13 of 96 fecal samples taken from broiler flock barns showed ciprofloxacin resistance in 2021.[129]

The estimated magnitudes (and surrounding probability distributions) of external contamination for general *Campylobacter* and for CIPR *Campylobacter* are identical due the assumption that CIPR *Campylobacter* will entirely outcompete ciprofloxacin-susceptible *Campylobacter* when initiating colonization of a bird and spreading through horizontal transmission. Consequently, in our model this created the division of all positive flocks into S

flock which were assumed to be 100% CIPS *Campylobacter* and R flock which were assumed to be 100% CIPR *Campylobacter*.

Intriguingly, *in vivo* transmission studies have shown that CIPR *Campylobacter* outcompetes ciprofloxacin susceptible *Campylobacter* in the broiler gut.[71,72] In many cases of antimicrobial resistant bacteria, a loss-of-fitness is documented when compared to the susceptible (i.e., wild type) bacteria in the absence of the antimicrobial in question.[133] In 2005, Luo *et al*. published striking findings that, contrary to what was expected, when live chickens were exposed to both wild-type and CIPR *C. jejuni*, the CIPR strain almost entirely became the only *Campylobacter* strain to colonize the bird's caeca.[71] These findings were repeated and confirmed by Han *et al*. in 2012.[72] While the exact cause of this remains unknown, these studies theorize that the *gyrA* mutation responsible for resistance to ciprofloxacin (see Section 1.3) additionally promotes the ability of the cell to replicate by reducing the amount of DNA supercoiling needed.[71,72] Ultimately, the experimental evidence shows that even in cases where initial exposure of CIPS and CIPR *Campylobacter* is 3:1, respectively, the replication advantage of CIPR *Campylobacter* is enough to completely dominate a living chicken gut. Due to these findings, the modeling decision was made that any on-farm flock showing CIPR *Campylobacter* would be contaminated with only CIPR *Campylobacter*.[71,72]

An additional assumption was made that CIPR *Campylobacter* has identical transmission characteristics due to a lack of evidence illustrating otherwise. Therefore, both S and R flocks were assigned the same data inputs and modeling relationships and consequently estimate the same magnitudes of external contamination on birds at depopulation.

4.1.2. Transportation

The transportation node of this model was adopted from the FAO & WHO and estimates added contamination to a flock from direct and indirect sources.[30] Direct contamination is due to fecal spread over the duration of transportation from other birds within the same flock. On the other hand, indirect contamination estimates contamination sourced from other flocks through cross-contaminated workers and crates and is primarily a function of the probability of a positive flock being transported with the same equipment earlier that day. Overall, this model estimates a 9.6% increase in prevalence among all birds that have any species or magnitude of external contamination following contamination. The fraction of all externally contaminated birds that have some amount of CIPR *Campylobacter* also increases from 12.3% of all positive birds at depopulation, to 14.1% of all positive birds at the conclusion of transportation.

The estimated increase in prevalence of birds with external contamination is primarily due to the indirect spread of *Campylobacter* to previously negative flocks (N flocks). As discussed in Section 4.1.1, virtually all birds within S and R flocks have some amount of external *Campylobacter* contamination at depopulation, logically indicating that a growth in overall prevalence comes from the introduction of *Campylobacter* to N flocks. A major knowledge gap impacting this modeling is the lack of better probabilistic data for the likelihood of a bird picking up any external *Campylobacter* contamination when there was a prior contamination flock transported with the same equipment.

Similarly, our fraction of all contaminated birds that have some amount of CIPR *Campylobacter* grew during transportation due to CIPR *Campylobacter* moving between flocks through indirect contamination. Although, this modeling has the same pitfalls since no precise data exist bridging the probabilities of cross-contamination between a positive flock being

transported and a downstream flock being contaminated specifically with those *Campylobacters* left behind.

Stern *et al*.'s 1995 data support the theory of between-flock cross-contamination being notable during transportation.[98] Of 10 monitored flocks before and after transportation, four showed no external *Campylobacter* contamination beforehand while only one flock remained without external *Campylobacter* after transportation. The recent literature review from Rasschaert *et al*. also notes the importance of transportation as a time of significant cross-contamination between flocks.[86]

Perez-Arnedo & Gonzalez-Fandos found between 3.6-3.9 $\log_{10}$ CFU/cm^2 on recently used, unwashed transport crates.[134] Even prior to being used for transportation and encountering a flock, these crates can have up to 2 $\log_{10}$ CFU/cm^2 of *Campylobacter* contamination.[86,134] A 2010 study from Ellerbroek, Lienau, & Klein directly compared used transport crates before and after cleaning and only noted a 7.8% reduction in *Campylobacter*-positive crates.[135] While it is clear transportation is crucial in between-flock cross-contamination (and our assumption of no crate contamination at the beginning of day is likely questionable) the mechanisms and associated probabilities relating the contamination on crates to the skin of live broilers remains elusive.

Overall, our model estimated a median of 5.94 $\log_{10}$ CFU/bird of total external *Campylobacter* contamination post-transportation. Several other studies enumerating *Campylobacter* on broilers entering the abattoir show counts per bird in the 5.5-7.5 $\log_{10}$ CFU/bird range.[122,136–138] Being soundly within this range is an encouraging sign that our magnitude of total contamination on positive birds entering the abattoir is on target, despite any

possible overestimation of contamination attributed specifically to between-flock cross-contamination.

Sahin *et al*. noted that in the United States approximately 20% of *C. jejuni* isolated from broilers at slaughter (directly after transportation but preceding scalding) show resistance to ciprofloxacin.[139] Dramé *et al*. reported approximately 11% of *Campylobacter* samples taken from across Canada at broiler slaughter were ciprofloxacin-resistant.[62] Our estimated 14.1% of *Campylobacter* post-transport being CIPR falls within these numbers. Our estimation falling on the low end of what has been reported may be due the constraints placed on between-flock cross-contamination, leading to an underestimation of the spread of CIPR *Campylobacter* from prior R flocks, to transport equipment to downstream flocks. Assuming the crates are entirely clean at the beginning of each day when they likely still carry some resistant contamination from R flocks transported on previous days is a likely explanation for this apparent underestimation.

4.1.3. Scalding and Defeathering

Our exposure assessment had simple scalding and defeathering nodes compared to the previous depopulation and transportation. Log changes and odds ratios from Dogan *et al*. were simply applied to 'before' counts and prevalence to estimate 'after' external concentrations per bird and overall prevalence of contaminated birds among the national flock.[91] Due to the lack of data, the assumption was made that CIPR *Campylobacter* has the same survivability characteristics as unspecified *Campylobacter*.

Our model estimated a 7.5% reduction in overall prevalence of birds with any external *Campylobacter* contamination post-scalding, no change in the proportion of positive birds with

CIPR *Campylobacter*, and an approximate 1.5 $\log_{10}$ CFU/bird decline per birds in average total *Campylobacter* contamination.

This drop in carcass contamination is in line with observational studies. Pacholewicz *et al*. documented between 1.17-1.58 $\log_{10}$ CFU per bird decline due to scalding, while Duffy *et al*. found approximately a 2 $\log_{10}$ CFU per bird decline.[122,140] In regard to prevalence of birds with *Campylobacter* contamination following the scalding process, a systematic review by Guerin *et al*. stated that few studies measured prevalence changes and those that did found between 20-40% reductions.[141] Overall, however, Zweifel, Althaus, & Stephan found an average prevalence of birds post-scald showing *Campylobacter* contamination to be 22.6%, in line with our estimate of 23.0% (90% CrI: 1.6, 35.5).[142]

Following scalding, birds undergo defeathering. Post-defeathering, our model estimated a slight rise in overall prevalence, to 23.4% (90% CrI: 1.9, 39.5), an accompanying slight rise is median contamination, to 4.32 $\log_{10}$ CFU per bird (90% CrI: 0.36, 7.61) and no change in the proportion of positive birds that are ciprofloxacin-resistant. A small fluctuation ($\leq$1 $\log_{10}$ CFU/bird) in either direction for these estimates due to defeathering is acceptable. Increases in external *Campylobacter* contamination are theorized to be caused by the rubber fingers of the machine putting pressure on the bird and causing fecal leakage and spread.[140,143,144] Less common but still occasionally seen is a decrease in average external contamination theoretically due to the rubber fingers picking up contamination.[142] Allen *et al.* reconciled these opposing findings (defeathering reducing vs. increasing external contamination) by postulating that the machine may remove significant contamination from one bird, but that these removed microorganisms are distributed to other birds both upstream and downstream.[145] The potential travel of *Campylobacter* between birds (especially from contaminated birds to those without

prior external contamination) due to defeathering is well accepted and mechanistic models are emerging that attempt to describe this spread.[99] Logically, in cases where *Campylobacter* is transferred to previously negative birds, the flock prevalence will rise as documented in observational studies and reported by our model.[144,146]

4.1.4. Evisceration

In our exposure assessment model, the evisceration node is the second and final place where explicit between-flock cross-contamination was quantified and estimated. In effect, this means the potential for significant changes for the CIPR *Campylobacter* population in S flocks and CIPS *Campylobacter* population in R flocks, as well the as the creation of mixed birds (both CIPS and CIPR *Campylobacter* on one bird) in N flocks. Prior to evisceration, our model excluded the possibility of mixed birds in N flocks because of the decision at transportation for one flock to potentially receive indirect contamination from only one prior flock, therefore birds in N flocks between transportation to defeathering could be contaminated with only CIPS *Campylobacter* or CIPR *Campylobacter*.

Our model estimated that 32.7% of all birds have some amount of external *Campylobacter* contamination with 15.0% of these positive birds possibly having some CIPR *Campylobacter*. Additionally, the average magnitude of total contamination is 4.84 $\log_{10}$ CFU/bird, a 0.52 $\log_{10}$ CFU increase from the median total contamination post-defeathering. As expected, this is the highest average contamination per bird since the birds entered the processing facility. Evisceration is frequently the stage at which contamination on broilers spikes due to damaged viscera and the spread of caecal contents downstream to other birds and other flocks.[86,141,147]

Pacholewicz *et al.* found an average increase of 0.75 $\log_{10}$ CFU in external contamination of broilers with *Campylobacter* at evisceration.[140] Seliwiorstow *et al*. also measured external contamination after defeathering and again after evisceration and recorded increases in mean contamination between 0.42 and 1.7 $\log_{10}$ CFU.[146] An earlier study from Seliwiorstow *et al*. reported within-flock increases between 0.20 and 0.80 $\log_{10}$ CFU, and one flock which showed an average decrease in external *Campylobacter* contamination by 0.07 $\log_{10}$ CFU.[148] Interestingly, Zweifel *et al*. also reported small average decreases in external *Campylobacter* contamination post-evisceration, although a consistent mechanism for this unexpected decrease has yet to emerge from the literature.[142] Small increases in overall prevalence of birds (0.05-4.5%) with external *Campylobacter* were also reported, including by Zweifel *et al*., likely indicating that the main driver behind the notable increases at evisceration is the amount of contamination being spread and not necessarily the number of birds it is being spread to.[141,142,144]

Mechanistically speaking, the source of additional contamination comes from evisceration equipment rupturing internal organs (specifically during cloacal excision) and contaminating the equipment itself and spreading caecal contents downstream.[14,149,150] Theoretically, if such damage could be prevented, evisceration would cease to be such a critical point along the farm-to-fork pathway, and perhaps the final *Campylobacter* load reaching the consumer would diminish. However, evisceration machines are set to expected sizes of birds, and not individually changed for individual bird measurements or even flock measurements, meaning their action can be imprecise.[86]

A different approach rather than eliminating damage during evisceration is to minimize the caecal concentration of *Campylobacter* to begin with. Our model used caecal concentration per 1 gram of contents data from Stern *et al*., which has a median 6.69 $\log_{10}$ CFU/g (Table 2.2).

Averages between 6-9 $\log_{10}$ CFU/g have been reported, in line with the data used here.[13,15,151,152] Effectively preventing *Campylobacter* colonization on farm has so-far proven challenging and unpredictable, as further discussed in Section 4.3.2 of this thesis. Alternatively, introducing a feed withdrawal period, where flocks are restricted from eating for some period of time (optimally 9-12 hours) before slaughter with the intention of clearing the gastrointestinal tract, has potential in lowering the caecal load.[124] However, this period of time is a tricky balance with no precise answer: too little and the GI tract does not clear leading to increased external contamination downstream, too much and the birds weight can decrease (causing economic loss for a company) and the tensile strength of the intestinal walls can diminish, increasing risk of rupture during evisceration.[86,124,153]

Hartnett identified a key knowledge gap connecting the concentration within the caeca and the concentration contaminating the subsequent birds.[99] The assumption proposed by Hartnett, and adopted by this model, was to apply a random chance that the concentration of *Campylobacter* spread is anywhere from 0 to 100% of the caecal concentration. Additionally, García-Sánchez *et al*. exemplified the persistence of *Campylobacter* on the evisceration equipment itself, with 78% of samples from the equipment testing positive.[149] Even after cleaning, 56.4% of these equipment surfaces were contaminated with *Campylobacter*.[149] Studies which identified 'negative' flocks (no *Campylobacter* colonization in gut) found external contamination of these birds rise notably by 1 $\log_{10}$ CFU from defeathering to evisceration when a positive flock was processing directly before, clearly indicating spread of the microorganisms downstream from the positive flocks.[13,146] Observational findings still remain too scattered and vague for a robust mechanistic model of risk of rupture and consequent down-stream

contamination, but findings are moving in the right direction for understanding evisceration's impact on *Campylobacter* spread through the abattoir.

4.1.5. Washing and Chilling

Washing and chilling are the major decontamination steps in the abattoir, which remove any fecal matter or other organic matter remaining on the carcass after defeathering and evisceration.[86,153] This model includes inside-out washing with chlorinated water. Washing effectiveness is highly dependent on water pressure and chlorine concentration and as a result the reported results of this stage can vary.[154] Generally, washing appears to create modest reductions in *Campylobacter* prevalence among birds and concentration on external skin when compared to post-evisceration measurements. Prevalence among birds may be reduced by approximately 5% while the external count may diminish by 0.5-1 $\log_{10}$ CFU/bird.[15,142,144,155] Our model estimated a 2.5% reduction in prevalence of birds with any external *Campylobacter* contamination and an average 0.46 $\log_{10}$ CFU/bird reduction from post-evisceration to washing. With no published data specific to CIPR *Campylobacter* it was assumed that the effects of washing were the same for CIPR and unspecified *Campylobacter*.

Chilling is typically accomplished either through air chilling or immersion chilling. In air chilling the carcasses are placed in a cold room for upwards of an hour, while immersion chilling involves placed the birds in tanks of cold water, usually chlorinated and possibly with a counterflow, for less than an hour.[141] A review and meta-analysis from Bucher *et al*. asserted that, in general, the chilling stage is effective at reducing the magnitude of *Campylobacter* contamination, but has variable outcomes in modifying prevalence.[156] Air chilling is commonly believed to create modest (<0.5 $\log_{10}$ CFU/bird) but consistent concentration changes on carcass skin.[15,141,150,157] Some believe that there is no possibility of cross-contamination between birds or

flocks due to the nature of air chilling whereby birds do not come into contact with each other.[30] While reasonable, Bucher *et al*.'s meta-analysis found a large increase in prevalence of birds with external *Campylobacter* contamination following air chilling (although the quality of studies included was rated as poor).[156] Meanwhile, immersion chilling with chlorinated water is believed to have greater effectiveness and eliminating *Campylobacter* (approximately 1 $\log_{10}$ CFU/bird reduction) but may potentially cause cross-contamination due to shared chilling water.[141,154,158–160]

Post-chilling is the point in almost all broiler QMRAs and observational studies where final determinations are made for *Campylobacter* contamination and therefore presents an opportunity for robust comparisons. In 2018 CIPARS reported a 24% recovery rate of *Campylobacter* from broiler samples taken post-chilling across Canada. Reports from the United States show slightly higher prevalence of post-chill birds with *Campylobacter* ranging from 30-44%.[136,161] The magnitude of *Campylobacter* found on these North American birds post-chill varies greatly from 1.98 to 5.65 $\log_{10}$ CFU/bird.[105,136] Our model estimated a median of 30.1% (90% CrI: 2.0, 62.1) of all broilers leaving Canadian abattoirs have some *Campylobacter,* with a median magnitude of contamination of 3.53 $\log_{10}$ CFU/bird (90% CrI: 0.00, 8.75). In addition, we estimated an average 3.27 $\log_{10}$ CFU/bird (90% CrI: 0.01, 8.05) of CIPR *Campylobacter* on approximately 4.7% (90% CrI: 0.3, 11.1) of all birds at the end of the processing stage.

Some studies have measured prevalence of birds that show ciprofloxacin-resistance post-chilling, providing an important opportunity to evaluate CIPR *Campylobacter* within the abattoir environment. In 2018, CIPARS reported 20% of *Campylobacter* isolates from birds post-chilling showed ciprofloxacin resistance, an increase from previous years (7% in 2015, 13% in 2014, 16% in 2013).[42,128] This is similar to our estimate of 15.3% of *Campylobacter*-positive birds

having ciprofloxacin resistance when leaving the abattoir. A unique study from the United States provides some insight into AMR profiles and chilling types. Sánchez *et al*. reported that only 18.2% of the *Campylobacter*-positive isolates recovered from after air-chilling were ciprofloxacin-resistant, this prevalence significantly increased to 58.3% of isolates from immersion water chilling.[159] This may be either from increased *Campylobacter* strain diversity accumulating in the chill tank water or the presence of chemical agents (e.g., chlorine) creating selective pressure that allowed resistant populations to flourish.[149] While an interesting finding, further discussion regarding processing procedures and their impact on CIPR *Campylobacter* populations specifically is not possible due to the dearth of similar research.

4.1.6. Post-Processing

Retail meat also provides a routinely studied moment for the broiler meat to evaluate bacterial contamination due to ease of sample collection and the proximity of the product that moves directly to the consumer, partitioning the farm-to-fork pathway nicely. This place is also where some of the richest CIPR *Campylobacter* prevalence and magnitude data are collected along this pathway.

Our model combined all cold storage time (whether on retail shelves or at home) but for ease of comparison published retail data were compared to the cold storage node in this model. We estimated 22.5% of all broilers had an average concentration of 2.13 $\log_{10}$ CFU/bird of any *Campylobacter*, while 3.5% of all birds (16.0% of all *Campylobacter*-positive birds) had an average 1.76 $\log_{10}$ CFU/bird of specifically CIPR *Campylobacter*. Surveillance reports from CIPARS and the Canadian Food Inspection Agency (CFIA) have very clear estimates of total retail products with *Campylobacter*, and helpfully, the fraction of those resistant to ciprofloxacin.

In 2018, the overall prevalence of *Campylobacter* was 25.4% of all retail samples taken.[94] This prevalence was generally consistent with recent years (25.3% in 2017, 26.6% in 2016, and 25.8% in 2015) as reported by CIPARS.[94] Our model estimation of overall prevalence following cold storage was very closely in-line with these the robust nation-wide surveillance data from CIPARS, adding notable external validity to our model. It is possible that our post-cold storage prevalence was slightly lower than what has been observed because of the extra time in chilled temperatures with at-home storage in our model.[162]

In 2018, CIPARS found that 13.6% of the *Campylobacter* isolated from retail broiler meat (overall prevalence of 25.4%) was resistant to ciprofloxacin, implying that 3.5% of all broiler meat at retail across the country had CIPR *Campylobacter* contamination.[94] This is exactly in line with the mean of our calculated probability distribution for birds with CIPR *Campylobacter* at cold storage. Other Canadian studies have reported approximately 12% of *Campylobacter* from retail broiler meat is CIPR.[62] A recent sample of retail chicken from North Carolina found 11.1% of *Campylobacter* contaminated birds were ciprofloxacin resistant.[128]

The CFIA completed a microbial baseline study in 2013 and found the concentration of total *Campylobacter* on retail meat to be an average of 2.86 $\log_{10}$ CFU/bird.[105] Australian studies have estimated average *Campylobacter* concentrations on retail chicken to be 1.82 – 2.49 $\log_{10}$ CFU/bird.[137,163] Unfortunately, similar microbial count estimations at retail have not been completed specifically for CIPR *Campylobacter*. One study did complete enumeration of CIPR *Campylobacter* specifically in Arkansas and found the median of total *Campylobacter* to be between 2 – 3 $\log_{10}$ CFU/carcass, while the median of CIPR *Campylobacter* was between 1 – 2 $\log_{10}$ CFU/carcass.[164] Albeit, this study was completed between 2001 and 2003 and ciprofloxacin resistance trends have changed in the intervening 20 years and may not be directly comparable to

today's context. This work does provide a helpful foundation for understanding CIPR *Campylobacter* at retail and that a concentration approximately 1 log lower per bird than total *Campylobacter* (as our model estimates) can be expected.[164]

Once the meat product has been purchased at a retail facility and brought into a domestic kitchen it becomes difficult to known exactly how it is handled. The best possible data regarding at-home preparation come in the form of consumer surveys, a less than perfect but still useful measuring system. Nonetheless, peer-reviewed data give insight into preparation habits when raw chicken is handled, cooking times and temperatures, and other demographic information.

Two mechanisms for ingestion of *Campylobacter* were included in this final stage of the exposure assessment: cross-contamination from raw meat and undercooking the meat. For the former, our exposure assessment adopted the drip-fluid model proposed by FAO & WHO in 2009.[30] This model is especially advantageous in modeling exposure through cross-contamination as it does not rely on the presence of another vehicle. Some cross-contamination models use salad or other vegetables as a vector the uncooked microorganisms attach to due to suboptimal sanitization in the kitchen.[83,165] The drip fluid model does not require a vector mediating between the broiler meat and consumption nor consideration of associated attachment rates with *Campylobacter*. However, unvalidated assumptions are made by the FAO & WHO regarding the volume of fluid diluting the bacteria and the fraction of cells which may be ingested.[30] These assumptions are employed here as well. To estimate microbiological changes due to cooking the thermal inactivation model used by Dogan *et al*. is adopted here and is a function of biologic parameters specific to *Campylobacter*.[91,104]

Generally, our post-processing module did an adequate job of representing reality. In a large literature review, Khalid *et al*. highlights three components of consumer behaviour that are

critical in preventing campylobacteriosis: storage, preparation, and cooking.[84] Our cold storage, cross-contamination, and undercooking nodes modeled each of these, respectively. Among these, Khalid *et al.* postulates that cross-contamination during preparation of raw poultry is the main factor leading to illness.[84] Observed data are likely to be more accurate as they avoid the pitfall of social desirability bias common with self-reported surveys. In 2017, 92.9% of participants in Canadian survey reported washing hands after handling raw meat.[103,166–168] Meanwhile, through direct observation, Bruhn estimates that only 38% of Americans properly wash their hands after handing raw chicken.[115] While the latter report may be closer to the truth, it still does not encapsulate the full probability of cross-contamination during preparation. The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) showed that utensil cleaning interventions reduced *Campylobacter* risk by 85% whereas hand washing interventions reduced risk by 1%, indicated far more cross-contamination occured through utensils than hands.[169] Perhaps future models should closely consider the mechanistic dynamics of cross-contamination in the kitchen, particularly through utensils and cookware.

Many believe that inadequate thermal inactivation from cooking is a less significant exposure route due to the sensitivity of *Campylobacter* to temperatures higher than its tolerant range (35-40°C).[83,84] While a thermal inactivation model was adopted from Dogan *et al*. and is based on biologic parameters (e.g., D-value, z-value), alternative models have been developed that hypothesize that only a portion of the meat in a 'protected area' is likely to experience lower temperatures, and therefore allow the survival of *Campylobacter*.[30,91,104] These models help provide a mechanistic explanation for the survival of *Campylobacter* throughout the cooking process despite the organisms heat sensitivity.[30] However, the data requirements are immense and predicated on assumptions which are not necessarily quantitatively supported.[30] Our

undercooking node does not require such parameters and assumes an equal likelihood of inactivation for the entire *Campylobacter* and CIPR *Campylobacter* populations, regardless of location on the meat. Approximately 33.2% of Canadians use a thermometer when preparing whole chickens and only 12.3% when preparing pieces of chicken (e.g., breast, thigh), leaving many home cooks vulnerable to the possibility of undercooked chicken.[167] Risk assessors should not underestimate the possibility inadequate thermal inactivation as a pathway to campylobacteriosis.

Unfortunately, no studies or models representing ciprofloxacin-resistant *Campylobacter* in home kitchens or during thermal inactivation have been published. Perhaps a recent finding that CIPR *Campylobacter* is more prone to biofilm formation may indicate a resistance to cross-contamination spread (lowered rate of detachment), however further research is necessary.[68]

4.1.7. Risk Estimates

Perhaps the best stage to validate a model is with the final outputs. Despite any upstream merits, if the final estimated results are not considered reasonably similar to real-life observations, then upstream decisions are irrelevant. The final risk estimates from our QMRA are presented in Table 3.2, as risk of infection or illness from *Campylobacter* from one serving of Canadian broiler chicken meat and number of infections or illnesses per 100,000 inhabitants, both overall and specifically from ciprofloxacin-resistant *Campylobacter* exposure.

Our median risk of campylobacteriosis from one serving is 1.5e-04, or 0.015%. The foundational QMRA from the FAO & WHO estimates the probability of illness from one serving a chicken from their baseline model is 1.18e-03.[30] Alternatively, they offer an estimated risk per one serving when the prevalence at retail is 30% (or baseline prevalence post-chilling is 30.1%)

of 1.6e-03.[30] Unfortunately, because the FAO & WHO report is intended to be relevant to the global community and does not specify several parameters like setting or population, the results are vague for our purposes. Based on the above estimates of risk from one serving from the FAO & WHO, our estimate of overall illness from one serving is approximately one order of magnitude lower. Habib *et al*. estimates the Australian campylobacteriosis risk specifically from cross-contaminated raw broiler chicken fluids in the kitchen and found the average risk per one serving to be 7.1e-04.[165] Lastly, a Danish QMRA from Rosenquist *et al*. estimated a risk of campylobacteriosis from one serving of broiler chicken meat to be 7.0e-05, approximately half the risk per one estimation than our own estimation.[170] Given that this measure of risk is not one easily measured or routinely collected through representative surveillance systems and these comparison also come from complex QMRA models, it is difficult to pinpoint why one estimate is larger while another is smaller. Readers are advised to keep in mind that the range of published risks per serving is wide, and the present risk of campylobacteriosis per one broiler meat serving falls within the extremes presented above.

Our novel approach estimated the risk of ciprofloxacin-resistant illness per one serving to be 2.0e-05, or 0.002%. However, no estimates of CIPR campylobacteriosis risk per one broiler serving exist to compare to. Our estimation that any one random serving of broiler chicken meat in Canada has a 0.002% chance of initiating treatment-resistant campylobacteriosis is therefore a significant contribution to the field and sets a precedent for future CIPR *Campylobacter* risk assessments.

A more widely reported metric and one that is directly comparable to surveillance data is number of illnesses per 100,000 inhabitants. Our model estimates a median of 1,101 campylobacteriosis illnesses per 100,000 inhabitants in Canada per year. The Dogan *et al*.

estimate was smaller at 274 cases per 100,000 American cases per year.[91] Meanwhile, Rosenquist *et al*. estimated 14,000 total campylobacteriosis cases in one year in Denmark, or scaled to 260 cases per 100,000 Danish residents for that year.[170,171] Similarly, in the Netherlands Nauta, Jacobs-Reitsma, & Havelaar estimated 12,300 total cases of campylobacteriosis specifically from servings of salad cross-contaminated with raw broiler chicken fluids in 2007, yielding an estimated incidence of 76 cases per 100,000 Dutch inhabitants.[172,173] However, these three estimates all come from individual and complex QMRA models. More useful comparison may perhaps be made against national surveillance data of campylobacteriosis, although this method of data collection is not without its own caveats.

FoodNet Canada, a federal surveillance program mandated with collecting and reporting on foodborne illness in select sentinel sites in Canada, reported 23 non-travel related campylobacteriosis cases in Canada per 100,000 inhabitants in 2018.[174] FoodNet (the surveillance program from the American Centers for Disease Control and Prevention [CDC], not to be confused with FoodNet Canada) reports the annual incidence of laboratory-confirmed campylobacteriosis cases in the USA at 19.5 per 100,000 inhabitants in 2019, an increase from 13 per 100,000 inhabitants in 2015.[26,175] Meanwhile, in Australia in 2015, OzFoodNet reported an incidence rate of campylobacteriosis of 139 per 100,000 population.[176] All three of these incidence rates are not only vastly different from our estimated median of 1,101 campylobacteriosis cases per 100,000 inhabitants, but they are also different from each other, despite similar socioeconomic and cultural settings. It is likely that differences captured between these countries is due to the structure of the surveillance systems, laboratory testing methods, and statistical modeling decisions.[177–179]

In Canada, the United States, and Australia campylobacteriosis is a notifiable disease (except for Australia's state of New South Wales).[179,180] However, despite this, cases are likely to be greatly underestimated. The course of illness for most campylobacteriosis cases is mild and self-limiting; often those infected will not seek medical attention or may not have the infection confirmed using laboratory procedures.[77,181] Ciprofloxacin-resistant campylobacteriosis cases may also be overrepresented those clinical cases that are captured because gastroenteritis initiated by CIPR strains may be more severe symptomatically and may have a higher chance of causing serious health events.[182–184]

Several studies have developed adjusted estimations to approximate the total number of community cases of campylobacteriosis to counteract underreporting. While there are different approaches, the most common involves using multipliers to scale up surveillance data based on underreporting estimates.[181,185] Using Canadian data, Thomas *et al*. estimated a total annual mean of domestically acquired foodborne *Campylobacter* illnesses of 145,350 (90% CrI: 95,686–212,971) and a mean incidence of 447 per 100,000 people (90% CrI: 294–655), after accounting for care seeking behaviour, symptomology, and domestic foodborne exposures, among other factors.[27] Our estimate of 1,101 illnesses per 100,000 people appears to be a slight overestimation compared to this work although within the plausible realm of reality.

Unfortunately, similar scaling studies do not exist to validate our ciprofloxacin-resistant campylobacteriosis incidence of 143 cases per 100,000 inhabitants, or approximately 13% of total cases (Table 3.2). Analysis from samples taken from diarrhetic individuals in Lethbridge, Alberta, indicates that I) the fraction of those ill with campylobacteriosis having a ciprofloxacin-resistant strain is increasing steadily by approximately 1.5% each year, and II) this CIPR proportion has increased from 2.4% in 2004 to 23.9% in 2018.[59] Similar findings have been

found in other studies examining clinical samples from human cases of campylobacteriosis in North America. Percent resistant can range from 10-50% and there is a general trend of increasing proportion of clinical samples being CIPR over the past 20 years.[61,186–188]

A final consideration when situating the results of this thesis in the broader context is that risk assessments, including this one, are often populated with worst-case data.[76,78] As exemplified throughout this chapter, QMRAs frequently create complex probabilistic models using sparse, biased, or otherwise imperfect data with multiple underlying assumptions.[30] Often, blatantly false assumptions must be made in cases of no better alternatives.[78] Due to the fact that the quantity and quality of data to perfectly populate a QMRA and create precise estimates of reality do not exist, these risk models are geared to err on the side of caution. The general wisdom in biological risk assessment is that it is better to overestimate a risk and be transparent in the modeling and limitations, than underestimate and allow for unexpected harmful consequences to a population.[189]

As the FAO & WHO write "Models are always incomplete representations of the system they are intended to model, but they can still be useful."[77] The aim of the present is not to precisely mimic reality but rather to allow for insights on how the *Campylobacter* populations change throughout the farm-to-fork pathway and how various interventions may alter the risk posed to Canadians.

4.2. Novel Dose-Response Modeling

The dose-response modeling in this QMRA consisted of one of the first applications of a novel technique to differentiate the dose-response relationship of an antimicrobial resistant strain of a bacterium from a susceptible counterpart, and the first use for *Campylobacter* with AMR.

The expansion of methods and theories into new territories will undoubtedly always be accompanied by inaccuracies and limitations. However, discussion of weak points of this model is necessary to improve the capacity AMR dose-response modeling. The introduction of a fraction of doses being antimicrobial resistant created quandaries in modeling. Understanding and quantitatively describing the transmission and infection initiation of foodborne microorganisms with AMR is an emerging topic in the scientific community, and several knowledge gaps had to be contended with and are further illuminated in this section.

In simple cases where the ingested dose of *Campylobacter* has an unknown susceptibility profile, there is compelling experimental and mathematical evidence that the parametric approximate beta-Poisson model form (Eq. 30) is a good fit for estimating the probability of *Campylobacter* surviving host defences and initiating infection.[76,78] This thesis endeavoured to extend this accepted model and include scenarios where ingested doses were entirely ciprofloxacin-resistant or the exposure was some mixture of both susceptible and resistant *Campylobacter*. This presented the challenges of I) estimating single-hit (i.e., one microorganism) probability of infection parameters for resistant strains of *Campylobacter* which have never undergone feeding challenge experimentation, and II) mechanistically describing what may happen when both susceptible and resistant *Campylobacter* are simultaneously ingested.

Many existing *Campylobacter* DRMs are based off single-hit probabilities, where the probability of a single organism initiating infection or illness is extrapolated from experimental feeding trial data.[190] This is more of a theoretical number useful in creating models than a biologically reality.[109,190,191] Considering that feeding trials, or other observed exposure-response studies, of CIPR *Campylobacter* have never been completed, a single-hit probability cannot be

deduced. A large knowledge gap exists here in that we do not know the likelihood of a CIPR *Campylobacter* CFU initiating infection or illness in a human, and because of this gap must assume it has the same single-hit probability as *Campylobacter*.

Chandrasekaran & Jiang proposed a methodology attempting to illustrate what could happen when susceptible and resistant strains of bacteria are ingested simultaneously.[110] At the time of publication, this work is the first known application and assessment of this novel AMR-DRM methodology to *Campylobacter*. As with all new steps forward in science this new model is not without its shortcomings, and in-depth discussion of its current merits and pitfalls will only serve to advance the field into new territory. Chandrasekaran & Jiang are transparent regarding the scenario they attempted to define: hosts with some ambient antimicrobial concentration in the body exposed to doses containing both resistant and susceptible microorganisms. The necessity of ambient drug in the host in the Chandrasekaran & Jiang model limits its application from a broader population. Most Canadians exposed to *Campylobacter* are not already taking a clinical course of fluoroquinolones and acceptable antimicrobial residue limits in food from animal sources are set extremely low.[192–195] Including some drug concentration in the body allowed the authors to estimate how the susceptible fraction of the dose and the resistant fraction of the dose would act differently (i.e., the drug would act on the susceptible fraction, diminishing its ability in some capacity to initiate infection).[110] There currently exists no other model that attempts to illustrate how an antimicrobial resistant foodborne bacterium may differ in a dose-response assessment compared to its susceptible counterpart simultaneously due to enhanced virulence, which in itself represents an exciting area for future research.

A crucial detail of the present dose-response model is the use of *Escherichia coli* pharmacodynamic (PD) data for ciprofloxacin because of the lack of applicable *Campylobacter*-

fluoroquinolone data. The methodology outlined by Chandrasekaran & Jiang to determine new alpha and beta parameters for the probability of survival of a susceptible microorganism in the presence of some drug concentration in the body necessitated values for E_{max} and EC_{50}.[110] Where E_{mx} is the maximum efficacy of the drug on the organism (often given as the maximum proportion of a population which will die given any concentration of the drug) and EC_{50} is the effective concentration of the drug which causes 50% of the organism population to die (or the concentration at which half of E_{max} is achieved).[196] It is worth discussing the authors' use of E_{max}. Their work applied the novel AMR DRM to gentamicin-resistant *E. coli* using a value of 1,224 $days^{-1}$ as the "maximum killing rate" of *E. coli* for this drug.[110] It can be argued that this parameter is in fact k_{max}, the maximum killing rate, which is a measurable value given in units of organisms dying over time.[112,197] The functional definition and application of E_{max} is somewhat ambiguous and the relationship between E_{max} and k_{max} may be described as E_{max} acting as a theoretical idea and k_{max} as a specific quantitative value, which is one of many that may be used to describe the efficacy of a drug on an organism.[198] Therefore, in the present model values for k_{max} are assessed and applied.

The k_{max} and EC_{50} of a fluoroquinolone acting on *Campylobacter* are not available in the current literature. The relationship between this drug-bacterium combination has, of course, been studied but published parameters are often in the form of MIC_{50} and MIC_{90}, which do not have a direct relationship with EC_{50} or k_{max}.[199–201] Therefore, out of necessity, a substitute drug-bug relationship had to be used. Ultimately, it was decided that *E. coli*-ciprofloxacin PD data from Schuck *et al*. would be a suitable replacement.[112] Some *E. coli* strains are also widespread foodborne pathogens with similar exposure mechanisms that can initiate gastroenteritis and are developing a growing resistance to CIP.[202] Evidently, this is not a perfect substitute and caution

has been raised in the past regarding the use of *E. coli* as an indicator species for *Campylobacter*.[203]

Additionally, to reflect these PD parameters, the ambient concentration was modified to stay consistent with the minimum inhibitory concentration (MIC) for ciprofloxacin on *E. coli*. Chandrasekaran & Jiang recommend setting the concentration of the drug in the body (C_{body}) to some small percentage of the MIC and given that the CIP MIC on *Campylobacter* is much smaller than that for *E. coli*, the consequent effects on probability of the bacterial survival would not have aligned.

The present work considers fluoroquinolone prescribing practices in human medicine and the probability of a consumer taking a course of antibiotics before chicken consumption. However, an alternative way of approaching the data requirement of C_{body} is to consider antimicrobial residues consumed through animal products, such as chicken meat. Ciprofloxacin and other fluoroquinolone antimicrobials have been found in very small quantities in both drinking water and animal food products elsewhere globally.[204–207] The FAO currently advises an acceptable daily intake (ADI) of enrofloxacin (used in poultry production and is metabolized to ciprofloxacin) of 0-2 μg/kg of bodyweight, which translates to 136 μg in a 150 lb person and 182 μg in a 200 lb person.[194] The concentration of ciprofloxacin in blood serum has a half-life ($T_{1/2}$) between three to five hours.[208,209] The maximum concentration of CIP in the blood serum (C_{max}) after a single oral dose is dependent on the magnitude of the dose but can be approximated at 0.5% of the oral dose.[208,210,211] Therefore, in cases where an individual recommended ADI of CIP is reached, the C_{max} that might appear in the blood serum shortly after might be between 0.68 and 0.91 μg/L for 150 and 200 lbs of bodyweight, respectively. Therefore, ambient concentrations of ciprofloxacin up to 1 μg/L appear plausible. In theory the ambient concentrations used in this

model of 0.325 μg/L appears reasonable. However, it must be noted that very little quantitative data exists regarding ambient concentrations of antimicrobials in the body due to residues in food.

Following the steps prescribed by Chandrasekaran & Jiang, k_{max}, EC_{50}, and C_{body} were used to determine α_S and β_S for the new beta distribution, with values of 0.2182 and 1088.37, respectively.[110] This new distribution is only used in our novel dose-response model in cases of ingestion of CIPS *Campylobacter* and fluoroquinolone use in the 45 days prior to ingestion. Although typical courses of fluoroquinolone prescriptions are between 10 and 14 days, the lasting consequences of these antimicrobials on the body, and particularly, the gut microbiota, may have a duration of up to 45 days.[113,114] Such changes in the microbiota and the lengthy period ostensibly required to re-establish baseline populations opens the door for opportunistic bacteria in this period, and as such may influence the infectivity of foodborne pathogens, like *Campylobacter*.[114] Due to restrictions in scope, however, this possibility was not modeled further.

As expected, the new parameters when used in a simplified DRM produced a general shift to the right, in comparison of the traditional distribution, the latter representing the resistant population in the presence of ciprofloxacin (Figure 3.2). This indicates that in a scenario of 100% CIPS *Campylobacter* at the set C_{body} concentration, the same dose has a smaller predicted probability of initiating infection. In other words, a larger dose of CIPS *Campylobacter* would be needed to initiate infection if there is a small concentration of ciprofloxacin in the body, as described by the associated ID_{50}s. Our model accounts for the rarity of there being an ambient ciprofloxacin concentration in the body prior to *Campylobacter* exposure and consequently this new distribution with α_S and β_S parameters is only used in certain cases. In the usual case where

there is no pre-existing ciprofloxacin concentration, our model assumes that CIPR and CIPS *Campylobacter* populations behave the same, including in cases of mixed doses when they are ingested together. Given the interesting finding from *in vivo* experiments that when co-inoculated in chickens CIPR *Campylobacter* will entirely outcompete CIPS *Campylobacter*, the validity of this assumption is called into question. However, no evidence currently exists to assert how this would play out in the human GI system, let alone enough data to construct predictive models. Future work may be interested in investigating the infection dynamics in humans when both CIPR and CIPS *Campylobacter* are ingested.

4.3. Model Analyses

4.3.1. Sensitivity Analysis

Sensitivity analysis can reveal where the most influential data points are in the model and, when external validity is achieved, where critical control points along the exposure pathway are. As demonstrated above in Section 4.1, this model operates with a reasonable level of external validity and our sensitivity analysis results may be interpreted in a broader context. The variability of three outputs were examined: the probability of any illness from one random serving ($P_{ill,overall}$), the probability of ciprofloxacin-resistant illness from one random serving ($P_{ill,CIPR}$), and the fraction of all illnesses that are resistant ($Fr_{ill,CIPR}$).

The data inputs that caused the most variation (i.e., swing) in $P_{ill,overall}$ and $P_{ill,CIPR}$ had generally the same ranking and magnitude of effect. A large overarching assumption made in the model is that CIPR *Campylobacter* has the same survivability and environmental tolerance characteristics as CIPS *Campylobacter*, making the final probability of CIPR illness also a proportional subset of the total probability of illness. Essentially, these two measures grow or

shrink together. If the overall risk from one serving increases so will the corresponding risk of CIPR illness in most cases. To assess what is driving the minority of cases where this proportionality is disturbed, $Fr_{ill,CIPR}$ was analyzed. These results are discussed further below.

The factors in our model which have the most impact on variability in $P_{ill,overall}$ and $P_{ill,CIPR}$ include the temperature after cooking at home (T_{cook}), the change in external contamination on a bird from within-flock sources during evisceration (LC_{EV}), and the probability of direct (within-flock) contamination during transportation (P_{DCT}). These results echo what other farm-to-fork QMRAs of *Campylobacter* have found.[84,91] There is some discordance with others regarding the most significant input variables in the sensitivity analysis, although other models may have more mechanistic processing nodes necessitating a different selection of input variables or omitted some variables used here (e.g., LC_{EV}, P_{DCT}) altogether.[127,212]

Consumer behaviour is frequently emphasized as one of the most important control points for minimizing *Campylobacter* contamination and reducing risk. [84,91] Our findings strengthen this claim, with the final internal temperature of the chicken at cooking leading to the most variation in probabilities of illness from one serving. The estimated risk of both overall illness and TxR illness decreases as the final internal temperature of the chicken rises, until approximately 66.7°C at which point no further reduction in probability of illness per serving are observed (Figure 3.4). Chicken is frequently recommended to be cooked to an internal temperature of 74°C (165°F), however this recommendation exists to capture a broader spectrum of contaminating bacteria that have different thermal inactivation points.[115,153] For example, *Salmonella* spp. has a thermal inactivation point at 70°C while *Campylobacter* has a thermal inactivation point of 60°C, lining up with the observation from our model that cooking

temperatures higher than 66.7°C does not decrease campylobacteriosis risk per serving (not considering cross-contamination).[84]

Average risk per serving also remains stable until LC_{EV} reaches values of 1.16 log_{10} CFU/bird and higher. This likely indicates that external contamination from within-flock sources (as opposed to cross-contamination from different flocks) at evisceration becomes a concern when moderate amounts of contamination are being transmitted but does not appear to increase risk when smaller amounts of *Campylobacter* are spread to birds (below 1.16 log_{10} CFU/bird). It is very interesting that we only observe one side of the distribution for LC_{EV} leading to a change in risk of illness per serving. At its lower end, LC_{EV} takes on negative values, indicating a decrease in contamination per bird. One would assume that if contamination per bird is decreasing then this may translate to decreased risk, but that is not the case in this model.

Understandably, because the median value of 0 for P_{DCT} is also its minimum value, there is no further reduction in risk that P_{DCT} can influence when conditioned to its low values and its influence on risk can only be one directional. When P_{DCT} reaches the upper limits of its distribution (in particular, higher than its 75th percentile of 0.4995), the average risk of total illness and TxR illness begins to increase. When the probability of within-flock cross-contamination during transportation is below 50%, excess risk of illness is not passed on to the consumer.

It is interesting that $Conc_{depop}$ (magnitude of external contamination on a bird at depopulation) shows very little influence on the final estimates of $P_{ill,overall}$ and $P_{ill,CIPR}$. As is shown by the results of the $Fr_{ill,CIPR}$ sensitivity analysis, the data inputs that signal the starting levels of contamination can be very influential in determining final risk estimates. However, the lack of variation caused by $Conc_{depop}$ in probability of illness from one serving may indicate that

there are more critical control points along the farm-to-fork pathway than initial magnitude of external contamination. This claim is further bolstered when considering that P_{DCT} was one of the most influential data inputs on probability of illness, highlighting how critical the external *Campylobacter* acquired during transportation is far more important in determining final probability of illness than the contamination on the bird at the time of loading on to the truck.

The relationship between $Fr_{ill,CIPR}$ and BFP_Z presents the most clear example of the median of an output varying after conditioning on percentiles of the inputs distribution. Unlike some of the relationships described above, BFP_Z is able to both increase and decrease the fraction of risk attributable to CIPR *Campylobacter* and does not pass a threshold where its effects are no longer influential. The distribution for BFP_Z used in this model is Beta(16+1, 122-16+1) and based on federal surveillance data (median: 13.5%; 90% CrI: 9.0–19.1%).[94] Logically, this finding indicates that decreasing the proportion of on-farm broiler flocks that are colonized with CIPR *Campylobacter* will lead to the greatest reduction in fraction of CIPR illness. Given the substantial data and knowledge gaps regarding CIPR *Campylobacter* along the farm-to-fork pathway (detailed in Section 4.1) it is expected that the amount of CIPR *Campylobacter* entering our model (as represented by BFP_Z) will end up having the greatest impact on the proportion of final risk that is CIPR. In future models with additional modeling and relationship specific to CIPR *Campylobacter* surveillance and behaviour, we may uncover other factors along the farm-to-fork pathway that are more influential in reducing the proportion of risk that is antimicrobial resistant. Focus should be paid to on-farm interventions or practices that modify this parameter, which was beyond the scope of this thesis research.

4.3.2. Scenario Analysis

While sensitivity analysis is advantageous at showing the inputs that most influence the final risk estimate, the scenario analysis allows for identification of potential consequences from specific changes from a risk management perspective. Seven scenarios were assessed for the percent change in estimated incidence of campylobacteriosis cases per 100,000 population ($N_{ill,100k}$), incidence of ciprofloxacin-resistant campylobacteriosis cases per 100,000 population ($N_{ill,CIPR,100k}$), and the fraction of cases that are CIPR ($Fr_{ill,CIPR}$).

In Scenario D, a hypothetical change was applied to the overall prevalence of contaminated birds post-chill, the final abattoir stage. The results of the scenario analysis show that a 90% reduction in the number of birds with any external *Campylobacter* contamination at the end of processing may reduce the overall incidence of illness by 97.4% (90% CrI: 90.2-99.3%) and the incidence of CIPR illness by 97.5% (90% CrI: 90.2-99.3%). This scenario resulted in the largest and most significant reduction in campylobacteriosis incidence among all the scenarios examined. Scenario D acts as a hypothetical target, rather than a specific policy option, and indicates that meaningful changes upstream from post-chilling which target the prevalence of birds with external *Campylobacter* could be very effective. A recent meta-analysis calculating changes in prevalence from abattoir interventions found interventions at the evisceration and post-wash pre-chill stages to cause the largest reductions.[213] Additionally, the study indicated that physical decontamination approaches (process realignment, cloacal plugging, slaughter style) to be more effective at reducing prevalence than chemical decontaminants, although the meta-analysis still found significant reductions in prevalence from such chemical processes.[213]

Interestingly, reducing the average concentration on a contaminated bird at post-chilling (Scenario E) was not nearly as effective as reducing the prevalence of birds contaminated at this

point. In Scenario E, the lowered magnitude of external contamination at retail was only sufficient to eliminate 10.8% (90% CrI: 0-83.3%) of total downstream illness in the model, compared to the estimated 97.4% (90% CrI: 90.2-99.3%) reduction in $N_{ill,100k}$ in Scenario D when prevalence was lowered. This may be because the 1 log reduction on all contaminated birds post-chilling in Scenario E still left a significant majority of these birds with enough *Campylobacter* to initiate illness. The scenario analysis from a consumer-based QMRA of *Campylobacter* risk from broiler meat also found that reducing the prevalence of contaminated birds was more effective at lowering risk than lowering the magnitude of concentration on birds, specifically through reduced transmission via cross-contamination in the kitchen.[165] While not specifically tested here, it is possible that reducing prevalence had such a high intervention efficiency because of its action through the cross-contamination exposure pathways in the kitchen in our model as well. Future interventions and policy changes should consider that lowering prevalence of total birds with any *Campylobacter* contamination may be more effective than targeting concentration on a bird.

Scenario A consisted of reducing the prevalence of *Campylobacter*-positive flocks across Canada by 50%, while maintaining the proportion of these flocks that were CIPR. A 50% reduction in total number of flocks colonized by *Campylobacter* while on the farm led to a 50.7% (90% CrI: 47.4-95.4%) reduction in overall incidence in the model and a 50.4% (90% CrI: 42.0-95.6%) reduction in CIPR incidence. Similarly, New Zealand reported a 50% reduction in notifiable cases of campylobacteriosis as well as hospitalizations following the implementation of strict on-farm policies meant to prevent the introduction of *Campylobacter* to a flock.[214]

Perfect understanding of *Campylobacter* transmission to a broiler flock is still unclear, therefore there is still debate on how to prevent it. In broad strokes, it is accepted that vertical transmission is not an important pathway and that human traffic and imperfect biosecurity measures most likely introduce the bacteria to the flock.[19,215–217] Genotyping of *Campylobacter* finds that strains colonizing a flock of interest are far more likely to match other flocks nearby, samples taken from humans (e.g., boots), and nearby environmental samples (e.g., puddles).[216–218] A likely pathway is that *Campylobacter* exists in other flocks or in environmental reservoirs (especially in warm and rainy months) and is spread to the previously uncolonized flock through human traffic and imperfect hygiene practices when staff enter and exit flock houses.[219,220] To control this, biosecurity measures for staff when entering and leaving flocks such as handwashing, boot covers, or a hygienic antechamber by the exit, should be strictly adhered to.[221,222]

In our model and scenario analysis, CIPR *Campylobacter* and consequent illness is often a proportional fraction of total *Campylobacter*, and this trend of proportional change continues in Scenario A. Recent research has suggested CIPR *Campylobacter* does indeed have individual risk factors for colonizing a previously negative flock. Antimicrobial use, including antimicrobials of other classes, during rearing is most likely to contribute to colonization with fluoroquinolone-resistant *Campylobacter*.[216,223,224] Caffrey *et al*. also found that presence of rodent traps on the farm and the lineage of broiler used in the flock were also associated with higher likelihood of colonization by fluroquinolone-resistant *Campylobacter*.[223] Ultimately, factors leading specifically to CIPR *Campylobacter* colonization of flocks are still generally unknown. Until these pathways become clearer and more differentiated, it is not unreasonable to proceed with biosecurity strategies targeting *Campylobacter* in general.

The third and final scenario assessed that reduced overall and CIPR-specific campylobacteriosis incidence in our model was Scenario F. In Scenario F, simultaneous 90% reductions in the likelihoods of undercooking and fluid cross-contamination in the kitchen show a very notable 90.0% (90% CrI: 88.9-90.0%) potential reduction in both total campylobacteriosis incidence and CIPR campylobacteriosis incidence (Table 3.5). Again, as with Scenarios D and A, Scenario F acts as a hypothetical target rather than a specific intervention.

In theory, at-home interventions appear to be the most logical and effective given their place at the final stage of the exposure pathway and direct attention to detail outside of a mass production environment. However, despite being a critical control point, what happens to a broiler chicken product in a domestic kitchen remains quite uncertain and difficult to evaluate, let alone modify.[84] There exists a certain disconnect between what the research community knows to be effective and safe chicken preparation and what happens in a Canadian kitchen. Health Canada currently recommends that chicken be placed on the bottom shelf of a refrigerator kept below 4°C, frozen if not prepared within three days of purchase, prepared on separate surfaces with dedicated utensils, cooked to an internal temperature of 74°C, verified with a meat thermometer, and that adequate handwashing and surface disinfecting happens throughout.[225] Van Asselt *et al*. have estimated that error-free cooking behaviour can lead to up to a 7.5 $\log_{10}$ CFU *Campylobacter* reduction, which would eliminate almost all exposure to *Campylobacter*.[226] Some postulate that pervasive knowledge gaps are what inhibits Canadians from handing raw chicken in a way that eliminates risk from undercooking and cross-contamination.[167] However, knowledge about food handling and attitudes towards food handling are separate motivational entities, and in fact it is the latter that has a greater impact on practices in the kitchen.[227] Indeed, self-report surveys often show a generally acceptable knowledge of food handling safety, yet

when observed, home cooks do not prepare raw chicken to a similar level of sufficient safety.[168,228] Human behaviour and motivation is complex and self-reported studies or scenarios where participants are aware they are being observed are likely to be rife with bias. An intervention which may help bridge that gap between sufficient knowledge and insufficient practice is including reminders, cues, or nudges to home cooks during chicken preparation.[84,229] Nauta *et al*. found including a reminder to take care in avoiding cross-contamination when preparing chicken reduced risk by 83%.[229] Additionally, given that human behaviour can be extremely difficult to change, there are industry changes that might be made which could aid in improving at-home food handling. Bruhn suggests that manufacturers should always include the display of current temperature for domestic refrigerators and recommends that meat thermometers should be more easily calibrated with large, visible numbers.[115] Ultimately, knowledge gaps persist in our understanding of both why Canadians fail to safely handle food and what interventions would be most effective at reducing risk of illness from chicken products.

As was done in the sensitivity analysis, to investigate any specific scenarios which might more effectively target CIPR *Campylobacter* in this model, the fraction of all illness that is CIPR ($Fr_{ill,CIPR}$) was included. Disappointingly, none of the seven scenarios showed a meaningful percent change in fraction of risk that was CIPR. This is most surprising regarding Scenarios B, C, and G.

Scenarios B and C reduce between-flock cross-contamination by 90% at transportation and evisceration, respectively, and represent the two mechanisms in our exposure assessment model where CIPR *Campylobacter* may spread widely across the national flock without regard to proportionality to overall *Campylobacter* counts. It is quite surprising that when limiting either of these pathways, which primarily enable the spread of CIPR *Campylobacter*, there is not a

subsequent decrease in the proportion of all illness that is CIPR, due to lower doses on fewer birds. Upon closer examination of the modified exposure assessments in Scenarios B and C it appears that when between-flock cross-contamination is limited either at transportation or evisceration, the spread of total and CIPR *Campylobacter* throughout the national flock still stabilizes to baselines levels by the post-chill stage.

Lastly, Scenario G lowered the probability of human fluoroquinolone use (P_{AMU}) by 90%, modifying the dose-response so that when CIPS *Campylobacter* is ingested is it far less likely to encounter FLQs in the body and be unsuccessful at initiating illness. It is surprising that when greatly reducing this selection pressure in the cases of mixed doses (both CIPR and CIPS *Campylobacter*) that the probability of CIPR illness, and therefore the fraction of all illness that is CIPR, does not increase. However, given that the baseline P_{AMU} was already exceptionally low (0.6%) it is understandable how a further reduction on this very small probability did not greatly affect the overall fraction of illness that is CIPR.

4.4. Knowledge Gaps

A recurring theme throughout this thesis, and indeed QMRAs in general, is the lack of precise data to model a specific phenomenon, or the lack of theoretical understanding behind a phenomenon. Farm-to-fork models like this create a fantastic opportunity to compile and outline the major knowledge gaps associated with the topic of interest. All data points and modeling techniques used in this thesis come with a set of uncertainty and assumption. Rather than focus on minute details at this stage, it is more helpful to look at the big picture of knowledge gaps which seriously hinder quantitative, mechanistic modeling. As such this section outlines major themes of uncertainty that remain in the field surrounding broiler-borne *Campylobacter* in

general and ciprofloxacin-resistant *Campylobacter* risk to humans with the hope of guiding future research initiatives.

There are two major knowledge gaps to be noted in the context of the farm. The first is the competition and colonization dynamics of CIPS versus CIPR *Campylobacter* in broiler chickens from a flock perspective. *In vivo* studies have found that in individual broilers CIPR *Campylobacter* will entirely outcompete CIPS *Campylobacter* in establishing colonization of the chicken gastrointestinal tract, even when at lower rates of exposure.[71,72] It is unclear if this finding persists when spreading through an entire commercial broiler flock. Extensive models of *Campylobacter* transmission dynamics within these large flocks have recently shown the complexity and possibilities of some birds indeed remaining uncolonized in a 'positive' flock.[230–233] Studies that measure and model the transmission and competition dynamics of CIPR and CIPS *Campylobacter* would be greatly beneficial. With this information a more accurate depiction of the prevalence of birds and average concentration per bird of CIPR *Campylobacter* within broiler gastrointestinal tracts could be formed, rather than the all-or-nothing approach used here.

Secondly, as explored in Section 4.3.2, enhanced biosecurity and control of organic matter coming in and out of the broiler house (via humans, rodents, insects, etc.) is key in preventing flock colonization, especially in warm and rainy months.[217] The roadblock in this realm is in achieving human compliance among farm and transportation staff and uniformity in the measures implemented.[220] Human behaviour can be notoriously difficult to modify, so despite mounting evidence that increased entry into a flock between placement and depopulation and breaching biosecurity measures when entering and leaving flock houses, this information needs to be put into practice in a way that is consistent, understandable, and easy to comply

with.[220] The exact mechanisms of preventing *Campylobacter* spread to a flock is not agreed upon, but ensuring that what measures are in place are followed correctly should be an item of priority for policy makers and researchers.

When attempting to mechanistically model Canadian poultry abattoirs, a major gap specific to Canada is lack of transparency and inconsistency in abattoir set-up and decontamination procedures. Current best practice in designing a farm-to-fork exposure assessment for chicken-based species include five abattoir stages: scalding, defeathering, evisceration, washing, and chilling.[83] Indeed, these are essential steps in the processing of the birds, but they also exclude key stages in the process that could lead to further contamination (e.g., slaughter, re-hanging, portioning) or additional decontamination (e.g., rinses between stages, chemical antimicrobials used). Abattoirs are private enterprises with set-up and practices that will understandably vary between facilities. A transparent report summarizing the specific stages, interventions, and chemicals used throughout will greatly aid future farm-to-fork QMRAs attempting to mechanistically model the transmission of foodborne pathogens through poultry production.

An additional knowledge gap pertaining to the realm of processing is priority of intervention efficacy studies versus surveillance and observational studies. A wealth of important information has been generated regarding *Campylobacter* prevalence and average concentrations at key stages within the abattoir in high-income countries.[134,135,141,142,160,234–236] This information serves as the backbone for data-driven QMRAs such as this one. However, there appear to be substantially fewer studies that measure and assess the efficacy of interventions on reducing *Campylobacter* prevalence and concentration as opposed to reporting baseline levels. Therefore, while clear understanding exists around the average levels of *Campylobacter* at various points in

the abattoir, much less data exist regarding how to further manipulate these averages. Fortunately, work in this area is emerging, including research which helpfully synthesizes intervention results.[91,156,213] Researchers are encouraged to continue quantitatively evaluating interventions and synthesizing results.

A major weakness in available Canadian data at the end of the farm-to-fork pathway is an understanding of chicken meat consumption patterns. A self-reported survey of chicken serving sizes in Canada, similar to what was done by Zheng *et al.* in Australia, does not exist.[106] The Canada Food Guide recommends one serving of chicken meat should be 75 grams.[237] This likely is not really the typical quantity being consumed in one serving, considering Zheng *et al*. reported approximately 150 grams per serving when self-reported.[106] Canadian data would be useful to clarify the discrepancy between what institutions recommend as a serving size and what is actually being consumed in order to accurately estimate exposure. Additionally, given that enumeration of *Campylobacter* on different types of broiler products at retail shows differences (e.g., whole birds, boneless-skinless, bone in-skin on), reporting on which forms of broiler meat being consumed would be helpful in estimating exposure of *Campylobacter* to the population.[105]

There is a serious lack of detailed data collection for CIPR *Campylobacter* along the processing pathway comparable to the data that exist for general *Campylobacter* contamination. Consequently, it is extremely difficult to make justifications if there are stages that influence survival and transmission of resistant *Campylobacter* populations differently from susceptible populations. Indeed, many others have noted the gaps that persist in the surveillance of AMR species in agricultural reservoirs.[90] There have been several studies that recovered *Campylobacter* samples from broiler skin at various distinct stages along the processing pathway, however the results are consistently aggregated and reported as an overall prevalence,

impairing any ability to monitor changes in CIPR *Campylobacter* along the pathway.[14,62,150,160,238,239] Additionally, no study to date has quantified the magnitude of CIPR *Campylobacter* on broiler skin throughout the abattoir. It is possible that assuming CIPR *Campylobacter* would contaminate birds in the same concentrations as general *Campylobacter* is reasonable. However, Chen *et al*. found that each processing step in the abattoir changes the bacterial diversity in different ways and recent research suggests that CIPR *Campylobacter* may be more adept at biofilm formation, augmenting its persistence throughout decontamination processes.[137] A detailed enumeration of CIPR *Campylobacter* specifically would bolster this assumption for future modeling, or indeed indicate that there is a meaningful difference in how antimicrobial resistant *Campylobacter* is transmitted from farm-to-fork. Future research should seriously consider testing isolates for antimicrobial susceptibility to aid in understanding the independent spread of CIPR *Campylobacter* throughout processing.

Like above, there is a stark lack of dose-response modeling methodology tailored specifically to foodborne species with AMR. Strategies that have been used to bridge this gap include employing the assumption that a susceptible and resistant strain have the same dose-response characteristics or using a posterior conditional probability to account for resistance.[80] Understandably, given the chance of initiating serious and untreatable illness, it is unethical to perform experimental feeding trials with human subjects and pathogens with AMR as has been done with foodborne pathogens in the past.[110,182] The proposed novel model that we developed based on the framework from Chandrasekaran & Jiang is a significant step forward in dose-response modelling tailored to antimicrobial resistant species. Future risk modellers are encouraged to both apply this framework in new contexts and to develop alternative modeling strategies tailored specifically to the foodborne AMR dose-response context.

4.5. Limitations

As result of certain modeling decisions, concessions and limitations were imposed on this model. These should be explored to both maintain transparency in the capabilities of the model and to guide future research. It should be noted that certainly the knowledge gaps discussed above do indeed act as limitations on this work. However, given the present objective was not necessarily to fill those gaps and they are beyond researcher control, they are not thematically classified as limitations here and are not discussed further.

An overarching consideration is the lack of differentiation between the major species of the *Campylobacter* genus that initiate human illness: *C. jejuni* and *C. coli*. Despite being often discussed together (as *Campylobacter* spp.) these two species do have their differences. *C. jejuni* predominates broiler colonization, external meat contamination, and human illness but *C. coli* is more frequently found to be ciprofloxacin resistant.[62,128] The former could possibly be due to the nature of broiler colonization dynamics, with *C. jejuni* predominating in earlier days of colonization and up until slaughter, whereas *C. coli* would predominate at later times had the bird lived.[162] Meanwhile, some mystery remains surrounding the cellular basis for *C. coli* showing higher rates of CIPR than *C. jejuni*, but it may be due to homogeneity in *C. coli*'s *gryA* gene, leading to more consistently expressed CIPR mutations.[67] Due to these epidemiological differences in *C. jejuni* and *C. coli*, differences in exposure and risk may exist, but are unaccounted for here. Future work may benefit from refining the species of interest or investigating how *Campylobacter* species and associated antimicrobial resistant strains interplay to cause gastroenteritis risk. However, the data gaps noted in Section 4.4 would likely be greatly exacerbated when refining by *Campylobacter* species.

Two limitations should be noted regarding the exposure assessment. The first is regarding the nodes which were chosen along the farm-to-fork pathway. Some stages of production were excluded from this pathway, most notably slaughter, portioning, and packaging, which have shown to have some impact on *Campylobacter* contamination during processing.[124,155,239] The majority of farm-to-fork QMRAs of broiler production also exclude these stages, meaning that the modeling infrastructure of these stages is limited.[83] It was out of scope of this project to develop sub-models for these nodes in what is already a complete model with several scenarios. However, despite a consistent exclusion of slaughter, portioning, and packaging, future research would benefit from estimating their influence on contamination of broilers during production. Additionally, it should be noted that our exposure pathway begins at the end of the flock tenure on a farm (depopulation) and does not model placement or interim flock management practices. Therefore, our model is unable to analyze on-farm rearing practices which may influence likelihood of colonization of a flock or magnitude of external contamination. Given the results of our scenario analysis point towards preventing initial gut colonization as a meaningful way of reducing downstream risk to humans, future mechanistic modeling of on-farm practices effects on *Campylobacter* would be beneficial.

Secondly, our model is generally simplistic when modeling cross-contamination of *Campylobacter* throughout the farm-to-fork pathway. Certainly, within the abattoir facility, evisceration is the most high-risk station for the spread of *Campylobacter* from bird to bird and from flock to flock.[86,146] The transmission dynamics of *Campylobacter* within the abattoir are complex and shared equipment and water-based processes (such as scalding, washing, immersion chilling) also present opportunities for substantial transmission throughout the abattoir environment.[138,146,149] Explicitly modeling cross-contamination only at transportation

and evisceration may have lead to an overall underestimation of the spread of bacteria, in particular AMR strains, between birds and flocks.

When interpreting the risk to the population our risk characterization was unable to make nuanced estimations regarding season, age, sex, gender, or other environmental or socioeconomic factors that may impact risk. Additionally, our risk characterization did not include a measure of severity of burden of illness (e.g., DALYs, QALYs), particularly for different virulence by fluoroquinolone resistance status. Serving sizes and frequencies of chicken meat consumption vary by age and gender, and various demographics also have individual probabilities of cross-contamination and safe handling.[106,167,184,240] A recent epidemiological study from Michigan, USA, reported higher probabilities of hospitalization from a CIPR case of campylobacteriosis for urban area dwellers, non-White people, and those over 40 years of age.[184] In additional to socioeconomic differences in burden of disease, our model does not assert on severity of illness, probability of sequelae, or burden on the healthcare system due to CIPR *Campylobacter* given our estimates in doses. Including such estimates would enrich future research and be useful in evaluating the impact of foodborne AMR illness in a larger context.

Lastly, our scenario analysis was limited in its ability to make specific recommendations regarding policy or procedure changes. In many instances explicit interventions or options were not modeled in the baseline model, rather the overall scenario was accounted for by using the recently published meta-analysis data from Dogan *et al*.[91] These data inherently include the outcomes and effectiveness of procedures currently being employed in processing broiler meat but is unable to be parsed apart and effectiveness and theoretical changes of procedures cannot be studied. Therefore, in conducting the present scenario analysis, we were unable to target

specific policy or procedure changes along the farm-to-fork pathway. Future work is needed to further investigate the directions and themes highlighted by our scenario analysis.

Chapter 5: Conclusion

This thesis accomplished four primary tasks: to model ciprofloxacin-resistant *Campylobacter* throughout the farm-to-fork pathway and estimate exposure to Canadians; to model probabilities of infection and illness from exposures to various doses of *Campylobacter* and CIPR *Campylobacter*; to estimate the risk of campylobacteriosis and CIPR campylobacteriosis from one serving and the incidences of these illnesses in the population; and to analyze the model for critical control points and risk-reduction strategies.

The exposure assessment closely resembles real-world observational data collected, including for CIPR *Campylobacter* when comparable data was available. Our exposure assessment modeled a large concentration reduction per bird after scald, moderate increases after defeathering and evisceration, and moderate reductions after washing and chilling, consistent with field studies.[141] The prevalence estimates made by our exposure assessment line-up closely to surveillance data from the surveillance program CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance). In 2018, CIPARS reported that 24% of birds tested at the end of processing (post-chilling) were contaminated with *Campylobacter* and that 19.7% of these *Campylobacter* samples were CIPR.[94] Similarly, at retail CIPARS reported a prevalence of 25.4% of chicken meat samples were *Campylobacter*-positive, with 13.6% being CIPR.[94]

Comparatively, in our model the prevalence of whole birds contaminated with any *Campylobacter* at the end of processing was 30.1% (90% CrI: 2.0-62.1) while after cold storage our estimation of total prevalence descended to 22.5% (90% CrI: 0.2-33.1). At both these stages, our model estimated 15.6% of contaminated birds are CIPR, 4.7% (90% CrI: 0.3, 11.1) of all birds post-chilling, and 3.5% (90% CrI: 0.0, 6.8) of all birds post-cold storage.

Additionally, the Canadian Food Inspection Agency reported that the average concentration of *Campylobacter* for whole birds for sale at retail is 0.72 CFU per gram of bird weight.[105] The average eviscerated weight of a broiler is 1,495 grams, indicating a rough average of 2.03 $\log_{10}$ CFU/bird.[87] Our model estimates an average of 2.13 $\log_{10}$ CFU/bird (90% CrI: 0.00-8.15) after cold storage. The closeness of these measures to data published by CIPARS and others indicates that this model is able to accurately represent the transmission and exposure of *Campylobacter* to consumers in Canada based on what is known at this time.

Utilizing our dose-response model (DRM), which included a novel component specifically developed to account for antimicrobial resistance, the total probability of campylobacteriosis from a single serving prepared at home was estimated to be 0.015% (90% CrI: 0.000082-3.1) while the probability of CIPR campylobacteriosis from a single serving was 0.002% (90% CrI: 0.000012-0.044). The incidences estimated from this risk characterization were 1,101 campylobacteriosis cases per 100,000 population (90% CrI: 6-223,000) and 143 CIPR campylobacteriosis cases per 100,000 population (90% CrI: 1-31,500). Recall, that true population incidence of campylobacteriosis, let alone CIPR cases, is very difficult to accurately measure because of its frequently mild and self-limiting nature and consequent underreporting.[28] A Canadian estimate that adjusts for underreporting suggested the community incidence of campylobacteriosis in Canada due to domestic foodborne exposures is 447.23 per 100,000 people.[27] The World Health Organization reported that the global incidence of campylobacteriosis, however, is 1,390 cases per 100,000 persons (95% UI: 752-2,576).[23] Our estimation of illness in the population is larger than those made for the Canadian context in the past, but given its difficulty to measure and aligning with the WHO's data-intensive estimate, the total incidence may be higher in Canada than previously thought.

The sensitivity analysis pointed towards the temperature of the meat after cooking, the probability of within-flock cross-contamination occurring during transportation, and the probability of within-flock cross-contamination occurring during evisceration as the most influential data points towards risk per serving. (Recall, CIPR *Campylobacter* is a subset of total *Campylobacter*, so measures that influence overall risk also influence CIPR *Campylobacter*.) The between-flock prevalence of birds colonized with CIPR *Campylobacter* (i.e., the proportion of colonized flocks on farm that are colonized with CIPR *Campylobacter*) was the driving data point influencing the proportion of total risk per serving that was CIPR.

The scenario analysis showed that reducing prevalence, rather than average concentration, of total contaminated birds leaving the abattoir, reducing the probabilities of undercooking and cross-contamination during preparation, and reducing the total number of flocks colonized with *Campylobacter* may most effectively lower the incidence of total and CIPR illnesses in Canada. No scenario studied in this analysis was effective at reducing the proportion of illnesses that were CIPR. Recommendations have been made by other researchers and institutions which echo these findings. Controlling *Campylobacter* entry to a flock can be elusive, but providing hygiene stations for staff, fly and rodent control of the broiler house, and reducing flock thinning practices are currently advised for on-farm practices.[221,241] Interventions that can be enacted in the abattoir include properly fitting and monitoring equipment (especially defeathering and evisceration equipment), crust-freezing the final chicken products, or steam treating the birds.[241] These options represent only a sample of possible implementations among the many that have studied and considered.[213,215,221,241] Risk managers implementing interventions should also consider the specific scenario in their locale; *Campylobacter* risks and transmission throughout the farm-to-fork pathway differ around the world and between types of

operations, there is not one-size fits all solutions and interventions should be tailored to the concern.

This research represents an important step forward in the field of risk modeling by establishing the first farm-to-fork QMRA for an *Campylobacter* species with AMR in Canada and the rest of the world. Indeed, QMRAs of any species with AMR remain very sparse due to gaps in data availability and the broad assumptions required to bridge these gaps. This model provides the structure for future AMR QMRAs attempting to study pathogens throughout broiler production for which data can be substituted or modified to suit the objective. Additionally, this work compiles the current quantitative data available regarding CIPR *Campylobacter* in commercial broiler processing in Canada and from other sources where required, and highlights where there are substantial data limitations, providing an excellent summary of the state of knowledge of exposure to and risk from CIPR *Campylobacter.* While the estimated CIPR campylobacteriosis risk cannot be compared to other estimates of this in the population, this model sets a precedent for future CIPR *Campylobacter* risk assessments.

There is a critical need for quantitative data estimating prevalence and magnitude specifically for CIPR *Campylobacter* on broiler products. Without such data, future risk assessments will have very little real-world data to be anchored in and be ill-informed. Subsequently, developing and validating mathematical equations predicting the CIPR *Campylobacter* colonization, transmission, and survival dynamics of broilers within the abattoir and during food preparation, and human infectivity with and without competing CIPS *Campylobacter* are needed. The farm-to-fork data for general *Campylobacter* are well detailed and attention should be paid towards the antimicrobial susceptibility of the *Campylobacter* contaminating chicken products. Additional future work should expand upon the present risk

characterization and further describe the severity of health consequences from CIPR *Campylobacter* exposures and the burden of this AMR illness on the wellbeing of the population and on the healthcare system.

Cases of ciprofloxacin-resistant campylobacteriosis are more likely to have longer duration and more severe set of symptoms.[181,241,242] One study estimated a 6-fold risk of invasive illness or death if a case of campylobacteriosis was resistant to quinolones rather than susceptible.[242] Additionally, antimicrobials like ciprofloxacin are historically effective options when treating campylobacteriosis in immunocompromised patients or those with more complicated or persistent courses of illness.[183] Recently, 77.4% of *C. jejuni* (and 79.8% of non-*C. jejuni* isolates) recovered from 79.9% of a pediatric cohort that became symptomatic were found to be CIPR, placing in stark relief the potentially devastating impacts loss of antimicrobial efficacy could have on vulnerable populations.[243] It should be noted, however, that in most cases a course of antimicrobials is rarely advised in treatment of campylobacteriosis.[244,245] Despite this, over one million recommendations for antibiotic treatment for cases of gastrointestinal infection were made in Canada in 2014.[246] More specifically, of the 2,690 campylobacteriosis cases captured by FoodNet Canada, approximately 19.7% were prescribed ciprofloxacin.[247] This drastic over-prescription of ciprofloxacin for campylobacteriosis is deeply concerning.

The other side of fluoroquinolone stewardship occurs in veterinary practice and especially of consequence here, use while rearing broiler flocks. Use of Category I (very high importance) antimicrobials (which includes fluoroquinolones among others) are currently prohibited for prophylactic use in broiler production in Canada.[45] More specifically, fluoroquinolones are not indicated for poultry use in Canada in any capacity, yet some off-label use has been reported.[73,94] The connection between on-farm fluoroquinolone use and subsequent

development of CIPR *Campylobacter* colonization and external contamination is well established.[248,249] Even more worrying, is the evidence that CIPR *Campylobacter* is able to persist in a barn environment and appear in future flocks even after the fluoroquinolones are no longer being used.[132,250] Ensuring proper and wise use is critical in limiting the development and spread of CIPR *Campylobacter*.

The consequence of CIPR *Campylobacter* is also substantial from an economic perspective. The Council of Canadian Academies estimates that all AMR illness cost the Canadian healthcare system $1.4 billion CAD in 2018 due to longer and more complex courses of treatment while simultaneously reducing the GDP by an additional $2 billion CAD in lost productivity.[53] The consequences of CIPR *Campylobacter* are already being felt clinically and economically. If the trends of *Campylobacter* antimicrobial-resistance described herein continue, there is a very real chance that the typically self-limiting campylobacteriosis could become a life-threatening illness.[53,243]

As the global community reckons with the possibility of a post-antimicrobial era, in which simple illnesses no longer have simple fixes, it is critical that researchers work in coordinated efforts. There are severe knowledge gaps in the realm of ciprofloxacin-resistant *Campylobacter* that should be addressed forthwith before meaningful predictive models of this antimicrobial resistant pathogen can be built. It is heartening that serious restrictions have been placed on the use of ciprofloxacin in both agricultural and human contexts, which will help curb the development of CIPR *Campylobacter*.[40,248,251] With swift action by government, researchers, and health professionals, we may yet avoid the darkness of a post-antimicrobial world.

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Appendices

Appendix A.1. Custom probability distributions

Variable	Description	Value	Source
$Conc_{depop}$	External contamination count at depopulation (CFU)	CustomPDF(1, 2340, 2510, 4470, 7590, 200000, 6.46e+06, 9.33e+06, 1.23e+07, 1.41e+07, 1.45e+07, 1.7e+07, 2.4e+07, 2.64e+07, 3.0483e-05, 5.68951e-05, 6.73027e-05, 2.81554e-05, 7.32378e-07, 2.2152e-08, 1.56418e-08, 2.44361e-08, 2.98064e-08, 6.63924e-08, 7.50021e-08, 2.24761e-08, 1.51899e-08, 2.97764e-08)	[98]
P_{DCT}	Probability of direct contamination during transportation (%)	CustomPDF(0.04, 0.05, 0.06, 0.08, 0.12, 0.13, 0.15, 0.17, 0.18, 0.2, 0.25, 0.27, 0.3, 0.32, 0.33, 0.34, 0.35, 0.36, 0.38, 0.39, 0.4, 0.41, 0.44, 0.45, 0.47, 0.5, 0.51, 0.54, 0.55, 0.57, 0.58, 0.59, 0.6, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.7, 0.72, 0.73, 0.74, 0.75, 0.76, 0.78, 0.79, 0.8, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.88, 0.89, 0.9, 0.93, 0.95, 1, 2, 1, 1, 3, 3, 3, 5, 5, 1, 2, 10, 2, 1, 2, 2, 4, 1, 1, 2, 2, 2, 2, 1, 1, 1, 11, 2, 1, 1, 1, 1, 3, 1, 1, 1, 2, 1, 3, 2, 2, 1, 1, 1, 1, 4, 2, 1, 2, 1, 4, 2, 4, 1, 1, 1, 1, 2, 1, 1, 2, 1, 50)	[99]
$Conc_{trans}$	External contamination count post-transport (CFU)	CustomPDF(1, 851138, 3.38844e+06, 6.30957e+06, 1e+07,2.29087e+07, 4.0738e+07, 4.67735e+07, 6.60693e+07, 1.12202e+08, 2.18776e+08, 3.01995e+08, 3.38844e+08, 5.62341e+08, 1.51356e+09, 4.57088e+09, 4.16869e+10, 4.58556e+10, 6.52722e-08, 3.27912e-08, 2.03559e-08, 1.68056e-08, 6.6938e-09, 3.61478e-09, 4.65585e-09, 4.38631e-09, 1.69821e-09, 1.09142e-09, 8.78148e-10, 9.25402e-10, 4.26782e-10, 9.45854e-11, 2.77186e-11, 2.76579e-12, 2.69134e-12, 1.33268e-11)	[98]
$Conc_{caec}$	Caecal concentration (Log_{10} CFU)	CustomPDF(3.699, 4.11, 5.11, 5.4, 5.65, 5.74, 6.28, 6.38, 6.58, 6.84, 6.88, 6.99, 7, 7.08, 7.2, 7.28, 7.34, 7.59, 9, 0.128057, 0.0746018, 0.0815993, 0.194932, 0.309598, 0.167084, 0.246711, 0.526316, 0.228833, 0.350877, 0.701754, 0.877193, 1.16959, 0.526316, 0.526316, 0.75188, 0.339559, 0.0634115, 0.0373274)	[30]
$P_{frozen,home}$	Probability of being sold fresh and frozen at home	CustomPDF(0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.99, 0.7, 0.5, 0.35, 0.25, 0.45, 0.45, 0.15, 0.7, 1.7, 1.52632, 0.777778)	[103]

t_{home}	Time the product is refrigerated for at home (days)	CustomPDF(2, 4, 7, 14, 0.111, 0.0542, 0.0052, 0.000428571)	[103]
T_{cook}	Temperatures the product is heated at (°C)	CustomPDF(25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 0, 0.00101, 0.00202, 0.0037, 0.00521, 0.00605, 0.00958, 0.01109, 0.01277, 0.02672, 0.04322, 0.041, 0.02232, 0.00857, 0.00437, 0.00169, 0.00068)	[91]
$w_{serving}$	Average weights of one serving of chicken meat (g)	0.5*CustomPDF(105, 171, 225, 0.0038, 0.0042, 0.0046) + 0.5*CustomPDF(78, 113, 156, 0.0071, 0.0064, 0.0057)	[106]

Appendix A.2. Probability of undercooking chicken

The original data was collected and published by Bruhn while Collineau *et al*. proposed the construction of a probability distribution around each variable and an overall probability of undercooking ($P_{undercook}$).[87,115] Undercooking was defined by Bruhn as when the end-point temperature was below 165°F. [115] Beta distributions were used to add uncertainty to the fractions, as done by Collineau *et al*.[87]

$$P_{undercook} = \begin{cases} Prob_{grill}, & Bern(Freq_{grill}) = 1 \\ Prob_{fry}, & Bern\left(\frac{Freq_{fry}}{1 - Freq_{grill}}\right) = 1 \\ Prob_{oven}, & Bern\left(\frac{Freq_{oven}}{1 - Freq_{grill} - Freq_{fry}}\right) = 1 \\ Prob_{boil}, & Bern\left(\frac{Freq_{boil}}{1 - Freq_{grill} - Freq_{fry} - Freq_{oven}}\right) = 1 \\ Prob_{PC}, & Bern\left(\frac{Freq_{boil}}{1 - Freq_{grill} - Freq_{fry} - Freq_{oven}}\right) = 0 \end{cases}$$

Variable	**Description**	**Original data**[115]	**Data with probability distribution**[87]
$Freq_{grill}$	Frequency of preparing chicken by grill	33/120	Beta(33+1,120-33+1)
$Prob_{grill}$	Probability of undercooking chicken by grill	17/33	Beta(17+1,33-17+1)
$Freq_{fry}$	Frequency of preparing by frying	46/120	Beta(46+1,120-46+1
$Prob_{fry}$	Probability of undercooking when frying	19/46	Beta(19+1,46-19+1)
$Freq_{oven}$	Frequency of preparing by oven roasting	33/120	Beta(33+1,120-33+1)
$Prob_{oven}$	Probability of undercooking when oven roasting	9/33	Beta(9+1,33-9+1)
$Freq_{boil}$	Frequency of preparing chicken by boiling on stovetop	7/120	Beta(7+1,120
$Prob_{boil}$	Probability of undercooking chicken when boiling on stovetop	2/7	Beta(2+1,7-2+1)
$Freq_{PC}$	Frequency of preparing by pressure cooking	1/120	Beta(1+1,120-1+1)
$Prob_{PC}$	Probability of undercooking when pressure cooking	0/1	0