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Campylobacteriosis in New Zealand: Causes and Control

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Ali Mohammed Y. Al-Sakkaf

Lincoln University

2020

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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by

Ali Mohammed Y. Al-Sakkaf

New Zealand (NZ) has a higher rate of reported campylobacteriosis cases than the rest of the developed world. The majority of human campylobacteriosis cases are attributable to the consumption of poultry products. The hypotheses explored in this study are: (i) NZ C. jejuni strains have a greater heat tolerance and hence are better able to survive cooking, (ii) NZ strains are more oxygen tolerant, (iii) secondary poultry processing practices increase the NZ chicken contamination with Campylobacter (iv) Food preparation home hygiene practices in NZ are poor compared with the developed world.

Quantitative microbial risk assessment (QMRA) was carried out by applying an advanced Bayesian approach to assess all the factors in the food chain, which impact the final campylobacteriosis risk estimation. Moreover, the current intervention practices applied at the poultry plant was investigated to find a better physical intervention method than the potential unsafe chemical intervention processes applied currently. Finally, an alternative approach to QMRA that could predict more accurately the annual campylobacteriosis risk in NZ was also investigated.

The kinetic parameters (D and z values) determined under the isothermal and dynamic condition in broths culture or on chicken skin for the most implicated strains in human campylobacteriosis cases in New Zealand were broadly in agreement with published international data. They did not indicate that NZ strains (ST 474, ST 190, ST 48, ST 45 from humans and poultry) were more heat resistant than the overseas strains. Similarly, the bacterial survival under oxidative stress showed that the oxygen tolerance of NZ strains was also similar to other internationally reported strains. A secondary practice (marination of chicken by needle injection) investigated at a poultry production plant did not significantly increase the chicken carcasses contamination level. The results confirm that the tested New Zealand C. jejuni strains do not have unusual characteristics, and the high rate of campylobacteriosis in New Zealand is not associated with the emergence of more environmental stress-resistant strains. This finding has a significant impact on the poultry industry, regulators, consumers and researchers as it confirms the scientific evidence to maintain the stipulated standards for the heat treatment practices at poultry plants, foodservices and at homes. Changes to heat

treatment practices can cause unpredicted loss to the poultry industry. It is important for the regulatory bodies to convey to all consumers, the critical message of the cooking temperature given the reluctance of NZ consumers to use food thermometers to verify the home cooking temperatures.

The results of the consumers' food handling practices survey revealed that the mean score of food safety practices and food safety awareness of the New Zealanders who participated in the survey was below the average questionnaire score of 10.5 out of 21 (the total score). The study confirmed that the awareness of basic food hygiene and safe food preparation practiced by people in New Zealander was lower than in other developed countries. Therefore, an innovative and more effective approach needs to be implemented for future consumer education strategy.

The QMRA conducted in this study indicated that hygiene has a significant impact on the total illness probability. The 'Bayesian hierarchal model', which provides a better insight into the food chain than the 'time series model' and is more informative as it incorporates all the factors that impact on the final risk estimation. The QMRA model was able to identify the consumer hygiene practices, the initial contamination prevalence at the farm and the practices at the processing plant, as the significant factors influencing the final risk estimate. The association between the reduction in birds/chicken carcases prevalence and improvement in home hygiene practice and the calculated reduction in risk of human disease was estimated to be 1:1, which agrees with international findings. Thus, QMRA model can easily determine the impact of any intervention in the food chain. Therefore, the effect of a new planned intervention such as consumer education on the final risk estimate should be clear for the policymakers, risk managers and health professionals. This can be achieved by altering the priors of the consumer hygiene practices in QMRA model. Similarly, other interventions at poultry plants and farms should also be assessed by altering the priors of farms and poultry plants. The Bayesian hierarchal model, which has been used in this study, was able to deliver the expected outcomes and to provide a fast response for policymakers and risk managers. The Auto-Regressive Integrated Moving Average (ARIMA) with an intervention model provided one of the best prediction of the annual campylobacteriosis risk in NZ from the ten models tested. ARIMA intervention model has the lowest forecast error with , only 9 % campylobacteriosis cases more than the actual notified cases. The Holt-Winters method, being local in nature, quickly adapts to the new post-intervention regime and also gives a good prediction which is comparable with the ARIMA intervention models. This method has the additional advantage that it is simple to calculate.

This study also highlighted the potential alternative physical interventions such as the immersion in hot water or hot water wash at poultry processing plants that could be employed with possible another physical intervention in case a ban on chemicals that are currently used to control campylobacteriosis is imposed in NZ.

Declaration

Some aspects of this thesis have been published/submitted for publication in international peer-review journals or as a commission report to the funding organisation (New Zealand Ministry of Primary industries). Some of the results have also been presented at conferences.

Paper Published

- Al-Sakkaf, A. 2020. Comparison of three modelling approaches to predict the risk of campylobacteriosis in New Zealand. *Microbial Risk Analysis*. 14:100077 https://doi.org/10.1016/j.mran.2019.06.001.
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Commissioned Report

- Al-Sakkaf, A., Jones, G., & Mawson, J. (2010). Survival of New Zealand relevant *Campylobacter* strains Final Report: N0174/06 Prepared for NZFSA available online http://www.foodsafety.govt.nz/elibrary/industry/campylobacter-strains/index.htm.

Paper to be Submitted

- Al-Sakkaf, A., Charles Brennan, Elizabeth Redmond and Ravi Gooneratne. (2020). Risk factors associated with consumer handing of raw poultry in the home: implications for campylobacteriosis in New Zealand.

Conference papers

- **July 2013 USA:** "Quantitative Risk Assessment for campylobacteriosis in New Zealand by the Bayesian Belief Network Approach" at the Annual Meeting of IAFP. (July 28 – July 31, Charlotte, North Carolina) https://iafp.confex.com/iafp/2013/webprogram/Paper3707.html
- **August 2010 USA** Oral presentation at 99th Annual Meeting of International Association of Food Protection (IAFP). The title of my paper was: "Application of Kinetic Models to Describe Heat Inactivation of Selected New Zealand Isolates of *Campylobacter jejuni*". (August 1 – August 4, Anaheim, California) https://www.hitpages.com/doc/5678762540662784/12
- **September 2009 Japan:** Oral presentations at the 15th International Workshop on *Campylobacter, Helicobacter* and Related Organisms (CHRO). Papers' titles: "Survival of some New Zealand *Campylobacter* strains under oxidative stress at different temperatures". (September 2-September 5, Niigata)
- **September 2009 Japan** Oral presentations at the 15th International Workshop on *Campylobacter, Helicobacter* and Related Organisms (CHRO). Papers' title "Thermal inactivation of *Campylobacter jejuni* on chicken skin". (September 2-September 5, Niigata).
- **August 2008 USA:** Poster at International Association for Food Protection (IAFP) "Thermal inactivation of *Campylobacter jejuni* in broth". (August 3-August 6, Columbus, Ohio).
- **June 2008 New Zealand** – Poster at Annual Conference of the New Zealand Institute of Food Science and Technology (NZIFST). "Application of kinetic models to describe heat inactivation of selected New Zealand isolates of *C. jejuni*".(June 24-June 26, Rotorua)

Acknowledgements

In the name of Allah, most benevolent, ever merciful

This PhD was a unique journey of tenacity and thanks to Almighty Allah, who has stood by my side all the way through. Thanks to many people for enabling me to complete the task of finishing this thesis. I appreciate the efforts of my supervisors Professors Ravi Gooneratne and Charles Brennan, as they always have been there when I needed them.

I would like to express my profound appreciation to Professor Geoff Jones from the School of Fundamental Science, Massey University, for his continued statistical support throughout this study.

I would like to thank my mother for her patient acceptance of absence from home for such a long period and her caring. I am grateful to my family in New Zealand, my wife and my children for their outstanding support, help and patient acceptance for my absence from home. My special thanks to my wife for her technical assistance in the laboratory for a few months.

It is hard to name and thank more than 30 individuals in New Zealand and overseas such as the experts and academics, laboratory managers, laboratory technicians, engineering and maintenance technicians, library staff and IT professionals who supported me throughout my PhD journey.

Abbreviations

BBN	Bayesian Belief Networks.
BHI	Brain Heart Infusion broth
CA	Columbia Blood Agar
CEB	*Campylobacter* Enrichment Broth
cfu	Colony-forming unit
D	The decimal reduction time (s)
d	The actual ingested dose of the bacteria or the pathogen.
d_{bf}	The probability of additional contamination on the chicken during slaughtering or transferring from farms to processing plants.
D_{ref}	The *D*- value at a reference temperature T_{ref} (s).
EC	European Commission
EU	The European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S.A Food and Drug Administration
g_{bs}	The number of chicken carcasses contaminated out of a number sampled in a survey or routine sampling plan.
g_{fs}	The number of chicken flocks contaminated out of a number sampled in a survey or routine sampling plan.
g_{its}	The number of people suffering campylobacteriosis from whatever source in a year.
GlnaFit	An Excel add-in to fit inactivation curves to various models.
ICMSF	The International Commission on Microbiological Specifications for Foods
k_T	A temperature-dependent death rate constant (per time unit).
m_b	The mean or expected value of the normal probability distribution describing on a logit scale the probability of a chicken carcase being contaminated (p_b)
MC	Monte Carlo simulation which uses random numbers in the simulation algorithm
mCCDA	modified Cefoperazone Charcoal Deoxycholate Agar
MCMC	Markov chain Monte Carlo.
m_f	The mean or expected value of the normal probability distribution describing on a logit scale the probability of a bird in the chicken farm being contaminated (p_f).
MH	Muller Hinton Agar.
MLST	multi-locus sequence typing system
MSE	The mean squared error.
N	The number of cells per unit mass or volume.
N_0	The initial count of bacteria.
n_{bs}	The number of carcasses samples and tested after processing for *Campylobacter* prevalence

	in produced chicken.
n_{cs}	The total number of households times 13, contributing to the total annual chicken consumption in NZ.
n_{csb}	A total number of chickens purchased by NZ households during the year.
n_{fs}	The number of birds samples and tested at a farm for *Campylobacter* prevalence in flocks.
n_{its}	The average population of New Zealand during the year.
N_{res}	The number of cells in a subpopulation of the total population N which are more resistant to the thermal treatment
N_t	The number of cells per unit mass or volume at time t (cfu ml-1).
NZFSA	New Zealand Food Safety Authority
p	An arbitrary (fitted) power parameter
p_b	Probability of a chicken carcase being contaminated after processing in a poultry plant.
p_{bs}	The probability that a processed chicken carcass being tested is found to be contaminated.
p_e	The probability of an exposure event for a household over a four week period.
p_{ey}	Probability of an individual being exposed during the one year period.
p_f	Probability of a bird in a chicken farm being contaminated
p_{fs}	The probability of contamination of a bird tested on a chicken farm.
p_h	Probability of cross-contamination from a contaminated broiler in a consumer's kitchen.
P_{hc}	Probability of bacterial transfer from one surface to another surface.
P_{hh}	Probability of bad hygiene behaviour by a consumer during chicken preparation/handling or cooking.
p_{ib}	The probability that an individual acquires campylobacteriosis due to broiler meat during the year
p_{ie}	The probability of illness given exposure to *Campylobacter*.
P_{in}	The probability of illness given infection.
p_{iq}	The attributable fraction of disease burden due to a known source.
p_{it}	The probability that an individual acquires campylobacteriosis during the year, due to any *Campylobacter* attributable source.
p_{its}	The probability that an individual acquires campylobacteriosis within one year, due to all *Campylobacter* attributable sources after incorporating the data.
p_{ne}	The probability that a single pathogenic organism causes infection.
QMRA	Quantitative microbial risk assessment
RA	Risk assessment
s_b	The standard deviation of the normal probability distribution describing on a logit scale Probability of a chicken carcase being contaminated after processing in poultry plant (p_b).
SE	The standard error.
s_f	The standard deviation of the normal probability distribution describing on a logit scale the probability of a bird in the chicken farm being contaminated (p_f).
s_s	The standard deviation of the possible error term between the p_{it} and p_{its} .
t_L	The lag time

USDA	The U.S. Department of Agriculture
WHO	World Health Organisation
z	The change of temperature (°C) required to reduce the *D*- value by tenfold.
η	A parameter describing the shape of the Weibull distribution function.
δ	A parameter describing the scale of the Weibull distribution function.
λ_c	The rate of chicken consumption in a household during a four-week period.
λ_{csb}	The total annual rate of chicken consumption in NZ.
λ_e	The mean rate of exposure to *Campylobacter* per four week period.

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|

1 Introduction

1.1 Problem description

New Zealand (NZ) has a higher rate (~160 cases per 100,000) of reported campylobacteriosis cases than the rest of the developed world (French et al., 2019). This is 12 times higher than the rate for the United States, four times higher than the rate for Canada, Germany, Netherland and nearly six times higher than the rate in Norway (Kaakoush et al., 2015). The reasons for this high rate are not known. Due to the large economic and health consequences of campylobacteriosis, intervention programmes to reduce the disease rates are required urgently. For these to be effective, the causes for this high rate need to be investigated and addressed.

Several international and New Zealand epidemiological studies have established the link between the consumption of poultry meat and campylobacteriosis. The two main risk factors (consumption of undercooked meat or cross-contamination) identified in these studies need to be investigated in the NZ context.

There is also ambiguity and a lack of data concerning the heat-resistance and survival under aerobic conditions at low temperatures and an ambient temperature of NZ *Campylobacter* strains and international strains (Lake et al., 2003, Sagarzazu et al., 2010, Oh et al., 2019). Thus, there is a need to more fully determine the temperature-dependent growth of *Campylobacter* strains, to provide reliable data for decision-makers (such as government authorities), industry and consumers in NZ.

1.2 Project aim

The aims of this PhD research were:

- To experimentally evaluate the thermal process lethality of NZ *C. jejuni* strains and to update the current knowledge of kinetic reaction parameters such as D and z values of *Campylobacter* strains in general.
- To study the survival of the NZ *Campylobacter* strains that are most frequently associated with poultry products during processing, storage and handling.
- To assess the processing practices and the interventions applied in NZ poultry processing plants such as needle injection and how *Campylobacter* infection possibly has been increased by any processing practices or could be minimised by alternative processing practices such as hot water immersion or hot water wash.

- To investigate the NZ context for cross-contamination during poultry preparation at home as a possible hypothesis to explain the high reported campylobacteriosis rate.
- To construct a model to accurately predict the number of campylobacteriosis cases in NZ.
- To present a structured approach for identifying, assessing, and summarising campylobacteriosis data. Such a structured methodology will provide the most relevant scientific research information to the food safety decision-makers.

Campylobacteriosis is an internationally recognised complex problem. It is important to investigate all the above aims to understand campylobacteriosis in NZ and thereby improve public health.

1.3 Hypothesis

The hypotheses for this research are:

- New Zealand *Campylobacter* strains from human and poultry (ST-474, ST-190, and ST-45) are heat resistant and survive normal cooking processes (chapter 4-6).
- New Zealand *Campylobacter* strains (ST474, ST190 and ST48) are oxygen tolerant (chapter 7).
- The secondary processing technique, marination by needle injection, may increase the *Campylobacter* contamination level in carcasses (chapter 8).
- The physical intervention by combining the hot water wash and hot water dipping would reduce *Campylobacter* level in carcasses, especially the heat-sensitive NZ strains (chapters 4 - 6). This is as an alternative to the EU banning of the use of chemicals in poultry processing in 2006 (chapter 9).
- Poultry farms practices, poultry processors practices, poultry cooking and handling practices in NZ homes are not effective in eradication *Campylobacter* (chapters 10, 12, 13).
- The use of time-series to forecast campylobacteriosis cases in NZ would be more accurate than the forecast provided by the risk assessment approach (chapter 11).

2 Literature Review

2.1 Background

Worldwide, *Campylobacter* causes more cases of bacterial foodborne illness each year than any other bacterial pathogen (Jonsson et al., 2010, Rushton et al., 2019). It is the most commonly reported foodborne disease in industrial countries (Mylius et al., 2007, Facciolà et al., 2017).

The symptoms of campylobacteriosis include abdominal pain, watery or bloody diarrhoea, headache, fever, nausea, muscle pain (Gölz et al., 2018). It is sometimes accompanied by vomiting and late complications such as reactive arthritis, Reiter syndrome (RS) and Guillain-Barré syndrome (GBS). In NZ, the most affected population groups are adults between ages 20 and 29 and children < five years with 1057 and 884 respectively (EpiSurv, 2019) (see Figure 2.1 which is extracted from Episurve website)

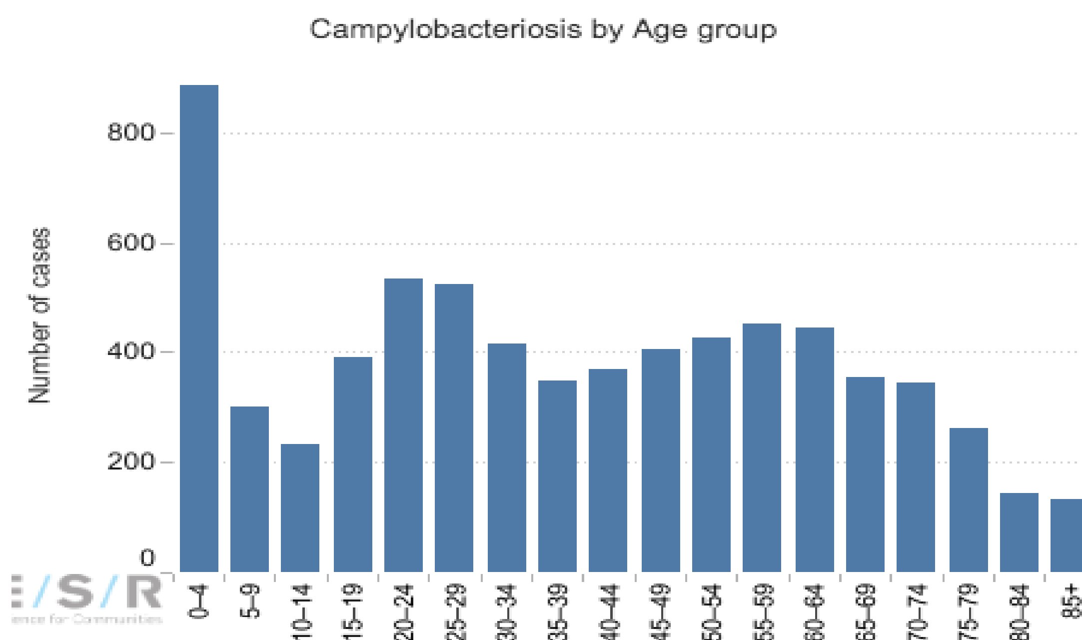

Figure 2-1 2018 Campylobacteriosis rates by age group in New Zealand.

In NZ, the estimated annual cost for campylobacteriosis is more than NZ$77 million per year (NZFSA, 2007). Several studies have estimated the burden of campylobacteriosis, expressed as disability-adjusted life-years (DALYs) (Babo Martins et al., 2017, Lackner et al., 2019). This refers to the number of years lost due to disability caused by sequelae of the infections that occur in GBS

and RS. Campylobacteriosis infection is considered the most expensive disease in Europe and NZ. The cost due to campylobacteriosis sequelae of the infections is estimated as 1568 DALYs (Lake et al., 2010).

In 1980, NZ legislated campylobacteriosis as a notifiable enteric disease. Since then, the incidence of reported campylobacteriosis has risen steadily, and it is believed that NZ has the highest rate of reported cases in the world. In 2006, the notification rate was 384 per 100,000, an increase of 56 % from 2001 and 14% since 2005. This is 35 times higher than the rate in the United States, four times higher than the rate in Australia and five times higher than the rate in the UK. (Baker et al., 2006a). The actual figures are estimated to be at least seven to eight times higher than the notified cases (Baker et al., 2006a). It is estimated (based on 7.6 multiplier factor commonly used in epidemiological studies) that the total number of actual campylobacteriosis cases as 120,000 per year (Baker et al., 2007). Another study reported that actual campylobacteriosis could be 30 times higher than the reported cases (Newman et al., 2015).

Baker (2006b) concluded that campylobacteriosis is a real public health burden, and it is not a surveillance artefact. The reason for this increase in the incidence is still not established, and many aspects of the epidemiology are not clear (Baker et al., 2006a). However, several international epidemiological studies have established the link between the consumption of poultry meat and campylobacteriosis (Wingstrand et al., 2006, Skarp et al., 2016). NZ epidemiological studies also indicate a similar link (French, 2008a, Mullner et al., 2009b, Baker et al., 2011, Nohra et al., 2018, French et al., 2019). In addition, the high level of prevalence and contamination of poultry meat (80 -100 %) with *Campylobacter* (French, 2008b) provide further evidence about this link. Moreover, the increase in the consumption of poultry is concomitant with the increase in the rate of reported campylobacteriosis. A dramatic drop in disease notification rate accompanied the withdrawal of poultry from the markets in Belgium and the Netherlands (Vellinga and Van Loock, 2002) also confirm such a link. The similar remarkable decline in the notification rate following the freezing of fresh poultry in Iceland indicated fresh chicken as the main risk factor for campylobacteriosis (Lake, 2006).

New Zealanders consume a slightly higher quantity of poultry per capita than Western European countries but less than the amounts consumed by North American and Australians as per the Food and Agriculture Organization of the United Nations (FAOSTAT) database for the year 2018 (Figure 2.2).

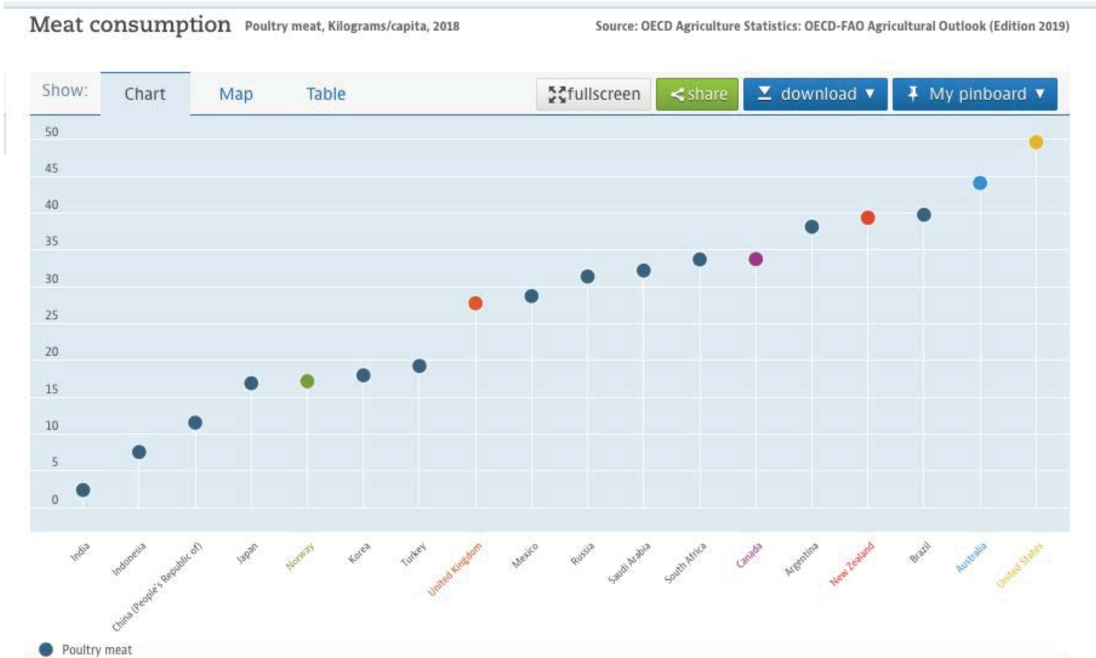

Figure 2-2 World poultry consumption per capita in selected countries

Poultry meat products are increasingly available to NZ consumers in a wide variety of formats: fresh or raw products (e.g. whole carcass, selected pieces, pre-prepared cuts), partially-processed raw products (e.g. frozen, marinated, coated, 'tenderised'), raw reconstituted meats (e.g. sausages, nuggets, patties, luncheon, bacon analogues) and many ready-to-eat (cooked) forms. Raw products are cooked by the home consumer or foodservice provider by a variety of methods including roasting, braising, grilling, frying and deep-frying.

It is important for processors, caterers and consumers to apply adequate heat treatment to destroy all food pathogens, including *Salmonella* and *Campylobacter* (Humphrey et al., 2007, NACMCF, 2007). Moreover, there is a possibility that undercooking may occur and/or a proportion of bacterial cells in some protected areas may survive the normal heating process. Thus, the consumption of undercooked chicken or poultry products is considered an important risk factor for campylobacteriosis (Butzler, 2004).

It has been reported that NZ *Campylobacter* strains differ genetically from international strains. The subtypes isolated in NZ have not been identified previously in other international databases, and some NZ strains are antimicrobial drug resistance (McKenzie, 2006, French et al., 2019). It is possible that local strains have a greater heat tolerance and thus are better able to survive cooking, an exposure route often discounted in risk assessments studies.

2.2 The pathogen

Most *Campylobacter* species are motile and possess a single flagellum at one or both poles. A majority

of *Campylobacter* are microaerophilic. The genus *Campylobacter* has changed dramatically since it was identified in 1963 (Sebald and V'Eron, 1963). At that time, the genus contained just two taxa, *C. fetus* and *C. bubulus*. *Campylobacter* contains 16 species and six subspecies (Boxall, 2005) (Table 2.1). However, there are currently 34 to 35 *Campylobacter* species recognised, but with the improved diagnostic techniques and genomic analysis, this number is expected to increase over time (Anonymous, 2017, Klein, 2016). Most human illnesses are caused by *C. jejuni*. *Campylobacter jejuni* and *C. coli* are considered the major species that threaten public health in NZ (Lake et al., 2003). A minority (1-5 %) of human campylobacteriosis cases are caused by other species (*C. upsaliensis* and *C. lari*).

Table 2-1 Different species of *Campylobacter*

Species	Subspecies	Biovar	Source
consisus			Human oral cavity
showae			Human oral cavity
curvus			Human oral cavity
rectus			Human oral cavity
gracilis			Human oral cavity, abscesses, lesions
sputorum		*sputorum*	Human oral cavity, abscesses, lesions, faeces, cows, sheep, pig's genital tract, aborted mater
		faecalis	Cow, sheep, faeces
		paraureolyticus	Cattle, human, faeces
hominis			Human oral cavity Pig intestine, oral cavity
fetus	*fetus*		Aborted fetus Placenta, stomach contents
	venerealis		Cows vaginal mucus, semen, prepuce
hyointestinalis	*hyointestinalis*		Pig intestine
	lawsonii		Pig stomach
lanienae			Human (abattoir workers)
jejuni	*jejuni*		Poultry, cows, sheep, human, Environment, faeces
	doylei		Human gastric tissue, diarrhoea, blood
coli			Pig faeces
lari			Birds, animals, water, shellfish, faeces
upsaliensis			Cats, dogs, faeces
helveticus			Cats, dogs, faeces
mucosalis			Pig intestine, oral cavity

Note: Adapted from (Boxall, 2005).

Campylobacter jejuni is a Gram-negative, slender, curved, and motile rod. It contains two subspecies that differ substantially in their ubiquity and to some extent ecology (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*). *C. jejuni* subsp. *jejuni* is often referred to as *C. jejuni* and represents the taxon first reported by Jones (Jones et al., 1931) as *Vibrio jejuni* isolated from bovine intestinal contents. Since the 1970s, this organism has been widely considered as the most commonly isolated bacterium that has caused human gastroenteritis (Facciolà et al., 2017). Its prevalence in food animals makes it one of the most important foodborne pathogens. Many studies (Dingle et al., 2001, Parkhill et al., 2000, Thépault et al., 2018) have reported its genetic diversity and genomic plasticity.

2.3 Survival and growth of *C. jejuni* in the environment

Campylobacter species exist in many wild and domestic warm-blooded animals (Adams and Moss, 2000). It does not survive well outside the gut, nor does it replicate readily. Most literature reports the best range for growth as 37- 43 °C with 42 °C as the optimum, similar to the chicken body temperature (Adams and Moss, 2000, CFSAN, 2008, FDA/CFSAN, 2000, Forsythe, 2000, Lake et al., 2003). Over the years, various studies targeted this pathogen to determine the environmental conditions necessary for growth and survival. The published values of temperature, pH, water activity and the optimal atmospheric conditions are summarised in Table 2.2.

Table 2-2 Growth conditions for *Campylobacter jejuni*

Parameter	Optimal Conditions	Growth Range
Temperature	42 °C	30.5 to 45 °C.
Generation time	~ 1 hour	
pH	6.5	4.9 to 9
Water activity	0.997	≥0.987
Gas composition	5% O_2, 10% CO_2, 85 N_2%	

Note; Adapted from Microbiological Specifications of Food Pathogens (ICMSF, 1996)

Laboratory studies suggest that *Campylobacter* species are sensitive to adverse conditions. They are unable to grow in the presence of elevated levels of oxygen (above 5 % O_2), difficult to multiply outside the animal host and are highly sensitive to environmental conditions such as drying, acidity, salting, freezing, chlorination, osmotic stress and pressure (Park, 2005, Burgess et al., 2016). Consequently, the organism can survive only for up to four to six hours on hands and moist surfaces (De Cesare et al., 2003, Humphrey et al., 2001). Moreover, *Campylobacter* is a poor competitor against other organisms in the environment (ICMSF, 1996). However, the pathogen can survive and remain in a viable but in a non-culturable state at 4 °C for up to four months and sometimes as high as seven months (Haddad et al., 2009). Others (Dasti et al., 2010) have reported the catalase activity, ATP generation, oxygen consumption, chemotaxis, and protein synthesis for the pathogen at the same temperature. Its viability at -20 and -70 °C, for 14 days and 56 days, respectively, has also been confirmed (Lee et al., 1998). Another study (Gunther Iv et al., 2015) reported that *C. jejuni* survived for 33 days at -20 °C. The survival ability of *Campylobacter* strains survival at low temperature remains poorly understood from the inconsistency of the results reported by the above studies and further research is required.

2.3.1 Atmosphere

Campylobacter strains survive well in modified atmosphere and vacuum packaging, but poorly at the atmospheric oxygen concentration (21%) (Kelly, 2008). The optimum atmosphere or gas composition for the growth is 5 % O_2, 10 % CO_2 and 85 % N_2 (ICMSF, 1996). Studies that have investigated the growth of *Campylobacter* under different gas compositions with different techniques and substrates are in general agreement with this proposed optimum composition (Gharst et al., 2013, Meredith et al., 2014).

Several investigators have studied the effect of temperature on oxidative stress reviewed by (Park, 2005). This review reported that the survival of *Campylobacter* strains in poultry and meat was not influenced by the storage atmosphere or the treatment condition at 4 °C, which is in agreement with other studies. Another study indicated that the exposure to atmospheric oxygen in broth culture for five and 15 hours had no effect on the *C. jejuni* growth and the count was similar to the *C. jejuni* incubated under micro-aerobic conditions (Mihaljevic et al., 2007). This is consistent with other reports, also regarding the growth and adaptation of *C. jejuni* to aerobic metabolism (Oh et al., 2015). There are studies which report that the presence of oxygen increased the death rate at 4 °C in broth and milk (Butzler, 2014). Yamasaki (2004) reported that initial *C. jejuni* inoculum (9.5 log) concentration declined by half log after the first six hours under aerobic conditions at 37 °C followed by another one log reduction in the following six hours. Yamasaki observed that a rapid

reduction by about 5 log occur between 12 - 15 hours of incubation. Others reported that *C. jejuni* could survive oxidative stress at 4 °C for several weeks (Chan et al., 2001, Garenaux et al., 2008, Oh et al., 2017). A NZ study revealed that *C. jejuni* could adapt to aerobic growth after repeated subculture. In this study, about 81 % of the human and 65% of poultry *C. jejuni* isolates that can grow under aerobic conditions in the nutrient agar, a cocktail of isolates (human, poultry, veterinary) survived aerobically for more than four weeks in poultry meat at 5 °C and for more than a week at 25 °C (Chynoweth et al., 1998). From all of the above studies, it is concluded that there is ambiguity about the behaviour of *C. jejuni* under oxidative stress. Evidently, *Campylobacter* causes more difficulties in standardization and handling than any other pathogen (Bergsma et al., 2007, Jasson et al., 2007).

2.3.2 Water Activity

Campylobacter is sensitive to drying, and its survival is dependent on the suspending substrate, the temperature and the extent of drying (ICMSF, 1996). The pathogen does not survive long in less moist environments compared to *Salmonella* and *E.coli*. For example, *Campylobacter* survives poorly on dishes with organic matter while *Salmonella* can survive for 24 h and *E. coli* even longer (Mattick et al., 2003). Coates et al. (1987) study reported a 3 - 7 log reduction in the pathogen numbers within two mins when the cells suspension were spread on fingertips. Handling of raw meat with moist hands enhances *Campylobacter* survival on fingertips which facilitates the cross-contamination or direct contamination during food preparation in the kitchen (Humphrey et al., 2001). Generally, moist hands transfer microorganisms more easily than do dry hands to skin, food and preparation surfaces (Mutters and Warnes, 2019). Others have reported that surface drying during air chilling in meat or poultry processing reduces water activity, retard bacterial growth and cause enough injury to pathogenic bacteria when pathogen levels on carcases are compared before and after air-chilling red meat and at poultry processing abattoirs. It has been suggested that drying is responsible for the reduction of the pathogen numbers on the surfaces (Sánchez et al., 2002, Lu et al., 2019).
Poultry is traditionally chilled by immersion in cold water, called the spin chiller, and are packed and stored in a high moisture environment which enables *Campylobacter* to survive longer. Many studies (Huezo et al., 2007, Sánchez et al., 2002, Lu et al., 2019) have considered that dry air chilling at the poultry processing plant is the best technology to reduce *Campylobacter* contamination level and to prevent the cross-contamination which is acquired in the wet chilling approach. Therefore, dry air chilling is implemented in many countries.

2.3.3 Temperature

Campylobacter cannot grow below 30°C (Jacobs-Reitsma et al., 2008), which means that it should

not multiply during food processing or storage at a typical ambient temperature. However, it survives better in foods under refrigeration than at ambient temperature (Bhaduri and Cottrell, 2004). It grows only slowly at the optimum temperature (42 °C) in the laboratory. *Campylobacter* typically takes 48 h for good colony formation of on agar plates. Plates may be incubated at 37 °C or 42 °C, but it is a common practice to incubate at 42 °C to minimise the growth of contaminants and to select *C. jejuni* and *C. coli* for optimal growth (Anonymous, 2017). However, the International Organization for Standardization recommends incubating plates at 41.5 °C (Jacobs-Reitsma et al., 2019). *Campylobacter* failed to grow above 47 °C (Baserisalehi et al., 2006) as a result of the irreversible denaturation of the proteins, the thermal breakdown of the plasma membrane and the most sensitive parts of ribosomes. The kinetics of microbial inactivation and its importance in food processing are explained in the next section (2.4). Information on the biological aspects and mechanisms of inactivation at a molecular level to understand what takes place from a physiological and genetic perspective are not yet completely understood, but the current knowledge has been summarised in various studies (Park, 2000, Park, 2002).
The methods for measuring the heat resistance of food pathogens can be classified by the heating method (direct or indirect) or by the method of sampling (successive sampling systems or multiple replicate unit systems) (Pflug, 1990, Downes and Ito, 2001).

2.4 Kinetic analysis of bacterial inactivation

2.4.1 Linear models

Bacterial inactivation is a function of residence time and temperature in the environment to which they are exposed to. It is commonly recognized that the number of organisms decreases exponentially with time. This implies that the rate of inactivation is proportional to the number of viable cells and that death follows first-order kinetics (Chick, 1910, Davis et al., Holdsworth, 1998, Prescot et al., 1996). This is mathematically described at constant temperature by:

$$\frac{dN}{dt} = -k_T N \qquad \text{2-1}$$

where N represents the number of cells per unit mass or volume (colony-forming unit; cfu/ml or cfu/g), t the total heating time at constant temperature (in s), and k_T a temperature-dependent death rate constant (per unit time)
On integration, this yields the following expressions:

$$\ln \frac{N_0}{N_t} = k_T t \qquad 2\text{-}2$$

$$\log_{10} \frac{N_0}{N_t} = \frac{t}{D} \qquad 2\text{-}3$$

Where N_0 is the initial count of bacteria, and *D* is the decimal reduction time. This is the time required to reduce the bacterial count by a factor of 10 and is equal to the number of seconds for the survival curve to traverse one log cycle.

The temperature dependence of the k_T or *D*- value can be expressed using the Arrhenius relationship or the *z* value model Equation 2.4.

$$\log_{10}(D_T) = \log_{10}(D_{ref}) + \frac{T_{ref} - T}{z} \qquad 2\text{-}4$$

where:

D_{ref} is the *D*- value at a reference temperature T_{ref} (s).

T is the temperature (°C).

z is the change of temperature (°C) required to reduce the *D*- value by 10-fold.

A summary of *D* and *z*-values for various pathogens and products can be found in a report from the FDA/CFSAN (FDA/CFSAN, 2000). Table 2.3 summarises *D* and *z*-values reported for *Campylobacter*.

Table 2-3Thermal inactivation parameter from *Campylobacter* heat resistance studies

Substrate	Temp °C	*D* value (min)	*z* value °C	**References** (as a group for each section)
Skim milk	48	7.2 - 12.8		(Doyle and Roman, 1981)
	50	1 – 7.3		(Christopher et al., 1982)
	52	5.2	2.8 – 5.3	(Skirrow, 1982)
	53	1.56 – 1.95		
	55	0.74 – 1.1		
Peptone	49	14.9 – 15.2		(Blankenship and Craven, 1982)
water/saline	50	0.88 – 1.63	6– 6.4	(Forsythe, 2000)
Buffer	51	4.90 – 7.02	4.94	(Humphrey and Lanning, 1987)
Scald water	51.5	2.45- 3.8	4-5.2	(Sorqvist, 1989)
	52	6.4 – 8.7		(Gill and Harris, 1982)
	52	1.96 -10.82		(Doyle and Roman, 1981)
	53	1.71 – 2.70		(Nguyen et al., 2006)
	53.5	0.72 -2.14		(Oosterom et al., 1983)
	55	0.64 – 1.09		(Sorqvist, 2003)
	55	4.6-5.3		(Al-Sakkaf and Jones, 2012)
	56	0.78		(Lahou et al., 2015)
	57	0.25		
	58	0.24		
	60	0.13		
	60	0.26 – 0.95		
	60	0.02-0.07		
	65	0.22		
	70	0.18		

Chicken Beef Lamb Smoked Salmon	49 50 50 51 53 55 55 56 56.5 57 58 60 60 100 109- 127	20.5 5.9 – 6.3, 5.9 – 13.3 8.77 – 9.27 4.85 – 4.89 0.96–1.26 2.12–2.25 0.62 - 0.96 22.5- 41.6 0.79 -0.98 0.25 0.21 – 0.26 11.2-18.0 1.90 1.95	4-8 8.7 - 10.2 7.6 - 11.3 12.3 2.8-5.8	(Blankenship and Craven, 1982) (Koidis and Doyle, 1983) (Bergsma et al., 2007) (de Jong et al., 2012) (Al-Sakkaf et al., 2010) (van Asselt and Zwietering, 2006) (Li et al., 2002) (Jackowska et al., 2008) (Lahou et al., 2015)

2.4.2 Alternative models of thermal inactivation

Many studies in the literature have reported that bacterial survival curves (i .e. log N or ln N vs t) are not always strictly linear (Figure 2.3). They could be sigmoid curves, curves with shoulders, tailings, and concavity upwards and downwards (Campanella and Peleg, 2001, Cerf, 1977, Peleg et al., 2000, van Boekel, 2002). Models have been proposed to fit these different survival curves. For example, a simple two-phase model was suggested by Whiting (1993), used for *Listeria monocytogenes* (Breand et al., 1998)

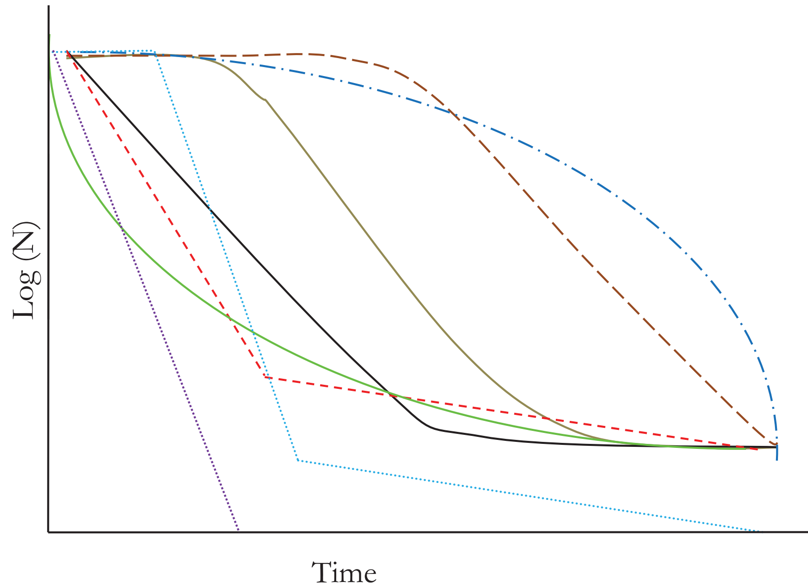

Figure 2-3 Different survival curves observed during heat inactivation of microorganisms (Geeraerd et al., 2005).

when $0 < t < t_L$

$$\log_{10} N_t = \log_{10} N_0 \qquad 2\text{-}5$$

when $t > t_L$

$$\log_{10} N_t = \log_{10} N_0 - \frac{1}{D}(t - t_L) \qquad 2\text{-}6$$

Where t_L is the lag time.

For the other forms of survival curves, a power-law modification of Equation (2-3) may be applied:

$$\log_{10} \frac{N_t}{N_0} = -\frac{t^p}{D} \qquad 2\text{-}7$$

Where p is an arbitrary (fitted) power; p = 1 gives the first-order model (Figure 2.4a), and when p>1 gives a convex shape (Figure 2.4b) results of p < 1 gives a concave curve (Figure 2.4c) (Huang and Juneja, 2001, Mafart et al., 2002).

Probabilistic models have also been developed in the last few years to describe the survival curve as a cumulative form of the temporal distribution of a lethality event (Peleg and Cole, 1998). For example, the Weibull frequency distribution has commonly been used to express the bacterial death

time or bacterial inactivation (Cunha et al., 1998, Fernandez et al., 1999, Mafart et al., 2002, Peleg, 2002, Peleg and Cole, 1998).

$$\log_{10} N_t = \log_{10} N_0 - \left(\frac{t}{\delta}\right)^{\eta} \qquad 2\text{-}8$$

η and δ are the two parameters relating to the scale and shape of the distribution function. The above model is another form of the power-law model (Equation 2.7) in which "D" value of Equation 2.7 is replaced by "δ"in the Weibull model. Most of the authors of these studies reported that the Weibull model as being successfully tested by using a variety of published heat resistance data and the authors have concluded that the Weibull model is considered a more suitable and simpler model to describe non-log-linear survival curve.

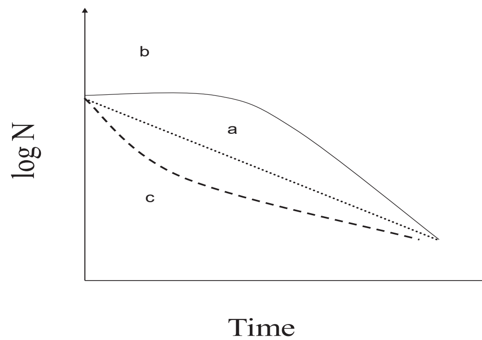

Figure 2-4 Most common survival curves which are applicable for the Weibull model

(a: linear model when η =1, b: convex curve when η >1, c: concave curve when η <1)

The non-linear models which were fitted for data in this study and covered most of the survival curves observed during the heat treatment shown below:

Shoulder and linear (Figure 2.3 brown dotted line), linear and tail (black straight line), shoulder linear and tail (straight brown line) (Geeraerd et al., 2000).

$$\log N = \log\left[(10^{\log(N_0)} - 10^{\log(N_{res})}\right] \cdot e^{-k_{max} t} \cdot \left(\frac{e^{k_{max} s_l}}{1+(e^{k_{max1} s_l} - 1) \cdot e^{-k_{max} t}}\right) + 10^{\log(N_{res})}$$

2-9

Where: N_{res} is the number of cells in a subpopulation of the total population N, which are more resistant to the thermal treatment (Cerf, 1977).

Weibull with a tail (Albert and Mafart, 2005)

$$\log N = \log[(N_0 - N_{res}).10^{(-(\frac{t}{\delta})^{\eta})} + N_{res}$$ 2-10

Biphasic (Cerf, 1977) (Figure 2.4 dotted red)

$$\log N = \log N_0 + \log\left(f \cdot e^{-k_{max1} t} + (1-f) \cdot e^{-k_{max2} t}\right)$$ 2-11

f is the fraction of the initial population in a major subpopulation, (1-f) is the fraction of the initial population in a minor subpopulation),
$f = N_{01}/N_0$ and $(f-1) = N_{02}/N_0$.

Biphasic with shoulder (Figure 2.4 dotted light blue) (Geeraerd et al., 2005)

$$\log N = \log N_0 + \log\left(f \cdot e^{-k_{max1} t} \cdot \frac{e^{k_{max1} s_l}}{1+(e^{k_{max1} s_l}-1) \cdot e^{k_{max1} t}} + (1-f) \cdot e^{-k_{max2} t} \cdot \left(\frac{e^{k_{max1} s_l}}{1+(e^{k_{max1} s_l}-1) \cdot e^{-k_{max1} t}}\right)^{\frac{k_{max2}}{k_{max1}}}\right)$$ 2-12

Generally, the non-linear models are based on experimental data without understanding the physiological or physical behaviour mechanisms of the microorganisms, and they are often over parameterised. In addition, the complexity of some of these models hampers their application in the calculation of the heat treatment as food industry practitioners and regulatory institutions are unwilling to abandon the *z* and *D*- value based method which through experience has demonstrated to be generally safe and reliable (Heldman and Newsome, 2003).

2.4.3 Handling bacterial survival data

Two major methods are employed for determining bacterial survival curves. The most common method follows from heating a number of replicate samples for different time intervals at a fixed temperature. The samples are plated, and after proper incubation, the number of colonies (colony

forming units; cfu) grown on plates is counted. The logarithm of the colony number is then plotted against the heating time to derive the survival curve.

The alternative negative fraction method is based on recording the presence or absence of bacterial growth (Pflug, 1990). Appropriate statistical and mathematical methods (e.g. fitting the observed data to pre-existing model or develop a new model) must then be applied to obtain the appropriate kinetic values (D and z). The final step is to validate the selected or developed model by comparing predictions from the models to observed growth, survival, death responses of bacteria in relevant foods. Detailed explanations of interpretation of survival data and modelling have been reported (Pflug, 1990, Pinheiro, 2000, Stumbo, 1973).

2.4.4 Non-isothermal models

Heat treatments employed in the food industry invariably results in temperature fluctuations during the heating or the cooling stages. In practice, the thermal effect during the time required to achieve a constant temperature (the come-up time) must be accounted for, and in many cases, it has a much larger effect than the holding time at a constant temperature. Moreover, kinetic parameters used under isothermal conditions should not be applied to variable processing conditions without validation under dynamic conditions (Welt et al., 1997). Therefore, many researchers have tried to apply non-isothermal methods as an alternative to the study of microbial inactivation kinetics. Dynamic models describing microbial inactivation are expressed as differential equations which incorporate the imposed or observed time-temperature profile as an input to the dynamic models selected which describe the thermal death of micro-organisms. The resulting equations can be solved numerically to obtain the number of surviving organisms at a given position in the time-temperature profile. If the rate of temperature increase can be described by a simple function, the dynamic model equation can be solved analytically (Miles and Mackey, 1994). Several procedures proposed to accomplish this integration are described in the literature (Patashnik, 1953, Stumbo, 1973).

The determination of kinetic parameters under dynamic conditions can better reflect a realistic processing scenario and the ‘come-up time’ is explicitly a part of the experimental design. That is, by specifying the heating rate and time-temperature profile there will not be a limited temperature range from which to choose sufficient points for the experiments, particularly for heat-sensitive organisms (Welt and Tong, 1993, Welt et al., 1997).

The models employed in predictive microbiology have most commonly been obtained by differentiating a modified Gompertz equation with respect to time, in combination with an Arrhenius-type equation, to describe the microbial load as a function of both time and temperature

(Geeraerd et al., 2000). From this, a class of models have been developed used to describe the inactivation of micro-organism kinetics at time-varying temperatures (Conesa et al., 2003, Fernandez et al., 1999, Geeraerd et al., 2000, Schellekens et al., 1995, Van Impe et al., 1992). The results obtained for the kinetic parameters by non-isothermal methods have differed from those obtained by isothermal methods (Conesa et al., 2009, Decordt et al., 1992). However, Valdramidis (2008) succeeded in obtaining similar results from both methods and concluded that non-isothermal kinetic parameters are more accurate and can be obtained with less experimental effort.

2.5 Factors affecting heat resistance of microorganisms

Many factors influence the heat resistance of a pathogen including between strain variation, presence of salt or acid, the growth phase of the cells, experimental conditions (e.g. heating media directly or indirectly), cooling after heating, and products or laboratory media used.

2.5.1 Strain variation

Strains within an individual *Campylobacter* sp. genotype or phenotype vary considerably in their ability to survive in the environment and along the food chain and can exhibit large differences in their stress response. The diversity within *Campylobacter jejuni* strains is well known and has been detected at both the phenotypic and genotypic level (Gölz et al., 2018). The number of typed strains is rapidly increasing within data banks as different typing methods such as microarrays, multi-locus sequence typing system (MLST), pulsed-field gel electrophoresis (PFGE), Penner & Lior and other DNA-based methods and porA typing based on the sequencing of the major outer membrane protein (MOMP) encoding gene (Garaizar et al., 2006, Petersen et al., 2001, Wieczorek and Osek, 2019). Different *Campylobacter* strains show different capacities to resist environmental stress (Habib et al., 2010, Alter and Scherer, 2006, Chan et al., 2001, Duqué et al., 2019). A few studies have addressed differences in strain behaviour under environmental stress. Chan (2001) reported the variation in the survival of 19 different *C. jejuni* isolates subjected to temperature stress. The focus of the study was on the survival of the pathogen at low temperatures (4 °C and -20 °C). The results of the study have been used in the epidemiological investigation as human isolates showed greater cold-tolerance than poultry strains, although the freezing tolerance was strain-specific and independent of the strain source.

Similarly, Cools (2003) tested the survival of some *C. jejuni* strains in drinking water and reported their recovery after 64 days at 4 °C. Poultry strains survived for longer periods than human, water and bovine strains. Murphy et al. (2003) suggested that the pathogen acid tolerance may be strain-dependent. The carrying of *Campylobacter* strains by poultry carcass during processing is also strain-

dependent with the more resistant strains remaining on the carcass at the end of processing (Newell et al., 2001).

A study of NZ strains by Gill and Harris (1982) investigated six *Campylobacter* strains from human cases, six from calf and sheep and they reported a *D* value of less than a minute at 60 °C in broth medium. No poultry strains were included, and the study was conducted with the available methods, instrumentation and techniques at that time with regard to strain typing, isolation, recovery, enumeration, inactivation and incubation.

2.5.2 Test or experimental conditions

The experimental conditions have a significant effect on the reported data of heat resistance. A variety of methods, used for determination of heat-resistance, have attempted to reduce the errors introduced by calculation of heat transfer (sample 'come up time' calculation), sample dilution and accuracy of time and temperature measurement. Sorqvist (1989, Sorqvist) compared the test tube types for the *D*- value determination and found marked differences between the capillary tube and other tube types. The *D*- value obtained by a test tube was up to 29 times higher than the *D*- value obtained by the capillary tube. Similarly, Miller et al. (2000) reported a lower *D*- value for *Listeria monocytogenes* by using a complete submerged system. A study by Line (2006) compared the type of enrichment vessels used for recovery of *Campylobacter*. Moore and Madden (Moore and Madden, 2000) compared the heat transfer prosperities of three flasks to select the best one which can be used later for their *Campylobacter* heat sensitivity experiment, and they concluded that stoppered glass diluent bottles were better than sealed glass universal bottles or universal plastic bottles in terms of their conductivity. Heat-resistance studies have used a range of equipment for heating including water bath, heating block, incubators, oil bath, thermoresistometer (Condon et al., 1993, Condon et al., 1989), submerged–coil heating unit (Cole and Jones, 1990), temperature gradient incubator (Elsgaard and Jørgensen, 2002), differential scanning calorimeter (DSC) heating unit (Brown, 1988), and Peltier units. The Petlier unit operates like a thermocouple in reverse and the junctions heat or cool as the voltage changes across them. A specially designed apparatus for heating food sample was developed for the Bug Death project (Foster et al., 2006). This apparatus had the ability to heat the surface of food from 8 to 120 °C in 14 s, cooling from 120 to 40 °C in 28 s and then to 8 °C in 300 s. A 1 ml volume aluminium cell was developed for investigating the kinetics parameters (Chung et al., 2008) and a modified thermos-resistometer with the ability for use during non-isothermal heat treatment (Conesa et al., 2009), fast-responding thermal-death-time tubes (Büchner et al., 2012) and the heating block system (HBS) (Kou et al., 2016). Detailed descriptions of all the methods and equipment used for the study of microorganisms' heat

resistance are in the literature cited above and in major texts books (Pflug, 1990).

2.5.3 Culturing media and recovery procedure

The isolation of *Campylobacter* and the methods used for its detection are well documented (Donnison, 2002, Line, 2001, Hsieh et al., 2018, Jacobs-Reitsma et al., 2019). Corry (1995) listed 40 liquid and solid selective types of media used for the isolation of *Campylobacter*. However, the detection and the recovery of heat-injured cells have not yet received much attention from any. Murphy (2005) concluded that there was a direct effect of the medium used on stress and survival of *C. jejuni*, and suggested that studies using different media may not be comparable. In that study, Murphy (2005) compared the growth of *C. jejuni* after acid stress in seven selected media and observed a remarkable difference in the growth of *C. jejuni* - up to a 5 log difference. Similarly, Jasson (2007) tried to describe a procedure for sub-lethal injury for *Campylobacter* stressed by different environmental factors (heat, cold, freeze, acid and oxidation) by comparing the number of the survival counts in selective (modified Cefoperazone Charcoal Deoxycholate Agar) and non-selective media (Columbia Blood Agar). The results showed that *Campylobacter* contributed to the high variation in test reproducibility and repeatability and that there were difficulties in standardization during handling the organism compared to other pathogens. Habib (2008) compared three selective media for *Campylobacter* (mCCDA, Karmali agar and CampyFood ID agar) and concluded that there were minor differences among the three selective media in terms of the reproducibility and repeatability. The mCCDA, which the International Organization for Standardization (ISO) has recommended has no major problem with the accuracy of the results obtained by using it. Hsieh et al. (2018) concluded that 37 °C is the optimal temperature for incubation and not 41.5 °C or 42 °C as reported in most other international studies (Jacobs-Reitsma et al., 2019). Others (Efimochkina et al., 2017) developed new media for detection and enumeration. Recently (Andritsos et al., 2020) compared the performance of four culture media used for enumeration and detection of *Campylobacter* species in chicken meat and concluded that the newly selected *Campylobacter* agar (CASA) developed by bioMérieux outperformed another recently developed agar by Oxiod BrillianceTM CampyCount agar (BCCA) and the ISO recommended mCCDA and Karmali agars.

2.5.4 Effect of pH and presence of acids

The effect of pH is dependent on the type of *Campylobacter* strain used, suspension medium, water activity and the test temperature. Generally, the heat resistance is at its maximum at pH 7 and decreases as the pH shifts from neutrality (Varsaki et al., 2015). Humphrey and Lanning (1987) reported that the *D*- value was 11.5 min at 52 °C in a hot water tank at pH 7, and this was reduced

to 0.4 min and 1 min when the pH was adjusted to 4 and 10, respectively. They also reported that adjusting the scalding water tank pH to 9 significantly reduced the bacterial numbers in the scalding water but not the bacterial count on the carcass. Another study (Berrang et al., 2011) indicated that adjusting the scalding water tank pH to 9.89 was more effective to lessen the prevalence and the number of *Campylobacter* on broiler carcasses. Recently, another study (McCarthy et al., 2018) developed and validated a scalding model that accounted for both pH and temperature.

Another study (Coşansu and Ayhan, 2010) dipped chicken leg and breast in 1-3% solution of lactic acid and acetic acid (pH 2.2 - 2.3) and achieved a 0.3 to 2 log reduction in bacterial numbers. The combination of different lactic acid concentration with hot steam treatment on chicken breast samples was the objective of another study (Cil et al., 2019). This study concluded that the treatment with 4% lactic acid (pH 2.85) achieved more than 4 log reduction. However, 2% lactic acid with 15 seconds of treatment time by steam at 97 °C also achieved more than 4 log reduction.

2.5.5 Effect of age (growth phase) of microorganisms

Usually, microorganisms in the stationary phase of growth are assumed to be the most resistant to heat stress (Kaur et al., 1998). However, a study by (Martinez-Rodriguez et al., 2004) reported that a strain isolated from the late stationary phase showed only a small increase in heat resistance over its parent. Kelly et al. (2001) showed that *Campylobacter* did not possess a greater heat resistance in the stationary phase and instead was more heat resistant in the exponential phase. However, these authors did not explain adequately why some cells were able to survive after a prolonged period of heat application of the cells in the stationary phase of growth although they reported that this phenomenon was due to the acquisition of resistance during prolonged periods of stress application.

2.6 Heat resistance studies

Many reports have addressed the response of *Campylobacter* under thermal stress conditions by using broth or liquid media (Table 2.3). Some of these studies investigated changes in heat resistance combined with changes in pH or gas composition. A review study (Juffs and Deeth, 2007) has summarised different milk pasteurisation time and temperature combinations to determine D and z values in milk and to investigate whether pasteurisation could adequately destroy *Campylobacter* present in milk. They concluded that the organism was inactivated by both batch and HTST pasteurisation, with a wide margin of safety. Peptone water or saline has been used widely as the heating medium to determine D and z values. Poultry meat slurry was used in modelling the combined effect of heat and high pressure (Lori et al., 2007). *Campylobacter* survives better under

aerobic and ambient temperature in biofilms (Tang and Schraft, 2000, Joshua et al., 2006). However, Trachoo and Brooks (2005) claimed that *Campylobacter* in biofilms is not heat resistant.

Bergsma et al. (2007) reported the highest *D*- value for *C. jejuni* in the fried chicken fillet, 1.95 min at a surface temperature between 109 to 127 ° C. de Jong et al. (2012) confirmed this and reported a *D*- value of 1.9 min at 100 °C in a boiled chicken fillet. Such extremely high D-values have not been confirmed by other studies. Gunsen et al. (2008) could not detect any *Campylobacter* colonies after cooking an artificially inoculated chicken drumstick for three to five min to reach a core temperature of 70° - 80 °C respectively in an oven at 200 °C. However, Sampers et al. (2010) found the 4.5 log cfu/g of *C. jejuni* inoculated to chicken burgers were reduced to below the detectable level (<10 cfu/g) after four min frying to an internal temperature of 57.5 °C and also 2.5 log cfu/g of naturally contaminated chicken burger reduced to below detectable level after four min frying to an internal temperature 52.1 °C. Moreover, *C. jejuni* was not detected after simulated home pan-frying of artificially inoculated steaks or fillets, hamburgers and meat strips (Lahou et al., 2015). All meat samples of this study (Lahou et al., 2015) were from various animal species (pork, beef, chicken, lamb and some turkey, horse, and kangaroo), inoculated with 4 log cfu/g of *Campylobacter jejuni*. Only after a further enrichment step, *C. jejuni* was detected in few samples of pork hamburger, horse steak and crocodile steak. This is probably due to the matrix effect of the meat in these animals.

C. jejuni could not be detected when meatballs were artificially inoculated with 5 log cfu/g and cooked in a deep fryer at 180°C for one, two and three mins, grilled for four, eight and 12 min to reach a core temperature of at least 72.1°C, or cooked in an oven for five, 10 and 15 min at 200°C to reach a core temperature of 98.4 °C (Bostan, 2001). In some poultry processing plant studies, Yang (2001) reported a reduction up to 2 log while Guerin et al. (2010) reported a reduction of 1.3 to 2.9 cfu/g in a commercial scalding operation at 49 °C and 55.4 °C respectively.

Several studies examined the treatment of poultry carcasses by immersion or spraying with hot water or steam to reduce the contamination level. The reduction in the carcass contamination level was to a maximum of 3.8 log (Corry et al., 2007, Cox et al., 2005, Purnell et al., 2004, Zhang et al., 2013). However, these treatments cause a change in the appearance of carcasses, mostly a likelihood of the skin to shrink and become more fragile and also cause a slight colour change in any exposed muscle (Zhang et al., 2013).

The survival of *Campylobacter* following poultry scalding and chilling (Yang et al., 2001) was modelled with the Weibull distribution (Yang et al., 2002). Results of another study (Purnell et al., 2004) showed that immersing carcasses in the water at 65–70 °C did not reduce *Campylobacter* contamination level in naturally contaminated carcasses but by increasing the temperature to

>75°C reduced contamination but caused skin damage, discolouration and thus changed the external appearance.

A predictive model was developed for *Campylobacter* inactivation by heat and high pressure (Lori et al., 2007). Survival of *Campylobacter* was also studied in butter (Zhao et al., 2000). Based on the peak campylobacteriosis notification rate in early 2006, a NZ study investigated the cooking time and temperature required to eliminate naturally contaminated livers (Whyte et al., 2006). The results showed that the total *Campylobacter* elimination of 4 log cfu/liver could be achieved by the pan-frying method if the internal temperature was > 70 °C and held at this temperature for two to three min. If the effect of heat inactivation has to be estimated for a risk model, it is difficult to choose the appropriate *D*- value from the list in Table 2.3.

The development of a precise and accurate mathematical model(s) able to describe the inactivation behaviour of micro-organisms on the food subjected to stress factors (e.g. high temperature, pH, water activity) is crucial if the food industries are to design efficient and reliable preservation systems, to predict the shelf-life of the product or to use the model for quantitative microbial risk assessment (QMRA). QMRA estimates the magnitude of microbial exposure at different steps in the food production chain to assess the risk of foodborne illness (Cahill, 2005).

2.7 The role of the consumer in food safety

Consumers play an important role in making certain the foods they prepare, handle and serve safe. Consumers can take steps to prevent cross-contamination, which is the transfer of bacteria directly or indirectly from a contaminated source to a non-contaminated product during food preparation which can potentially cause foodborne illness. Cross-contamination is also considered as the most critical food handling practice in the transmission of campylobacteriosis (Kapperud et al., 2003, Nauta et al., 2008, Kosa et al., 2015). *Campylobacter* is considered heat-sensitive, unable to grow in the presence of air, unable to multiply or grow < 30 °C (Jacobs-Reitsma et al., 2008).

Quantitative risk assessment studies, which will be described later, consider the development of campylobacteriosis to be due to direct exposure to *Campylobacter* spp. in animal feed and water. Many of these risk assessment studies consider cross-contamination during food preparation as the major pathway that causes campylobacteriosis (Brynestad et al., 2008, Hartnett et al., 2001, Lindqvist and Lindblad, 2008, Mylius et al., 2007, Nauta et al., 2007).

Many consumer reports associate the foodborne illnesses to the food consumed outside of the home, such as at retailers, restaurants, fast food outlets, bars and cafes (Redmond and Griffith, 2003). However, many studies have confirmed that up to 87% of the foodborne illnesses are related to food usually eaten at home due to malpractices in the domestic kitchens (van Asselt et al., 2009,

Humphrey et al., 2001, Redmond and Griffith, 2003, Hillers et al., 2003, Lake et al., 2007). It is now believed that improper food handling practices contribute to at least 40 – 60% of foodborne illnesses (Cogan et al., 2002, Humphrey et al., 2001). A NZ analysis of gastroenteritis disease outbreaks for the period of 1998 to 2000 revealed that 39.3% of the cases were acquired at home (Gilbert et al., 2007). Another New Zealand study (UMR Research, 2007) revealed that between 2003 and 2005, about 20% of the participants experienced foodborne illness. However, only 6% of those participants indicated that their illness was caused by food cooked at home. In 2007, 30% of NZ study participants reported foodborne illnesses.

There are review papers that discuss home food safety practices throughout the world (Patil et al., 2005, Redmond and Griffith, 2003). Some of these studies (Langiano et al., 2012, Frewer et al., 2016) addressed consumer beliefs about hazards, their knowledge about food safety and their self-reported practices. There are observational studies of consumer behaviour in a laboratory kitchen and in real environmental conditions. A few of the observational studies linked specific consumer behaviour to the microbial contamination of select artificially or naturally contaminated meals (Redmond et al., 2004). Models for cross-contamination have been designed (Kusumaningrum et al., 2004, Perez-Rodriguez et al., 2006, Rosenquest et al., 2003, Schaffner, 2004b, Mylius et al., 2007, van Asselt et al., 2008, Fravalo et al., 2009) and validated by quantitative observational studies (van Asselt et al., 2009).

It appears that cross-contamination contributes to the high notification rate in NZ. Understanding of the relationship between consumer knowledge, attitudes and food handling practices, and discussions of the discrepancy between the predicted consumer behaviour and the observed behaviour are key issues that need to be addressed in the campylobacteriosis epidemic in NZ. Moreover, investigation of the best hygiene measures that can be used is vital to provide public health, risk managers and regulatory authorities with the scientific support for health promotion plans that will discourage consumers' risky, unsafe food preparation practices.

2.8 The role of the food regulatory body

Generally, the main role of any food regulatory body is to identify the most successful and realistic interventions and measures to eradicate or reduce the pathogen level at significant points in the food chain and ensure their removal.

2.8.1 Consumer education

The aim of health professionals and risk managers is to encourage consumers to practice desirable and safe practices and discourage improper or unsafe ones. A study of consumer knowledge,

attitude and behaviour can help in designing an effective health promotion initiative. Therefore, research and consumer education regarding the risk of food safety malpractices is an important element of precluding food-borne disease. Traditionally, the communication process is a transfer of information or education process with one way flow of objective scientific information from experts (government, academia, authorised organisation or industry) to the public considering the public as lacking knowledge and skills necessary to assess and judge the food risk appropriately (Hansen et al., 2003). Moreover, it is vital to investigate the best hygiene measures that can be used to provide public health professionals, risk managers and regulatory authorities with the scientific support for future health promotion plans that will discourage consumers from risky, unsafe food preparation practices. Laws and regulations cannot enforce controls and measures to prevent unsafe practices at home, as is the case for commercial food processors and retailers. The most logical way to change risky consumer practices is by educating consumers.

2.8.2 Control of the poultry industry

A regulatory body is responsible for developing and setting targeted risk-based control measures throughout the food chain. The regulatory body has to audit and monitor in general the food safety system in place (food code for hygienic practice) for compliance, effectiveness and success. Moreover, the regulatory body has to identify and provide guidance about the most successful and practical interventions to reduce the risk of campylobacteriosis in any step in the food chain. A simplified poultry processing steps at a plant is shown in the appendix E2.

Generally, good hygienic practices alone during slaughtering and processing of broilers cannot totally eradicate the contamination level of *Campylobacter* from poultry carcases, and poultry meat as the good hygiene practices can only reduce the *Campylobacter* contamination level (Boysen and Rosenquist, 2009, Cui et al., 2005). There are many review papers detailing poultry processing interventions (Hendricks et al., 2000, Keener et al., 2004, Oyarzabal, 2005, Loretz et al., 2010, Lu et al., 2019). The aim of all interventions such as the chemical (organic acids, chlorine-based and phosphate-based treatments), physical (hot water, freezing, irradiation, and steam) and biological (bacteriophages and bacteriocins) is to achieve a further reduction of raw chicken contamination level.

Chemical intervention with chlorine- and phosphate-based treatment of broiler carcasses has been used in the USA for several years (Oyarzabal, 2005) and in European countries and other countries for decontamination of poultry but it is not as successful as required (Boysen and Rosenquist, 2009). The new regulation regarding the strict use of chemicals in EU (EC) No. 853/2004 implemented in 2006 permits the use of a chemical, only after the European Food Safety Authority

(EFSA) has provided a risk assessment about the possible impact of the intended chemical (Hugas and Tsigarida, 2008, EFSA, 2011b). Thus far, no such chemicals have actually been authorised by the EU with the exception of EU regulation EU2016/672 (EU, 2016) which allows the application of peroxyacetic acid to be used in poultry processing but subject to compliance with certain specifications and conditions. Therefore, only few poultry processors in Europe have used peroxyacetic acid due to the strict conditions and most European consumers have no appetite for chicken washed in chemicals (Siekkinen et al., 2012, Korzen et al., 2011, Boysen and Rosenquist, 2009). EU experts have debated against the use of chemicals because of the adverse health effects for consumers due to formation of toxic compounds during the processing, and they emphasised that the use of chemicals should not replace the good hygienic practice required to be implemented in all poultry processors and that there was no indication that antimicrobial resistance would develop (Hugas and Tsigarida, 2008, Koolman et al., 2014).

The physical methods to decontaminate carcasses during poultry processing have acquired new importance as a result of 1998 EU regulations and the ban of imports of all US poultry carcases which were treated by chemicals. Irradiation techniques have succeeded in achieving 3 log reduction of bacterial contamination level from the chicken carcass, but consumers do not readily accept the use of such technology and the European Commission discouraged the use of this technology in 1994 and enforced strict regulation about the labelling of irradiated food in 1999. European Commission (EC) allowed initially only a limited range of products such as aromatic herbal, seasonings and a few other products with a legal absorbed irradiation dose to be marketed in EC (Kume et al., 2009, Wilcock et al., 2004). However, 2011 EC legislation (EFSA, 2011a) stipulated that any irradiated food or food ingredient, even if it is present in trace amounts in a non-irradiated compound food, has to be labelled as "irradiated" or "treated with ionizing radiation" in order to allow consumers an informed choice. European consumers are reluctant to take any risk and generally have a negative public attitude toward food irradiation.

Ultrasound with a steam treatment (Boysen and Rosenquist, 2009, Hansen and Larsen, 2007) achieved more than 2.5 log reduction, but the adverse quality effect on the treated carcases such as skin splits between the breast and thigh, pitted appearance of the outside of the thighs and drums and skin shrinkage can hinder the commercialisation of this combination treatment (Biggs, 2009).

Freezing to – 20 °C for five weeks has been used in Norway to reduce *Campylobacter* contamination level (Sandberg et al., 2005). Generally, freezing can achieve 1.3 -2.2 log reduction in naturally contaminated chicken carcasses and it is also used in Ireland and Denmark (Boysen and Rosenquist, 2009, Georgsson et al., 2006, Hofshagen and Kruse, 2005). However, it is not a feasible option due to the consumers' satisfaction, especially for those with a higher income and their

demand for chilled fresh chicken and poultry products in the market. Steam under atmospheric pressure can reduce up to 3.8 log in artificially contaminated carcasses or chicken parts, but the boiled appearance of the treated products has prevented its use in the industry (James et al., 2007, Loretz et al., 2010, Whyte et al., 2003).

Many studies reported a 0.9 - 2.1 log reduction mainly in artificially contaminated carcasses or chicken parts by using hot water immersion or spray as a decontamination method (Corry et al., 2007, De Ledesma et al., 1996, Göksoy et al., 2001, Loretz et al., 2010, Purnell et al., 2004). James (2007) combined immersion in hot water and crust freezing and achieved 3.2 log reduction in artificially contaminated chicken carcasses. There are new physical promising interventions such as electrolyzed water, ozone water and cold plasma treatment which are summarised in the literature (Lu et al., 2019).

In NZ, there is no data published regarding any poultry physical intervention method except those related to freezing (-12°C and - 20°C) which can achieve a 3.3 - 3.5 log reduction in artificially contaminated chicken portions after six weeks (McIntyre, 2008, McIntyre, 2009).

2.8.3 Risk assessment (RA)

Risk assessment is a scientifically based process of identifying and characterising the hazards and risk factors associated with a given food system. The risk posed by a particular hazard or process is calculated. Thus, risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards. This approach employs mathematical models that are used to explain: (i) the introduction of pathogens into food, (ii) the replication of pathogens in food over time, (iii) the destruction of pathogens by heat treatment or other techniques, (iv) consumption of pathogens in food, and (v) subsequent probability of illness. The core model is the risk pathway which starts at the source of the hazard and ends at the adverse health consequence. This pathway is called 'farm to fork' pathway where the hazard source is usually the farm "biological hazards reservoir" and end with the dose consumed by an individual or a number of the individuals becoming ill from the consumption of the biological hazard.

Quantitative microbial risk assessment (QMRA) uses the best measurements about microbe behaviour to identify where they can become a danger and estimate the risk. QMRA helps in assessing an intervention applied to the food chain steps by providing insight into microbial hazard dynamics which allows identification of targeted control strategies in order to reduce the risk of foodborne illness (Havelaar et al., 2007, Membré and Boué, 2018). QMRA describes the food chain step by a mathematical model where the change in model inputs will cause a change in the model output (Membré and Boué, 2018). Thus, if a new intervention is applied or a desired hypothetical

intervention is introduced to any step in the food chain, the expected outcome of that intervention can be examined and evaluated (Membré and Boué, 2018). QMRA is thus the scientific aspect of the risk analysis framework, which also comprises risk communication and risk management as defined by FAO/WHO (2007).

Risk management involves the development of policies and executing them to reduce the risk explained by the risk assessment study. The risk manager considers the social, economical and political factors during the evaluation of the risk and develops the policies to tackle the health impact on the community. Risk communication is all about the communication between the risk assessors, risk managers, and targeted stakeholders or individuals. The risk managers' (government officials) task is to use the risk assessment findings to establish a practical limit for the hazard if total elimination was impossible when developing a new policy or a standard according to the findings of the risk assessment (Tuominen, 2009).

Many countries have developed QMRA to comply with the sanitary and photo-sanitary agreement (SPS) of the World Trade Organisation. The agreement in 1994 allowed member countries to give priorities to food safety over international trade only if a sound scientific risk assessment has been conducted to justify their regulatory measures. The goal was to protect consumers and facilitate international trade in a transparent and reliable approach in spite of the limited resources of national governments as QMRA is a labour-intensive process with a data gap in many areas and it requires extra time and financial cost (Membré and Boué, 2018).

The Codex Alimentarius Commissions set standards for food in international trade and provide a framework for conducting risk assessment (Codex Alimentarius Commission, 1999) to harmonise and promote the methodology internationally. The first published QMRA was in Canada for *E.coli* 0157 in-ground beef hamburger (Cassin et al., 1998) and the first QMRA for *Campylobacter* was also in Canada (Fazil et al., 1999). QMRA methodology is used internationally by the World Health Organisation (WHO), Food and Agriculture Organisation (FAO), and The Food and Drug Administration (FDA), US Department of Agriculture, EFSA, Food Safety and Inspection Service (USDA-FSIS). Furthermore, the QMRA methodology is used nationally and regionally or on a smaller industrial scale for particular pathogens in a particular product or process. The use of QMRA in investigations of human health risks from poultry meat has increased in recent years and especially for *Campylobacter* as it causes more cases of foodborne illness each year than any other bacterial pathogen. Table 2.4 summarises several published QMRA.

Table 2-4 A list of some published quantitative risk assessments of foods

Pathogen	**Food**	**Country**	**Year**	**Source**
E. coli 0157	Ground hamburger	Canada	1998	(Cassin et al., 1998)
Salmonella enteritidis	Shell and egg products	USA	1998	USDA/FSIS
Campylobacter jejuni	Chicken	Canada	1998	(Fazil et al., 1999)
Salmonella	Shell egg	USA	2000	(Whiting et al., 2000)
Campylobacter jejuni	Chicken	U.K	2001	(Hartnett et al., 2001)
Listeria	Ready to eat	International	2001	FAO/WHO
Salmonella	Pasteurised liquid eggs	USA	2001	FDA/USDA
Campylobacter spp *(fluoroquinolone-resistant)*	Poultry	USA	2001	FDA/Centre for Veterinary Medicine
Vibrio parahaemolyticus-us	Row molluscan shellfish	USA	2001	USDA/Centre for Food Safety Applied Nutrition
Vibrio spp	Seafood	USA	2002	FAO/WHO
Salmonella	Sheep meat	NZ	2002	(Sabirovic, 2002)
Salmonella spp.	Eggs and chickens	International	2002	FAO/WHO
Thermophilic *Campylobacter* species	Chicken	Denmark	2003	(Rosenquest et al., 2003)
Listeria	Ready to eat	USA	2003	FDA/USDA
Campylobacter jejuni	Chicken	France	2003	(Mégraud and Bultel, 2003)
Salmonella	Pork products	Italy	2004	(Giovannini et al., 2004)
Campylobacter	Broiler meat, other routes	Netherlands	2005	(Nauta et al., 2005)
Campylobacter spp	Poultry meat	Belgium	2006	(Uyttendaele et al., 2006)
Campylobacter spp	Poultry food chain	New Zealand	2007	(Lake et al., 2007)
Campylobacter	Poultry food chain	France	2008	(Albert et al., 2008)
Thermophilic Campylobacter spp	Chickens	Sweden	2008	(Lindqvist and Lindblad, 2008)
Campylobacter spp	Chicken	Germany	2008	(Brynestad et al., 2008)
Campylobacter jejuni	Chicken	Italy	2008	(Calistri and Giovannini, 2008)
Campylobacter spp	Broiler chickens	International	2009	FAO/WHO
Campylobacter spp	Broiler chickens	EU	2011	(EFSA, 2011b)

2.9 QMRA approach description

The concentration of the pathogen is the variable the QMRA quantifies in order to follow the dynamics of the pathogen through the food chain. The final pathogen concentration is usually dependant on the variable describing the concentration at a previous stage in the food chain and other process parameters such as temperature, time, pH, and water activity.

Generally, QMRA separates the food chain into a specific number of modules which is subdivided into sub-modules. Each module or sub-module can describe a stage, process and part of the real-world problem. Those modules are expressed by functional relationships containing the process parameters and the bacterial concentration at a previous stage as an argument of the functions (Smid et al., 2010). The final model is calculated by linking the modules, transferring information from one module to the subsequent ones (Smid et al., 2010). In QMRA, the consumer phase model (CPM) is an essential link between previous phases in the food chain and the dose-response model. Several CPMs have been developed (Signorini et al., 2013, Lopez et al., 2015, Pouillot et al., 2012, Evers and Chardon, 2010) and reviewed widely in the literature (Chapman et al., 2016, Nauta and Christensen, 2011).

Generally, a QMRA model is defined as a schematic description of a system, theory or phenomenon that considers its known or deduced properties and may be used for further study of its characteristics (Tuominen, 2009). The main objective of the modelling process is to organise the data and relevant knowledge in a coherent framework. This can lead to a better ability to estimate the human risk and to use the model as a tool to assess the input variable changes and consequently assess the intervention which may lead to modify the output of the model and eventually reduce the risk (Lake et al., 2007). The model input variables or parameters can be changed during the execution, and the model equations can quantify the change in the observed variables in terms of other variables. The variables can be represented by one numerical value or point estimates. If all variables are expressed by one value, then the model is called a deterministic model (Vose, 2008). Usually, differential equations are used to express the rate of decay which depends on the initial count of bacteria and other variables and parameters. This can sometimes be solved to give an explicit algebraic formula for the number of surviving bacteria. However, the accuracy of the deterministic approach is questionable as it does not provide any information about uncertainty (the lack of knowledge of the system) or variability (heterogeneity of population).

Usually, uncertainty about the error in the parameters used in the model due to measurement, sampling, simplification or assumptions of realistic processes should be identified clearly to derive scientific results (Lindqvist et al., 2002, Neves et al., 2018). Uncertainty can be reduced by

increasing knowledge or improving the measurement, but variability cannot be reduced further as it is a natural variation of the system.

Stochastic models account for the uncertainty and variability by expressing the results or the parameters used in the models with probabilistic statistical distributions. Thus, stochastic models are preferred and widely used in QMRAs (Cahill, 2005, Vose, 2008, Neves et al., 2018). These distributions contain all possible values of the estimated parameter or the possible values of the expected results by using simulation techniques (for example Monte Carlo) which calculates model output multiple times with different initial values of input parameters. Each time a model calculates with a particular initial value called iteration. A single simulation consists of one to any number of iterations, to obtain a series of possible distributions considering the random variation in the input variable.

2.9.1 Monte Carlo simulation

Monte Carlo (MC) simulation is the random sampling of each input of the probability distribution of a variable or a parameter in the model for each iteration of a simulation model (Vose, 2008). The main idea of MC method is to represent the solution of a mathematical problem by a parameter of a true or hypothetical distribution and to estimate the value of this parameter by sampling from this distribution reflecting the uncertainty and/or the variability of the parameter (Lewerenz, 2002, Hadjicharalambous et al., 2019). All probability distributions of variables, which are independent variables, are sampled per iteration to form the shape of the future distribution (Mataragas et al., 2010). The model outcome reflects the range of possible model inputs and evaluates each possible outcome by the probability of its occurrence based on the frequentist theory, which depends on the availability of the data. To obtain reliable results by the MC method may require many iterations to get accurate results which may be a time-consuming process. However, MC simulation of these models is relatively easy to implement using commercial software to automate the tasks, and they produce acceptable results (Robert and Casella, 2004, Huang et al., 2018). Another drawback of MC is that this technique performs poorly when modelling rare events (Vose, 2008). Other methods of calculating outcome distributions for QMRA exist and Bayesian belief networks have gained increased interest by researchers in recent years.

2.9.2 Bayesian belief networks (BBN)

Bayesian Networks or Bayesian Nets technique is based on Bayes' theorem as described by Thomas Bayes in 1763 (Moore et al., 2009) as:

Prior Information + Evidence → Posterior Information (refined and updated information)

BBN is a method to express the relationship between causes and consequences represented by nodes and arcs in a directed acyclic graph. Each node describes an uncertain variable (cause/sequence) expressed by a probability distribution. Each arc or line describes the direction of the relationships (influential or casual) between nodes. Usually, the model is built from prior distributions consisting of the joint probability distributions of all the nodes derived from the prior distributions. Bayesian analysis, which is revising the assigned probabilities in the light of actual measured or observed data, is solved analytically by one of several software packages described by Uusitalo (2007). Sometimes, the complicated integrals of arbitrary probability distributions can be solved by combining with another Bayesian-based analysis method called Markov Chain Monte Carlo (sampling) which can cope with a larger class of posteriors than the Gaussian or any other fixed form distribution (Uusitalo, 2007).

BBN is a popular method for modelling in engineering, environmental management, image processing, decision analysis, gene sequencing, financial predictions, neural networks, robotics, geology, geography, forensic science, ecology, complex epidemiological model, text analysis, evaluation of scientific evidence and medical diagnoses (Smid et al., 2010, Uusitalo et al., 2005, Bromley et al., 2005, Yuen, 2010). More than 600 published papers on Bayesian method are listed on the Agenarisk web site (https://www.agenarisk.com). However, Bayesian approach has rarely been used in food science or in QMRA, and only a few studies have been published (Albert et al., 2008, Barker et al., 2002, Maijala et al., 2005, Ranta et al., 2005, Tuominen et al., 2006, Delignette-Muller et al., 2006, Mikkelä et al., 2016, Evers and Bouwknegt, 2016).

The Bayesian approach has many advantages such as being an appropriate method in handling missing data, accounting for the uncertainty in all estimated parameters, enabling data combination with the expert prior belief or knowledge. They allow back-propagation of prior variables with the observed data, assist understanding of underlying relationships between variables to provide a method to avoid overfitting of data and offer satisfactory prediction accuracy (Smid et al., 2010). Bayesian networks are not yet a preferred methodology in MRA research field due to the complexity involved in constructing and assigning probabilities from expert knowledge or prior information, linking the nodes, discretising the data, complex software and solving the resultant joint probability distributions. However, they are currently slowly gaining interest in food science and are likely to increase their relevance as one of the standard methods of QMRA. Therefore, the application of the Bayesian approach will be valuable in NZ as a NZ QMRA model (Lake et al., 2007) to use the MC simulation techniques which fail to predict the actual notified campylobacteriosis cases correctly. The NZ model prediction is about ten times more than the

actual notification value. The main drawback of the NZ adapted model is that it relies on the international literature data which are not reflected in the actual scenario in NZ poultry processing and does not consider the implemented interventions by the industry and the government. Moreover, the challenges faced in general in the probabilistic QMRA approach used in the NZ model have, to some extent, contributed to the current model inaccuracy (Havelaar et al., 2008). However, there are limitations to QMRA in general, since inadequate and inaccurate data can result in false predictions in any type of modelling. Moreover, QMRA model development can be resource-intensive and time-consuming and require an additional cost to fill the data gaps and update the model accurately with correct and appropriate data (Chung, 2004, Membré and Boué, 2018).

2.10 An alternative approach for campylobacteriosis cases prediction

Predicting campylobacteriosis have become a matter of considerable concern in NZ. Moreover, the intervention strategies selected by policymakers to combat the high rate of the disease in the country can be evaluated by the cost and benefit in terms of campylobacteriosis cases prevented. Therefore, there is a need to develop a model to predict the number of campylobacteriosis cases in NZ accurately. Time-series forecasting methods have been successfully applied in the fields of engineering, science, sociology and economics (Brockwell and Davis, 2002). Other studies have shown that a time-series analysis is an appropriate methodology to clarify trends of campylobacteria, salmonella and listeria outbreaks to forecast future cases and to test the impact of interventions on the burden of food-borne diseases (Maertens de Noordhout et al., 2017, Weisent et al., 2010).

2.10.1 Time-series

Finding the most appropriate time-series model for NZ data has many practical considerations. It is a complex task given the seasonality and the change of pattern in response to any implemented interventions. The classical time-series modelling approach is considered as an appropriate technique to predict campylobacteriosis in NZ. A reliable method for making time-series predictions would provide a great benefit to epidemiologists and public health officials (Altizer et al., 2006, De Greeff et al., 2009, Nobre et al., 2001).

2.10.2 Time-series modelling techniques

Decomposition methods

These decompose the time-series into several components as any time-series may contain four

components; i) trend ' T ': increase or decrease in a time-series over a long period of time, ii) cycle ' C ': smooth fluctuations around the trend line, iii) season ' S ': yearly repeated fluctuation (not necessarily an identically repeated fluctuation), iv) Error ' E ': random variations which remain after the removal of all of the above components from the time-series. Estimates of those components can be used for forecasting (Makridakis et al., 1998).

The decomposition method assumes that the time-series can be expressed as a sum (additive model which is mainly used where the time-series exhibits constant seasonal variation) or product (a multiplicative model which is mainly used where the time-series exhibits increasing or decreasing seasonal variation) of up to three or 45 components only for a more practical decomposition by ignoring the cycle component. The decomposition method has been used successfully in predicting campylobacteriosis risk in the USA (Weisent et al., 2010).

Regression-based methods

These methods use a simple model for the trend, which represents the average level that changes over time. This is a linear trend model (straight line growth or decrease), but other possibilities are quadratic trend (curvilinear) and the pth-order polynomials model trend (with one or more reversals in curvature). Least squares point estimates of the parameters in these models are obtained by using standard regression techniques. These assume that the error term E represents random fluctuations that are independent and identically distributed. Variables are retained if they improve predictive value (R^2), or indicate significance ($p>0.05$), i.e. with significant regression coefficients. This is a “global” model in the sense that it assumes the trend model and seasonal factors are the same throughout time, and all data points contribute equally to estimating the fixed parameters.

Holt’s techniques

The exponential smoothing method without a trend or seasonal components which rely on simple updating equations to predict or forecast can be generalised to handle a time-series with a trend and season components (Chatfield, 1978). These techniques give a “local” model in which the components are constantly changing over time, and data points are given more weight in the estimation when they are close to the time of estimation. The Holt Winter method is a simple method to implement and is widely used in practice (Chatfield, 2004).

Moving average (MA)

In a time-series with a MA process, the model describes how each observation is a function of the previous errors. In the MA, errors are the average of this period’s and the last period’s random errors. Thus, the observed series is a weighted moving average of the unobserved series (Armstrong, 2001).

Autoregressive(AR): A simple way to model dependence on past observations is to use ideas from

regression. AR model expresses the current time-series observation as a linear function with its past observed values. It is also called a Markov process (Chatfield, 2004).

Box and Jenkins model

This involves identifying an appropriate model to fit the data and then using the fitted model for forecasting. ARMA models, combining MA and AR components described by Box and Jenkens (1976) have been specifically developed for the analysis of time-series, and the advantages of these models are well documented (Benschop et al., 2008, Reichert et al., 2004). However, the drawback of the above ARMA model is that it is valid only for stationary time-series, i.e. when there is no systematic change in mean (a constant mean and constant variance in time with no trend). Thus, to use the ARMA model, it is necessary to remove the non-stationary source of variation such as trend and seasonal variation to model the residuals of the time-series as those are more suitable for modelling as a stochastic process. In some cases, the trend and the seasonality of the time-series may have more interest than the residuals of the time-series. To transform into a stationary-series, the observations usually first need to be filtered (removing of trend and seasonality) usually by subtracting consecutive observations from each other to achieve stationarity. The resulting model is capable of relating certain types of non-stationary series and is known as an integrated model or ARIMA as the stationary model that is fitted to the differenced data summed or integrated to provide a model for the original non-stationary data (Chatfield, 2004).

Thus, each ARIMA process has three parts: AR, the integrated (I), and MA parts. The models are written as ARIMA(p, d, q) where p describes the AR part, d the integrated part and q the MA part. Series with seasonal components also need equivalent terms at seasonal lags (S), leading to the full seasonal ARIMA model written as ARIMA(p, d, q)× (P, D, Q)S. A multiplicative form of the ARIMA can be obtained by making a logarithmic transformation of the data, fitting an ARIMA to the transformed data, and then back-transforming the predictions.

Intervention models

These models are mainly used when exceptional, sudden, external events affect the variable to be predicted. Possible examples of those events are worker strikes, disasters, and policy changes, to name a few (Coshall, 2005). However, the ARIMA with intervention is considered an advanced method which requires further knowledge of statistics and the use of additional complex software. New Zealand has applied interventions in 2007 and in 2008 to reduce the rate of campylobacteriosis. Thus, those models were considered to accommodate interventions by the use of an intervention variable which is used to build the intervention model. Such variables are called step functions, impulse functions or response functions. There are different types of response functions. Historically, an improvement in forecasting has been noticed by many authors after

applying the ARIMA intervention model (Goh and Law, 2002, Min et al., 2010, Rashed et al., 2017). These studies have shown that ARIMA intervention models had the lowest forecast error.

2.11 Scope of this work

Due to the increase in notified cases of campylobacteriosis in NZ, especially when compared with other developed countries, it is vital to investigate and to understand the reasons and the risk factors for this increase in order to develop intervention strategies to protect consumers and lower the rate of human illness associated with *Campylobacter*. The strategies are intended to identify the preventive steps within the food production chain. There are many hypotheses for this increase, and more than one have contributed to this staggering historical worldwide notification rate record, as outlined below:

- NZ has more heat resistant strains to survive normal cooking.
- NZ has more oxygen tolerant strains.
- NZ poultry processing interventions are less effective than those used elsewhere, or the secondary processing practices may increase the contamination level.
- New Zealanders have the worst home hygiene practices than the citizens of other developed countries.

Campylobacteriosis is an internationally recognised complex problem, and it is important to investigate all the above hypotheses to solve New Zealand's public health, economical and historical crisis. The thermal treatment destroys *Campylobacter* and is one of the primary interventions used to ensure the safety of foods. Therefore, the survival and heat-resistance of *Campylobacter* under different conditions need to be investigated.

D values are not always determined under the same conditions as are encountered in the actual situation or scenario. Moreover, the physiological state of the bacteria and the specific contaminating strain present is often unknown. It is then unclear which *D*- value to use from the large datasets available and whether to include factors that may influence heat resistance. When there is an effect reported for a factor (e.g. food product), this has usually been tested in only one laboratory for a few variable conditions and a few strains. It is unclear whether such effects are relevant overall in comparison with other conditions. Therefore, the determination of the *D* and z-values for NZ strains is a high priority research task. Under-cooking in food manufacturing facilities is not currently causing widespread food safety problems (Lambertz et al., 2012). The multistate European survey has confirmed that manufacturing cooking is not a food safety concern (EFSA, 2016). Most of the surveys of cooked poultry products showed only negligible prevalence and concentration or no contamination (Egan, 2006). However, continued development of new

products and processes, the possible emergence of new heat resistant strains and the significantly higher reported *D*- value values for *Campylobacter* by Bergsma (2007) and de Jong (2012) require investigation. Moreover, the on-going regulatory changes regarding food safety codes and NZ *Campylobacter* strains which genetically differ from international strains (McKenzie, 2006) necessitate a practical stance in ensuring appropriate evaluation of thermal process lethality and in updating the current knowledge of kinetic parameters of the different strains. It is important to focus future research on the most prevalent poultry, human, and animal strains to identify specific risk factors that may be linked to greater heat resistance strains with the usage of all the best methods, techniques, and instrumentation. This will lead to a better understanding of the survival of *Campylobacter* in the food chain and provide improved targeted information on health and food risks.

Further research is needed to develop growth, survival and death models specifically for *Campylobacter* either on or in food under dynamic temperatures. Most studies conducted for *Campylobacter* until now were not designed to generate sufficient data to develop such models (Grigoriadis et al., 1997, Svedhem et al., 1981, Bergsma et al., 2007, de Jong et al., 2012, Sampers et al., 2010). Rather, most of the models developed were static models, which are applicable only for static or invariant environmental conditions. In addition, most of these models were developed on broth and not on the solid foods with which *Campylobacter* is mostly associated with.

To properly assess the risks and risk minimisation strategies require: i) accurate knowledge of the survival and heat resistance during processing, storage and handling of the NZ strains that are most frequently associated with poultry products and humans, ii) evaluation of the processing practices and the interventions applied in NZ poultry processing plants, iii) investigation of the cross-contamination route in the NZ context as a major risk factor for the high rate of campylobacteriosis, iv) provision of insight into consumers' knowledge, attitude and behaviour which can help in designing an effective health promotion initiative, v) advising decision-makers on the current state of knowledge for designing an effective health promotion strategy by a structured approach to identify, assess, review and summarize data on the most relevant scientific research, as consumer information intervention may lead to prevention of campylobacteriosis by increasing consumer protection.

Cumulatively, this PhD research was planned to solve the mystery of a very complex problem, the high rate of NZ campylobacteriosis and to provide the means for control and prevention by finding the optimal industry and consumer interventions to combat the disease. Moreover, this study is intended to develop a model to predict accurately the number of campylobacteriosis cases in NZ as the control strategies selected can be evaluated by the cost and benefit in terms of

campylobacteriosis cases prevented. Thus, this study was tailored to serve as a scientific response to address a major public health problem in NZ and to provide a fast response and a guide in analysis of the causes of the problem and recommend relevant solutions with scientific evidence in an acceptable time frame.

2.11.1 The structure of this thesis

Chapter one includes the introduction. Chapter two contains the literature review. Chapter three describes the materials and methods used in the microbiological experimental chapters (a total of six chapters of the thesis). Chapters four, five and six report on the investigations of heat resistance characteristics of most relevant NZ *C. jejuni* isolates from human cases and in poultry. Chapter four shows data on the heat resistance of those isolates in broth medium. Chapter five reports the heat resistance studies on the chicken skin. Chapter six tests the heat resistance under dynamic conditions, simulating processing scenarios such as cooking. Chapter seven evaluates the survival of *C. jejuni* isolates under oxidative stress conducted in various ways and under different temperatures simulating storage and handling temperatures. Chapter eight identifies the effect of secondary poultry processing practices at the plant level, such as needle injection, on final contamination level and prevalence in poultry carcasses and poultry products sold in NZ. Chapter nine explores a safe alternative physical decontamination intervention that could be applied in poultry plants to replace the unsafe chemical decontamination intervention currently applied in NZ, but it is banned in the EU. Chapter 10 reports on the quantitative risk assessment for campylobacteriosis in NZ by the Bayesian approach. Chapter 11 assesses the time-series models for predicting campylobacteriosis risk in NZ. Chapter 12 addresses the cross-contamination route in the transmission of *Campylobacter* spp and highlights the most important unsafe consumer practices. Chapter 13 shows the data on the consumers survey about their poultry handling practice at home and their basic food safety knowledge. Chapter 14 discusses the thesis findings and presents conclusions and future research.

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3 Microbiological experiments materials & methods

In this chapter, materials and methods used in all microbiological experiments (in chapters four to nine) are described.

3.1 Campylobacter isolates

All the *Campylobacter* multilocus sequence typed (MLST) isolates used in this study were provided by the Hopkirk Molecular Epidemiology Laboratory in Palmerston North. The MLST method provides a discriminatory molecular profile, is reproducible and easy to interpret (Maiden, 2006). Use of this method has resulted in the recognition of major genetic lineages or clonal complexes in *C. jejuni* populations from human infections and animal and environmental sources (Dingle et al., 2002).

Human faeces submitted to MedLab Central, Palmerston North that were positive for *Campylobacter* by ELISA (ProSpecT R, Remel, USA) were sent to the Hopkirk Molecular Epidemiology Laboratory over a three year period from 1st March 2005 to 29th February 2008 (Mullner, 2009). Human faecal swabs were cultured on modified cefoperazone charcoal deoxycholate agar (mCCDA) plates (Fort Richard, Auckland) and in Bolton broth (Lab M, Bury, England) and incubated at 42 °C in a microaerophilic atmosphere (85% N_2, 10% CO_2, 5% O_2) for two days. A single colony resembling *Campylobacter* was subcultured in Blood Agar (BA) (Fort Richard, Auckland) and incubated micro-aerophilically at 42 °C for two days before DNA preparation for MLST. Cultures were frozen at −80 °C in glycerol broth (Difco, USA).

Over the same period (1st March 2005 to 29th February 2008), 12-18 fresh whole poultry carcasses from different poultry suppliers in the region were sampled each month from retail outlets in Palmerston North, with the number of samples collected per supplier reflecting market share. Chickens samples were analysed for campylobacter. Single colonies of *Campylobacter* species were subcultured to BA and incubated micro-aerophilically at 42 °C for two days before DNA preparation for MLST. Bacterial cultures were frozen at −80 °C (Mullner, 2009). A total of 62 different MLSTs were detected in human and poultry samples. The major isolates frequently detected in human and poultry samples according to Mullner et al. (2009), namely ST - 474, ST - 48, ST - 45 and ST 190 were used in this study.

3.2 *Campylobacter* culture growth and enumeration

Campylobacter strain cultures were stored at -80 °C on beads (Microbank, Pro-Lab Canada). They were sub-cultured onto CA and incubated at 41.5 °C for 48 h in a microaerobic atmosphere within

a specialized workstation (Forma System model 1024, USA) supplied with continuous optimal atmospheric conditions conducive for *Campylobacter* growth, 5 % O_2, 10 % CO_2 and 85 % N_2. This atmosphere was used in all subsequent incubations in this study. Colonies grown on CA were sub-cultured on to another CA plate and incubated for 48 h in a micro-aerobic atmosphere at 41.5 °C to obtain a plate with prolific growth for inoculum preparation. Each inoculum for testing heat resistance was prepared by transferring all colonies from a CA plate into Brain Heart Infusion broth (BHI). The final culture concentrations used for inoculation were in the range of 10^7 to 10^9 cfu ml^{-1}or per portion/drumstick. The determination of inoculum concentration was conducted by a serial dilution of the inoculum in BHI followed by plating in triplicate on mCCDA by the Surface Plate Method (Downes and Ito, 2001). Plates were incubated micro-aerobically at 41.5 °C for 48 h as described above.

3.3 Thermal inactivation of *C. jejuni* in broth

The typed isolates of *C. jejuni* examined were ST-474, ST-190 and ST-45 isolated from both poultry and humans. These three strains were implicated in more than 42 % of campylobacteriosis cases in a Manawatu study (French, 2008a). An isolate from a wild bird for strain 45 was also tested at two different temperatures (53.5 and 56 °C) in this study.

3.3.1 *C. jejuni* heat inactivation procedure

The bacterial suspensions obtained from above were heated to a predetermined temperature using a submerged coil heating apparatus (Protrol instruments Ltd., U.K). This comprised of a stainless-steel coil fully submerged in a thermostatically-controlled water bath which allows microbial suspensions to be heated within a short time to achieve a rapid temperature equilibration. Samples were taken at predetermined intervals (from 2 s -200 s with a total heating time range from 12 s – 1050 s, depending on the investigated temperature and the isolate heat resistance) automatically and collected in a series of vials in an automated carousel (Cole and Jones, 1990). The inoculum population and the number of surviving organisms in suspension after heat treatment were determined by dilution in BHI followed by plating on mCCDA using an automated spiral plater (Don Whitley, Yorkshire, United Kingdom) and triplicate plates used at each dilution. The inoculated plates were incubated micro-aerobically at 41.5 °C for 48 h.

3.4 Thermal inactivation of *C. jejuni* on chicken skin

The typed isolates of *C. jejuni* examined were ST-474 and ST-48 isolated from both poultry and humans.

3.4.1 Chicken skin sample preparation and inoculation

The chicken skin was purchased from a local butchery. Discs of chicken skin (18 mm diameter and 4 mm thick) were prepared using a stainless steel circular cutting tool. The prepared chicken skin discs were sent for gamma irradiation (25 -32 kGy) at the irradiation plant (Schering-Plough Animal Health, Wellington) to ensure that *Campylobacter* no longer exists in the tested sample before the artificial inoculation. Skin samples were stored at 4 °C until use. Each irradiated chicken disc was equilibrated to room temperature and placed aseptically in an aluminium test cell (Appendix B, Figure B1) specially designed for kinetic studies (Chung et al., 2008). An inoculum of 20 µl was pipetted to the chicken skin centre in the lower part of the aluminium cell. After inoculation, the cell was left for 60 min to allow *C. jejuni* attachment to chicken skin to mimic natural contamination.

3.4.2 *C. jejuni* heat inactivation procedure.

Eight aluminium cells were placed in a rack submerged in a temperature-controlled water bath (Grant, UK) maintained at a 56.5 to 65 °C temperature range for a predetermined time period (Appendix B, Figure B2). One aluminium cell was removed at each prescribed time interval and then transferred immediately to an ice bath for *C. jejuni* survivor analysis. All aluminium cells were equipped with a wire holder for ease and speed of handling during the heating period in the water bath. The time it took for the chicken skin sample to reach the equilibration temperature ('come up time') was measured by a very fast response thermocouple sensor with one second response time (Labfacility, UK). The temperature was recorded with a Measurement Advantage USB-TEMP logger (Measurement Computing, USA). A graph of the temperature profile is shown in Appendix B (Figures B3-B6). The 'come-up time was 55 s from a number of experiments for both the investigated temperatures 56.5 °C and 60 °C. There was a significant amount of variation caused by i) the chicken skin moisture and fat content both of which affect the thermal properties of the chicken skin, and ii) shrinkage of the chicken skin during heating inside the cell which may have created an air gap and slowed down the heat transfer from the aluminium cell walls to the chicken skin (because the air can form an insulation layer). All data points used in the calculation of the kinetic parameters were considered to estimate the come-up time.

3.4.3 Enumeration of *C. jejuni* survivors

Each chicken sample was transferred from the aluminium cell to a vial containing 4 ml BHI and the aluminium cell was flushed out with a further 5 ml BHI to remove any survivors from the internal surface of the cell. The sample was mixed using a vortex mixer and serially diluted in BHI. All the samples remained in BHI for at least two hours to allow recovery of injured cells, followed

by plating in triplicate on mCCDA by the Surface Plate Method, a 200 µl spread by L shaped sterilised spreader (Downes and Ito, 2001). Plates were incubated microaerobically at 41.5 °C for 48 hours as described above. The average of the logarithm (base 10) of three plate counts at each sampling time was used for modelling and analysis.

3.5 Thermal inactivation of *C. jejuni* on chicken skin under dynamic conditions

The typed isolates of *C. jejuni* examined were ST-474 and ST-48 isolated from both poultry and humans.

3.5.1 Meat sample preparation and inoculation

Same as detailed above in section 3.4.1

3.5.2 Heat inactivation procedure

For each run, 16 aluminium cells containing inoculated chicken skin samples were placed in a programmable water bath (developed locally at the university, described in Appendix C, (Figure C1). The water temperature was programmed to increase linearly to reach 65 °C for a period of either 8 or 18 min simulating the scenarios that can occur in retail food service, industry and in domestic cooking (Persson et al., 2002, van Asselt et al., 2009, NACMCF, 2007). Duplicate samples (two aluminium cells) were withdrawn at different predetermined intervals and transferred immediately to an ice bath until analysed for the counts of surviving culturable *C. jejuni*. All aluminium cells were equipped with a wire holder for ease and speed of handling during the heating inside the water bath. The come-up time for the chicken skin sample within the cell to equilibrate with the external medium was short for both heating regimes (8 and 18 min). The difference at any given time between the temperature of the skin and the bath temperature did not exceed 0.25 °C for 18 min heating regime or 1 °C for the eight min heating regime. For most of the heating interval of the eight min heating regime, the difference was between 0.3 and 0.7 °C. These values were measured using a very fast response thermocouple sensor (1s response time; Labfacility, UK) and the temperature was recorded by a Measurement Advantage USB-TEMP logger (Measurement Computing, USA). The temperature profiles for both regimes were embedded in the individual survival curves and are shown in the results section.

3.5.3 Enumeration of *C.jejuni* survivors

As described in 3.3.2, the average of the $\log_{10}$ counts for four plates at each sampling time was used

for modelling in contrast to the three plates used in section 3.3.2. This was to improve the accuracy of the results of this experiment because less number of experiments are required to conduct under the dynamic conditions.

3.6 Survival of C. jejuni under oxidative stress at different temperatures

The multilocus sequence typed strains ST-474, ST-48, ST-190 were used in this study.

3.6.1 Exposure of *C. jejuni* to oxygen in broth

Inoculums of 3 ml were added to 27 ml BHI which was then dispensed to form a shallow layer of 30 ml in a 125 ml conical flask equipped with a special bung (Bug stopper, Whatman) that allows air exchange with the surrounding atmosphere but prevents microbial contamination. This flask was allowed to equilibrate at the appropriate temperature and atmosphere (aerobically or microaerobically) by gentle shaking using an electrical shaker (Lab-line Junior Orbit Shaker, U.K). The number of surviving organisms in samples withdrawn from each flask at predetermined intervals after exposure to the controlled atmosphere conditions at each temperature (4, 10, and 20 °C) was determined by dilution in BHI followed by plating on mCCDA. Duplicate plates were made at each dilution. The plates were incubated micro-aerobically within a specialised workstation (Thermo scientific, USA) continuously supplied with 5 % O_2, 10 % CO_2 and 85 % N_2 at 41.5 °C for 48 h.

3.6.2 Exposure of *C. jejuni* to oxygen on agar plates

50 µl of the culture suspension in BHI was spread on CA plates (blood-free 16 to 40 plates for each isolates at the specified incubation temperature based on a previous primary trial data collected before the actual experiment) using a spiroplater (Don Whitley, Yorkshire, U.K) and incubated aerobically at 4 °C and 25 °C for one to five weeks. The CA control plates containing the culture spread were simultaneously incubated under microaerobic conditions at 4 °C and 25 °C using anaerobic plastic jars with a microaerobic atmosphere generating system (Pack MicroAero, Mitsubishi Gas Chemical Co. Inc). Two plates from each aerobic condition were sampled every day and incubated at 41.5 °C for 48 h in a microaerobic atmosphere within a specialised workstation (MACS VA 500, Don Whitley Scientific U.K).

3.6.3 Exposure to H_2O_2

Each culture suspension prepared in BHI was spread by a swab on Muller Hinton Agar plates and allowed to grow under microaerobic conditions for 48 h. Subsequently, filter discs (6 mm) inoculated with 10 µl of 1 mM, 10 mM, 100 mM, or 1 M hydrogen peroxide were placed onto the

plates (Disc Diffusion Method) (Fields and Thompson, 2008). These plates were then incubated at 41.5 °C under microaerobic conditions for 48h.

3.7 Evaluation of processing practices contributing to *Campylobacter* contamination in New Zealand chicken and chicken products

One MLST isolate was used in this study, namely ST-474, which was isolated from poultry.

3.7.1 Procedure

Two brine solutions were tested. One, solution A, is usually used for frozen products and contained 5% w/v marination powder mix (salt (0.5-1%), mineral salt (451), hydrolysed vegetable protein (soy), thickeners (415, 412), vegetable oil (canola, antioxidant 319), anti-caking agent (551) and maltodextrin). The other brine solution (B; 'Tenderpaste' marinade) is usually used for fresh products and contain 15% powder mix (salt (6-7.5%), mineral salts (450, 451, 452), thickeners (412, 415) and sugar. Two 50 ml portions of each brine solution were prepared according to the manufacturer's instructions and placed in sterile 125 ml flasks and inoculated with *C. jejuni* at 10^6 cfu $ml^{-1}$and the flasks held at 7 °C. A 1 ml sample of each brine solution was withdrawn for enumeration of *C. jejuni* at the start of incubation, and another 1 ml was withdrawn at predetermined intervals from Solution A (0.5 -1 % salt) and for Solution B (6-7.5% salt), and again after 24 h for both brines.

Serial 10-fold dilutions of each sample were also prepared in BHI by transferring a one ml sample to nine ml of sterile BHI. The number of surviving organisms in the sample suspensions was determined by plating on mCCDA as described above. Duplicate plates were made at each dilution. The plates were incubated micro-aerobically within a specialized work station as described above. The number of *Campylobacter* is expressed as log count (mean + SE) for the two duplicates counts for all experiments.

3.7.2 Survival of *C. jejuni* in injected chicken products at the plant

Fresh chicken drumsticks were purchased from the local supermarket and were sealed in a vacuum packaging bag and irradiated with a 25 kGy dose at Schering –Plough Animal Health plant located in Wellington (NZ) and stored at 4 °C until use. The irradiated chicken drumsticks were injected with brine during normal processing operations at a commercial plant processing. The needle injection machine conveyor carries the drumsticks to the injection chamber, and the brine is injected through needles. The brine was prepared in a mixing tank and was pumped through a permanent line to another feeding tank and from there circulated continuously through a

refrigeration unit and filtration unit to remove chicken meat particles. The brine was maintained at 6 -8°C. Ten irradiated and injected chicken drumsticks were processed during the production run (at four hours) and another ten irradiated and injected drumsticks were processed at the end of the production run (eight hours) for the two brine types commonly used in the processing plant (solutions A and B).

3.7.3 Recovery of *C. jejuni* from surfaces of the injected drumsticks

Five irradiated injected chicken drumsticks from each brine concentration at the end of the run and another five drumsticks from the middle of the run from each brine solution (giving a total of 20 drumsticks) were examined by removing 10 g of skin aseptically, using scalpels with sterilised disposable stainless-steel blades. The skin sample was placed in a stomacher bag with a filter containing 90 ml *Campylobacter* Enrichment Broth (CEB) without blood (Lab m135) and pummelled for two min by Colworth Stomacher model 400 (A.J. Seward & Co. Ltd, London), then 1 ml of stomacher fluid was pipetted from inside the filter sleeve for plating. 200 µl of the same liquid was separately plated on duplicate mCCDA plates. Serial dilutions were performed for the stomacher fluid by transferring one ml fluid to nine ml CEB without blood and plated as described above. The final results were also expressed as cfu per portion (~10g) as the whole skin surface was used in each sample. Confirmation that the colonies were *C. jejuni* was achieved by subculturing to CA and incubating at 37 °C aerobically. Additionally, the hippurate test was performed later.

3.7.4 Recovery of *Campylobacter* from the internal muscles of the injected drumsticks

Five irradiated injected chicken drumsticks from each brine concentration collected at the middle of the run and another five at the end of the run for each brine solution (a total of 20 drumsticks) were dipped in boiling water for 15 s. The initial studies confirmed a reliable recovery of internal injected inoculum after the 15 s treatment with boiling water and those studies also confirmed that the irradiated chicken drumsticks were free from *Campylobacter* from the surface and from the internal tissues. Using a special tool, the injected chicken drumsticks were removed in batches of three from the water and placed individually in a new sterilised bag to cool to room temperature. The skin and about a 3-4 mm layer from the surface were aseptically removed with a scalpel with sterilised disposable stainless steel blades and sterilised forceps. Strips of 2 mm thick were cut from the deep internal muscles using the scalpel and the sterilized forceps and transferred to a weighed stomacher bag with a filter, which contained 90 ml of CEB without blood, to obtain a 10 g sample and the cells were enumerated as described above. The final results were expressed as cfu per portion for consistency with the surface samples described above in spite of the fact the 10 g sample did not represent the whole internal tissue of the drumstick. We assumed this to represent

the whole internal tissue of that drumstick layer.

3.7.5 Survival of *C. jejuni* in injected chicken carcasses and chicken products in the retail market

A total of 20 injected drumsticks (of the same drumstick type as those injected in the plant) were randomly selected between the periods of mid-June to mid-July 2009 from different local retail shops. All products were analysed for internal and external contamination as described above. In addition, ten fresh whole chickens injected with solution B were analysed for *Campylobacter* presence by rinsing for two min with 400 ml CEB without blood and proceed by plating as described above in section 3.7.3.

3.8 Evaluation of *Campylobacter* mitigation strategies by physical methods in a poultry processing plant

One MLST isolate was used in this study, namely poultry isolate ST474,

3.8.1 Sample preparation and treatment

The rough surface of chicken skin facilitates attachment and survival of *C. jejuni* during multiple stages of processing. Therefore, we used chicken drumstick with skin as a model to represent the whole carcass for testing the efficacy of the hot water dip that is used against *C. jejuni*. Fresh chicken drumsticks were purchased from the local supermarket and were stored at 4 °C until use. Each drumstick was allowed to condition at room temperature for at least ten h before a 200 ul of inoculum was introduced on the skin surface and was left for 60 min to allow *C. jejuni* to allow for chicken skin attachment to mimic the natural contamination scenario. Chicken drumsticks were held by tongs and submerged in a temperature-controlled water bath (GD 100; Grant, UK) maintained at various temperatures in the range 56.5 to 70 °C for a predetermined time interval. The chicken drumsticks were removed at each prescribed time interval and then transferred immediately to an ice bath until analysis for *C. jejuni* survivors. The following treatment temperatures, based on the *D* and *z* value obtained in previous sections and the literature, 70, 65, 63, 60, 56.5 °C were applied. A combination of 11 different times and temperatures were used to investigate the best time and temperature combination to achieve a high *Campylobacter* log reduction with the minimum adverse effects on the visual appearance of the treated chicken skin or chicken drumstick.

The time it took for the chicken skin sample to reach the equilibration temperature ('come up time') was measured by a very fast response thermocouple sensor with one-second response time

(Labfacility, UK). The temperature profile was recorded with a Measurement Advantage USB-TEMP logger (Measurement Computing, USA). A graph of the temperature profile is shown in Figure 3.1. Ten drumsticks were used for each treatment, and five for the control group, which was only immersed in a different water bath GD 100 (Grant, UK) maintained at room temperature (20°C) for a similar time interval to the heated drumsticks in order to account for the wash effect (Smith, 1992). Two repeated trials were carried out for only one time-temperature combination.

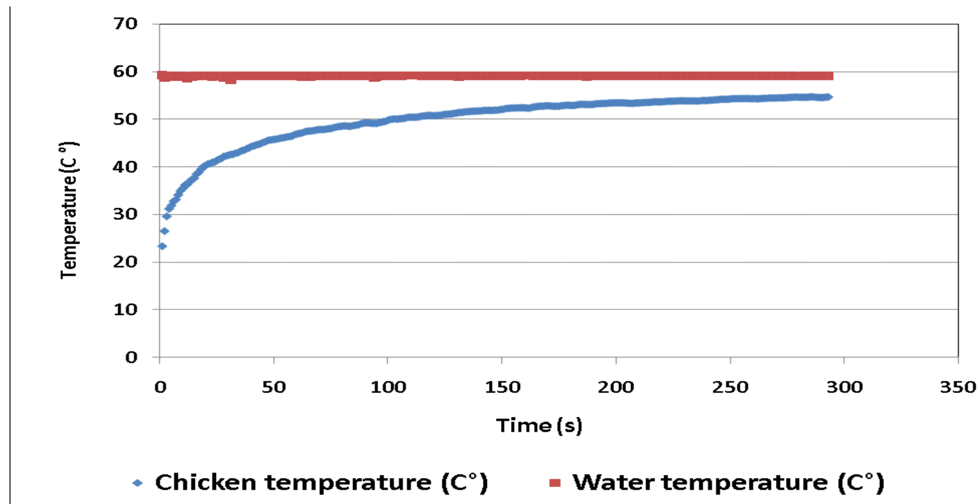


Figure 3.1 Temperature profile of water and chicken during heating the water bath to 60°C.

3.8.2 Enumeration of *C. jejuni* survival.

The treated drumsticks and control drumsticks were examined by removing the whole of the skin aseptically using scalpels with sterilised disposable stainless steel blades. The skin sample was placed in a stomacher bag with a filter containing 90 ml *Campylobacter* Enrichment Broth (CEB) without blood (Lab m135) and pummelled for two minutes using the Colworth Stomacher model 400 (A.J. Seward & Co. Ltd., London). Then 1 ml of stomacher fluid was pipetted from inside the filter sleeve for plating, and 200 µl of the same liquid was separately plated on triplicate mCCDA plates. Serial dilutions were performed for the stomacher fluid by transferring one ml fluid to nine ml CEB without blood and plated as described above. Confirmation that the colonies were *C. jejuni* was achieved by subculturing to CA and incubating at 37 °C aerobically. Additionally, the Hippurate test for *Campylobacter* identification was also performed.

4 Thermal inactivation of *Campylobacter jejuni* in broth

4.1 Introduction

Campylobacter causes more cases of food-borne gastroenteritis each year than any other bacterial pathogen worldwide (Jonsson et al., 2010, Samuel et al., 2004a, Schönberg-Norio et al., 2006, Schönberg-Norio et al., 2004, Rushton et al., 2019). The reason for this high incidence is still not established, and many aspects of the epidemiology of the infection are not clear. However, several international epidemiological studies have established the link between the consumption of poultry meat and campylobacteriosis (Eberhart-Phillips et al., 1997, Ikram et al., 1994, Kapperud et al., 2003, Michaud et al., 2004, Skarp et al., 2016, Neimann et al., 2003) and the high level of contamination of poultry meat in NZ (~80 %) with *Campylobacter* supports this assumption (French, 2008a).

It is important that processors, caterers and consumers apply heat treatments that are effective at destroying all food pathogens. In general, the heat treatments required to eliminate *Salmonella* will also destroy *Campylobacter* (NACMCF, 2007). However, there remains a possibility that undercooking may occur or a proportion of cells in some protected areas may survive the normal heating process leading to ingestion of an infectious dose. Moreover, the remarkable high *D* value for *C. jejuni* reported ($D_{100°C}$ value of about two min, (de Jong et al., 2012)) may indicate the emergence of heat resistant strains. Therefore, it is appropriate that the current knowledge of thermal inactivation of NZ *Campylobacter* strains is updated and expanded.

The objective of this study was to determine the thermal inactivation kinetic parameters (*D* and *z*) for NZ-relevant strains of *C. jejuni* isolated from both humans and poultry. The effects of different time-temperature combinations on survival and variations in survival within the same typed strain (isolated from different sources) and between different typed strains were evaluated.

4.2 Materials and methods

4.2.1 *Campylobacter* culture growth and enumeration

Campylobacter strain cultures of ST-474, ST-190 and ST-45 were prepared as it was described in section 3.2 in details to obtain a plate with prolific growth for inoculum preparation. Each inoculum for testing heat resistance was prepared by transferring all colonies from a CA plate into Brain Heart Infusion broth (BHI). The final culture concentrations used for inoculation were in the range of 10^7 to 10^9 cfu ml^{-}. The determination of inoculum concentration was conducted by a serial

dilution of the inoculum in BHI followed by plating in triplicate on mCCDA by the Surface Plate Method (Downes and Ito, 2001).

4.2.2 *C. jejuni* heat inactivation procedure

The bacterial suspensions obtained from above were heated to a predetermined temperature using a submerged coil heating apparatus (Protrol instruments Ltd., U.K). As described in detail in section 3.3 that samples were taken at predetermined intervals (from 2 s -200 s with a total heating time range from 12 s – 1050 s, depending on the investigated temperature and the isolate heat resistance) automatically and collected in a series of vials in an automated carousel (Cole and Jones, 1990). The inoculum population and the number of surviving organisms in suspension after heat treatment were determined by dilution in BHI followed by plating on mCCDA using an automated spiral plater (Don Whitley, Yorkshire, United Kingdom)

4.3 Modelling and survival data fitting

Survival curves were constructed by plotting the log of surviving bacterial counts against heating time for each temperature. These were then fitted using a range of kinetic models. The log-linear kinetic model (Equation 4.1) has been used extensively in the calculation of the thermal inactivation parameters, *D* and z. However, non-linear survival curves have also been reported (Cerf, 1977, Peleg and Cole, 1998) and several other models have been proposed to describe the patterns observed (Cerf, 1977, McKellar and Lu, 2004).

4.3.1 Log-linear model

D values were estimated by two regression methodologies: (i) the classical two-step regression, and (ii) one step regression or global regression. With classical regression, the *D* value was calculated from the negative reciprocal of the slope of the linear regression line of the plot of log counts versus heating time (Equation 4.1). The z-value of each organism was subsequently calculated from the negative reciprocal of the slope of the plot of log *D* versus temperature (Equation 4.2).

$$\log_{10}(N_t) = \log_{10}(N_0) - \frac{t}{D} \qquad \textbf{4-1}$$

Where:

N_t represents the number of cells per unit mass or volume at time t (cfu ml^{-1}).

N_0 is the initial count of bacteria (cfu ml^{-1}).

D is the decimal reduction time (s).

t is the total heating time at constant temperature (s).

$$\log_{10}(D) = \log_{10}(D_{ref}) + \frac{T_{ref}-T}{z} \qquad 4\text{-}2$$

Where:

D_{ref} is the D value at a reference temperature T_{ref} (s).

T is the temperature (°C).

z is the temperature (°C) required for a one log D reduction.

In the one-step regression method, all the data are considered as a whole, and the parameters D (at a reference temperature, usually taken as the middle of the range of the temperatures investigated) and z are estimated by incorporating Equation 4.2 into Equation. 4.1 to express the temperature dependency of the D value (Equation4.3). By using numerical optimisation methods, the D and z values were estimated simultaneously using all the data (Valdramidis et al., 2004). The advantage of this method is that the error in estimating the D value is incorporated appropriately into the estimation of the z value. By contrast, in the classical method, the error in the estimation of D from Equation 4.1 is not included in Equation 4.2 for the calculation of the z value. Therefore, one-step regression is better in preventing the accumulation of fitting errors.

$$\log_{10}N_t = \log_{10}N_0 - \frac{t}{D_{ref}10^{(T_{ref}-T)/z}} \qquad 4\text{-}3$$

4.3.2 The non–linear models

There are many reports in the literature which indicate that bacterial survival curves (e.g. log N vs. t) are not always strictly log-linear as described by Equation 4-1. Sigmoidal curves, curves with shoulders, tailing, and concavity upwards and downwards have all been reported (Campanella and Peleg, 2001, Cerf, 1977, Peleg et al., 2000, van Boekel, 2002). Various models have been proposed to fit these different survival curves.

The non-linear models which were tested for this study data are described in the literature review chapter two (section 2.4.2):

1. Shoulder before log-linear decrease.
2. Tail after a log-linear decrease.
3. Shoulder and tailing behaviour.
4. Concave or convex curves.

5. Concave or convex carves followed by tailing.
6. Biphasic inactivation kinetics.
7. Biphasic inactivation kinetics preceded by a shoulder.

The Weibull model has been the most widely studied and for this model bacterial inactivation is described by Equation 4-4 (Cunha et al., 1998, Fernandez et al., 1999, Mafart et al., 2002, Peleg, 2002).

$$\log_{10} N_t = \log_{10} N_0 - \left(\frac{t}{\delta}\right)^{\eta} \qquad 4\text{-}4$$

In Equation 4.4, η and δ are the parameters describing the shape and scale of the Weibull distribution function, respectively. The shape parameter gives a convex curve when η>1, or a concave curve when η<1. When η is equal to 1, a straight line corresponding to the log-linear model is obtained (Figure 4.1). This model has been successfully tested using a variety of published heat resistance studies in which it was concluded that the Weibull model was both more suitable and simpler for describing non-log-linear survival curves than other models (van Boekel, 2002).

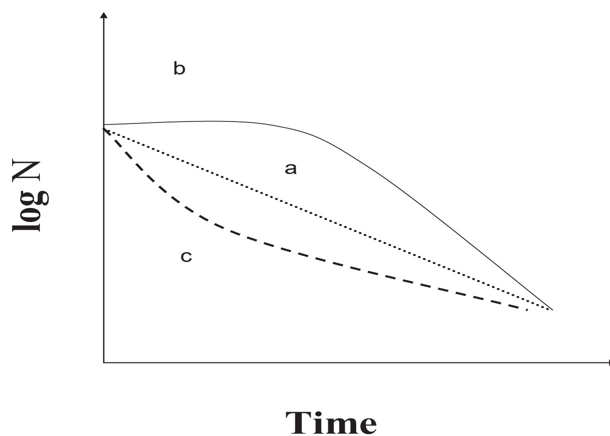


Figure 4-1 Typical inactivation curves for the Weibull model.

(a) a straight line when the shape parameter *η* =1, (b) a convex curve when *η* >1, (c) a concave curve when *η* <1. (Adapted from (Cunha et al., 1998)).

The secondary models describe the dependency of one or more of the parameters of the primary models on a change in environmental conditions. In this study, the classical simple *z* value model was also used to describe the evolution of the Weibull model's scale parameter's (*δ*) dependency on temperature (van Boekel, 2002).

The temperature dependency of the scale parameter δ for the popular Weibull model is expressed

by the *z* value model (Eq4-2) in the following form (by substitution of log δ by a, $T - T_{ref} = T$ and $z = 1/b$):

$$\log_{10}\delta = a - bT \qquad 4\text{-}5$$

4.4 Statistical analysis and software

The software tool which was used to fit the data is *GlnaFit*, v 1.5 (Geeraerd et al., 2005). An example of the *GlnaFit* output is shown in Appendix A (Figure A1). The statistical criterion of the mean squared error (MSE) which was automatically reported for each model and the Akaike criterion were compared for the fitting of each survival curve using a simple ranking analysis (1 = best fit, 2 = 2nd best fit, etc.). The model with the minimum rank-sum value after fitting all the data sets was considered the best fitting model. The software has some built-in logical constraints on the parameter values to be estimated and will deliver an error message when selecting a model which seems not to be substantiated with the tested data. The R software (version 2.9.1) was used to calculate the *D* and *z* value by one step regression which was applied only for the log-linear model. The codes used for both parameters calculation are detailed in appendix A. For comparison purposes, the linear regression analyses for all data sets were performed using the Statistical Analysis System v.9.1 (SAS Institute Inc, USA) to estimate the *D* value from the negative reciprocal of the slope along with the *D* values obtained by GlnaFit software.

4.5 Results and discussion

4.5.1 The log-linear model

The survival curves at different temperatures for the six isolates were obtained. A typical survival curve is shown in Figure 4.2. Most of the survival curves were fitted by the log-linear model, and the r^2 values were > 0.90 for all regressions with only a few showing a small lag (shoulder) during heating at a temperature ranges from 51.5 to 60 ^{0}C. However, due to the heat sensitivity of ST 474 to 60 ^{0}C temperature no sufficient data points were collected for both isolates of this strain. Thus, no measured *D* value at 60 ^{0}C was reported for these strains but its *D* value was estimated from the *z* value and by one step regression technique. The non-linear model fitting is discussed later in this section.

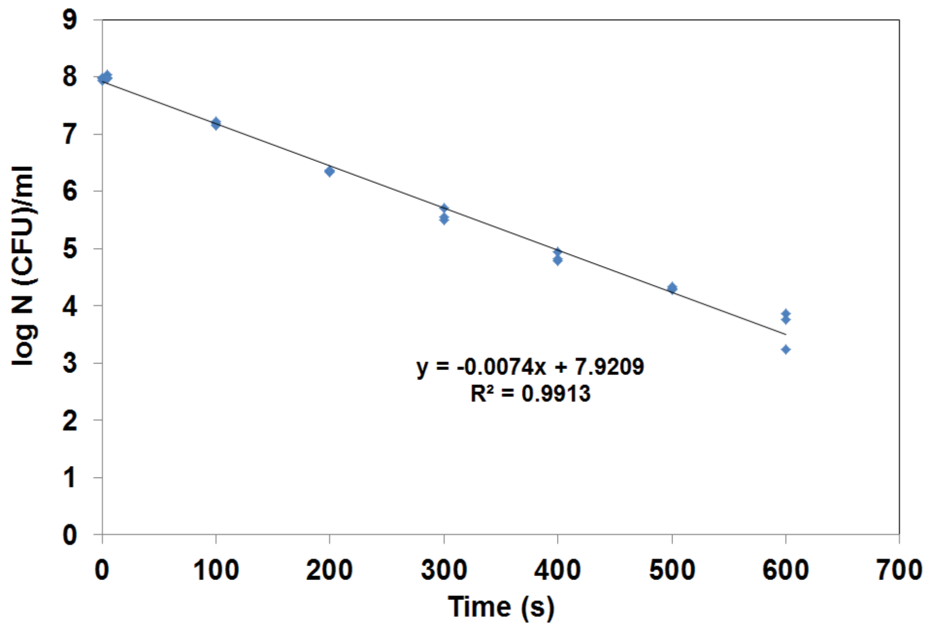


Figure 4-2 Survival curve for *C. jejuni* (ST-190P) at 53.5 °C.

The linear model was fitted to all the experimental data and the *D* and *z* values obtained were 1.5 - 228.3 s, 4.1 - 4.7 °C respectively, by the two-step approach (Table 4.1, Figures 4.2, 4.3), and 1.3 - 187.8 s and 4.0 - 5.2 °C respectively by the one-step approach (Table 4.2, 4.3, appendix A for the model code in R software and the model output). These results are in agreement with international published data (Li et al., 2002, Ray, 2014, Juffs and Deeth, 2007). In fact, the *D* values for all NZ isolates at 60 °C (1.3 - 4.2 s)(Table 4.1) is slightly lower than the minimum *D* value of the published international data which range from seven to 23 s (ICMSF, 1996). Similarly, the *z* values calculated for all isolates from Table 4.1 or Table 4.2, ranged from 4.0 -5.2 °C were within the range of the published *z* values (2.8 - 5.8 °C) (Sorqvist, 2003). It is clear from Tables 4.1- 4.3 and Figure 4.4 that the *z* values did not vary significantly for each isolate.

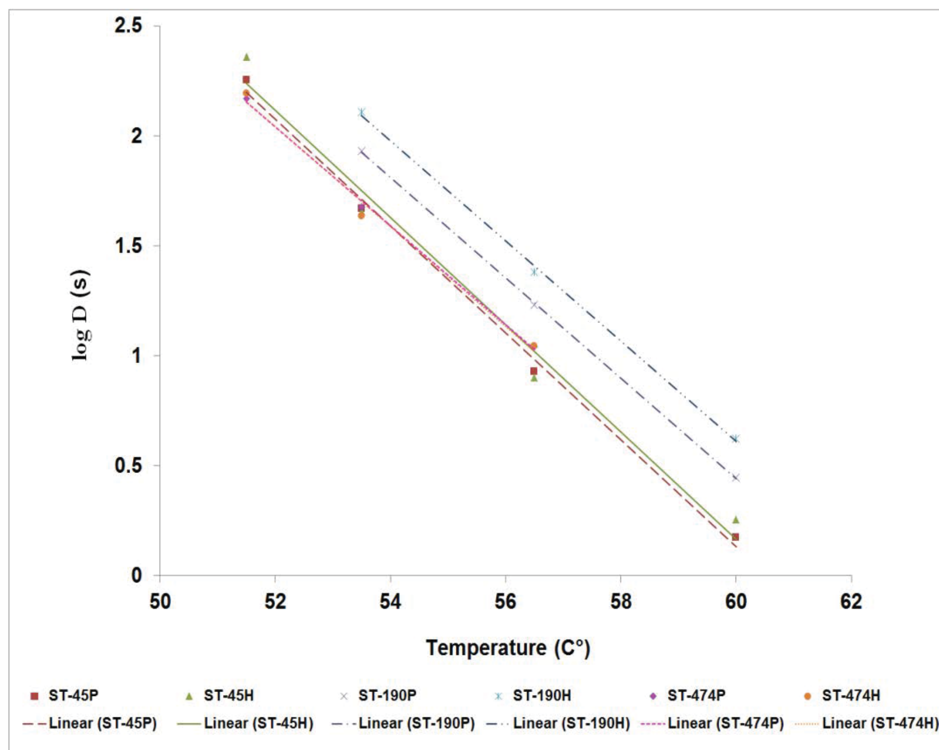


Figure 4-3 *z* values for six isolates of *C. jejuni*.

The fact that the minimal *D* values estimated here are lower than published values may be due to many factors such as the strains used in the study, experimental conditions (e.g. heating media directly or indirectly), cooling after heating, equipment or laboratory media used, presence of salt or acid (pH), the growth phase of the cells and other factors (Pflug, 1990). Moreover, most published data for *Campylobacter* were obtained during the early 1980s with the available methods, instrumentation and techniques available at that time with regard to strain typing, isolation, recovery, enumeration, inactivation and incubation. The only *Campylobacter* D value reported by (Scanlon et al., 2015) in an experiment which used similar methodology and heating equipment to this study, reported higher D values at 60°C in the range of 42.4 s to 74.7 s. However, the study by Scanlon et al. (2015) investigated four different *Campylobacter* species (*C. coli, C. helveticus, C. concisus, C. fetus* subsp. Fetus) *and* also *Arcobacter butzleri*. Most of these species have not been found in NZ campylobacteriosis patient clinical samples in New Zealand, according to a NZ epidemiological study (Nohra et al., 2016). This epidemiological study screened 1,601 of *Campylobacter* clinical isolates and found only 47 *C. coli* (2.9 %). Moreover, the difference between this study D values at 60°C and Scanlon et al. study (2015) is not remarkable.

Our data showed only a minor variation as the standard errors for the estimated *D* and *z* and values were from 0.3 - 1.1 s and 0.04 - 0.13 °C respectively. This was mainly achieved by using the one-

step regression approach due to the increased number of degrees of freedom (van Boekel, 1996). Strains ST-474 and ST-45 had similar *D* values, and they are slightly more heat-sensitive than ST-190. In general, the source of the isolate, either poultry or human, do not significantly affect the *D* value of the strain. Only the human isolate of ST-190 was (to a limited extent) more heat resistant than the poultry isolate of the same strain at all of the measured temperatures. Human strains have previously been reported to be more tolerant of chilling temperature than poultry strains (Chan et al., 2001). In addition, the isolate of ST-45 from a wild bird was more heat resistant than the human and poultry isolates of the same strain and its *D* value was similar to the *D* value of the poultry strain ST-190 (data not shown). The poultry and human isolates of ST-474 and ST-45 showed source-independent variations in their observed *D* values due to random biological variation. This random biological variation was observed to be more significant at the lowest heating temperature of 51.5 °C. This phenomenon has also been observed by others. For example, Doyle (1981) reported that as the temperature increased the range of rates of inactivation among the different strains narrowed. This was also observed in our study, particularly for strain ST-190 and ST-45, as shown in Tables 4.1, 4.2. and 4.3.

Table 4-1 Estimated *D* and *z* values for *Campylobacter* isolates by two-step regression at different temperatures (SE in square brackets, P = poultry isolate, and H = human or clinical isolate, n.r = not reported).

Strain	D value (s) at Temperature (°C)				z value (°C)
	51.5	53.5	56.5	60	
ST-45P	178.9 [2.95]	46.8 [2.4]	8.5 [0.18]	1.5 [0.06]	4.1 [0.18]
ST-45H	228.3 [6.91]	45.3 [1.69]	8.0 [0.25]	1.8 [0.08]	4.1 [0.39]
ST-190P	n.r	85.2 [2.47]	17.0 [0.32]	2.8 [0.15]	4.4 [0.17]
ST-190H	n.r	128.2 [2.28]	24.1 [0.28]	4.2 [0.13]	4.7 [0.41]
ST-474P	147.5 [3.25]	47.5 [0.76]	11.0 [0.32]	n.r	4.4 [0.05]
ST-474H	156.5 [3.96]	43.3 [2.83]	11.1 [0.20]	n.r	4.4 [0.14]

Table 4-2 Estimated *D* and *z* values for *Campylobacter* isolates by one-step regression at different temperatures (P = poultry isolate and H = human or clinical isolate).

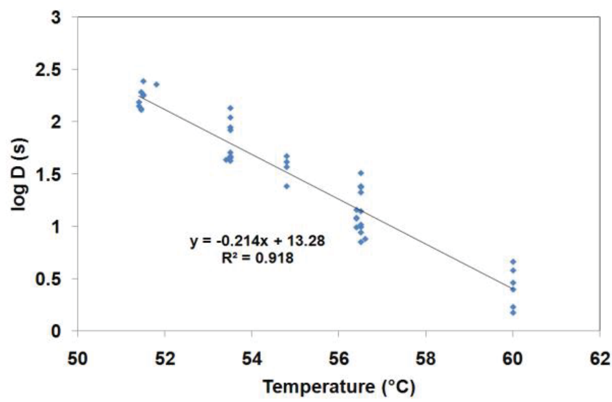
Strain	Temperature (°C)	D value (s)	SE	*z* value (°C)	SE
ST-45_P	55.8	14.7	0.31	4.0	0.05
ST-45_H	55.8	15.8	0.45	4.0	0.06
ST-190_P	56.8	17.1	0.47	4.5	0.11
ST-190_H	56.8	22.5	0.34	4.4	0.05
ST-474_P	54.0	46.1	0.79	5.2	0.13
ST-474_H	54.0	40.8	1.06	4.4	0.09

Table 4-3 Calculated *D* and *z* values for *Campylobacter* isolates at different temperatures based on the *D* and *z* values estimated by one-step regression in Table 4.2 for a comparison with the values indicated in Table 4.1.

Strain	*D* value (s) at Temperature (°C)				*z* value (°C)
	51.5	53.5	56.5	60.0	
ST-45$_P$	174.7	55.2	9.8	1.3	4.0
ST-45$_H$	187.8	59.4	10.6	1.4	4.0
ST-190$_P$	n.r	92.5	19.9	3.3	4.5
ST-190$_H$	n.r	126.5	26.3	4.2	4.4
ST-474$_P$	139.5	57.5	15.2	n.r	5.2
ST-474$_H$	151.0	53.0	11.0	n.r	4.4

4.5.2 The non–linear models

In general, for any given run, one or more of the non-linear models produced better fits to the inactivation data than the log-linear model based on the goodness of fit criteria employed. This is in agreement with other studies (Campanella and Peleg, 2001, van Boekel, 2002, Keklik et al., 2011, Cebrián et al., 2019) which reported that non-linear models fit the survival data sets better. An example of a few data sets is shown in Table 4.4. Overall, the Weibull model (Equation 4.4) was the only non-linear model which could be successfully fitted to all data sets. The *GInaFit* program identified unlikely or unreliable parameter estimates for the other non-linear models for at least some data sets. Therefore, the Weibull model was further used to determine the effect of temperature on the scale and shape parameters (δ, η). The scale factor (δ) showed a similar log-linear dependency on temperature as the *D* value (Figure 4.4 and Figure 4.5). This temperature dependency of the scale factor has also been reported by others (Mishra and Puri, 2013, Farakos et al., 2014, Kaur and Rao, 2017, Li et al., 2014) However, the shape factor (η) did not depend on the temperature systematically but varied significantly across the data sets (Figure 3.6). This is in agreement with most of the published data (van Boekel, 2002, Farakos et al., 2014).



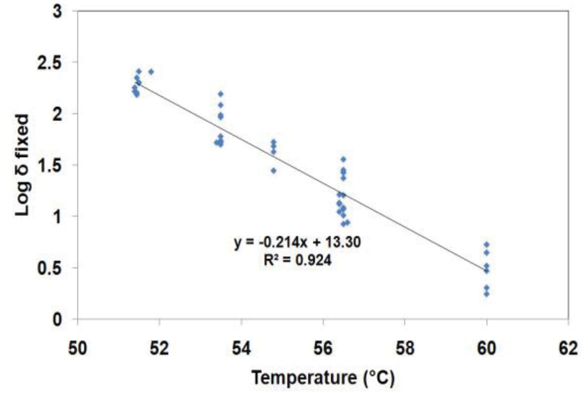


Figure 4-4 *D* value as a function of temperature for *C. jejuni* inactivation

Figure 4-5 δ value as a function of temperature for C. *jejuni* inactivation

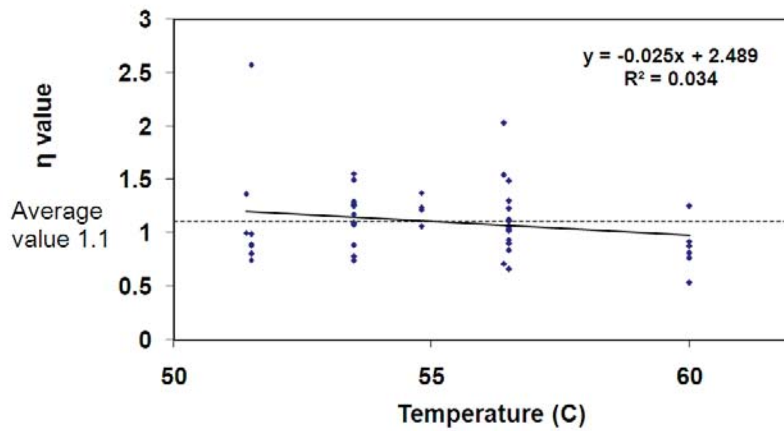


Figure 4-6 Variation observed in the Weibull shape factor η fitted to *C. jejuni* survival curves as a function of temperature.

Thus, it seems worthwhile to fix the η value. The mean value of η was selected as a characteristic of *Campylobacter* for the overall data sets (Mafart et al., 2002). The new generic model, therefore, can be represented as:

$$\log_{10}N_t = \log_{10}N_0 - \left(\frac{t}{\delta}\right)^{1.1} \qquad 4\text{-}6$$

When the mean shape factor (η =1.1, SE= 0.053) was employed, the goodness of fit to the survival data was poorer than for the Weibull model when fitting η and δ individually (Table 4.4) and was also poorer than the linear model for many data sets, providing a better fit for only 50 % of the data sets. The highlighted mean squared error values and Akaike criteria values in Table 4.4 indicate the best goodness of fit for the Weibull model followed by the linear model which has equal goodness of fit to the 'Weibull with fixed parameter' model.

Table 4-4 Selected examples for illustrating the goodness of fit for nine models fitted to the survival data of selected strains of *C. jejuni*. (P =poultry isolate and H = human or clinical isolate).

	Model	The goodness of fit (MSE/AIC)			
		$ST\text{-}474_P$ 53.5 °C	$ST\text{-}45_H$ 51.5 °C	$ST\text{-}190_H$ 53.5 °C	$ST\text{-} 474_P$ 56.5 °C
1	Linear	0.095 -65.731	0.048 -95.124	0.023 -85.376	0.093 -73.561
2 u	Linear + shoulder	0.032	n/a	n/a	0.045
3	Linear + tail	n/a	0.020	0.018	n/a
4	Linear + shoulder + tail	n/a	0.020	n/a	n/a
5	Weibull	0.042 -91.789	0.035 -107.071	0.018 -92.390	0.037 -105.503
6	Weibull + fix	0.062 -78.517	0.064 -85.949	0.038 -73.119	0.071 -82.4129
7	Weibull + tail	n/a	0.020	n/a	n/a
8	Biphasic	n/a	n/a	0.025	n/a
9	Biphasic + shoulder	n/a	0.018	0.016	n/a

The recalculated scale factor (δ') with fixed η showed a similar dependency on temperature as the D value in the log-linear model, and the z value 4.7 °C was unchanged (z' = 4.7 °C) (Figures 4.4 and 4.5). The time required to achieve a given process extent (e.g. a 7 D reduction) was similar for the 'Weibull with fixed parameter' model and the log-linear model, as shown in Table 4.5.

Table 4-5 Comparison of heating time(s) required to achieve a 7 D reduction in the number of bacterial cells using the Log-linear (Equation 4.1) and the Weibull model (Equation 4.4) with pooled data D and δ values at selected temperatures.

Temperature (°C)	7 D time based on Weibull	7 D time based on log-linear
51.5	1114.99	1270.86
53.5	416.17	474.35
56.5	94.90	108.17
60	16.91	19.28
65	1.44	1.64
70	0.12	0.14

4.6 Conclusions

The D_{60} and z values in broth medium for the most important strains of *C. jejuni* in human cases of campylobacteriosis in NZ were within the range from 2 - 5 s and 4 - 5.2 °C, respectively. These values are broadly in agreement with the published international data and do not indicate that the tested NZ *Campylobacter* strains are more heat resistant than other strains. The Weibull model fitted the survival data better than the log-linear model if the shape factor was allowed to vary between runs. When the mean shape factor was employed and the scale factor obtained by re-fitting the model, the goodness of fit was poorer. This 'Weibull with fixed parameter' model was only as good a fit as the log-linear model. That is not so strange if the value is 1.1, i.e. very close to 1. The scale factor of the Weibull model showed a similar dependency on temperature as the D value in the log-linear model and the time required to achieve a given process extent (e.g. a 7 D reduction) was similar for both models. The likelihood of a systematic error in using the log-linear model in quantitative risk models, therefore, appears to be low. It is important to verify that the D and z values obtained in broth are similar to those obtained in food samples to confirm that these NZ strains are not heat resistant and the next chapter addresses this point.

5 Thermal inactivation of *C. jejuni* in food

5.1 Introduction

Different values of *D* and *z* for many pathogens have been reported in the literature (as summarised in Table 2.3). One major factor influencing the observed kinetics is the composition of the matrix with which the bacterium is associated with during the heat treatment (Stumbo, 1973). *Campylobacter* heat resistance has not been studied as extensively by researchers as other food pathogens probably because it is generally regarded as heat-sensitive. Nevertheless, due to the variation in reported kinetic parameters, it is difficult to choose a value for risk assessment and HACCP plans for any specific cooking process from the reported values in the literature (Bergsma et al., 2007, de Jong et al., 2012, Forsythe, 2000, Nguyen et al., 2006).

It is also known that *Campylobacter* attachment to chicken skin increases its heat resistance (Yang et al., 2001). The chemical environment around microorganisms during the heating can increase or decrease heat resistance (Doyle et al., 2001). Many studies have reported the importance of determining the kinetic parameters *D* and *z* in food matrices rather than in liquid media. WHO and the local regulatory bodies always emphasise that the evaluation of process lethality should be based on food data or real substrates contaminated with the target organism (Gaze et al., 1989).

Therefore, the objective of this study was to determine the kinetic parameters (*D* and *z* values) of selected NZ *C. jejuni* strains on chicken skin which is often significantly contaminated (Scherer et al., 2006). The finding of this study will confirm whether NZ *Campylobacter* strains are more heat resistant than overseas strains, which has been proposed as a reason for the high rate of campylobacteriosis in NZ.

5.2 Materials and methods

5.2.1 *Campylobacter* culture growth and enumeration

As described in detail in section 3.2 *Campylobacter* strain cultures of ST-474 and ST-48 were grown on CA and sub-cultured on to another CA plate and incubated for 48 h in a micro-aerobic atmosphere at 41.5 °C to obtain a plate with prolific growth for inoculum preparation. Each inoculum for testing heat resistance was prepared by transferring all colonies from a CA plate into Brain Heart Infusion broth (BHI). The final culture concentrations used for inoculation were in the range of 10^7 to 10^9 cfu ml$^-$ The determination of inoculum concentration was conducted by a

serial dilution of the inoculum in BHI followed by plating in triplicate on mCCDA by the Surface Plate Method (Downes and Ito, 2001). Plates were incubated micro-aerobically at 41.5 °C for 48 h.

5.2.2 Meat sample preparation and inoculation

The chicken skin was prepared as described in details in section 3.4.1 and was equilibrated to room temperature and placed aseptically in an aluminium test cell (Appendix B, Figure B1) specially designed for kinetic studies (Chung et al., 2008). An inoculum of 20 µl was pipetted to the chicken skin centre in the lower part of the aluminium cell. After inoculation, the cell was left for 60 min to allow *C. jejuni* attachment to chicken skin to mimic natural contamination.

5.2.3 *C. jejuni* heat inactivation procedure.

AS described in detail in section 3.4.2, the eight aluminium cells were placed in a rack submerged in a temperature-controlled water bath (Grant, UK) maintained at a 56.5 to 65 °C temperature range for a predetermined time period (Appendix B, Figure B2). One aluminium cell was removed at each prescribed time interval and then transferred immediately to an ice bath for *C. jejuni* survivor analysis. The time it took for the chicken skin sample to reach the equilibration temperature ('come up time') was measured by a very fast response thermocouple sensor with one second response time (Labfacility, UK). The temperature was recorded with a Measurement Advantage USB-TEMP logger (Measurement Computing, USA). A graph of the temperature profile is shown in Appendix B(Figures B3-B6). The 'come-up time was 55 s from a number of experiments for both the investigated temperatures 56.5 °C and 60 °C. All data points used in the calculation of the kinetic parameters were considered to estimate the come up time.

5.2.4 Enumeration of *C. jejuni* survivors

Each chicken sample was transferred from the aluminium cell to a vial containing 4 ml BHI and the aluminium cell was flushed out with a further 5 ml BHI to remove any survivors from the internal surface of the cell. The sample was mixed using a vortex mixer and serially diluted in BHI. All the samples remained in BHI for at least two hours to allow recovery of injured cells, followed by plating in triplicate on mCCDA by the Surface Plate Method, a 200 µl spread by L shaped sterilised spreader (Downes and Ito, 2001). Plates were incubated microaerobically at 41.5 °C for 48 h as described above. The average of the logarithm (base 10) of three plate counts at each sampling time was used for modelling and analysis.

5.3 Modelling and survival data fitting

All the modelling and survival data were carried out as for the broth data in the previous chapter, section 4.3.

Survival curves were constructed by plotting the log of surviving bacterial counts against heating time for each temperature. These were then fitted using a range of kinetic models.

5.3.1 The log-linear model

D values were estimated by two regression methodologies: (i) the classical two-step regression, and (ii) one step regression or global regression. With classical regression, the *D* value was calculated from the negative reciprocal of the slope of the linear regression line of the plot of log counts versus heating time (Equation 4.1). The z-value of each organism was subsequently calculated from the negative reciprocal of the slope of the plot of log *D* versus temperature (Equation 4.2).

In the one-step regression method, all the data are considered as a whole, and the parameters *D* (at a reference temperature, usually taken as the middle of the range of the temperatures investigated) and z are estimated by incorporating Equation 4.2 into Equation. 4.1 to express the temperature dependency of the *D* value (Equation4.3). By using numerical optimisation methods, the *D* and z values were estimated simultaneously using all the data (Valdramidis et al., 2004).

5.3.2 The non–linear models

Various models have been proposed to fit these different survival curves.

The non-linear models which were tested for this study data are described in the literature review chapter two (section 2.4.2):

The Weibull model has been the most widely studied and for this model bacterial inactivation is described by Equation 4-4

The secondary models describe the dependency of one or more of the parameters of the primary models on a change in environmental conditions. In this study, the classical simple z value model was also used to describe the evolution of the Weibull model's scale parameter's (δ) dependency on temperature (van Boekel, 2002).

The temperature dependency of the scale parameter δ for the popular Weibull model is expressed by the z value model (Eq4-2)

5.4 Statistical analysis and software

The software tool which was used to fit the data is *GInaFit*, v 1.5 (Geeraerd et al., 2005). The R software (version 2.9.1) was used to calculate the *D* and z value by one step regression which was

applied only for the log-linear model. The codes used for both parameters calculation are detailed in appendix A. For comparison purposes, the linear regression analyses for all data sets were performed using the Statistical Analysis System v.9.1 (SAS Institute Inc, USA) to estimate the *D* value from the negative reciprocal of the slope along with the *D* values obtained by GInaFit software.

5.5 Results and discussion

5.5.1 The linear model

A typical survival curve is shown in Figure 5.1. Most of the survival curves were linear, and the r^2 values were > 0.92 for all regressions with only a few showing a small lag (shoulder, r^2 >0.85) during heating at the investigated temperature range (56.5 to 65 °C). Therefore, the linear model was chosen to fit all the experimental data. All isolates were rapidly inactivated at 65 °C and hence no data for that temperature. Sufficient data were obtained at 56.5 °C and 60 °C. The extrapolated *D* values at 65° for all the isolates were obtained from the measured *D* values at 56.5 °C and 60 °C. The calculated D_{65} values were comparable with the *D* values obtained for at 65 °C by the dynamic method in the next chapter.

The *D* values at the temperature range 56.5 - 60 °C were 42 - 12 s when estimated by one step regression (see Table 5.1) and 42 - 11 s by the two-step regression (see Table 5.2). These results are lower than the published *D* values for *C. jejuni* on chicken skin, 90-130 s at 55 °C and 30 s at 60 °C (Yang et al., 2001).

However, the *z* values of all studied isolates (ranging from 7.6 - 11.3 °C, Table 5.1) were higher than some (extrapolated) published data, viz. 6 - 6.4 °C (Forsythe, 2000). However, they are in agreement with the extrapolated *z* value of 12.3 °C published by another study (van Asselt and Zwietering, 2006). Discrepancies between *z* values are well documented in the literature. For example, one study reported a *z* value for Listeria as 29 °C (Fang and Lin, 1994) when most textbooks reported a range of 5 - 12 °C (Ryser and Marth, 2007). The *D* and *z* values we report here are 2- to 3-fold higher than the values obtained for the same isolates in broth in the previous chapter, confirming the importance of making measurements in food.

The lower *D* values found in this study compared to published data for chicken may relate to the strains used in the study, experimental conditions (e.g. mechanism of heating of media), method of cooling after heating, equipment or laboratory media used, presence of salt or acid (pH), or the growth phase of the cells (Pflug 1990). Most published data for *Campylobacter* were acquired during the early 1980s with the then available methods, instrumentation and techniques used for strain typing, isolation, recovery, enumeration, inactivation and incubation. *Campylobacter* has been

described as having a 'capricious' nature because of the high variation detected between tests, which cause more difficulties in standardisation and handling this pathogen than with any other pathogen (Bergsma et al., 2007, Jasson et al., 2007). This study data also shows variations as the standard errors for the estimated *D* values were from 0.8 to 4.8 and 0.6 to 4.7 for the *z* values. The diversity within *C. jejuni* strains is well known and has been detected at both the phenotypic and genotypic level (Gölz et al., 2018).

There was a small but statistically significant difference in the *D* values between the poultry and human isolates of strain 474. However, there was no significant difference between the poultry and human isolates of strain 48 or between the human isolates of both strains with only a minor but statistically significant difference between the *D* values of both poultry strains. This statistical difference does not have any practical importance as the more heat resistant strain 48 is less attributed to campylobacteriosis in NZ than strain 474 which accounts for ~30 % of the cases (Müllner et al., 2010). Generally, the low *D* values obtained for both strains confirmed that NZ strains tested in this study are more heat-sensitive than the international strains and should be easily eliminated by normal cooking or processing treatment. Cross-contamination has been stated as the most important factor for the high rate of campylobacteriosis internationally (Luber, 2009), and it seems likely that the same situation applies in NZ.

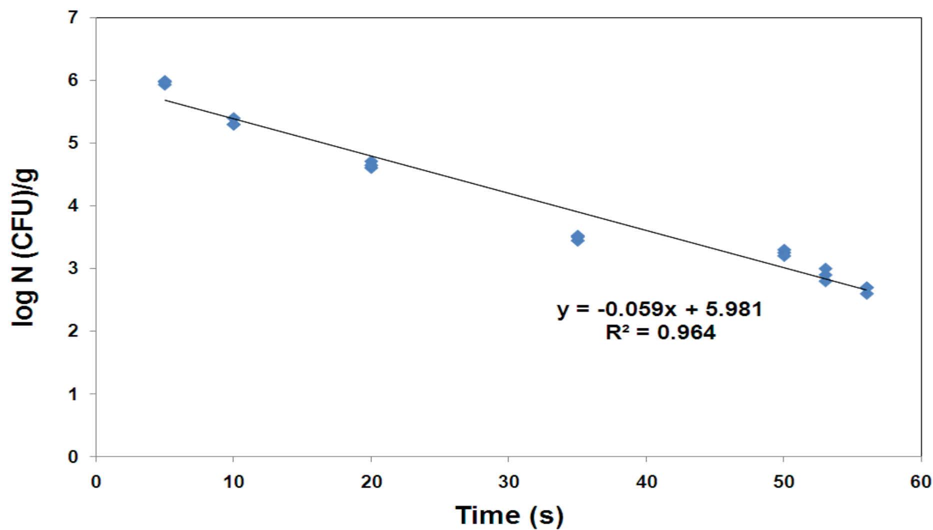


Figure 5-1 Survival curve for *C. jejuni* strain ST-474$_H$ inoculated on chicken skin and heated at 60 °C.

Table 5-1 *D* and *z* values for *C. jejuni* isolates estimated by one step regression at different temperatures.

	Strain	D value (s)		*z* value (°C)	Se
		at 58.25 °C	Se		
1	$ST\text{-}474_P$	19.0	0.8	7.6	0.6
2	$ST\text{-}474_H$	23.2	1.3	11.1	1.7
3	$ST\text{-}48_P$	27.3	4.8	9.6	4.0
4	$ST\text{-}48_H$	22.7	3.4	11.3	4.7

Table 5-2 Calculated *D* and *z* values for *C. jejuni* isolates at different temperatures based on the *D* and *z* values estimated by one-step regression in Table 5.1 for a comparison purpose with the values indicated in Table 5.3.

Strain	*D* value (s)			*z* value (°C)
	Temperature (°C)			
	56.5	60.0	65.0	
$ST\text{-}474_P$	34.7	12.1	2.7	7.6
$ST\text{-}474_H$	33.3	16.1	5.7	11.1
$ST\text{-}48_P$	41.6	18.0	5.4	9.6
$ST\text{-}48_H$	32.5	15.9	5.7	11.3

Table 5-3 Estimated *D* and *z* values for *C. jejuni* isolates by two-step regression at different temperatures

	D value (s) at Temperature (°C)		*z* value (°C)
Strain	56.5	60	
$ST\text{-}474_P$	22.5 (0.31)	11.2 (0.43)	8.8 (3.77)
$ST\text{-}474_H$	33.3 (2.45)	16.1 (1.39)	11.1 (2.15)
$ST\text{-}48_p$	41.5 (11.07)	14.6 (0.12)	10.0 (5.14)
$ST\text{-}48_H$	29.5 (1.27)	15.9 (1.12)	11.1 (6.93)

5.5.2 The non–linear models

In general, for any given run, one or more of the non-linear models produced a better fit for the inactivation data than the linear model based on the goodness of fit criteria employed. Overall, the Weibull model (Equation 4.4) was the only non-linear model which could be successfully fitted to all data sets. The *GlnaFit* program identified unlikely or unreliable parameter estimates for the other non-linear models for at least some data sets. Therefore, the Weibull model was further analysed to determine the effect of temperature on the scale and shape parameters (δ, η). The scale factor (δ) showed a similar log-linear dependency on temperature as the D value (Figures 5.3 and 5.4). However, the shape factor (η) did not depend on the temperature systematically and varied significantly across the data sets (Figure 5.2). This is in accordance with most published data (van Boekel, 2002).

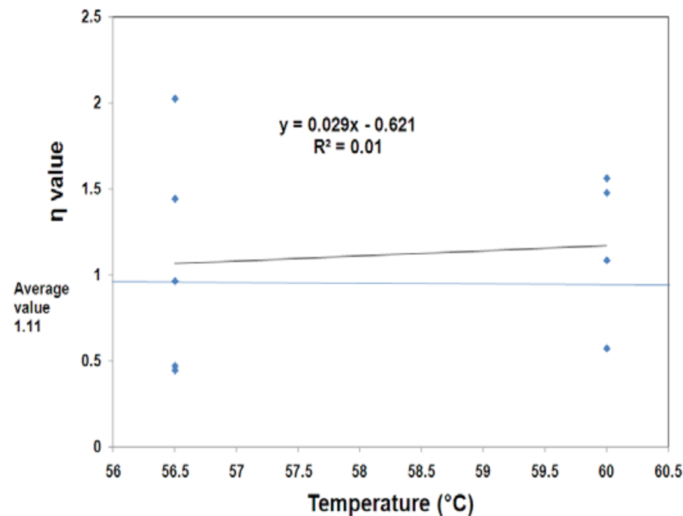


Figure 5-2 Variation observed in the Weibull Shape factor η fitted to *C. jejuni* survival curves as a function of temperature.

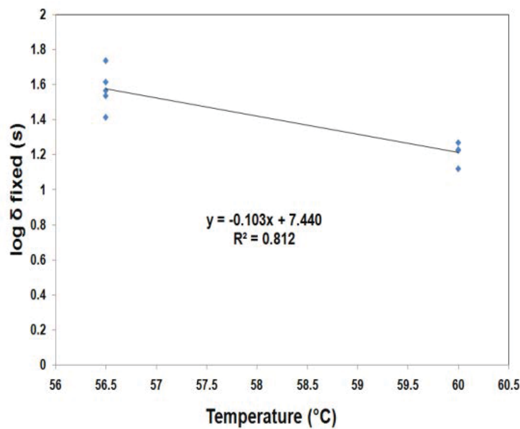


Figure 5-3 δ value as a function of temperature for *C. jejuni* inactivation

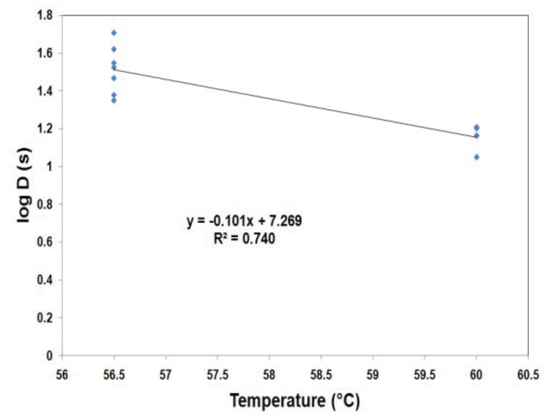


Figure 5-4 D value as a function of temperature for *C. jejuni* inactivation

Thus, it seems worthwhile to fix the η value. The mean value (1.11, SE=0.18) of η was selected as characteristic of *C. jejuni* for the overall data sets (Mafart et al. 2002). Hence, a new generic model is described below.

$$\log_{10} N_t = \log_{10} N_0 - \left(\frac{t}{\delta}\right)^{1.11} \qquad 5\text{-}1$$

When the mean shape factor (η = 1.11) was employed, and the Weibull model was refitted to the survival data, the goodness of fit was poorer than for the Weibull model when η and δ were fitted individually. However, it was still better than the linear model for >60 % of the data sets. The recalculated scale factor (δ') with fixed η showed a similar dependency on temperature as the D value in the log-linear model (z' = 9.7 °C, Figure 5.3; z = 9.9 °C, Figure 5.4).
The time required to achieve a given process extent (e.g. a 7 D reduction) was quite similar at the highest temperature for the Weibull fixed model and the log-linear model (Table 5.4). This finding suggests that both models give similar results. However, the Weibull fixed model should be investigated further as the pooled data approach may not represent the individual thermal characteristics of each *C. jejuni* isolate or strain. The primary comparison of the time for 7 D at 65 °C by the linear model, for example for ST-474_P was 19 s from Table 5.2, whereas the pooled data value in Table 5.3 was 39 s. Therefore, Table 5.4 illustrates how important it is to select an appropriate model to fit inactivation survival data.

Table 5-4 Comparison of heating times (s) required to achieve a 7 *D* reduction in the number of bacterial cells using the Log-linear and the Weibull model.

Temperature (°C)	7 *D* time based on Weibull	7 *D* time based on log-linear
51.5	736.67	956.31
53.5	462.67	595.11
56.5	230.29	292.14
60	102.04	127.38
65	31.90	38.91
70	9.97	11.89

The maximum contamination level with *C. jejuni* in NZ is below 10^6 cfu per carcass (Chrystal et al., 2008) and inactivation at this level would be easily achieved by cooking to the temperature recommended by The Food Safety and Inspection Service for poultry products (75 – 80 °C). In fact, this level of contamination would be inactivated by far lower cooking temperatures than the recommended cooking temperature.

Overall, the objective of this experiment was to determine if the food matrix and the cell attachment can alter the heat resistance of *Campylobacter* significantly and it seems that the food matrix significantly increases the heat resistance of *Campylobacter* but not to the same extent as reported by a Netherlands study (de Jong et al., 2012)). The two *Campylobacter* strains tested here, which are the most commonly implicated strains of campylobacteriosis in NZ, should still be considered as heat-sensitive microorganisms. Moreover, another study confirmed that *C. jejuni* is heat sensitive as it was not detected after simulated home pan-frying of artificially inoculated with 4 log cfu/g of *Campylobacter jejuni* steaks or filets, hamburgers and meat strips (Lahou et al., 2015).

5.6 Conclusions

The presence of a food matrix altered the kinetic parameters for *Campylobacter* heat resistance. Nevertheless, the D_{60} and z values calculated for the most important strains of *C. jejuni* on chicken skin in NZ were within the range from 11 - 18 s and 8 - 11 °C, respectively. These values are broadly in agreement with the published international data and do not indicate that NZ *Campylobacter* strains are more heat resistant than other strains. However, it is important to verify that the D and z values obtained by isothermal conditions and the D and z values obtained under non- isothermal conditions reflect the real scenario in practice as the temperature is not always constant in a processing environment.

6 Thermal inactivation of *C. jejuni* in food under dynamic conditions

6.1 Introduction

Kinetic parameters describing thermal inactivation of micro-organisms are most commonly determined under nominally isothermal conditions for the apparent theoretical and practical simplicity. These kinetic parameters obtained under isothermal conditions should not be applied to variable processing conditions without validation under dynamic conditions. In practice, the thermal effect is incurred during the time required to achieve a constant temperature. This must be accounted for to avoid errors in parameters calculation. This time is called the 'come up time' which considers the heat transfer phenomena. As a result of excluding the come up time period from the heat treatment time, there may be a limited temperature range from which to choose sufficient points for the experiments, particularly for heat-sensitive organisms (Welt and Tong, 1993).

Dynamic models describing microbial inactivation are expressed as differential equations which incorporate the imposed or observed time-temperature profile as an input to the dynamic model selected which describes the thermal death of micro-organisms. The resulting equations can be solved numerically to obtain the number of surviving organisms at each time and temperature regime. If the rate of temperature increase can be described by a simple function, the dynamic model equation can be solved analytically (Miles and Mackey, 1994). Several procedures have been proposed to accomplish this integration (Patashnik, 1953, Stumbo, 1973).

The determination of kinetic parameters under dynamic conditions can better reflect realistic processing scenarios and the 'come-up time' is explicitly a part of the experimental design, for example by specifying the heating rate and time-temperature profile (Welt et al., 1997). In general, estimating the kinetic parameters under dynamic conditions has been hampered by the mathematical complexity of the calculations involved and the instruments required to conduct the experiments. However, modern software and rapid development in technology and instrumentation have helped to overcome these two drawbacks. Therefore, interest in mathematical modelling and analysis of dynamic processes have increased in the last two decades, and dynamic models have been developed for microbial growth and inactivation, heat transfer and changes in texture and sensory properties (Peleg, 2003, Conesa et al., 2003, Dolan et al., 2007, Valdramidis et al., 2008, Pesciaroli et al., 2019, Garre et al., 2018, Tarlak et al., 2020).

As *Campylobacter* is considered a heat-sensitive microorganism, the data for non-isothermal kinetic parameters are not available. Therefore, the objective of this study was to determine the microbial kinetic parameters (*D* and *z*) under dynamic conditions and to compare the data generated with the *D* and *z* values obtained under isothermal conditions for the most commonly implicated strains in campylobacteriosis cases in NZ.

6.2 Materials and methods

6.3 *Campylobacter* culture growth and enumeration

Campylobacter strain cultures of ST 474 and ST 48 were prepared as described in detail in section 3.2 grown on CA were sub-cultured on to another CA plate and incubated for 48 h in a micro-aerobic atmosphere at 41.5 °C to obtain a plate with prolific growth for inoculum preparation. Each inoculum for testing heat resistance was prepared by transferring all colonies from a CA plate into Brain Heart Infusion broth (BHI). The final culture concentrations used for inoculation were in the range of 10^7 to 10^9 cfu ml^{-1}. The determination of inoculum concentration was conducted by a serial dilution of the inoculum in BHI followed by plating in triplicate on mCCDA by the Surface Plate Method (Downes and Ito, 2001).

6.3.1 Meat sample preparation and inoculation

The chicken skin was prepared as described in details in section 3.4.1 and was equilibrated to room temperature and placed aseptically in an aluminium test cell (Appendix B, Figure B1) specially designed for kinetic studies (Chung et al., 2008). An inoculum of 20 µl was pipetted to the chicken skin centre in the lower part of the aluminium cell. After inoculation, the cell was left for 60 min to allow *C. jejuni* attachment to chicken skin to mimic natural contamination.

6.3.2 Heat inactivation procedure

As described in detail in section 3.5.2 For each run, 16 aluminium cells containing inoculated chicken skin samples were placed in a programmable water bath (developed locally at the university, described in Appendix C, Figure C1). The water temperature was programmed to increase linearly to reach 65 °C for a period of either 8 or 18 min simulating the scenarios that can occur in retail food service, industry and in domestic cooking (Persson et al., 2002, van Asselt et al., 2009, NACMCF, 2007). Duplicate samples (two aluminium cells) were withdrawn at different predetermined intervals and transferred immediately to an ice bath until analysed for the counts of surviving culturable *C. jejuni*.. The temperature profiles for both regimes were embedded in the individual survival curves and are shown in the results section.

6.3.3 Enumeration of *C.jejuni* survivors

As described in 3.3.2, the average of the $\log_{10}$ counts for four plates at each sampling time was used for modelling in contrast to the three plates used in section 3.3.2. This was to improve the accuracy of the results of this experiment because less number of experiments are required to conduct under the dynamic conditions.

6.4 Modelling and survival data fitting

The dynamic modelling technique requires the application of a one-step regression approach (Valdramidis et al., 2008). The first-order kinetic model (Equation 6.1) was selected to model the survival data as it has been shown to provide an adequate fit and for its simplicity and global applicability. It is a common approach (Pflug, 1990)to incorporate the Bigelow model for temperature dependence by defining the parameter D value as a measure of the rate of microbial inactivation instead of the kinetic rate coefficient K. By incorporation of Bigelow model (Equation 6..2) and with K expressed in the following format:

$$\frac{dN_t}{dt} = -kN \qquad 6\text{-}1$$

$$k = \frac{ln10}{D_{ref}} e^{ln10/z(T-T_{ref})} \qquad 6\text{-}2$$

The final model can be expressed as follows:

$$\frac{dN_t}{dt} = -\frac{ln10}{D_{ref}} e^{ln10/z(T-T_{ref})}.N \qquad 6\text{-}3$$

The measured time and temperature profile were incorporated as an input to the above model to obtain the *D* and *z* values at a reference temperature. Following the common convention, this was selected to be the average temperature of 56°C of the experimental temperature range. This is midway between the initial inactivation temperature of the pathogen 47 °C for *C. jejuni* (Hazeleger et al., 1998) and the final temperature selected for the heat treatment 65 °C. This was to minimise the uncertainty in the estimation of *D* value (Poschet et al., 2004)).

6.5 Statistical analysis and software

Parameter optimisation was accomplished using the MATLAB Optimisation Toolbox (MathWorks Inc. Natick, MA, USA). The Trust-Region-Reflective algorithm was used to

estimate the kinetic parameters. This is an iterative technique that locates the minimum of a multivariate function expressed as the sum of squares of non-linear real-valued functions. Initial guesses are made for the true value of the parameters. (Please see the appendix D for the MATLAB code used in this study). The standard errors of the estimated parameters were calculated by the Jacobian matrix (Valdramidis et al., 2008).

6.6 Results and discussion

The D and z values calculated at the reference temperature of 56 °C (the mid-point selected from the experimental values of 47 - 65 °C) were in the range of 30 - 40s and 8.7 - 10.2 °C, respectively. The results are summarised in Table 6.1 and Figures 6.1 - 6.4.

Table 6-1 D and z values estimated from inactivation data at 56 °C with two different heating time points (8 & 18 min).

Strain	$D_{56°C}$ (s)	SE	z (°C)	SE	N_0 (log cfu g^{-1})	SE
ST-474$_P$	37.6	3.9	10.2	1.3	8.6	0.3
ST-474$_H$	30.8	1.8	9.2	1.1	9.3	0.2
ST-48$_P$	39.8	2.2	8.7	0.7	9.5	0.2
ST-48$_H$	38.3	2.3	9.6	0.8	8.9	0.2

These results are in general agreement with the results obtained for the same isolates by the isothermal approach (Tables 5.1, 5.2) and do not indicate that the isolates are heat resistant. Therefore, the heat resistance of key NZ *C. jejuni* strains appears broadly to agree with most internationally reported values (Adams and Moss, 2000, Forsythe, 2000, Ray, 2014, Juffs and Deeth, 2007).

However, the z values listed in Table 6.1 (ranging from 8.7 - 10.2 °C) are higher than the isothermal published data (5 – 8 °C) (Forsythe, 2000, Juffs and Deeth, 2007) but are lower than the isothermal extrapolated z value (12.3 °C) published in the study by (van Asselt and Zwietering, 2006). The variation of z values reported internationally is well recognised, and in one study the z value of Listeria was reported as 29 °C (Fang and Lin, 1994) which is three to four times higher than the values published in the literature (Ryser and Marth, 2007).

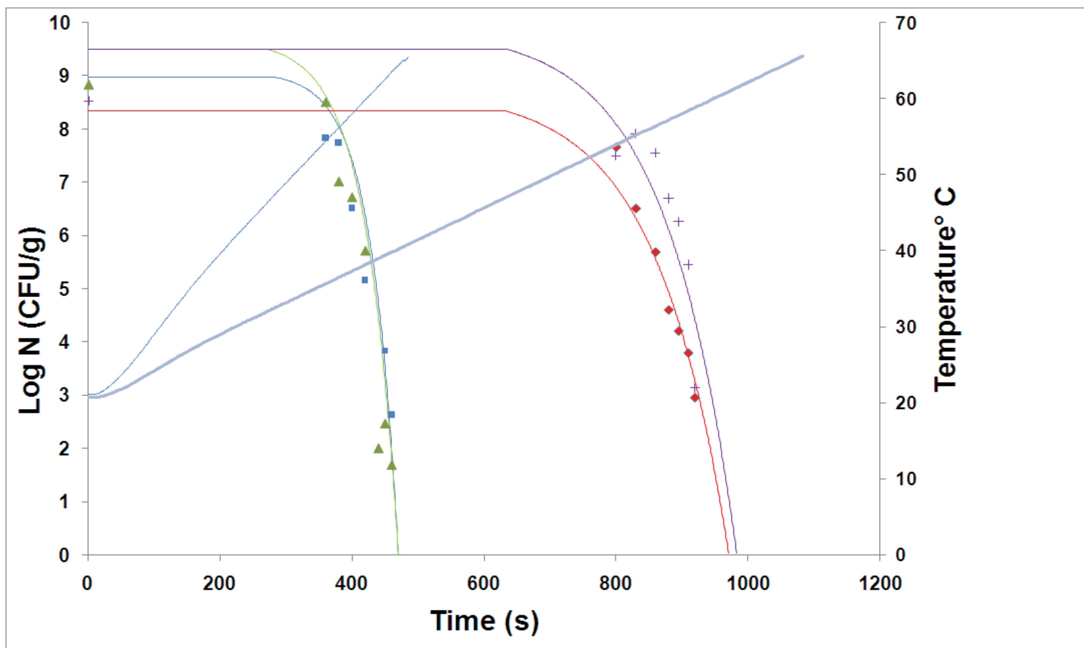


Figure 6-1 Thermal inactivation of *C. jejuni* ST-474P under dynamic conditions with two different heating profiles, 8 and 18 min (two straight lines).

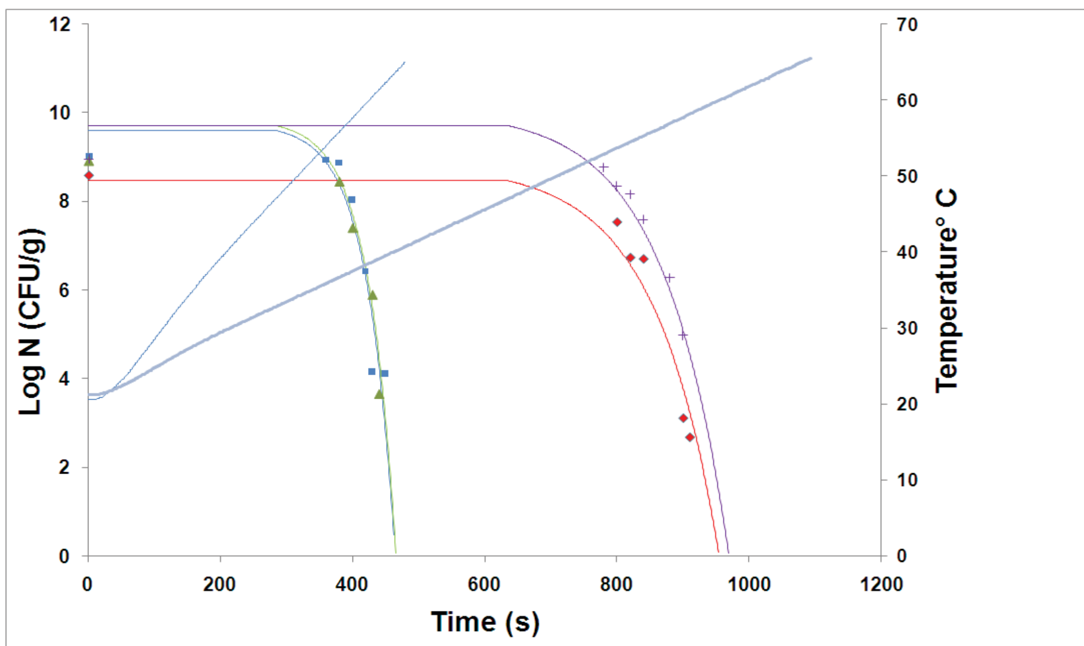


Figure 6-2 Thermal inactivation of *C. jejuni* ST-474H under dynamic conditions with two different heating profiles, at 8 min and 18 min.

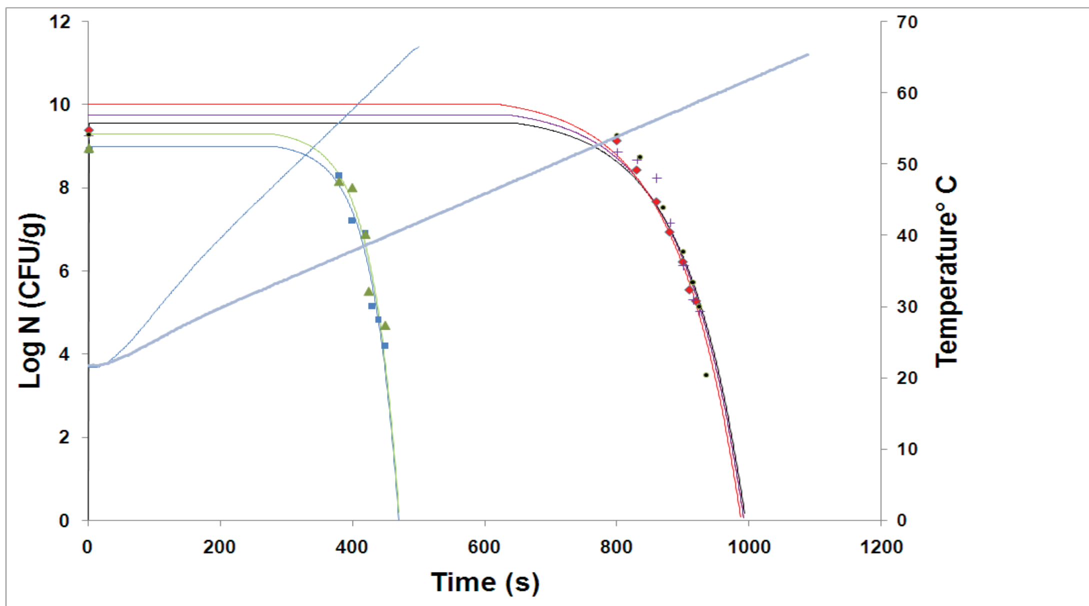


Figure 6-3 Thermal inactivation of *C. jejuni* ST-48P under dynamic conditions with two different heating profiles, 8 and 18 min.

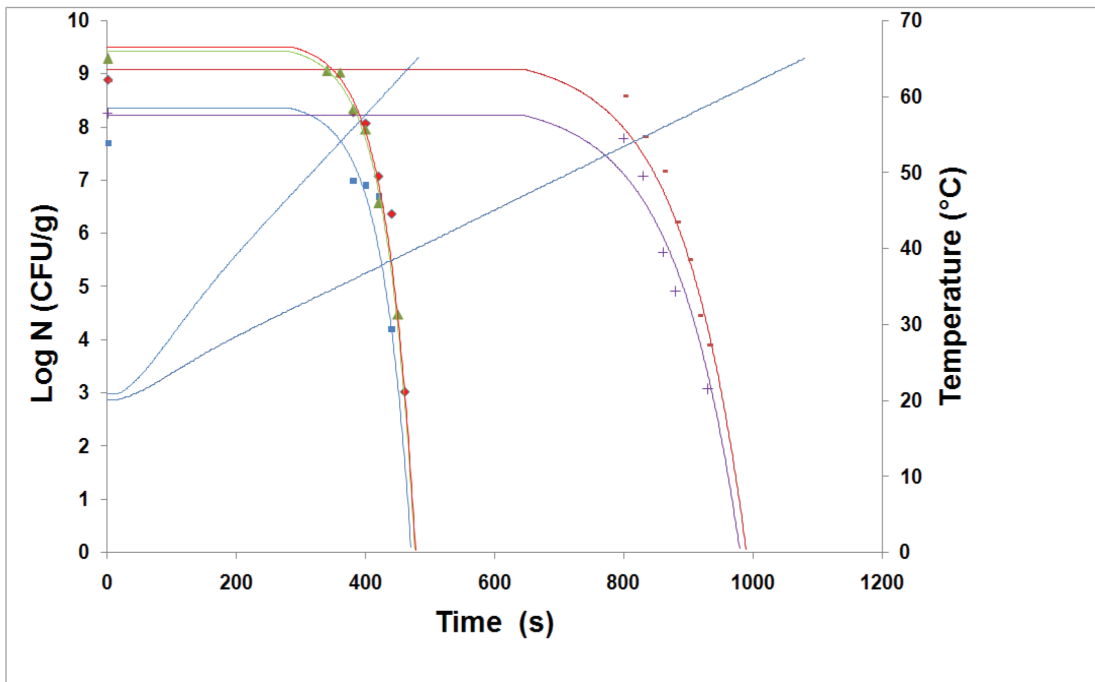


Figure 6-4 Thermal inactivation of *C. jejuni* ST-48H under dynamic conditions with two different heating profiles, 8 and 18 min.

This partial disagreement of the results of this study with the international isothermal data in terms of the estimated *z* values is due to many factors such as the strains used in the study, experimental

conditions (e.g. heating media directly or indirectly), cooling after heating, and the equipment or laboratory media used (particularly if in the presence of salt or acid), the growth phase of the cells, and other factors (Pflug, 1990). Most published data for *Campylobacter* were reported during the 1980s with the then available methods, instrumentation and techniques with regard to strain typing, isolation, recovery, enumeration, inactivation and incubation. The standard errors observed in this study for *D* and *z* values are 1.7 - 3.8 s and 0.7 - 1.2 °C respectively. Few recent studies have addressed the heat resistance of *Campylobacter jejuni* but none of these studies have reported the D values neither at constant temperature nor at dynamic temperature.

Sampers et al. (2010) found the 4.5 log cfu/g of *C. jejuni* inoculated to chicken burgers were reduced to below the detectable level (<10 cfu/g) after four min frying to an internal temperature of 57.5 °C and 2.5 log cfu/g of naturally contaminated chicken burger reduced to below detectable level after four min frying to an internal temperature 52.1 °C. Moreover, *C. jejuni* was not detected after simulated home pan-frying of artificially inoculated steaks or filets, hamburgers and meat strips (Lahou et al., 2015). It is noteworthy to indicate that this study (Lahou et al., 2015) has reported D values for the tested *C. jejuni* strains in broth medium only before inoculating the tested strains to the different meat types. All meat samples were from various animal species such as the pig, cattle, chicken, lamb and some turkey, horse, crocodile and kangaroo were inoculated with 4 log cfu/g of *C. jejuni*. Only after a further enrichment step *C. jejuni* was detected in few samples of pork hamburger, horse steak and crocodile steak. This is probably due to the different nutritional composition (e.g. fat content), texture (e.g. fibre structure) of the different meat types.

The *D* and *z* values estimated under dynamic condition are considered to be more precise and straightforward as they are i) derived from a complete temperature range, ii) estimated by one step regression approach with less uncertainty (smaller SE values), and iii) obtained with less experimental effort (Dolan et al., 2007). However, more validation is required before the estimated parameters are incorporated in subsequent thermal treatment calculation since the industry has a very good record on the use of the thermal parameters derived from the isothermal approach.

The estimation of the parameters under dynamic conditions overcomes the problem of calculating the 'come-up time' or the temperature transient (conduction) as the sample temperature is raised gradually from ambient temperature to minimises the delay that occurs in heating the sample.

6.7 Conclusions

The obtained *D* and *z* values for NZ strains under dynamic conditions are broadly in agreement with the published international isothermal data. Hence the tested NZ *Campylobacter* strains do not indicate that they are more heat resistant than other strains. The parameters obtained under

dynamic conditions are more robust than the isothermal parameters and such information can be derived with less experimental effort. A question that needs to be addressed is, are the NZ strains similar to the international strains in terms of their heat resistance, and then what their ability is to survive under other environmental conditions such as atmospheric gas atmosphere. It is known that *Campylobacter* is strictly microaerophilic, and this question is addressed in the next chapter.

7 Survival of *C. jejuni* under oxidative stress at different temperatures

7.1 Introduction

Campylobacter is considered as the most common bacterial causative agent of gastrointestinal disease in humans (Allos and Acheson, 2001). *Campylobacter* strains survive well in modified atmosphere and vacuum packaging but poorly at atmospheric oxygen concentration (~21%) (Kelly, 2008). *Campylobacter* spp. are harboured in many wild and domestic warm-blooded animals. These pathogens do not survive well in the environment when compared to other bacterial foodborne pathogens, such as Listeria and Salmonella. These organisms are unable to grow in the presence of air, and traditionally, a microaerobic mix (5 % O_2, 10 % CO_2 and 85 % N_2) and high temperature (42°C) have been used for the isolation of the thermotolerant *Campylobacter spp.* (*C. jejuni, C. coli, C. lari* and *C. upsaliensis)* (Garenaux et al., 2008, ICMSF, 1996). Many studies have investigated the pathogen's survival under oxidative stress, which is defined as cell damage leading to cell death caused by excessive levels of oxidant and free radicals in the environment including both free oxygen and compounds such as H_2O_2, hydroxyl radicals and superoxide anions. These are summarized in the literature, for example, the publication of the International Commission on the Microbiological Specifications for Foods (ICMSF, 1996) . Studies that have investigated the growth of *Campylobacter* under different gas compositions with different techniques and substrates are in general agreement with the proposed optimum composition (5 % O_2, 10 % CO_2 and 85 % N_2) (Grigoriadis et al., 1997, Koidis and Doyle, 1983). However, it remains unclear exactly how this pathogen survives the high oxygen level in the atmosphere and the low ambient temperatures during transmission to its human host.

There are other studies that have reported the survival of *Campylobacter* strains on poultry and meat is not influenced by the storage atmosphere or the treatment condition (vacuum packing) at 4°C (Hanninen et al., 1984, Wesley and Stadelman, 1985). Few studies reported that the presence of O_2 increased the death rate at 4°C in broth and milk (ICMSF, 1996). A New Zealand study revealed that *C. jejuni* could adapt to aerobic growth after a repeated subculture. In this study, about 81% of human, 75% of water, and 65% of poultry *C. jejuni* isolates were capable of growth under aerobic conditions in Nutrient Agar, and a cocktail of isolates (human, poultry, veterinary) survived aerobically for more than four weeks in poultry meat at 5°C and for more than a week at 25°C (Chynoweth et al., 1998). It was reported that *C. jejuni* isolates (10 human and nine poultry) could

survive up to four weeks at 4°C aerobically in Mueller Hinton Broth and sterilised chicken rinse with a strain variation from one week to four weeks (Chan et al., 2001).

Yamasaki (2004) reported that one log of the initial *C. jejuni* inoculums (9.5 log) decreased in concentration after the first six hours under aerobic conditions at 37C° followed by another one log reduction at the end of next six hours. They also observed that a rapid reduction by about 5 log occurred between 12 and 15 h of incubation. Another study reported the survival in broth and agar plates for poultry and human isolates at three different temperatures (4, 25, and 42 °C) but the data were collected only for a one week period (Garenaux et al., 2008). Another study also indicated that the exposure to atmospheric oxygen in broth culture for five and 15 hours did not affect the *C. jejuni* growth and the count was similar to that of *C. jejuni* incubated under microaerobic conditions (Mihaljevic et al., 2007). This is consistent with other reports regarding the growth and adaptation of *C. jejuni* in aerobic metabolism (Harvey and Leach, 1998, Jones et al., 1993). One study investigated the survival of *C. jejuni* under oxidative stress in broth and agar plates but only at 37°C (Kaakoush et al., 2009).

From all of the above studies, it is concluded that there is ambiguity about the behaviour of *C. jejuni* under oxidative stress. This may confirm the capricious nature of such bacterial organisms due to the high variation found between tests and discrepancies between the reported results of the different studies. Evidently, *Campylobacter* causes more difficulties in standardization and handling of this pathogen, than are seen with any other pathogen (Bergsma et al., 2007, Jasson et al., 2007). It is believed that NZ has the highest rate of reported campylobacteriosis cases in the world. In 2006, the notification rate was 422 per 100,000, thus an increase of 56% from 2001, and 14% since 2005. This is 35 times higher than the rate for the United States, four times higher than the rate for Australia, and five times higher than the rate for the U.K. One possible reason for the high rate of campylobacteriosis in NZ could be that local strains have a greater ability to survive under processing, storage and handling conditions in the country (Hansen et al., 2003).

The objective of this study was to investigate the behaviour of selected NZ isolates of *C. jejuni* under varying conditions of oxidative stress and temperature. The strains chosen were those commonly found in human cases of campylobacteriosis and contaminated food samples in NZ. Thus, the study will focus on those relevant isolates, in order to provide a fast response for the scientific community and risk managers in NZ, with regards to the isolates' aero-tolerance and resistance to oxidative stress. The provision of a fast response by this study will confirm the scientific, academic capacity of the University in NZ, as the response of a non-academic scientific institution in NZ can be usually obtained in a timely manner. A comprehensive study using more NZ isolates and international isolates is planned if the chosen isolates showed aero-tolerance or

resistance to oxidative stress. Tough NZ biosecurity measures regarding the importation of biological hazards and the resulting possible delays in the administrative process have prevented the inclusion of any international isolates in this study.

7.2 Materials and methods

7.2.1 *Campylobacter* culture growth and enumeration

Campylobacter strain cultures of ST- ST-474, ST-48, ST-190 were prepared as it was described in section 3.2 in details to obtain a plate with prolific growth for inoculum preparation. Each inoculum for testing heat resistance was prepared by transferring all colonies from a CA plate into Brain Heart Infusion broth (BHI). The final culture concentrations used for inoculation were in the range of 10^7 to 10^9 cfu ml^-. The determination of inoculum concentration was conducted by a serial dilution of the inoculum in BHI followed by plating in triplicate on mCCDA by the Surface Plate Method (Downes and Ito, 2001).

7.2.2 Exposure of *C. jejuni* to oxygen in broth

As it was described in detail in section 3.6.1 an inoculums of 3 ml were added to 27 ml BHI which was then dispensed to form a shallow layer of 30 ml in a 125 ml conical flask equipped with a special bung (Bug stopper, Whatman) that allows air exchange with the surrounding atmosphere but prevents microbial contamination. This flask was allowed to equilibrate at the appropriate temperature (4, 10, and 20 °C) atmosphere (aerobically or microaerobically) by gentle shaking using an electrical shaker (Lab-line Junior Orbit Shaker, U.K). The number of surviving organisms in samples withdrawn from each flask at predetermined intervals after exposure to the controlled atmosphere conditions at each temperature was determined by dilution in BHI followed by plating on mCCDA.

7.2.3 Exposure of *C. jejuni* to oxygen on agar plates

As it was described section 3.6.2 in details, a 50 µl of the culture suspension in BHI was spread on CA plates) and incubated aerobically at 4 °C and 25 °C for one to five weeks. The CA control plates containing the culture spread were simultaneously incubated under microaerobic conditions at 4 °C and 25 °C using anaerobic plastic jars with a microaerobic atmosphere generating system (Pack MicroAero, Mitsubishi Gas Chemical Co. Inc). Two plates from each aerobic condition were sampled every day and incubated at 41.5 °C for 48 h in a microaerobic atmosphere.

7.2.4 Exposure to H_2O_2

As described in detail in section 3.6.3, each culture suspension prepared in BHI was spread with a swab on Muller Hinton Agar plates and allowed to grow under microaerobic conditions for 48 h. Subsequently, filter discs (6 mm) inoculated with 10 µl of 1 mM, 10 mM, 100 mM, or 1 M hydrogen peroxide were placed onto the plates (Disc Diffusion Method).

7.2.5 Statistical analysis

All bacterial counts were log10 transformed prior to statistical analysis. The dataset was analysed using R software (version 2.9.1). Statistical differences between isolates, incubation atmosphere, time and temperature were undertaken by ANOVA, with P = 0.05 used as the statistical threshold for significance for only the survival experiment in BHI due to large amount of data generated from the experiment. The appropriate analysis of the data set as provided was to determine how the treatments were allotted and therefore linked to the observed values. The different treatment combinations were allotted to different number of plates and replicates. Measurements were therefore taken on plates and/or replicates in an unbalanced manner for the model that incorporated both fixed and random effects. The linear model was expressed graphically, and the slope of these lines (for each temperature and the individual isolates) were identified.

7.3 Results and discussion

7.3.1 Exposure to oxygen in broth.

The results from exposure to oxygen in BHI from the duplicate plates at all temperatures (4°C, 10°C and 20°C) are shown in Figure 7.1 - 7.5, where Figure 7.1 is for the human isolates and Figure 7.2 for the poultry isolates. All constructed linear model slopes for each data set are shown in Table 7.1. The results from the ANOVA analysis using R software is shown in Table 7.2. At every temperature, the poultry isolate survived longer than the human isolates. The human isolates did not show differential survival between the atmospheres, suggesting they were less sensitive to oxygen than the poultry isolate.

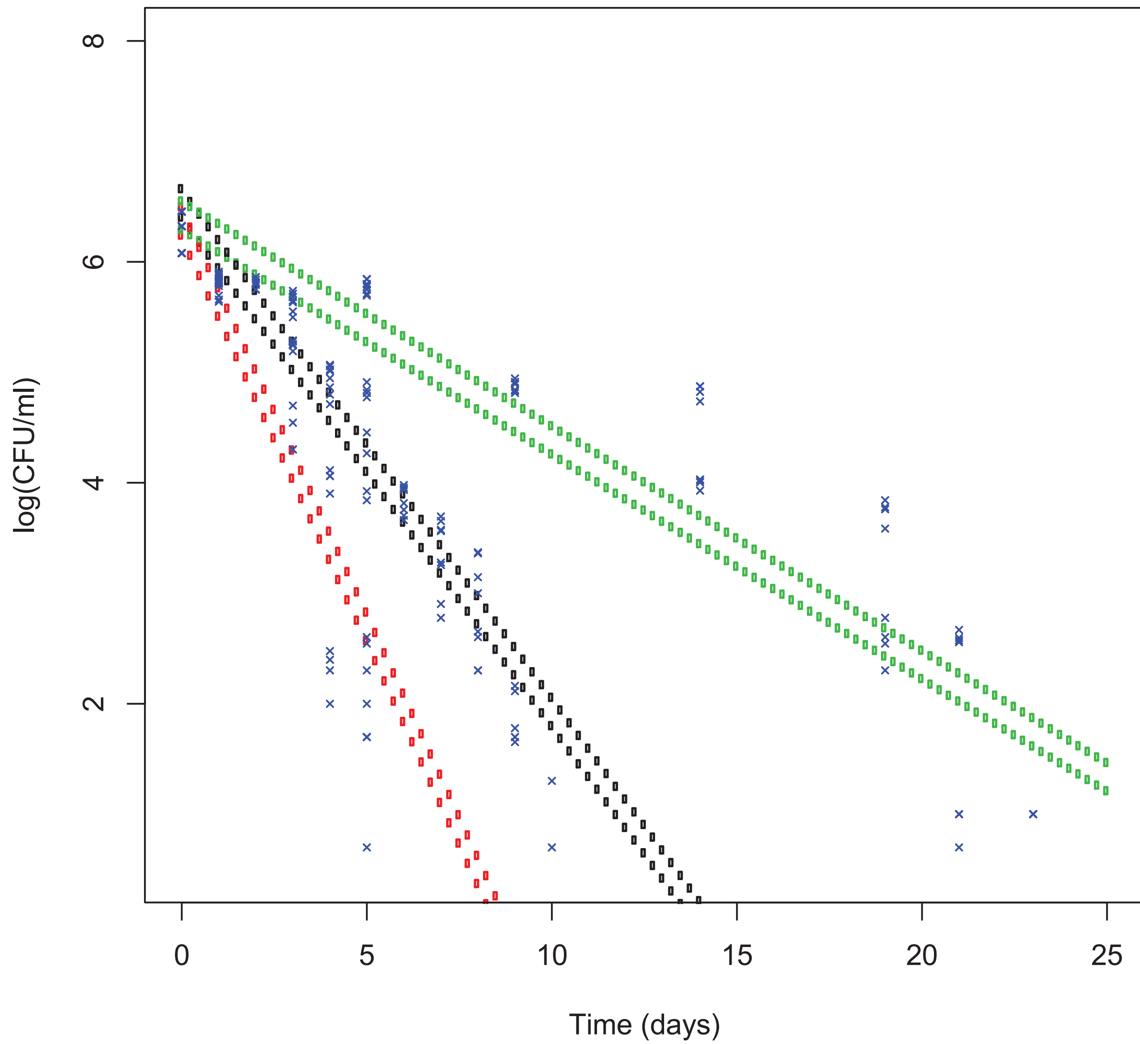


Figure 7-1 The linear model fitted to the observed data for survival of human *Campylobacter jejuni* isolate (474) in broth stored at three different temperatures (green colour for 4°C, black for 10°C and red for 20°C) and two different atmospheres (the upper line of the two lines for microaerobic and the lower line for aerobic).

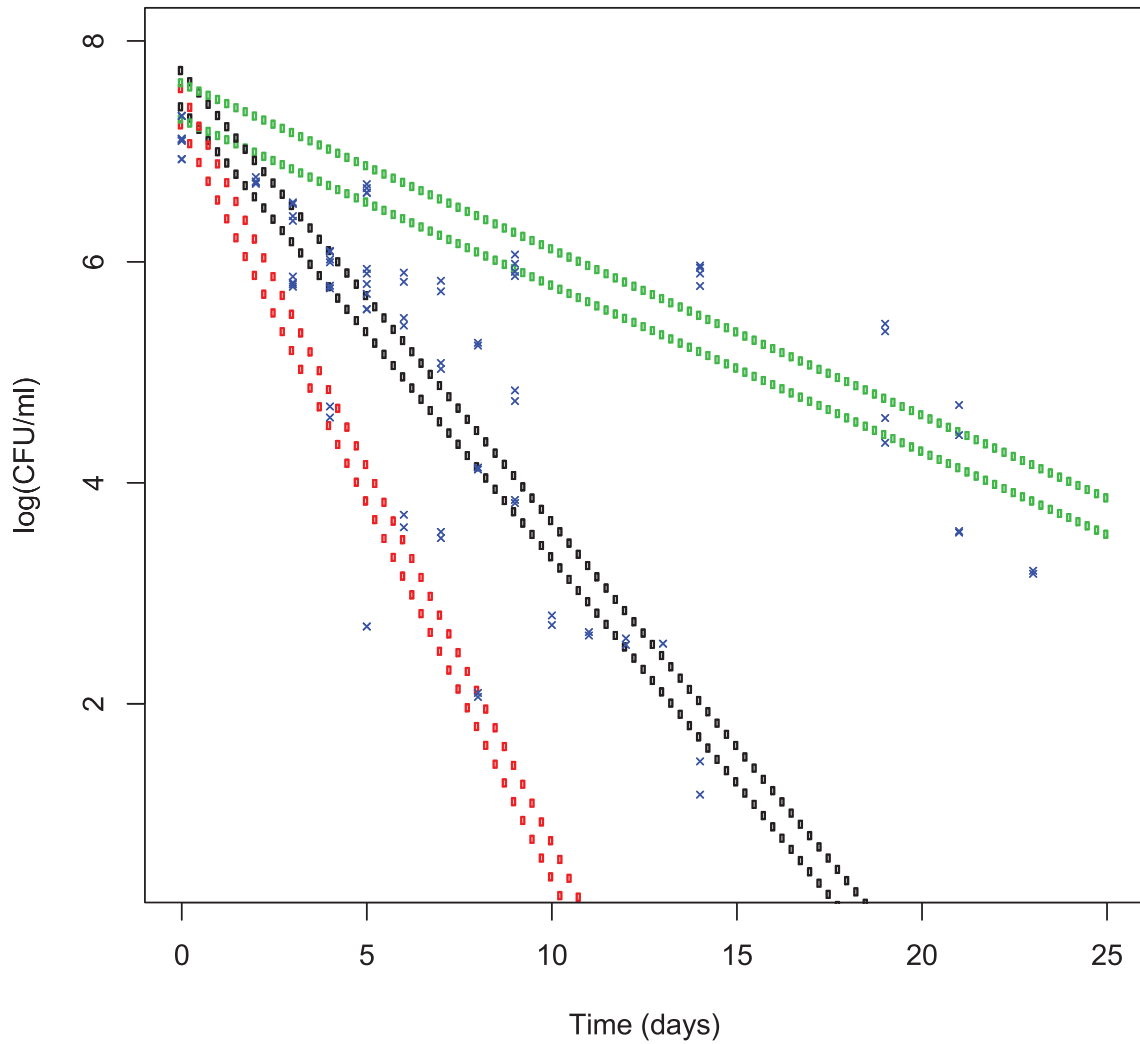


Figure 7-2 The linear model fit to the observed data for survival of poultry *Campylobacter jejuni* isolate (474) in broth stored at three different temperatures and two different atmospheres.

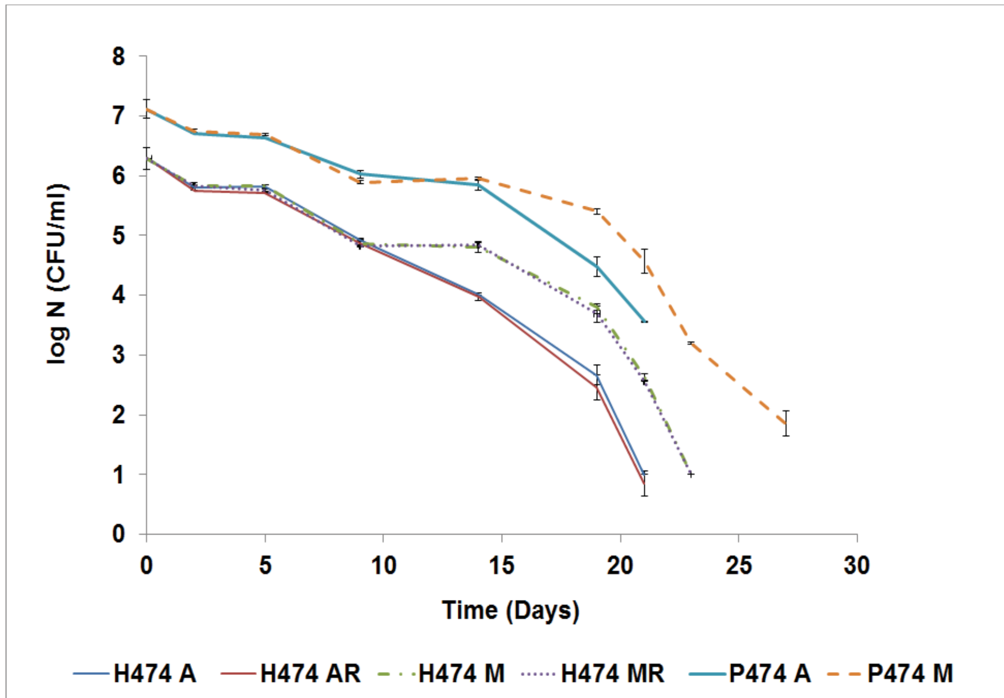


Figure 7-3 Survival of *C. jejuni* isolates at 4°C (H474 refers to human isolates 474, P474 refers to poultry isolates 474, A aerobic incubation, M refers to microaerobic AR/MR to aerobic/ microaerobic replicate data).

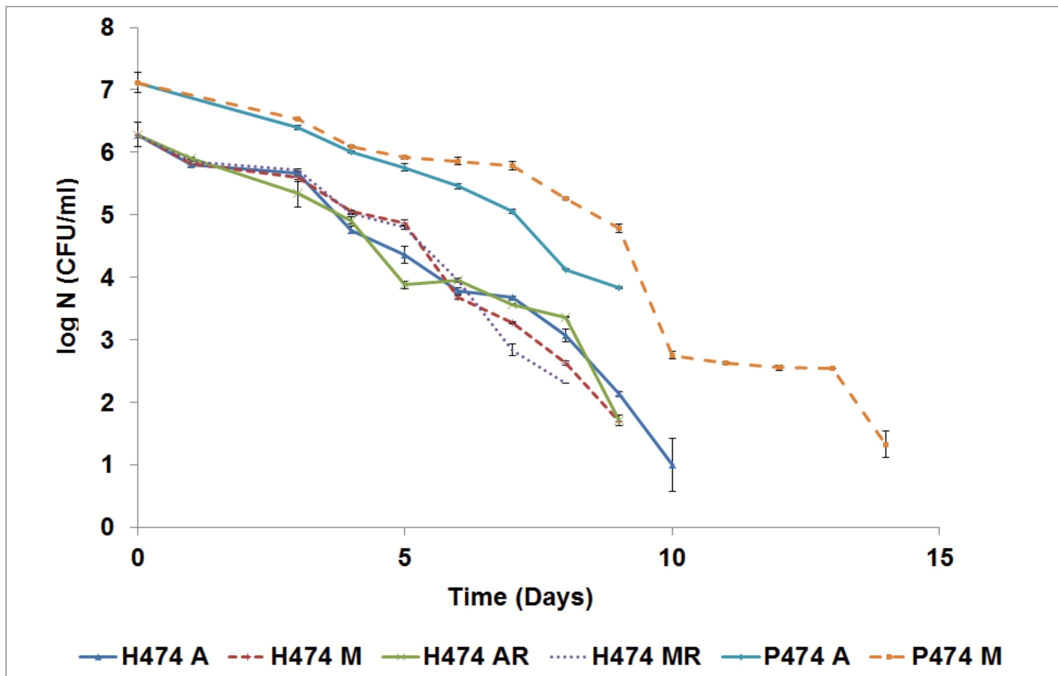


Figure 7-4 Survival of *C. jejuni* isolates at 10°C

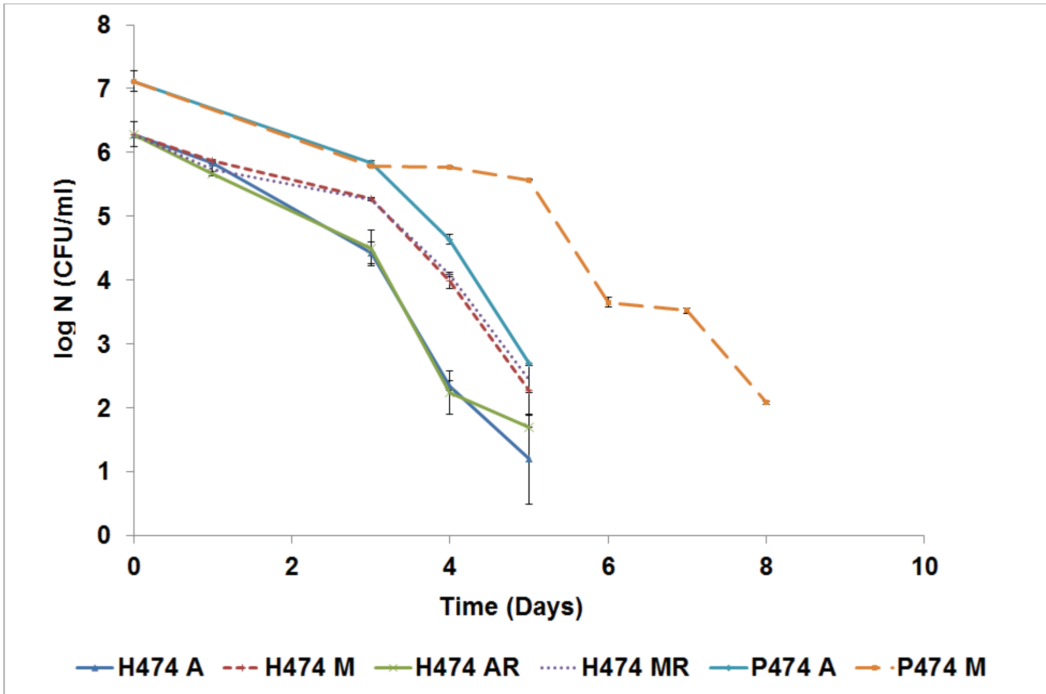


Figure 7-5 Survival of *C. jejuni* isolates at 20°C

Table 7-1 Slope values for the linear model shown in Figure 7.1 and Figure 7.2

Temperature (°C)	**Human**	**Poultry**
4	-0.20336	-0.15033
10	-0.46046	-0.40743
20	-0.7338	-0.68077

Table 7-2 Results of two-way analysis of variance for effects of storage atmosphere, isolates, and temperature on survival of *C.jejuni* in broth.

	numDF	denDF	F-value	p-value
(Intercept)	1	228	20015	<.0001
Isolates	1	54	160	<.0001
Atmosphere	1	54	2	0.1638
Temperature	2	54	40	<.0001
Time	1	228	1411	<.0001
Isolates:Atmosphere	1	54	3	0.1145
Temperature:Time	2	228	219	<.0001
Isolates:Time	1	228	14	0.0002

7.3.2 Exposure to oxygen on agar plates.

The results from exposure to air in agar plates confirmed that survival was significantly longer at low temperatures compared to high. However, on agar, both poultry and human isolates survived longer microaerobically than aerobically. The data are summarised in Table 7.3. There was no significant difference between the survival of the poultry and human isolates when differences in the initial concentrations are accounted for.

Table 7-3 Effect of temperature and storage atmosphere conditions on survival (in days) of selected New Zealand *C. jejuni* strains on agar plates

Isolates/strain	At 4 °C		At 25 °C	
	Aerobic	Microaerobic	Aerobic	Microaerobic
ST-474$_{H}$	21	>32*	6	7
ST-474$_{P}$	18	23	4	7
ST-190$_{H}$	22	>32	5	6

* The experiment was ended at 32 days. (ST-474$_{H}$ refers to human isolate and ST474$_{P}$ refers to poultry isolate)

7.3.3 Exposure to H_2O_2.

The exposure to H_2O_2 revealed that all the selected NZ *C. jejuni* strains (ST-474, ST-48, and ST-190) tested were sensitive to all the concentrations tested, except for the lowest concentration of 1 mM (Figure 7.6).

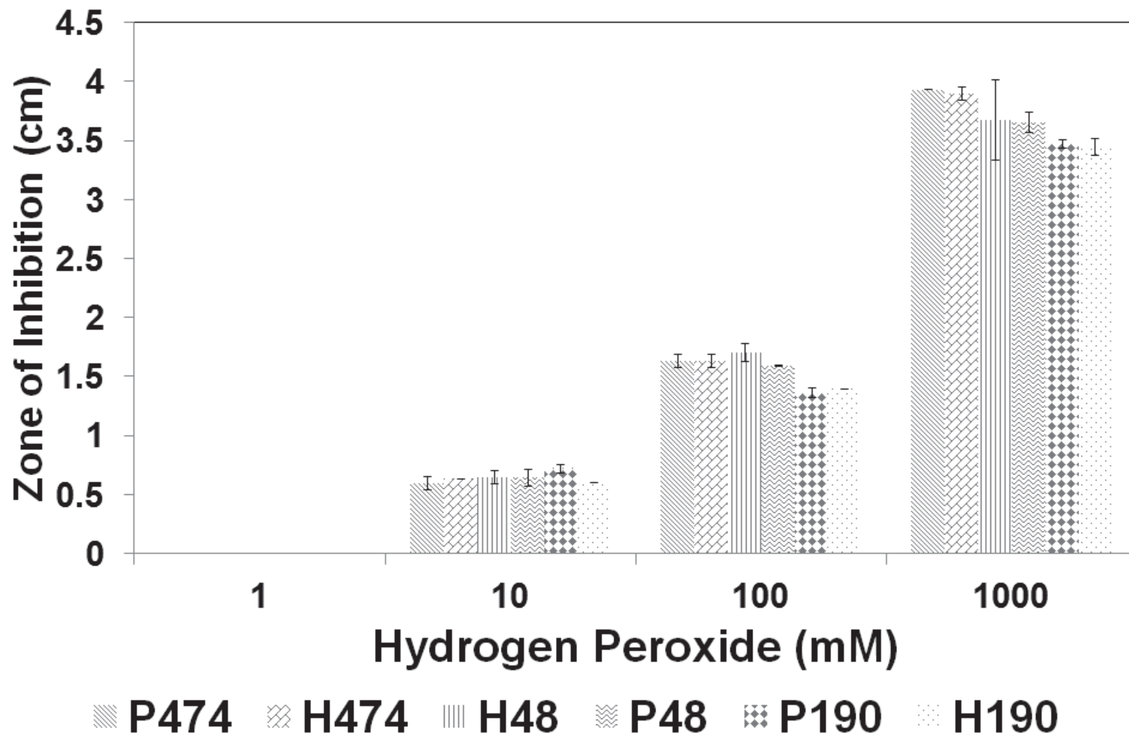


Figure 7-6 Susceptibility of C. *jejuni* isolates to different hydrogen peroxide concentrations in filter discs on MH agar.

Most of the data reported and the *Campylobacter* survival studies in the ComBase database for predictive microbiology were either for a short period exposure to a specified temperature or investigations of more than two factors (temperature, atmosphere) targeted by this study, such as the addition of NaCl, CO_2 and other stress factors. Therefore, the comparison of our results to the international data which used similar experimental conditions used in my study is limited. The results obtained in this study are in agreement with the findings of (Garenaux et al., 2008) especially at 25°C and at 4°C for the first-week data and also with those of (Chan et al., 2001, González et al., 2009) at 4°C. The results obtained in this study at 4°C are somewhat in agreement with the results of Yoon (2004) in broth at the same temperature aerobically in terms of the first reduction which occurred after nine days of incubation compared to eleven days in Yoon (2004) study. This marginal difference of two days is possibly due to the agitation employed in this study which was not practised in Yoon's study. It is well known that the agitation creates strictly aerobic conditions

which may enhance the pathogen death rate (Meredith et al., 2014, Butzler, 2014). Yoon's study was planned to simulate semi aerobic conditions or mixed aerobic and anaerobic conditions. Moreover, this study results are in agreement with a NZ study for chicken (Chynoweth et al., 1998) although the chicken meat used by Chynoweth et al. (1998) as a substrate or a medium may have influenced the results to some extent of that study. In fact, NZ strains survive less than the international strains, especially in within the 20 - 25°C range. Blankenship and Craven (1982) reported that survival of sterilised ground chicken at 23°C was for > three weeks whereas NZ strains investigated in my study survived aerobically at 25°C for only four to five days in the agar plates and up to 7 days at the same temperature in microaerobic environments. This may be due to the different strain and different matrices used in the two studies.

In general, there are not many differences in H_2O_2 sensitivity between the poultry and human isolates of each strain. These results are in agreement with a study published internationally with regards to the diameter of the inhibition zone (Fields and Thompson, 2008) Despite a report that *C. jejuni* has the good adaptive ability to H_2O_2 stress (van Vliet et al., 2002), this study results confirmed that the tested NZ *C. jejuni* strains were sensitive to H_2O_2.

The investigation of the NZ strains tested in this study by three methods revealed that the air tolerance of the NZ strains was not different to that of the international strains (Chan et al., 2001, Garenaux et al., 2005, ICMSF, 1996) and the previously tested NZ strains (Chynoweth et al., 1998). The results of this study did not support the hypothesis that the *C. jejuni* survival was not influenced by the storage atmosphere aerobically or anaerobically, as the survival of *C. jejuni* was shorter in the agar and the broth experiments under aerobic conditions than when subjected to microaerobic conditions at all temperatures tested, except in the broth experiment at 10°C. After the fifth day, the survival rate was more or less similar at aerobic or anaerobic conditions. This is consistent with other reports regarding the growth and adaptation of *C. jejuni* to aerobic metabolism (Oh et al., 2015). However, the best known and fundamental hypothesis is that *Campylobacter* is a microaerophilic microorganism that requires a specific gas composition for its growth and survival (Butzler, 2014, Kelly, 2008, Gharst et al., 2013, ICMSF, 1996).

It is not yet completely understood the biological aspects and mechanisms responsible for microaerobic growth or better survival at a molecular level and the processes that occur at the physiological and genetic level. It is assumed that due to the inhibition of enzymes by a higher concentration of O_2 (more than the maximum level 10%), the vulnerability to reactive oxygen species (ROS) and /or metabolic generation of free radicals are all capable of reacting with molecular components of cells to disturb their function in addition to producing more toxic components (Krieg and Hoffman, 1986, Kaakoush et al., 2009). The poultry isolates survived

longer than the human isolates especially at the incubation temperatures of 10° and 20°C, but there was no significant difference between the survival of the poultry and human isolates when differences in the initial concentrations were accounted for at the incubation temperature 4°C.
It is revealed from this study that the survival of *C. jejuni* from oxidative stress is more influenced by the temperature rather than the incubation atmosphere. At a low temperature of 4°C, there was less effect on *C.jejuni* survival and was able to survive for more than four weeks in both broth and agar experiments. This was assumed by Gareneux (2008) and Hazelegar (1998) to be due to a less active metabolism and a decrease in catalytic activity, or oxygen being less toxic for cells at 4°C. Despite the fact that this study was conducted with chicken meat, the combination of three methods to address the oxidative stress on *C. jejuni* increased the validity of the results reported by this study and saved time, resources and cost. Practically, international data on the survival of *C. jejuni* have been reported mostly for short periods and for above freezing temperatures for two days (Solow et al., 2003, Zhou et al., 2011) or for one to two weeks (Bhaduri and Cottrell, 2004, Garenaux et al., 2008, Davis and Conner, 2007, Oyarzabal et al., 2010). Moreover, the results obtained by this study are in agreement with the results reported in NZ with chicken mince and chicken nuggets (Chynoweth et al., 1998). A few studies (Byrd et al., 2011, Meredith et al., 2014) evaluated the effects of modified storage atmosphere and chilling on the survival of naturally occurring *Campylobacter* in raw poultry. For Byrd et al. study (Byrd et al., 2011) the modified packaging atmosphere applied for the stored chicken (n = 16) at 2 °C were 100% O_2, normal air, 85% N_2+10% CO_2+ 5% O_2 and 100% CO_2. *Campylobacter* was not detected after 14 days of storage in 13 out of 16 chicken samples stored with 100% O_2(Byrd et al., 2011). Similarly, *Campylobacter* was detected in the 12 out of 16 chicken exposed to normal air and in 9 out of 16 chicken samples treated with 85% N_2+10% CO_2+ 5% O_2..(Byrd et al., 2011). But, only in 7 out of 16 chicken samples, the *Campylobacter* was detected in chicken treated with 100% CO_2 (Byrd et al., 2011). Therefore, the comparison and discussion of this study are limited because there is only a limited amount of international data which had used similar experimental conditions to this study. Similarly, the results obtained by Garenaux et al. (Garenaux et al., 2008) using the Colombia agar plates were similar to the results of (Bhaduri and Cottrell, 2004) at 4°C using chicken mince or chicken skin. Both studies reported that at 4°C *Campylobacter J.* survived for one week. In fact, the implications of the spoilage of chicken meat samples or chicken skin samples during storage of the samples above 4° C hinders the use of a food matrix in long survival studies. Moreover, the use of irradiated skin or chicken meat samples eradicates the microflora (pseudomonads, micrococci, Staphylococci), which are naturally found in poultry and inhibits the growth of *Campylobacter* (Mai, 2003). The conclusion from such studies is that irradiated chicken meat must be treated with

caution due to the absence of microflora. Thus, the results of this study rejected the hypothesis investigated in this study, that the high rates of campylobacteriosis in NZ may be due to the emergence of unusual new strains with more oxygen tolerance, or that they have a unique survival ability at varied storage or handling temperatures (4, 10, 20, and 25 °C). Given the infectious dose of illness is about 500 cells (Robinson, 1981) and the length of time that *Campylobacter* can survive as revealed by this study for NZ strains which is more than four weeks This is a significant finding from a health perspective as the shelf life of a fresh chicken is eight days at 4°C. Thus, the contamination level of fresh poultry products will persist without any significant reduction until it reaches the kitchen of a consumer, and this poses a risk to the consumers. Poultry processing plants should apply the necessary intervention to ensure that chicken carcasses and poultry products are released to the retail market with as low a contamination as practically possible, and theoretically, the counts should not exceed 500 cells per serving of chicken portion.

The linear model constructed by this study is acceptable given the empirical nature of the Weibull model with its two parameters (shape η and scale δ), and the Weibull model, with a fixed parameter shape model, is only as good a fit as the log-linear model and only partially succeeded in the prediction of the tested *Campylobacter* data (Oyarzabal et al., 2010, Al-Sakkaf and Jones, 2012).

7.4 Conclusion

The results obtained from all the oxidative stress conditions are similar and do not indicate that the NZ strains differ in oxygen tolerance when compared with most of the other internationally reported strains at the investigated temperatures. At 20 or 25°C, NZ selected strains survived in media only up to one week aerobically, but at 4°C, they survived aerobically for more than three weeks. New Zealand higher rate of reported campylobacteriosis compared to other developed countries is possibly due to other factors, and these are explored in the next chapter(s).

Chapters 8 and 9 Embargoed until 31 December 2022

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10 Quantitative microbial risk assessment for campylobacteriosis in New Zealand by the Bayesian approach

10.1 Introduction

For more than a decade, QMRA has been an important tool for food safety strategic planning and control. It has been promoted by international organisations and national organisations worldwide such as WHO, FAO, EFSA, USDA, FDA, WTO, and Codex Alimentarius. QMRA provides a unified approach to a public health problem. QMRA not only predicts the consequences of the disease acquired by the consumption of contaminated food but also provides a prior assessment of any possible intervention in the food chain. However, QMRA predictions of human health cases have always been higher than the number of actual reported cases and the estimates of epidemiological studies. This is due to the uncertainties of estimating QMRA parameters. For example, the lack of a reliable dose-response model, failure to account for the variability of immunity within the population, and the estimation of prevalence/concentration of pathogens through all the steps or pathways of the food chain, all of which influence the accuracy of the QMRA outcomes (2008). Many QMRAs were conducted using the Monte Carlo (MC) approach. Many iterations are required to obtain reliable and accurate results from the MC method. This can be a time-consuming process. However, simulation of these models by the Monte Carlo approach is relatively easy to implement using commercial software to automate the tasks and can produce acceptable results (Robert and Casella, 2004). Another drawback of the MC approach is that this technique performs poorly when modelling rare events (Vose, 2008).

Bayesian belief networks, approaches and inference have gained increased interest from researchers in recent years to calculate the outcomes of QMRA. Bayesian inference has many advantages such as being able to accommodate missing data, accounting for the uncertainty in all estimated parameters, enabling the combination of data with the experts' prior belief or knowledge. The Bayesian approaches allow for the backpropagation of prior variables with the observed data, assist in understanding underlying relationships between variables, and provide a method for avoiding overfitting of data (Smid et al., 2010). The Bayesian approaches offer satisfactory prediction accuracy (Smid et al., 2010). The Bayesian inference prediction is consistent with the surveillance data, where MC techniques fail to predict the actual notified campylobacteriosis cases correctly. Several authors have reviewed the literature regarding the published QMRA models for *Campylobacter* (Nauta et al., 2009, Nauta and Christensen, 2011, Chapman et al., 2016). The NZ

QMRA model prediction was about ten times more than the actual notification value. Therefore, the application of the Bayesian approach and inference is valuable for NZ, since the NZ QMRA model (Lake et al., 2007) used MC simulation which prevents the inclusion of surveillance data necessary to evaluate the benefit of any future intervention in the food chain in terms of the change in the number of illnesses. The main drawback of the NZ adapted model is that it relies heavily on the data of international literature, which does not reflect the actual NZ scenario in poultry processing and also does not take into consideration the implemented interventions by the industry and the government.

Moreover, the challenges faced in general by the traditional QMRA approach used in the NZ model have probably contributed to the current model's inaccuracy (Havelaar et al., 2008, Smid et al., 2010). The objective of this study was to conduct QMRA by using the Bayesian Belief Network and inference approach in spite of the complexity involved in constructing and assigning probabilities from expert knowledge or prior information, linking the nodes, discretising the data, complex software and solving the resultant joint probability distributions. Hence, the priority will be to assess, illustrate and present the Bayesian approach for tackling the problem of campylobacteriosis in NZ. A simplified model representing the entire food chain from the 'farm to fork' was used.

10.2 Methods

10.2.1 The modelling approaches

The model developed by (Albert et al., 2008) in France for *Campylobacter* was adopted. The construction of the model and risk estimates are based on the classical Bayes' theorem, which can be presented in the case of two events as:

$$P(A \mid B) = \frac{P(B|A)P(A)}{P(B)} \qquad \textbf{10-1}$$

Where P(A|B) is the posterior probability (updated knowledge) of event A (unknown parameter), given that event B happens (observed data).

P(B|A) is the conditional probability of B given A, and it is the likelihood function for event A for a realised event B.

P(A) is the prior probability (prior knowledge) of event A indicating uncertainty about A before knowledge about B (data at hand).

P(B) is the normalizing factor ensuring the posterior probabilities sum to 1, and it is also the marginal probability of B. The posterior distribution P (A|B) is largely important as it expresses a

belief about A after obtaining data. It provides a solution to the problem of how our prior knowledge or estimate about an unknown parameter is improved using data.

Equation (10-1) can also be written as:

$$\text{Posterior distribution} = \frac{\text{likelihood x prior distribution}}{\sum \text{likelihood x prior distribution}} \qquad \textbf{10-2}$$

The objective of the Bayesian inference is to assess the total uncertainty based on all the available data and prior knowledge if it exists. The interpretation achieved by Bayesian inference can balance the model by completing gaps in the data. The Bayesian Belief Network model, as developed by Albert et al. (2008), consists of six sub-models, as shown in Figure 10.1. These are the chicken farm model, broiler production model, hygiene model, consumption model, exposure model and the illness model. Each sub-model is characterised by its variates, parent variates, complementary co-variates and parameters which are listed in Table 10.1. It is important to specify the joint distribution of the model using conditional independencies between some model parameters in constructing the typical Bayesian Network acyclic graph. Then the marginal distributions (priors) of the variates, complementary covariates and parameters must also be specified. Finally, the variates of interest are estimated from their posterior distributions after introducing the data to the model.

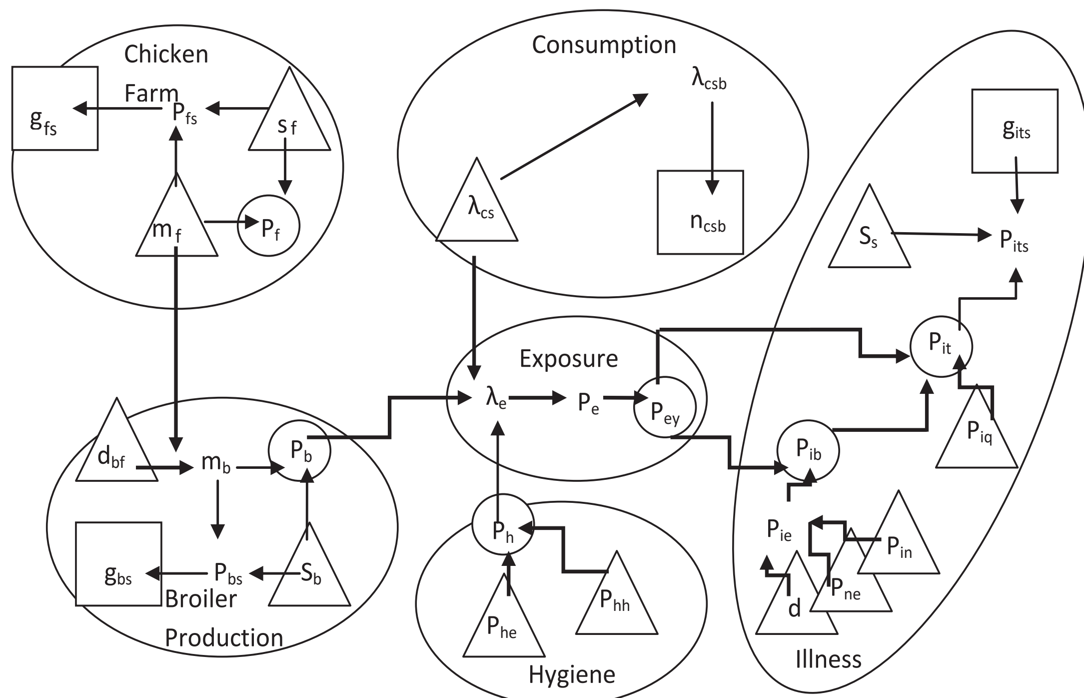

Figure 10-1 The complete model and the augmented model

adapted from Albert et al. 2008 with the variates (circled), parameters (triangles), variables and complementary variables expressed conditionally by their parent variates and linked by arrows (see Table 10.1 for the details)

10.2.2 Data sources

All of the data incorporated in this study were extracted from the scientific literature published in peer-reviewed journals for the priors rather than the exclusive expert opinions which include subjectivity and bias that may impact the validity of the QMRA and consequently affect the decisions made by the risk managers or policymakers based on the QMRA results. Reliance purely on expert opinion was avoided where possible or reduced as much as possible. Mostly NZ based studies were included for the priors, and international studies were included when the NZ data was either lacking or was of poor quality. However, NZ surveillance data or data from the daily microbiological monitoring programme, such as the National Microbiological Database (NMD) were used to improve the priors used in the core model. The parameters of interest were then conditionally estimated from their posterior distributions by the newly incorporated observed data (NMD, or surveillance data). The incorporation of the new data may require additional modelling to link them with the core model.

Table 10-1 Description of most variates (vi), parameters (pa), covariates (cv), data (da) and constant(co) used in the models depicted on Figure10.1

Class	Variate	Parent (s)	Distribution/Relationship
Vi	p_f	m_f,s_f	logit (p_f) ~ N (m_f , s f)
Vi	p_b	m_b, s_b	logit (p_b) ~ N (m_b, s $_b$)
Vi	p_h	p_{hc}, p_{hh}	= p_{hc} * p_{hh}
Vi	λ_c	-	Gamma(8, 2)
Vi	p_{ey}	p_e	=1 - (1 - p_e)^13
Vi	p_{ib}	p_{ey}	= p_{ey} * p_{ie}
Vi	p_{it}	p_{ey}, p_{ib}, p_{iq}	= p_{ib} / (1 - (1 - p_{iq})*(1 - p_{ey}))
Cv	m_b	m_f, d_{bf}	= m_f + d_{bf}
Cv	λ_e	p_b, λ_c, p_h	= p_b* λ_c* p_h
Cv	p_e	λ_e	= 1 - exp (-λ_e)
Cv	p_{ie}	d,p_{ne},P_{in}	$= 1 - (1 - p_e)^d) * p_{in}$
Pa	m_f	_	~ N (0,0.22), (0.511,20.66)
Pa	s_f	_	~ Uniform (0,0.2)
Pa	d_{bf}	_	~ N (0.1,0.0696)>0, (0.15,206.6)I(0,)
Pa	s_b	_	~ Uniform (0,0.2)
Pa	p_{hc}	_	~ Beta (8,8)
Pa	p_{hh}	_	~ Beta (22,20) & ~ Beta (166,345)
Pa	d	_	~(1,2,10,100,300)with p =(0.5, 0.163, 0.222, 0.097, 0.018)
Pa	p_{ne}	_	~ Beta (0.024, 0.011)
Pa	P_{in}	_	=0.33
Pa	p_{iq}	_	Beta (39,12)
Da	g_{fs}	p_{fs}	~ Binomial (n_{fs}, p_{fs})
Da	g_{bs}	P_{bs}	~ Binomial (n_{bs}, p_{bs})
Da	n_{csb}	λ_{csb}	Poisson (λ_{csb})
Da	g_{its}	p_{its}	~ Binomial (n_{its}, p_{its})
Cv	p_{fs}	m_f , s_f	logit (p_{fs}) ~ N (m_f , s_f)
Cv	p_{bs}	m_b, s_b	logit (p_{bs}) ~ N (m_b, s_b)
cv	λ_{csb}	λ_{cs}	= n_{cs} * λ_c
cv	p_{its}	p_{it}, s_s	logit (p_{its}) ~ N (logit (p_{it}), s_s)
pa	s_s	-	=1
co	n_{fs}	-	188,150,148,195,…….
co	n_{bs}	-	75,60,60,72,60,…….
co	n_{cs}	-	21037900
co	n_{its}	-	4292350

The new model with better data is called the augmented model, and its variates are described in Table 10.1 above the core model variates. Most of the model and sub-model details are summarised below. The priors or the scientific literature data are explained first before the surveillance, or the more reliable data are given in each model. More details about the model code and the calculations are also provided in Appendix E or by the cited literature.

Farm model

The prior for the probability of a bird in a chicken farm being contaminated (p_f) is expressed as a random variable with the logit normal distribution function centred at m_f with a value of 0.5 as an average for the values reported by Boxall (2005) where it was indicated that farm prevalence was 25%. Once any flock is discovered positive for being colonised with *C. jejuni*, the flock prevalence increases to 77%. The 0.5 value is also in agreement with the international value used by Albert et al. (2008). The data used to update the above prior come from the actual *Campylobacter* prevalence. This was obtained from the caecal sampling programme set up by the NZFSA under the *Campylobacter* in Poultry Risk Management strategy 2006-2009 for NZ broiler flocks at each batch in order to determine the prevalence of *Campylobacter*. A few examples of the data set are summarised in Table 10.2. The Poultry Industry Association of NZ (PIANZ) kindly provided the data.

Table 10-2 Prevalence of *Campylobacter* in New Zealand birds.

	Plant A			Plant B		
Month	No.samples collected	detected	% positive	No. sample collected	detected	% positive
Mar-08	188	133	71	181	66	36
Apr-08	150	108	72	160	140	88
May-08	148	102	69	172	106	62
Jun-08	195	128	66	229	133	58
Jul-08	170	119	70	141	107	76
Aug-08	309	131	42	168	114	68
Sep-08	211	142	67	181	121	67
Oct-08	144	107	74	176	119	68
Nov-08	145	102	70	1596	142	89
Dec-08	158	117	74	211	154	73
Jan-09	107	95	89	163	126	77
Feb-09	153	117	76	157	137	87
Mar-09	150	125	83	140	132	94
Apr-09	181	148	82	176	153	87
May-09	160	122	76	146	98	67
Jun-09	204	137	67	151	83	55
Jul-09	57	20	35	67	17	25
Aug-09	52	12	23	61	15	25
Sep-09	62	17	27	61	20	33
Oct-09	84	34	40	70	19	27
Nov-09	87	24	28	64	16	25
Dec-09	40	31	78	65	27	42
Jan-10	83	50	60	60	23	38
Feb-10	54	50	93	66	34	52
Mar-10	84	63	75	75	55	73
Ave.			64			60

Poultry plant model

Similarly, the probability of a chicken carcase being contaminated (p_b) is expressed as a logit distribution function (p_b) centred at m_b with a value of 0.5 with the limit $m_f<m_b$. It is possible that the carcasses become more contaminated during transport and slaughtering. This additional contamination was represented as a portion (d_{bf}) in the model structure (Figure 10.1) and expressed by a truncated normal distribution (0.1, 0.0696)I(0, ∞) from expert opinion used by the Albert et al. (2008) Chrystal et al. (2008) reported that 45% of 163 whole chicken samples collected from the retail market in NZ were positive. However, in a relatively old but large survey conducted in 2003-2004, 89% of 230 chicken meat samples were found to be positive for *Campylobacter* (McIntyre et al., 2010). The survey by Chrystal et al. (2008) was probably conducted after the intervention applied by the poultry industry, and this is the reason for it being included in this study. Mullner et al. (2009a) reported that 80% of the samples were positive (454 out of total 652 chicken samples) over the three years from 2005 to 2008, but that study did not report the prevalence each year to better visualise the change in the prevalence over a 3-year period of the chicken survey. Therefore, the values reported by Mullner et al. (2009a) and McIntyre et al. (2010) could be tested for sensitivity analysis to test their effect on the global model output. The actual data of the prevalence of *Campylobacter* in chicken carcases were obtained from the whole chicken carcasses sampling programme for NZ broiler. This programme aimed to survey the prevalence of *Campylobacter* and the contamination level (cfu) and to monitor compliance with the *Campylobacter* Performance Target (CPT) which was set up by NZFSA under the National Microbiological Database programme in February 2007. Therefore, all the microbial data for two major plants in NZ were included in this study, and a few examples of the data set are summarised in Table 10.3. The PIANZ kindly provided the data.

Hygiene model

International studies have shown that undercooking plays a negligible role in campylobacteriosis, and cross-contamination is the main reason for the disease. The consumer studies (van Asselt et al., 2008, van Asselt et al., 2009) confirmed that mishandling practices by the consumers lead to cross-contamination during food preparation in the kitchen.

Nauta and Christensen (2011) compared eight different consumer phase models in QMRAs. Most of them expressed the consumer mishandling practices and the bacterial transfer rate from contaminated raw chicken to equipment, hands and ready-to-eat or cooked food separately. International quantitative data for mishandling practices are scarce and are usually expressed as distributions derived from limited consumer observational studies or consumer self-reported surveys, where actual consumer mishandling practice may be far worse than the reported practice.

A NZ study (2007) has indicated that 28-41% of New Zealanders allow cross-contamination to occur in their kitchen. However, the same study reported that 61% of consumers do not wash their hands before food preparation, 78% do not wash their hands between food preparation steps, and 95% do not wash their hands after food preparation. Moreover, 73.6% do not wash their hands correctly (using hot water, soap, and drying them). Thus, different priors for poor home hygiene (P_{hh}) could be tested in order for the model to reflect the available NZ data with beta distributions, as the parameters of the beta distribution are chosen to reflect the plausible range of parameter values; (166,345 for 28-41%) (mean=0.77, CI 95%=[0.64,0.87]), (22,20 for 28-74%). In spite of the possibility of measuring the bacterial transfer rate to food or utensils and vice-versa, there is a significant discrepancy in the values of transfer rates reported in the literature. These values have been discussed briefly previously in this thesis and by others (Gilbert et al., 2006). The studies by Luber et al. (2006), Kusumaningrum et al. (2004) and van Asselt et al. (2008) are considered to be the main studies that have addressed *Campylobacter* solely. However, different transfer rates are reported in each study. Thus, similar to poor home hygiene practice, different priors could be tested for the model to reflect the available data for the cross transfer rate (Phc) representing the least reported transfer rate value of 5% and the possible highest value of 100%. However, the reported transfer rates (3-63%; Beta 8,8) by Luber et al. (2006) have been used extensively (Albert et al., 2008), as the naturally contaminated chicken was used in that study and therefore reflects the actual consumer preparation scenario. Use of artificially inoculated bacteria or high inoculums in other studies disqualified them from inclusion in this study (Montville and Schaffner, 2003, Purnell et al., 2004, Whyte et al., 2003). Therefore, Luber et al. (2006) transfer rate value was selected to be used in this model. A NZ primary transfer study (Gilbert et al., 2006) reported a low transfer rate values from 0 - 6% only, but the actual study was not later conducted, and so the findings of the primary study were not confirmed. Thus, NZ transfer data has been excluded for use in this study. The total probability of cross-contamination from a contaminated chicken in the home (P_h) is expressed as the product of P_{hc} and P_{hh}, as has been expressed in other international models (Albert et al., 2008, Lindqvist and Lindblad, 2008).

Table 10-3 Prevalence of *Campylobacter* in New Zealand whole chickens.

		Plant A			Plant B		
	Month	No.samples collected	No. detected	% positive	No.samples collected	detected	% positive
1	Mar-08	75	34	55	n.a	n.a	n.a
2	Apr-08	60	31	48	n.a	n.a	n.a
3	May-08	60	37	38	n.a	n.a	n.a
4	Jun-08	72	31	57	n.a	n.a	n.a
5	Jul-08	60	32	47	n.a	n.a	n.a
6	Aug-08	60	26	57	n.a	n.a	n.a
7	Sep-08	60	35	42	60	30	50
8	Oct-08	57	31	46	54	29	46
9	Nov-08	60	32	47	60	23	62
10	Dec-08	72	45	38	69	29	58
11	Jan-09	60	34	43	42	15	.64
12	Feb-09	57	26	54	57	27	.53
13	Mar-09	57	34	40	60	24	60
14	Apr-09	72	52	28	72	32	56
15	May-09	60	31	48	60	36	40
16	Jun-09	72	41	43	72	39	46
17	Jul-09	60	37	38	60	34	43
18	Aug-09	60	36	40	60	46	23
19	Sep-09	60	37	38	57	39	32
20	Oct-09	60	46	23	72	47	35
21	Nov-09	72	60	17	60	40	33
22	Dec-09	57	36	37	75	55	27
23	Jan-10	72	43	40	60	36	40
24	Feb-10	60	27	.55	55	39	29
25	Mar-10	72	21	71	75	32	57
	Average			44			45

Consumption model

Consumption data play an important role in any QMRA. However, poultry consumption surveys are insufficient in NZ. Moreover, the available data were not designed to be used or incorporated in a QMRA. The main parameters, such as, how frequently New Zealanders purchase chicken or how many chickens they purchase each time over a defined period of time, were scarce. Therefore, an alternative procedure was followed in order to overcome this data gap by expressing the purchase of chicken by New Zealanders as a Poisson random variable with intensity λ_c. In order to consider the variability of λ_c over the NZ population, it was modelled by a gamma probability distribution (8,2) (CI 95%=1.7,7.2) which has its mean at four chicken per month and a standard deviation of sqrt (2) = 1.414214. Consumption data were collected from the PIANZ as the total number of annual birds slaughtered in NZ (Figure 10. 2). The average value of the total annual birds slaughtered (79, 821 000 birds) for the two years 2008 and 2009 was used since the only microbial data available for this study also covered two years, 2008 and 2009. The number of households in NZ was obtained from Statistics NZ for the years 2008 and 2009, and the average value for both years was 1,609,500 households. The number of chickens consumed per year was divided by 13 to express the consumption over a four-week period.

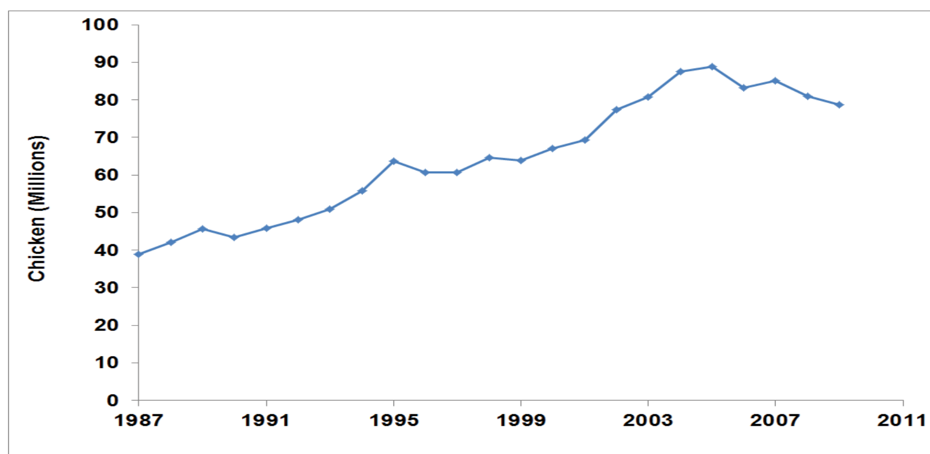

Figure 10-2 New Zealand chicken production data provided by the PIANZ.
(Poultry Industry Association of NZ).

Exposure model

For assessing the risk to human health from *Campylobacter* at purchase and preparation of chicken in the home, the potential of exposure to the pathogen during a specific period of the year or the number of times per year that chicken was brought home needs to be estimated. It was suggested to express the number of times the household was exposed to chicken by a random binomial

variable (X,p) as the binomial process describing a repeated trial (X) with a constant number or probability of success p (Albert et al., 2008). The probability of success p was modelled as a product of the probability that the chicken brought home is contaminated (p_b) times the total probability of cross-contamination occurring from a contaminated chicken in the home (P_h).

This can be mathematically expressed as follows:

$$\lambda_e=\lambda_c*P_b*P_h \qquad 10\text{-}3$$

Where λ_e represents the mean value of an exposure event.

The exponential distribution was used in the literature to express the probability of exposure to a hazard in order to represent the uncertainty and variability of the exposure event (Vose, 2008, Albert et al., 2008). Thus, the probability of exposure for the whole exposure event for New Zealanders over four weeks during a year is as follows:

$$P_e=1-\exp(-\lambda_e) \qquad 10\text{-}4$$

Then, the probability of exposure for a home in one year is as:

$$P_{ey}=1-(1-P_e)^{13} \qquad 10\text{-}5$$

Illness model

In the last sub-model, it is necessary to predict the probability of illness as a consequence of poultry consumption or purchase in a year. Conventionally, this model is built using the combination of the exposure model and a dose-response model. However, the exposure model and all the preceding models through the food chain were without the inclusion of bacterial counts due to the restrictions of the available data in NZ. All the farm samples were categorised into only positive/negative results, and poultry plant results were reported unfortunately in grouping methodology ((number of samples less than 1200/ 6000/10000/100000/750000 cfu per carcasses with missed values on a few occasions). Thus, the predicted ingested dose, which is the most uncertain variable in any QMRA, was represented in this study by a distribution derived from Section four of a CARMA report (Table 4.2.) (Nauta et al., 2005). Although there are questions about Table 4.2, the CARMA report was published in a peer-reviewed journal and satisfied the minimum criteria for the inclusion of the data in this study model. The values published in the CARMA report have also been used by Albert et al. (2008) in their model. It was considered by Albert et al. (2008) as the best information available to them as well. Due to the restriction of time and the lack of data for our knowledge, the CARMA report data was the only available option for use in this study as well. However, it is important to point out our reservations and concerns about the values reported in the CARMA report, and that it is considered as an area of investigation and

a priority for improving the model in the near future.
An estimate of the probability of illness, given the exposure to *Campylobacter* (P_{ie}), was derived from the simple binomial equation for the probability of infection as indicated in WHO guidelines (FAO/WHO, 2003) as follows:

$$P_{ie} = 1-(1-p_{ne})^{d} \qquad 10\text{-}6$$

Where p_{ne} represents the probability that a single pathogenic organism can cause the infection. A Beta (0.024,0.011) distribution has been used by Albert et al. (2008) to express this probability with α and β values estimated by Teunis et al. (2005). d is the dose or the number of pathogenic organisms in the exposure event and is expressed by the distribution based on the CARMA report (Table 4.2)

The multiplication of P(inf/d) by the probability of illness given the infection P_{in} (0.33) which has been derived from the only human dose-response study in the literature,(Black et al., 1988) estimates the final probability of illness given the exposure (Albert et al., 2008) It was reported by (Black et al., 1988) that only a third of the volunteers of his study became ill after they had ingested the pathogenic *Campylobacter* cells. As the surveillance data indicates the total reported cases of campylobacteriosis, but with no confirmation about the number of cases attributed to poultry consumption, the model has to consider how to incorporate the total reported cases in the model while only tracking the poultry source. The equation (11.8) which has been used to account for the flaw in surveillance data regarding the number of notified cases due to poultry was the same equation used by (Albert et al., 2008). That equation needs the relative contribution of poultry and non-food sources for acquiring campylobacteriosis in NZ. The determination of what proportion of human campylobacteriosis are acquired from poultry (P_{ib}) has been obtained from a NZ study (Mullner et al., 2009a) which estimated it as 80%. The other sources which can contribute to campylobacteriosis were calculated in this study in order to provide decision-makers with valuable information about the total probability of illness from campylobacteriosis within one year, whatever the source of infection (P_{it}), to estimate the total possible campylobacteriosis cases in one year and to prioritise targeted interventions. Usually, source attribution is extrapolated from surveillance, outbreaks and epidemiological studies. New molecular biology tools such as multilocus sequence typing (MLST), polymerase chain reaction (PCR) assays and the DNA microarray have been used to provide the relative importance of each source in acquiring human diseases (Bumgarner, 2013). Mullner et al. (2009a) in a molecular-based study, reported that 80% (379 out of 474 cases) of human cases in NZ were attributed to poultry, which is translated into

Beta (39,12) (mean=0.77, CI 95%=[0.64,0.87]). Taking into account all of the literature, this is a high population attributable fraction, and it is only close to the value reported in Scotland (Sheppard et al., 2009). An expression used by Greenland and Drescher (1993) was used to define the attributable fraction (p_{iq});

$$P_{iq} = \frac{\text{Pr (disease)} - \text{Pr(disease|no exposure)}}{\text{Pr(disease)}} \qquad \text{10-7}$$

After the substitution and rearrangement, the final total probability of illness was calculated as follows (Albert et al., 2008):

$$P_{it} = \frac{\text{p.ey} * \text{p.ie}}{(1 - (1 - \text{p.iq}) * (1 - \text{p.ey}))} \qquad \text{10-8}$$

The actual reported number of campylobacteriosis cases for the years 2008 and 2009 were obtained from annual surveillance reports prepared by the Institute of Environmental Science and Research Limited (ESR) (NZPHO, 2010). There was no significant difference between the reported cases for the years 2008 and 2009, 6693 and 7160, respectively. Thus, the average value (6926) was included in this study.

10.2.3 Computing tools

The software, WinBUGS version 1.4.3 (Lunn et al., 2000) was used for numerical computation. It was developed from the original code by Albert et al. (2008) and ran for 700,000 iterations after a burn-in period of 240,000 iterations using Markov chain Monte Carlo (MCMC) sampling algorithms. The convergence of the algorithms was monitored by using the general methods for assessing the convergence of Markov Chain Monte Carlo iterative simulations (Toft et al., 2007). Three chains have been simulated in parallel, each with different starting values which are overdispersed relative to the posterior distribution. Convergence is assumed when the output from the three chains is similar, and the BGR convergence diagnostic scale reduction factor stays close to 1. The resulting burn-in of 240,000 iterations was discarded, and the remaining iterations summarised to provide the posterior distributions. The error of each posterior distribution standard deviation has to be less than 5% (Tuominen et al., 2006) all the variates or parameters have to behave reasonably, and the results have to be convincing to experts. The coda results produced by the WinBUGS simulations were processed using the statistical R software package to better represent the results of the WinBUGS software.

10.3 Results and discussion

The statistical output of the prior distribution and posterior distribution are summarised in Table 10.4. The Monte Carlo error (MC error) for each parameter, which assesses the accuracy of the posterior estimates, represents the difference between the mean of the sampled values (which are used as the estimate of the posterior mean for each parameter) and the true posterior mean, (Huang and McBean, 2007) are shown in Table 10.4. The statistics table also reports the sample standard deviation (SD) with the confidence level at 2.5% and 97.5%. The primary results from running the core model without incorporating the most relevant data, such as the surveillance data or robust data from the daily microbiological monitoring programme conducted by the poultry industry and monitored by the NZFSA were similar to that obtained by the Monte Carlo Simulation method based solely on the prior information available in the core model using WinBUGS. These prior distributions, which are depicted in Figure 10.3 (by dashed lines) can provide valuable information when relevant data is lacking. The incorporation of the augmented model has produced posterior distributions of the variates of interest which have a different shape to the prior distributions, as the data forced the change of the shape of the prior distributions, except for hygiene, farm and plant models, where the differences between both the distribution's shapes were marginal. The spread of the posterior distribution has been narrowed mostly as it was depicted in Figure10. 3. However, it is important to indicate that this narrowing cannot yet establish a comprehensive response to the question of risk, as the distributions still have a large spread to reflect all the variability and uncertainty of all parameters, variates, co-variates and data used in the developed model. The resultant probability of illness derived from the priors' distributions without incorporating the observed data, was significantly higher than the surveillance data value by 100 times as was the case with the previous model developed in NZ by (Lake et al., 2007). The prediction of campylobacteriosis cases was calculated crudely by multiplying the mean probability of illness from all sources ($p_{.it}$ value shown in Table 10.4 of the results obtained from WinBUGS for each model with the total NZ population to estimate the possible campylobacteriosis cases given the available information from the priors and the most relevant data, respectively in the model. Evidently, the prediction for campylobacteriosis cases for the year 2010, by the Bayesian inference with the data, indicated 9,250 cases from all the sources. This prediction was close to the value reported by the surveillance in NZ in 2010, which was 7333 cases.

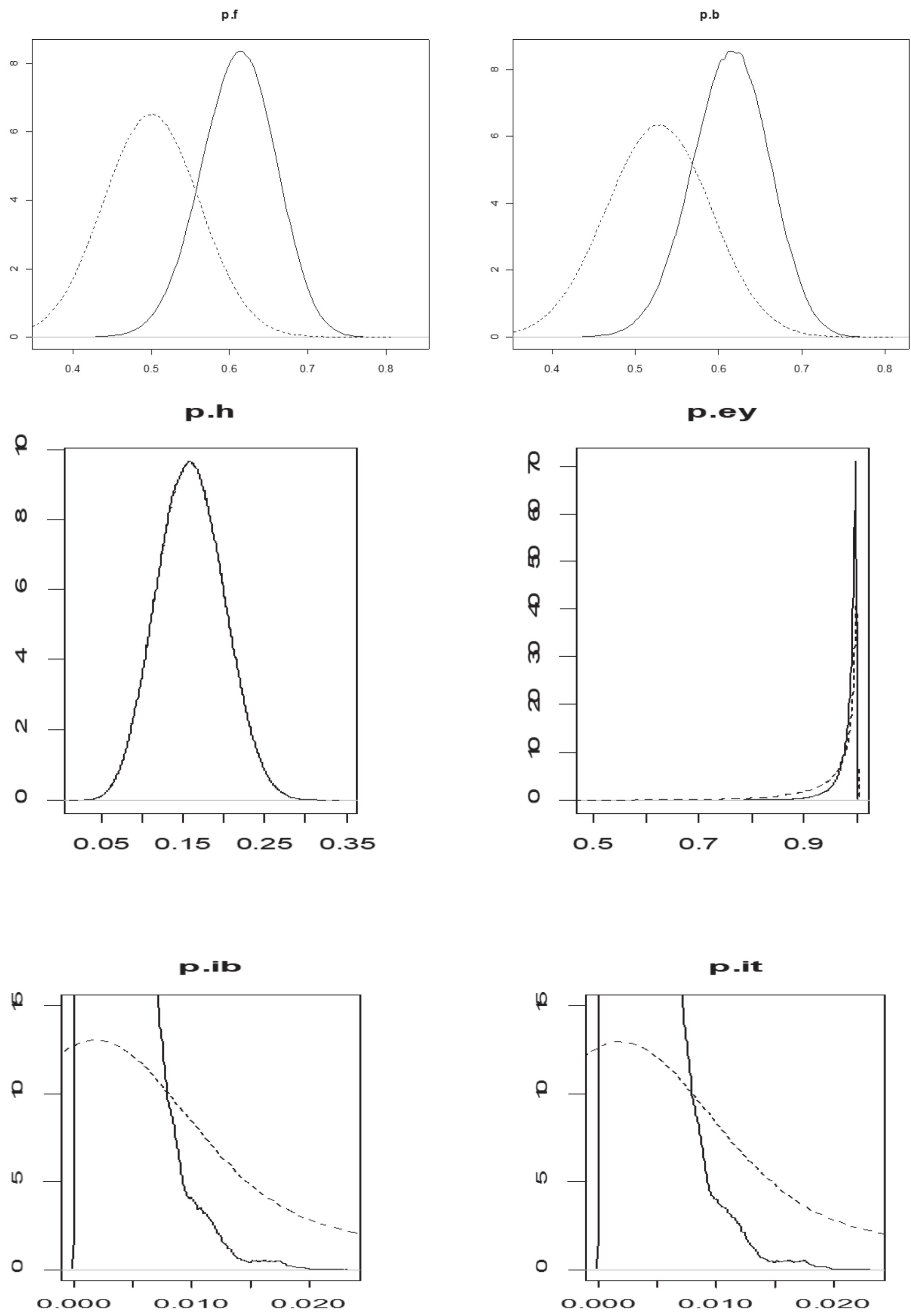

Figure 10-3 Posterior marginal kernel densities

(solid lines) and its prior densities (dashed lines) of the six variates defined in Table 10.4. for the main model.

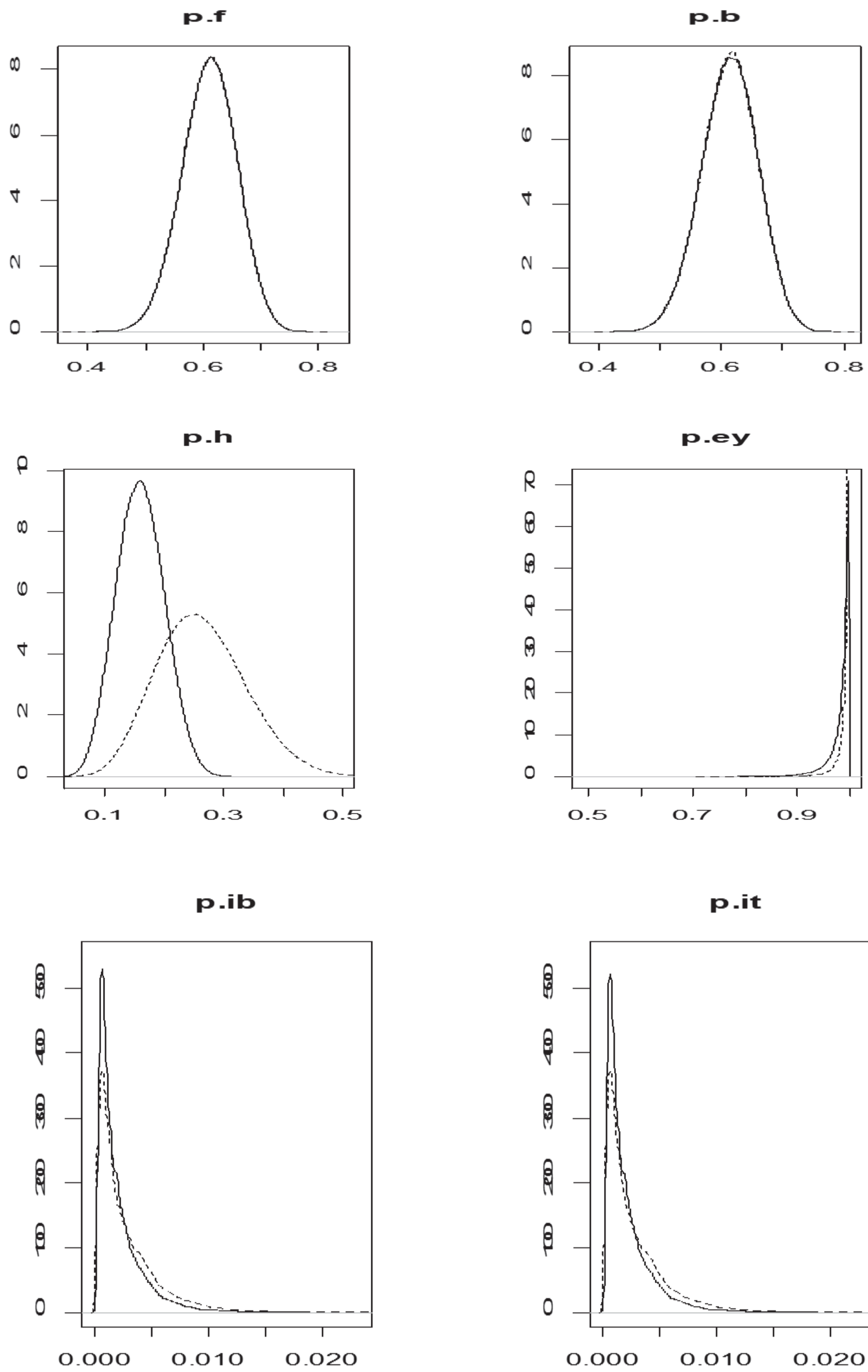

Figure 10-4 Posterior marginal kernel densities

(solid lines) for the main model and posterior marginal kernel densities for the hypothetical model with a 50 % increase in hygiene value priors (dashed lines) of the six variates defined in Table 10.4.

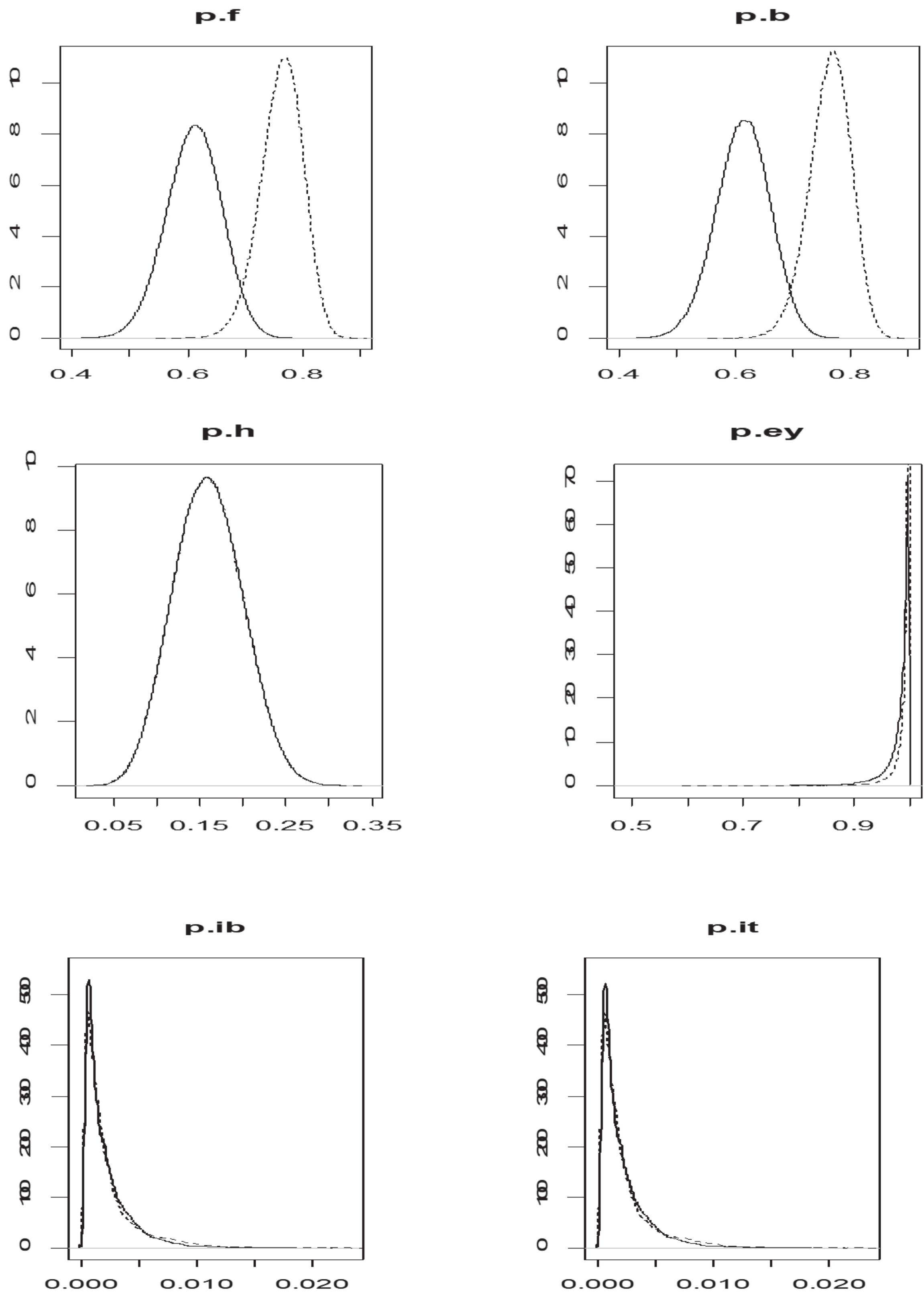

Figure 10-5 Posterior marginal kernel densities

(solid lines) for the main model and posterior densities for the hypothetical model with 25 % increase in the values of the farm and the poultry plant priors (broken lines) of the six variates defined in Table 10.4.

Table 10-4 Statistical summary for the most important variates of models used representing the model with and without the use of the data along with possible two plan interventions (+50% in hygiene prior,+25% in farm & plant priors).

The main model								
node	**mean**	**SD**	**MC error**	**2.50%**	**median**	**97.50%**	**start**	**sample**
p.b	0.6139	0.04616	0.00009293	0.5208	0.6149	0.7012	240000	1380000
p.ey	0.9825	0.02462	0.00004153	0.9137	0.991	0.9993	240000	1380000
p.f	0.6109	0.04743	0.00004136	0.5152	0.6119	0.7006	240000	1380000
p.h	0.1593	0.03998	0.00004215	0.08375	0.1587	0.2386	240000	1380000
p.ib	0.002126	0.002101	0.00004517	0.0002515	0.001419	0.007876	240000	1380000
p.it	0.002155	0.002128	0.00004578	0.0002552	0.001438	0.007982	240000	1380000
The model with a 50 % increase in hygiene prior								
p.b	0.6138	0.04598	0.00009261	0.5212	0.6149	0.7009	240000	1380000
p.ey	0.997	0.008878	0.00002981	0.9771	0.9995	1.000	240000	1380000
p.f	0.6108	0.04744	0.00004116	0.5153	0.6119	0.7007	240000	1380000
p.h	0.2619	0.07457	0.00008285	0.129	0.2577	0.4186	240000	1380000
p.ib	0.002899	0.003173	0.00006833	0.0002396	0.001859	0.01119	240000	1380000
p.it	0.002901	0.003175	0.00006838	0.0002398	0.00186	0.01119	240000	1380000
The main model with priors only (without the data)								
p.b	0.5272	0.06267	0.00005304	0.4035	0.5277	0.6489	240000	1380003
p.ey	0.9568	0.06395	0.00005397	0.7688	0.9822	0.9999	240000	1380003
p.f	0.5001	0.06114	0.00005223	0.3803	0.5001	0.6198	240000	1380003
p.h	0.1594	0.04007	0.00003404	0.08368	0.1587	0.2387	240000	1380003
p.ib	0.1953	0.142	0.0001227	0.0000115	0.284	0.3298	240000	1380003
p.it	0.1972	0.143	0.0001235	0.00001161	0.291	0.3299	240000	1380003
The priors only for the 50 % increase in hygiene								
p.b	0.5272	0.06265	0.00005328	0.4034	0.5277	0.6486	240000	1380003
p.ey	0.9877	0.03112	0.00002609	0.9004	0.9986	1.000	240000	1380003
p.f	0.5	0.06111	0.00005176	0.3803	0.5001	0.6197	240000	1380003
p.h	0.262	0.07467	0.00006367	0.129	0.2577	0.4189	240000	1380003
p.ib	0.2018	0.1457	0.000127	0.00001192	0.3072	0.33	240000	1380003
p.it	0.2024	0.146	0.0001272	0.00001195	0.3099	0.33	240000	1380003
With 25% increase in pf and dbf								
p.b	0.7647	0.03586	0.00007092	0.6893	0.7667	0.8295	240000	1380003
p.ey	0.9924	0.01499	0.0000666	0.9538	0.9972	0.9999	240000	1380003
p.f	0.7627	0.0364	0.00003248	0.6862	0.7646	0.8285	240000	1380003
p.h	0.1593	0.04006	0.00006607	0.08336	0.1587	0.2387	240000	1380003
p.ib	0.002416	0.002816	0.00006079	0.0002332	0.001475	0.0101	240000	1380003
p.it	0.00242	0.002821	0.0000609	0.0002337	0.001478	0.01011	240000	1380003
Priors only for 25% increase in pf								
p.b	0.6578	0.05704	0.00004848	0.5399	0.6601	0.763	240000	1380003
p.ey	0.976	0.0449	0.00003799	0.8434	0.9935	1.000	240000	1380003
p.f	0.6234	0.05745	0.00004864	0.5058	0.6251	0.731	240000	1380003
p.h	0.1593	0.04002	0.00003431	0.08357	0.1587	0.2386	240000	1380003
p.ib	0.1992	0.1443	0.0001223	0.00001173	0.2976	0.33	240000	1380003
p.it	0.2003	0.1449	0.0001228	0.0000118	0.3022	0.33	240000	1380003

Therefore, the prediction produced by Bayesian inference was of acceptable accuracy, given the uncertainty of the surveillance data due to under-ascertainment in the notifiable disease in general. Under-ascertainment can occur as a result of factors associated with a patient who may decide to seek treatment, the general practitioner who may request a sample, and/or the laboratory which analyses the sample. The under-ascertainment of disease and its implication have been discussed in the literature (Doyle et al., 2002) suggesting that the completeness of notification varies between areas, for different diseases, and during outbreaks. Thus, the rate of under-reporting of campylobacteriosis cases in NZ or internationally becomes a complex task. A NZ study (Lake et al., 2009) revealed that only 0.4 % of NZ community cases of acute gastrointestinal illness are notified to national surveillance. However, that study also indicated that their findings could not apply to the notification rate of campylobacteriosis, giardiasis or the norovirus infection, and presented a factor of 7.6 used internationally as an alternative to estimate the possible campylobacteriosis incidences. There were also some reservations concerning this factor.

10.3.1 Sensitivity analysis

Testing the model with the two consumers' hygiene priors is fundamental to determine the constancy of the model outputs as part of the sensitivity analysis to change the different model priors, parameters and variates to evaluate their marginal significance and also to validate the model (Huang and McBean, 2007, Albert et al., 2008). However, only the main factors, such as consumer hygiene practice at home, and the farm, and processer intervention, were investigated in this study. This was due to the significant practical and possible benefits for regulators, policymakers, industry, public health professionals and researchers which may be gained from the outcomes of the indicated model priors by altering their input values. As discussed previously, cross-contamination plays a major role in the risk of campylobacteriosis (Kapperud et al., 2003, Mylius et al., 2007) in NZ Previous studies and next chapter indicate that most New Zealanders scored worse than those in other developed countries (Gilbert et al., 2007, Redmond and Griffith, 2003, Al-Sakkaf, 2012). Therefore, the first model was tested with the lowest value reported by Gilbert et al. (2007) and supported by the findings of the next chapters as a prior for poor hygiene, where 28 - 41% of New Zealanders allowed cross-contamination to occur in their kitchen, which resulted in an unpleasant prediction for the year 2010 as mentioned above. The other higher percentage of New Zealanders, who allow cross-contamination to occur in their kitchen was derived as an aggregate of the 78% of New Zealanders who did not wash their hand before food preparation, and the 74% who do not wash their hands correctly and the 95% who do not wash their hands after food preparation. This is represented by a beta distribution reflecting the 74%, as this value is considered more

reasonable and acceptable. Moreover, the 74% value was approximately 50% higher than the lower reported value. This can serve as a planned target for intervention by policymakers and risk managers and should be considered in their long term strategy to combat the disease in NZ. It is noticed from Table 10.4 that hygiene has a significant impact on the total probability of illness, as the increase of the hygiene percentage by approximately 50 % reflected an increase of approximately 50% in the probability of illness (from ~ 0.002 to ~ 0.003). The prediction by the model used in this study, with the use of a higher percentage for the hygiene prior (28-74 %) increased the total campylobacteriosis cases for the year 2010 to 12,452 cases, which is approximately 70% more than the notifiable incidences or surveillance value (7,333 cases). This may include the number of unreported cases also. This finding with the other evidence discussed previously confirms the hypothesis that poor hygiene can be the cause of the high rate of campylobacteriosis in NZ. While the other hypothesis regarding NZ *Campylobacter* strains may have extreme characteristics or NZ poultry plant malpractices increases the contamination level of poultry were eliminated as explained in the previous chapters. It is also important to indicate that there is a data gap in NZ regarding consumers' hygiene practices since new relevant data can improve the current model output as the current model relies only on the information of the priors or the limited data available regarding New Zealanders' hygiene practice. However, the finding of Bayesian inference model argues for the regulatory, policymakers, risk managers, health professionals and educators to review and to assess their current consumer education strategy plans. They should motivate all parties involved and responsible for protecting consumers health and for developing a more effective education strategy to improve consumer hygiene practices to avoid the unsafe practices which can lead to the contamination of their food by *Campylobacter*. This would reduce the campylobacteriosis cases and possibly other foodborne diseases in NZ. Unfortunately, *Campylobacter* has the remarkable capability of disseminating easily from raw poultry, directly onto cooked chicken meat or some other component of the meal or indirectly onto all utensils (knives, cutting boards, taps, kitchen cloths, etc.) or hands that have been in direct contact with raw meat or contaminated packages if consumers do not follow safe handling practices. Despite the fact that the importance of the microorganism cross-contamination route in preventing the risk of food poisoning and most of the international published QMRAs have considered it as the major or the only pathway that enables the development of campylobacteriosis (Christensen et al., 2005, Hartnett et al., 2001, Mylius et al., 2007, Nauta et al., 2007, Rosenquest et al., 2003), several of QMRA studies (Dogan et al., 2019, Akil and Ahmad, 2019, Jeong et al., 2019, Premarathne et al., 2017) have not conducted sensitivity analysis regarding the uncertainty of the hygiene model parameters. Especially the frequency of improper hygiene behaviour by consumers,

and its impact on the final risk estimate, as has been performed in this study and other studies (Calistri and Giovannini, 2008, Pang et al., 2017) which performed a sensitivity analysis as recommended in the literature (Zwietering and van Gerwen, 2000). Therefore, the effect of a new intervention, for example, an education plan, was clear for the policymakers, risk managers and health professionals before the implementation of the new intervention. This can be achieved by altering the priors of consumers' hygiene practice.

The results obtained from increasing the prior of the poultry farm model (the probability that a bird in a poultry farm has been contaminated or positive, pf) and the prior presenting the possible increase in the d_{bf} (the contamination prevalence due to the transportation of flock and slaughtering process) by 25% in the model (N~ 0.511, 20.66 and (0.15, 206.6) revealed a similar increase of approximately 25% in the final probability of illness (p.it, p.ib). The 25% increase in the actual microbiological data for birds prevalence (g.fs) and chicken prevalence (g.bs) incorporated in the model was performed to avoid any impact of the actual data on the shape of the resultant posterior distribution of the final probability of illness estimated by the model computation. The finding of the 25% increase in (pf, d_{bf}) priors and data (g.bs, g.fs) has confirmed the significant impact of the two priors in altering the final risk estimation. This is in agreement with another international QMRA model (Rosenquest et al., 2007, Huang et al., 2018, Vigre et al., 2016).

The selection of the value of 25% was justified by the practical future pragmatism as a planned intervention. It was not practical to further reduce the contamination in poultry to 50% given the status and circumstances of the poultry industry in NZ in terms of the prevalence and load of the counts on birds and on slaughtered chicken in 2006. The contamination level/count loads reported in 2006 was from 2.6 up to 6.8 log (Chrystal et al., 2008) on slaughtered chicken and the prevalence was 80 -100 % (French, 2008a) where the prevalence in birds varied from 27 up to 100% (NZFSA, 2009, Boxall, 2005). However, to our knowledge, there is no reported data regarding the counts in bird caecal samples in NZ, and all the data available in NZ were based only on positive/negative analysis.

Moreover, the poultry industry reported that they implemented interventions across the industry that have seen *Campylobacter* detection rates drop by 25% between 2007 and 2010 in response to a NZFSA strategy set by (PIANZ, 2011). This supports the reasonable selection of the 25% value for use in the model to test the change in the final risk estimate output of the model in contrast to the 50% value selected for the consumer's hygiene variate prior, which has been discussed above.

Therefore, the 25% level of contamination was a practical target for the poultry industry to achieve.

The association between the reduction in flock/ poultry prevalence and reduction of poor hygiene practice and the calculated reduction in risk of human disease was estimated to be 1:1 which is in

agreement with international findings (Rosenquest et al., 2003, Vigre et al., 2016). The overall performance of the simplified proposed model was acceptable and fulfilled the primary proposed objectives such as the use of the Bayesian technique in QMRA for its strength, as was recommended (Smid et al., 2010, Nauta et al., 2009). The model proved to be useful in characterising particular risk mitigation strategies once they have been planned and expressed (Smid et al., 2010, Neves et al., 2018). A source attribution study in NZ noted that 74% of the decline in notification rate in 2007 and 2008 was attributed to poultry (Sears et al., 2011). This may support the finding of this risk assessment study by the Bayesian Belief Network and inference. The hypothetical interventions were selected in order to reduce consumer malpractice by 50% and improve the level of poultry contamination at farms and plants by 25%, which adds up to 75%. The model also highlighted areas of data gaps which need to be improved in the future. The model used in this study needs further improvements, such as the incorporation of the contamination level in terms of the bacterial count, since the current model was based only on the analysis of presence or absence due to the lack of count data in NZ, especially for live birds. The availability of data for other model variates parameters, variables and co-variables will improve the model globally since it is known that QMRA is a data-hungry process, requires enormous resources and takes a long time to complete. However, it is important to clarify that a QMRA model simplifies a complex reality system by the use of mathematics and making assumptions and hypotheses. The simplified model used in this study was able to identify consumer hygiene practice, the initial prevalence at the farm level, and the processing practice at the plant level, as the major factors influencing the final risk estimate. A QMRA model can provide additional insight into the system and identify relevant data gaps, but it cannot provide additional knowledge itself except for the provision of an evaluation of control measures all through the food chain in terms of human health risks (Nauta et al., 2009, Membré and Boué, 2018). Therefore, before making any decision regarding the allocation of resources to gather the data required for a more comprehensive QMRA model in NZ, it is important to ask: is it worth collecting more data? What is the cost-benefit? And what analysis could be done with the currently available data, and possibly with the data available shortly, with limited time and resources? (Vose, 2005). In spite of the short time frame and the limited resources, the outcomes of this study are better than the QMRA model developed earlier in NZ (Lake et al., 2007) which was guided from abroad (Denmark and U.K) (Nauta et al., 2009) and whose main function was to guide future research.

Moreover, the MC modelling technique was used for the old NZ model, ignored the farm step due to the lack of data, and tested only two scenarios out of the three scenarios listed for the flock contamination (Nauta et al., 2009). The Bayesian technique deserves further investigation, and the

convergence of the model needs to be treated with caution and be tested and validated with other software. There are new software packages which may perform better than WinBUGS and which conduct a complete sensitivity analysis as an automated routine (Smid et al., 2010). The Bayesian inference technique has more advantages than the MC technique, (Albert et al., 2008, Smid et al., 2010, Tuominen, 2009) although it is always the purpose of the model to impact the decision of the appropriate technique to be used for the model. The Bayesian inference technique is more complex than the MC technique, and the MC technique can fit the purpose for use as well (Smid et al., 2010).

10.4 Conclusions

The Bayesian proposed technique can provide better information for QMRA than MC technique for its ability to incorporate the data. The Bayesian approach can provide better insight into the food chain, is more informative and can easily understand the impact of any intervention in the food chain. However, Bayesian methodology is a complex task and its use in QMRA it still in its infancy. Bayesian approach deserves further investigation, and the convergence of the model needs to be treated with caution and tested and validated with other software or packages. A simplified model used was able to identify the consumer hygiene practice, the initial prevalence at farm level and the processing practice at the processing plant level as the major factors influencing the final risk estimate. This study provided a fast response to regulatory, policymakers, risk managers regarding the main risk drivers in NZ campylobacteriosis epidemic and provided additional supportive evidence for the conclusions stated in the cross-contamination chapter regarding the possibility of the poor hygiene practice as the reason for campylobacteriosis in NZ. Future research needs to be conducted to improve the model, data and the understanding of the BBN technique and its feasibility for NZ QMRA studies and to use it as a tool to enhance food safety in NZ. However, predicting campylobacteriosis cases for the year 2010 by the Bayesian approach failed to provide an accurate estimation of the number of actual notified campylobacteriosis cases in 2010. Therefore, there is a need to explore other methods to predict campylobacteriosis more accurately. The next chapter has addressed this point.

11 Comparison of time-series models for predicting campylobacteriosis risk in New Zealand

11.1 Introduction

Campylobacter causes more cases of bacterial foodborne illness each year than any other bacterial pathogen worldwide (Schönberg-Norio et al., 2006, Schönberg-Norio et al., 2004, Facciolà et al., 2017, Samuel et al., 2004b). It is the most commonly reported foodborne disease in industrialised and European countries (Hilmarsson et al., 2006, Johnson et al., 2006, Jore et al., 2010, EFSA, 2018). The symptoms of campylobacteriosis include muscle pain, headache, fever, watery or bloody diarrhoea, abdominal pain, and nausea, sometimes accompanied by vomiting with late complications such as reactive arthritis, RS and GBS.

The estimated annual cost for campylobacteriosis in NZ is more than $77 million per year, not including its effect on New Zealand's reputation in the world and the financial loss to the tourism industry (Mataragas et al., 2010). In 1980, NZ legislated campylobacteriosis as a notifiable enteric disease. Since then, the incidence of reported campylobacteriosis has risen steadily, and it is now believed that NZ has the highest rate of reported cases in the world. In 2006, the notification rate was 380 per 100,000, an increase of 56 % from 2001 and 14% since 2005. This rate is 35 times higher than the rate for the United States, four times higher than the rate for Australia and five times higher than the rate for the U.K. (Baker et al., 2006a). Because of under-reporting, the actual figures are estimated to be at least seven to eight times higher than the notified cases (Lake et al., 2007) or possibly up to 30 times higher (Newman et al., 2015).

Based on above, campylobacteriosis in NZ is considered as a national epidemic, raising a public demand for urgent action and in particular control of chicken contamination to protect New Zealanders and to reduce the economic impact of the disease in the country. The NZ Food Safety Authority (NZFSA) has developed a comprehensive risk management strategy aimed at achieving a significant reduction in *Campylobacter* levels in chicken meat by employing interventions at appropriate points in the food chain. In April 2007 NZFSA established a microbiological monitoring programme (NMD) to estimate on a national basis the prevalence of *Campylobacter* in flocks (sheds) of poultry at slaughter and the prevalence and numbers of *Campylobacter* on poultry carcasses after processing. All poultry processors have to collect three samples every processing day for monitoring of the prevalence and the contamination level in flocks before slaughtering and on carcasses before distribution to the market for each day. Thus, the producers have been able to

reduce the contamination level as the result of the daily analysis as monitored by NZFSA.

In April 2008, NZFSA imposed the Poultry *Campylobacter* Performance Target (CPT) of the maximum contamination level in chicken carcasses as 6000 cfu/carcase when released to the market. This probably forced the poultry processor in NZ to increase their efforts to implement more interventions throughout the processing lines to further reduce the contamination level in chicken carcasses. It should be noted that the European Union performance target is only 1000 cfu.

The intervention strategies implemented in New Zealand and reported by Sears et al. (2011) appear to have led to a noticeable reduction in incidence. However, a careful statistical analysis is necessary to evaluate the evidence that these interventions have resulted in a change-point in disease incidence and to evaluate their cost and benefit in terms of campylobacteriosis cases prevented.

Predicting campylobacteriosis cases has become a matter of considerable concern in NZ. The Dutch Microbial Risk Assessment Model used to predict the number of campylobacteriosis cases in NZ (Lake et al., 2007) failed to predict the accurate number of actual cases, giving a prediction about threefold higher than the actual notified cases for 2006 and about nine-fold higher than the actual notified cases in 2008. The main drawback of this NZ adapted model was that it relied on the international literature data which did not reflect the actual scenario in NZ poultry processing and also did not consider the interventions implemented by the industry and the government. Moreover, the challenges faced in general by the probabilistic QMRA approach and used in the NZ model have probably contributed to the current model's accuracy (Havelaar et al., 2008).

Here in this chapter, it was explored the classical time-series modelling approaches for predicting campylobacteriosis in NZ. Such time-series have been successfully applied in fields as diverse as engineering, science, sociology and economics (Brockwell and Davis, 2002). A reliable method for making time-series predictions would be of great benefit to epidemiologists and public health officials (Altizer et al., 2006, De Greeff et al., 2009). A study in Belgium, showed that a time-series analysis is an appropriate methodology to clarify trends of campylobacteriosis, salmonellosis and listeriosis, forecast future cases and test the impact of interventions on the burden of food-borne diseases (Maertens de Noordhout et al., 2017). A US study (Weisent et al., 2010) examined time-series forecasting of *Campylobacter* rates, comparing a variety of time-series models. They found that a seasonal decomposition model provided the fastest, most accurate, user-friendly method to capture the temporal patterns in disease risk based on their main evaluation criteria of evaluating mean absolute percentage error, mean squared error and coefficient of determination (R^2). Finding the most appropriate time-series model for NZ data has additional practical considerations given a possible structural change, i.e. a specific and sudden change in the underlying nature of the time-

series, in response to the implemented interventions.

In this study, it has been considered the decomposition, smoothing and ARIMA methods reported in a US study (Weisent et al., 2010) and added an ARIMA with interventions to explicitly model the effect of an intervention. The developed models used the monthly disease incidence of campylobacteriosis calculated from NZ surveillance data from 1997 to 2008. Predictive accuracy was evaluated and compared using the data for 2009.

11.2 Methods

11.2.1 Data collection

Campylobacteriosis monthly notification reports were obtained from surveillance data published on the official Public Health surveillance website (Episurv) on behalf of the NZ Ministry of Health, from January 1998 to December 2009. Rates of notification were calculated using annual end-of-year population estimates obtained from the governmental Statistics NZ web site, with spline interpolation used to estimate the monthly population figures. These risk estimates were presented as the number of cases/100 000 persons. The data from 1988 to 1996 were disregarded as the monthly notification figures were not available, with only the yearly notification totals obtainable (NZPHO, 2010). The data from the years 1998 to 2008 were used to model the time-series, with the year 2009 held out of the dataset for model validation. The best two models were then fitted to the full 1998-2009 data and used to predict for each month of 2010. The best two models were then fitted to the full 1998-2009 data and used to predict for each month of 2010 (Figure 11.1). A total of 144 points were used in this study, which is generally considered to be sufficient for time-series methods (DeLurgio, 1998). The statistical analyses were performed in Minitab ® version 15 statistical software (Minitab Inc, USA) and R software (version 2.9.1).

11.2.2 Time-series decomposition techniques

These methods decompose a time-series into several components regarded as operating at different time scales. Generally, any time-series is considered to contain up to four components: i) trend 'T ' or the increase or decrease in a time-series over a long period of time; ii) cycle ' C ' or the smooth fluctuations around the trend line; iii) season ' S ' or yearly repeated fluctuation, not necessarily an identically repeated fluctuation; iv) error ' E ' or random variations which have a transitory effect on the time-series. Estimates of the first three components can be obtained by decomposition of the series and used for forecasting (Makridakis et al., 1998). The decomposition method assumes that the time-series can be expressed as a sum (additive model) or product (multiplicative model) of the above components, as shown in Equation 11-1 (see below). A multiplicative model denotes

proportional variation when the time-series exhibits increasing or decreasing seasonal variation.

$$Y_t = T + C + S + E \text{ or } Y_t = T \cdot C \cdot S \cdot E \quad \text{11-1}$$

It is often practical to ignore the cycle component or to subsume it into the trend component. There are two main approaches to decomposition, which are discussed below:

Regression-based methods

These methods assume a simple regression model for the trend, which represent the average level that changes over time, as follows:

$$T = \beta 0 + \beta 1 t \quad \text{11-2}$$

This is a linear trend model (straight line growth or decrease), but other possibilities are a quadratic trend (curvilinear) and a *p*th-order polynomial trend (with one or more reversals in curvature). The seasonal component S is modelled by using a different fixed value for each month, or perhaps periodic functions such as sine and cosine. Least squares point estimates of the parameters in these models are obtained by using standard regression techniques, assuming that the error term E represents random fluctuations that are independent and identically distributed. Variables are retained if their corresponding regression coefficients indicate significance ($p>0.05$). This is a "global" model in the sense that it assumes the trend model and seasonal factors are the same throughout time, and all data points contribute equally to estimating the fixed parameters.

Smoothing techniques

The basic exponential smoothing method without a trend or seasonal components uses simple updating equations to decompose a series into a stochastic, smoothly varying level component (L_t) and a random component. It is generalised in the Holt-Winters method to handle a time-series with both trend and seasonal components (Chatfield, 1978). The updating equations employ user-specified smoothing parameters α, β, γ as shown below for the multiplicative case (there are analogous formulae for the additive case).

For the level component:

$$L_t = \propto \left(\frac{x_t}{S_{t-s}}\right) + (1 - \propto)(L_{t-1} + T_{t-1}) \quad \text{11-3}$$

For the trend component

$$T_t = \beta(L_t - L_{t-1}) + (1-\beta)T_{t-1} \quad 11\text{-}4$$

For the seasonal component

$$S_t = \gamma \frac{Y_t}{L_t} S_{t-s} + (1-\gamma)S_{t-s} \quad 11\text{-}5$$

Then the forecasting equation for h periods into the future is:

$$F(h) = (L_t + hT_t\,)\,S_(t - s + h) \quad 11\text{-}6$$

These techniques give a "local" model in which the components are constantly changing over time, and data points are given more weight in the estimation when they are close to the time of estimation. The Holt-Winters method is a simple method to implement and is widely used in practice (Chatfield, 2004).

11.2.3 ARIMA modelling

Moving average

In a time-series with the MA process, the model describes each observation as a function of the previous random errors. The observed series is represented as a weighted moving average of the unobserved error series up to a specified lag q (Armstrong, 2001).

$$Y_t = \theta_1\varepsilon_{t-1} + \cdots + \theta_q\varepsilon_{t-q} \quad 11\text{-}7$$

Where ε_t represents the random error at time t and θ_i are constants.

Autoregressive (AR)

A simple way to model dependence on past observations is to use ideas from regression. The AR model expresses the current time-series observation as a linear function of its past observations, plus a random error. It can also be regarded as a Markov process (Chatfield, 2004).

$$Y_t = \emptyset_1 Y_{t-1} + \emptyset_2 Y_{t-2} + \cdots + \emptyset_p Y_{t-p} + \varepsilon_t \qquad 11\text{-}8$$

$$\{\varepsilon_t\} \sim iN(0, \sigma_\varepsilon^2)$$

ARIMA models

ARMA and ARIMA models were described by Box and Jenkins in 1976 as giving a very flexible framework for the analysis of time-series. The advantages of these models are well documented in the literature (Benschop et al., 2008, Reichert et al., 2004). The ARMA model can be expressed as follows:

$$Y_t = \emptyset_1 Y_{t-1} + \cdots + \emptyset_p Y_{t-p} + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \cdots + \theta_q \varepsilon_{t-q} \qquad 11\text{-}9$$

Where $\emptyset_1$ …, $\emptyset_p$ and θ_1 , …, θ_q are constants and ε_t represents the random error at time t.

This model combines the features of the MA and AR models. However, a practical drawback of the above ARMA model is that it is valid only for stationary time-series with a constant mean. To extend the ARMA model, it is necessary to consider how non-stationary sources of variation such as trend and seasonal variation can be removed. To transform to a stationary series, the observations usually first need to be filtered (removing of trend and seasonality) by subtracting consecutive observations from each other or subtracting observations at seasonal lags. The resulting combination of filtering to achieve stationarity and ARMA modelling of the stationary series is known as an integrated ARMA model or ARIMA as the stationary model that is fitted to the differenced data has to be summed or integrated to provide a model for the original non-stationary data (Chatfield, 2004).

Thus, each ARIMA process has three parts: (AR), integrated (I) and (MA) parts. The models were written as ARIMA(p,d,q) where p describes the AR part, d the integrated part and q the MA part. Series with seasonal components also need equivalent terms at seasonal lags (S), leading to the full seasonal ARIMA model written as ARIMA(p,d,q)× $(P,D,Q)_S$. A multiplicative form of the ARIMA can be obtained by making a logarithmic transformation of the data, fitting an ARIMA to the transformed data, and then back-transforming the predictions.

ARIMA intervention models

These models can be used when an exceptional, sudden, external event affects the time-series at a particular point in time, for examples strikes, disasters, policy changes, etc. (Bonham and Gangnes, 1996). The NZ campylobacteriosis series experienced an intervention in 2007 when an attempt was made to reduce the rate of campylobacteriosis. ARIMA intervention models try to

accommodate such intervention by the use of an intervention variable which is included in the basic ARIMA model. This variable may be a step function for representing a permanent change or an impulse function to model a temporary effect. Historically, an improvement in forecasting has been noticed by several authors after applying an ARIMA intervention model (Goh and Law, 2002, Min et al., 2010, Rashed et al., 2017). In some cases, estimating the effect of the intervention is the main goal of the analysis.

11.2.4 Model evaluation

The candidate models were fitted using the training data from 1998 to 2008, and quantitatively evaluated based on their predictive ability on the 2009 holdout data using mean square error (MSE) and mean absolute percentage error (MAPE). MAPE measures the average size of the prediction error in relative terms, whereas the MSE penalises the worst predictions more heavily.

11.3 Results and Discussion

Inspection of the NZ campylobacteriosis series (Figure 11.1) confirmed that the peak of the annual incidence data was observed during the hot summer months for all the years incorporated in this study, which is in agreement with most international data (Louis et al., 2005, Tam et al., 2006, Fleury et al., 2006, Hartnack et al., 2009). This may be due to human behavioural activity in summer such as picnics, barbeques, other outdoor activities, or possibly the presence of flies (Jepsen et al., 2009, Naumova et al., 2000). This indicates that seasonal models are required.

A significant decline in campylobacteriosis notifications was noticed in 2007 (Figure11.1) as expected, possibly due to the improved consumers' safety practice at home and reduced level of contamination of chicken carcasses brought to consumers' kitchens. This suggests, as mentioned above, using an intervention variable at this point in the series. The analysis suggested that the best ARIMA was an ARIMA $(2,0,0)\times(0,1,1)_{12}$. When this was fitted with an intervention for 2007, using the R software, the intervention constant was found to be significantly negative, indicating evidence of a reduction in the underlying mean number of cases at this time. For the implementation of the Holt-Winters method, the smoothing parameters were left at their default values of 0.2, as this study wanted to investigate the performance of a simple "off-the-shelf" method.

The Holt-Winters and ARIMA with intervention models were found to be the best models for predicting campylobacteriosis in NZ. Both additive and multiplicative versions were considered.

The results for the best models identified for each technique for the training data and the test data are summarised in Table 11.1 with the monthly forecasts of campylobacteriosis incidence by all techniques tested for 2009 given in Table 11.2. The MSE and MAPE for ARIMA and Holt-Winters

method were better than the regression-based decomposition method for both the training data and the test data.

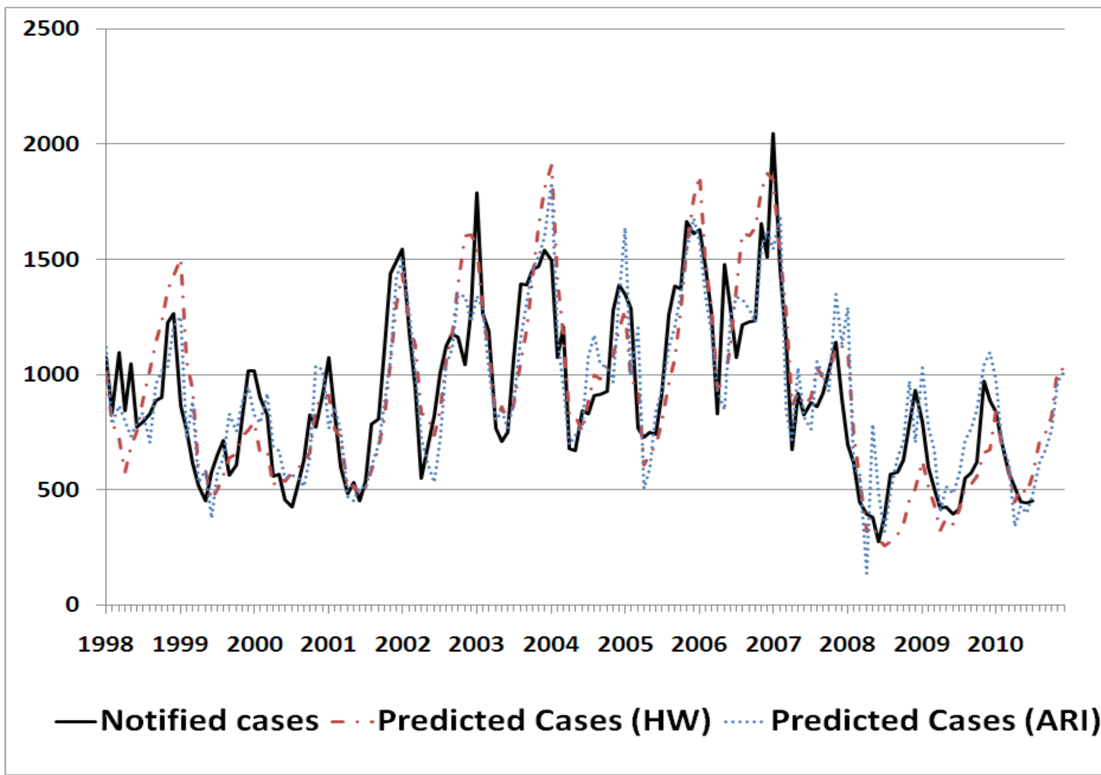

Figure 11-1 Campylobacteriosis notifications in New Zealand from 1998 to 2010 with Holt Winter and ARIMA intervention analysis and forecast.

Table 11-1 Time-series statistical model comparisons for campylobacteriosis forecast in New Zealand.

	Training (1997-2008)			Test (2009)	
Method	R^2	MSE	MAPE	MSE	MAPE
Decomposition (multiplicative)	0.3773	44.84	26.60	142.93	88.34
Decomposition (additive)	0.3778	44.81	26.63	138.65	88.77
Holt-Winters (multiplicative)	0.6228	27.16	18.13	8.99	14.71
Holt-Winters (additive)	0.6604	24.46	18.88	16.24	29.57
Arima	0.7724	16.39	14.11	35.17	40.16
Arima with intervention	0.6959	21.90	17.21	7.69	17.86
Arima (mult.)	0.7875	15.30	12.77	38.65	45.77
Arima (mult.) with intervention	0.7981	14.54	12.09	16.35	30.69

MSE = Mean square error, MAPE = mean absolute percent error

Table 11-2 Time-series model comparisons for campylobacteriosis rate (per 100000) forecasts in NZ for 2009.

Month	TRUE	DCa	DCm	HWa	HWm	ARa	ARm	AR_Ia	AR_Im
jan	18.46	33.40	34.94	19.77	14.60	24.54	23.24	23.24	22.63
feb	13.91	29.13	29.03	13.21	12.02	18.82	19.58	16.98	18.52
mar	11.78	23.58	24.11	9.03	10.14	15.94	17.20	14.67	16.63
apr	9.82	17.06	17.02	2.43	7.51	9.89	12.67	8.17	12.11
may	9.85	19.55	17.25	5.53	8.71	13.49	14.64	10.58	13.24
jun	9.19	19.40	19.49	4.38	8.14	13.15	14.05	9.84	12.63
jul	9.62	21.24	21.14	5.98	9.55	15.45	16.20	11.75	14.29
aug	12.68	23.18	23.18	9.46	11.76	19.25	19.39	15.09	16.78
sep	13.18	24.64	24.39	10.33	12.13	20.83	20.86	16.30	17.85
oct	14.34	27.77	28.41	11.76	12.94	23.00	23.08	18.20	19.60
nov	22.41	32.89	32.73	16.34	15.25	27.97	27.71	22.70	23.09
dec	20.38	32.72	33.70	16.70	15.51	28.94	28.92	23.99	24.50

DCa = Decomposition (additive), etc.

Table 11-3 Forecast performance of the number of cases for the most successful models for the year 2010.

Month	TRUE	HWm	AR_Ia	AR_Im
jan	839	841.8	982.7	912.4
feb	705	686.1	703.2	744.6
mar	571	585.4	610.8	670.5
apr	510	447.2	341.6	494.7
may	449	509.9	432.1	538.5
jun	452	482.9	400.2	514.2
jul	442	564.1	479.6	579.4
aug	628	715.1	623.1	687.7
TOTAL	4596	4832.6	4573.3	5142.0

The basic ARIMA and ARIMA with intervention easily out-performed all other methods in the training data, but the basic ARIMA performed poorly on the test data, presumably because it failed to accommodate the intervention in 2007 and subsequently over-estimate of future values. The Holt-Winters method, being local in nature, quickly adapts to the new regime post-intervention and gives good predictions, comparable with the ARIMA intervention models. A multiplicative model is preferable for the Holt-Winters method, but the choice is less clear for the ARIMA intervention model.

To investigate further, the performance of the Holt-Winters multiplicative and ARIMA intervention methods was also tested for the currently available data of the year 2010. The results are summarised in Table 11.3 and depicted in Figure 11.1 It can be seen that prediction by the additive ARIMA intervention method was slightly better than the prediction of Holt-Winters multiplicative method for the total number of cases over the eight data points currently available, whereas the Holt-Winters forecasts track the monthly figures slightly better. The Holt-Winters method has the additional advantage that it is a simple method to use (Chatfield, 2004), whereas the ARIMA intervention model requires extra statistical expertise to conduct it.

From the above, it is confirmed that classical time-series techniques such as ARIMA with intervention and Holt-Winters can provide a good prediction performance for campylobacteriosis risk. Regression-based decomposition might perform well in a stable situation, but such a global approach is likely to fail when there are structural changes in the series. The NZ campylobacteriosis data pattern, in particular, the sudden drop in incidence following an intervention, may have contributed to the weak performance of this decomposition method.

The general strength of the Holt-Winters forecast method is that it managed to predict the monthly campylobacteriosis cases in NZ accurately. Despite an apparent structural change in the series, and that it can be used easily with a minimum of statistical expertise with the currently available software (Rolfhamre and Ekdahl, 2006). The ARIMA intervention model, despite its much greater complexity and sophistication, did not lead to markedly better forecasts, except for the total number of cases in 2010. Its main advantage is that it can estimate and test the significance of any structural changes expected from a planned intervention. Given the development of appropriate software and the variety of forecast methods available and reviewed (Box et al., 2008, Brockwell and Davis, 2002), evaluation of such interventions can be conducted promptly. These time-series techniques can be useful to health professionals and policymakers in preparing for a predicted epidemic (Myers et al., 2000). The results reported by this study could be useful to the NZ Health and Safety Authority's efforts in addressing the problem of the campylobacteriosis epidemic. NZ has been requested by official international organisations such as Codex Committee on Food Hygiene (CCFH) Codex for NZ to lead the world in developing standards to combat campylobacteriosis. This is possible as a recognition of the initial strategy for targeting the campylobacteriosis epidemic in NZ. However, the number of reported cases in New Zealand is still higher than in other developed countries. Thus, the selected industrial interventions and the consumers' education programme need to be re-evaluated and reviewed to control the disease and achieve a comparable rate with other developed countries.

11.4 Conclusions

An accurate monthly prediction for campylobacteriosis cases based only on previous surveillance data in NZ was achieved using a simple method. The provision of valuable information for public health professionals and policymakers was also be achieved. Evaluation of the selected implemented interventions to combat campylobacteriosis was possible based on the results described above. The study could provide an early alert for the possible repeat increase of campylobacteriosis cases in NZ. The classical time-series analysis could provide an accurate prediction for campylobacteriosis in NZ with minor adjustments.

The Holt-Winters technique was slightly more successful in prediction than the ARIMA with intervention techniques for a general monthly prediction. ARIMA with intervention outperformed Holt-Winters on the total yearly prediction. The summer seasonal peak pattern for NZ data is comparable with international data. The drastic decline in NZ campylobacteriosis reported cases when the study was conducted in 2007, and 2008 hindered the straightforward application of time-series analysis.

12 Evaluation of food handling practice among New Zealanders and citizens of other developed countries as the main risk factor for campylobacteriosis rate

12.1 Introduction

The previous studies revealed that the pathogen characteristics including heat resistance or oxygen tolerance, were not unusual and that poultry industry practices were neither more unsafe nor did they significantly contribute to a higher level of chicken contamination. Thus, the most likely remaining hypothesis is, do New Zealanders have poorer home hygiene practices during food preparation than the people of other developed countries? If so, this would cause cross-contamination during food preparation in the kitchen. Internationally, cross-contamination is considered to be the main factor in campylobacteriosis transmission (Kapperud et al., 2003, Mylius et al., 2007). Thus, it is necessary to test whether the high rate of campylobacteriosis in NZ has a significant link to poorer practices by New Zealanders in their home kitchens. Cross-contamination (transfer of bacteria directly or indirectly from a contaminated source to a non-contaminated product during food preparation) has been a growing food safety concern for the last 20 years (Cogan et al., 2002, Gorman et al., 2002, Luber et al., 2006). *Campylobacter* spp. are heat sensitive and cannot multiply or grow below 30°C. Therefore, cross-contamination that occurs during food preparation becomes the most critical food handling risk factor that enables intestinal tract infection of campylobacteriosis in humans (Nauta et al., 2008).

Quantitative risk assessment studies consider the development of campylobacteriosis to be due to direct exposure to *Campylobacter* spp. in animal foods and water. Many of these risk assessment studies consider cross-contamination during food preparation as the major or the only pathway for campylobacteriosis (Brynestad et al., 2008, Lindqvist and Lindblad, 2008, Mylius et al., 2007, Nauta et al., 2007). Reported studies indicate that many cases of foodborne illnesses are related to the contamination of food eaten at home. Van Asselt et al. (2008) and Redmond and Griffith, (2003) reported that up to 87% and 10 - 50% respectively of the cases were related to infections from food usually eaten at home. A lower percentage reported by Redmond and Griffith (2003) is possibly due to the practices in different countries and/or due to the under-reporting of home foodborne illnesses. It is now believed that improper food handling practices contribute to at least 40 – 60% of cases of foodborne illnesses (Cogan et al., 2002). A NZ analysis of gastroenteritis outbreaks for the period between 1998 to 2000 revealed that 39.3% of cases were acquired at home

(Gilbert et al., 2007). Another NZ study (UMR Research, 2007) reported in the same year showed that about 30% of their participants reported foodborne illnesses in 2007. In the years 2003 and 2005, about 20% of the participants had experienced foodborne illnesses (UMR Research, 2007). However, only 6% of those participants indicated that their illness was caused by food cooked at home (UMR Research, 2007).

Several review papers have discussed food safety practices in homes throughout the world (Patil et al., 2005, Redmond and Griffith, 2003). These studies addressed consumer beliefs about hazards, their knowledge about food safety and their self-reported practices. There are also observational studies on consumer behaviour in both laboratory kitchens and in real environmental conditions. (kitchen). In these studies, consumers were asked to prepare and cook a contaminated meal which was either purposely or naturally contaminated (Evans and Redmond, 2018) Few of these observational studies linked specific consumer behaviour to the microbial contamination of the prepared meal (Redmond et al., 2004). Models for cross-contamination were designed and validated by quantitative observational studies (van Asselt et al., 2009).

The aim of this study was to (i) investigate the reason for the high number of cases of campylobacteriosis in NZ, (ii) provide insights into the relationship between consumer knowledge and food handling practices, and (iii) to discuss the discrepancy between predicted consumer behaviour and observed behaviour. Moreover, the study investigated the best hygiene measures that can be used to provide public health professionals, risk managers and regulatory authorities with the scientific support for future health promotion plans that will discourage consumers from risky, unsafe food preparation practices. Laws and regulations cannot enforce controls and measures to prevent unsafe practises at home, unlike as for the commercial food processors and retailers. The most logical way to change risky consumer practices is by educating consumers.

12.2 Methods

An extensive search of Scopus, ISI web of knowledge, and Google Scholar databases was conducted for publications concerning consumer food handling practices or self-reported practices, consumers' knowledge or perception about food safety and consumers' observed practices. Valuable data was also gained through attendance at international conferences (NZIFST 2008, IAFP 2008, CHRO 2009, and IAFP 2010), personal communication with the science group at the NZ Food Safety Authority (the funding organization of another *Campylobacter* related project, reference number (N0174/06 in 2006) and with *Campylobacter* lead scientists from the ESR. The available data provided an understanding of current consumer behaviour and the perception that can be used to plan effective education strategies. The search focused on consumer-based studies

that used the following methods.

12.2.1 Survey of consumers' knowledge

Personal interviews and self-completed questionnaires were used to collect quantitative and qualitative data. This method provides baseline data about consumers' perception of food safety. Surveys of consumer's knowledge are also used to evaluate the efficacy of health promotion initiatives or before a new health promotion can be formalized or issued (Redmond and Griffith, 2003). The amount and accuracy of consumers' knowledge do not always predict their behaviour towards food safety. However, correct knowledge provides consumers with informed choices about their practices or actions.

12.2.2 Survey of consumers' food handling practice

The consumers' food handling practice survey uses the same technique as the consumers' knowledge survey for collecting data (e.g. interviews and questionnaire). This approach is used mainly to investigate how consumers handle food at home and also to explore why consumers use such handling procedures. The disadvantage of this method is that self-reported practice surveys may or may not reflect actual consumer practice. Usually, consumers report the perceived correct practice rather than their actual practice (Redmond and Griffith, 2003).

12.2.3 Observational study

The direct observational study is considered to be more reliable and accurate for studying consumer behaviour. The observational study can be conducted in either a controlled environment (laboratory) or an uncontrolled environment (home kitchen). Advantages and disadvantages of observational studies are discussed in detail by Redmond and Griffith (2003), and Evans and Redmond (2018). A video camera was used to observe consumer behaviour, or an observer watched the consumer preparing meals and then documented the steps used in the preparation of food. Observational studies have linked cross-contamination practices with the actual microbial (*Campylobacter*) contamination of prepared food in domestic kitchens and the contamination level of the kitchen environment.

12.2.4 Bacterial transfer

A series of microbial studies targeting bacterial transfer from raw food or contaminated sources to cutting boards, hands, or surfaces in kitchens, were also initiated a long time in advance of the fully quantitative, actual kitchen observational studies or interdisciplinary studies (Scott and Bloomfield, 1990, Coates et al., 1987, Deboer and Hahne, 1990).

The simplest and most common equation to calculate the bacterial transfer rate, which is defined as the percentage of cells transferred from the donor surface to the recipient surface (Chen et al., 2001) is expressed as follows:

Transfer rate = 100 x (cfu recipient / cfu donor)

cfu – Colony-forming units of bacteria counted or measured.

12.3 Results and discussion

A few reports have applied a different approach to analyse or combine the data obtained from literature or questionnaires, such as the Meta-Analysis (Patil et al. (2005). Some surveys used the Delphi techniques to rank consumer behaviour (Hillers et al., 2003), while other surveys applied a descriptive analysis (Byrd-Bredbenner et al., 2007a, Kwon et al., 2008, Sanlier, 2009, Nesbitt et al., 2009).

The main purpose of this study was to inform regulatory authorities regarding consumers' knowledge and their critical or risky food preparation practices at home that may be contributing to the high rate of campylobacteriosis cases in NZ. This information is essential so that a successful health communication strategy can be designed to provide an effective message to consumers to change unsafe food preparation practices if any, in the future. Therefore, the main findings and conclusions of only the reviewed papers are classified and summarised below:

12.3.1 Survey of consumers' food safety knowledge

The purpose of such surveys is to determine what consumers knew about food safety. It was reported that 75% of U.S. consumers (study participants) understood the concept of cross-contamination In the U.S., 55% of the consumers participating in the study correctly answered questions about cross-contamination (Byrd-Bredbenner et al., 2013), and in Australia, 38-49% (Jay et al., 1999a). In another U.S. study, 79% of consumer participants reported that they avoid cross-contamination from raw meat juices (Meer and Misner, 2000) and in Canada, the majority of the participants were aware of separating raw food and cooked food during preparation or storage (Nesbitt et al., 2014).

However, an European study indicated that 75% of consumers were not informed /aware of potential risks and hence stored poultry and raw meat on the upper shelves of a refrigerator. U.K. studies reported that 64-100% of consumers knew about the use of different utensils for raw and cooked or ready-to-eat food, to prevent raw food from contaminating cooked or ready-to-eat food (Griffith et al., 2001, McCarthy et al., 2007).

In NZ, only 10-18% of the consumers (study participants) agreed that it is better to store cooked food above raw food in the refrigerator (Hodges, 1993, Kerslake, 1995). 38% of New Zealanders were not aware of the need to use separate or clean utensils for the preparation of raw and cooked food (Hodges, 1993). Moreover, it was reported that 28% of New Zealanders cut raw and cooked food with the same knife. The knife was not washed and was only wiped with a "clean" cloth (Kerslake, 1995). A study in NZ (Gilbert et al., 2007) revealed that 89% of the consumers believed that the consumption of chicken might cause foodborne illnesses. 63% of NZ consumers were concerned about *Campylobacter,* and 77% about *Salmonella* (UMR Research, 2007, Lake et al., 2007). Gilbert et al. (2007) indicated that 90% of the consumers had associated *Salmonella* with chicken, and only 70% associated *Campylobacter* with chicken. However, the same study reported that 20% of the consumers were not concerned that raw chicken carried bacteria.
As reported, 95% of consumers in the U.K. (Lader, 1999) and 86% of consumers in the U.S. (Altekruse et al., 1996) believed that washing hands before preparing food is very important. Another U.K study revealed that 100% of consumers identified when and how it was necessary to wash hands (Griffith et al., 2001). Similarly, in the U.S. 79% knew the six occasions (before, during and after preparing meals, before eating, after using the restrooms, before and after caring for children, after touching an animal or sick person, and when the hands are dirty) for washing hands (Redmond and Griffith, 2003).

12.3.2 Survey of consumers' practice

The first survey of consumer practices was conducted in 1975 (Redmond and Griffith, 2003). Hillers et al. (2003) listed 40 consumer practices that could cause 13 foodborne illnesses. Four practices, in particular, were shown to prevent campylobacteriosis, and these are listed below:

- Use a thermometer to assure that the food is cooked to the recommended temperature (75-80 °C).
- Wash food preparation surfaces, knives and cutting boards and sinks with hot water and soap after contact with raw poultry.
- Wash hands with warm soapy water before and after handling poultry.
- Drink only pasteurised milk.

Most surveys indicate that consumers do not use, or seldom use, thermometers to check if the food has reached the adequate heating temperature (Nesbitt et al., 2009). A U.S. survey indicated 76% of surveyed consumers did not use a thermometer. However, in another U.S. survey, it was reported that 12% use a thermometer for meat, and only 6% used a thermometer to check the

doneness of the cooked food. In another survey, the number of consumers using a thermometer was 3% (Takeuchi et al., 2006). In Canada, 13.7% of consumers use a thermometer to check whether meat is cooked (Nesbitt et al., 2009). There is no NZ data for this practice. The U.K and European studies indicate that 81% of surveyed consumers tended to cook poultry longer than recommended, and 85-92% 'ensured that the food was piping hot'. It has been reported that 28% of American consumers consider the internal pink colour of a burger as sufficiently cooked (Redmond and Griffith, 2003). In NZ, 40% of those surveyed indicated that roasting or baking chicken was the preferred method for cooking chicken and 37.5% of them preferred chicken to be well-done, whereas 35.2% preferred very well-done and only 27.3% preferred medium for roast chicken (Gilbert et al., 2007). Another NZ study revealed that 90% of New Zealanders always check that chicken has been cooked right before they eat it. New Zealander's methods of cooking and preferences for chicken negate the hypothesis that undercooking is considered as a factor for acquiring campylobacteriosis in NZ. Moreover, the heat sensitivity of the most implicated strains are reported in previous chapters and by (Al-Sakkaf and Jones, 2012) support the hypothesis that cross- contamination is the main risk factor responsible for campylobacteriosis in NZ.

With regard to the frequency of washing food preparation surfaces, 80-93% of surveyed consumers in the U.K and the U.S. reported that they always wash the chopping board and utensils after cutting raw chicken. 56-90% of consumers in the U.K., Netherlands, U.S. and Canada use different utensils or cutting boards or the other side of the board for raw and ready-to-eat food (Nesbitt et al., 2009, Redmond and Griffith, 2003, Nauta et al., 2005). In the Netherlands, only 3% of consumers surveyed used the same side of the cutting board for cutting meat and vegetables, and 97% wash the cutting board or use another board or use the other side of the cutting board (Nauta et al., 2005). Only 25% of surveyed consumers in the U.S used the same cutting board after cutting raw food without cleaning it first (Byrd-Bredbenner et al., 2013). Another U.S. study reported that 89% of surveyed consumers always wash the chopping board, put it in a dishwasher and/or use another cutting board (Roseman and Kurzynske, 2006). In Ireland, 78% of surveyed consumers reported that they washed the used cutting board with hot water, detergent and bleach, or used another clean cutting board (Jackson et al., 2007).

In NZ, 76.1% of surveyed, reported cleaning chopping boards at least once daily. Other equipment that was reported to be cleaned once daily is as follows: 54.9% clean sinks/tap, 51.4% kitchen sponge, 44.5% dishcloths, 47.5% dish brushes and 43.8% tea towels. Hand towels were cleaned two to three times per week by 46.2% of the surveyed consumers (Gilbert et al., 2007). This same study estimated that 28-41% of New Zealanders would treat knives and surfaces improperly or allow cross-contamination (re-use of knives and surfaces without washing between preparation of

raw and cooked food). Moreover, 5.3% of surveyed New Zealanders placed cooked meat into a raw meat container during a barbeque. The surveys in the U.K, U.S., Europe and Australia indicated that 72-93% of surveyed consumers (Redmond and Griffith, 2003, Nauta et al., 2005) washed their hands with soap and water before handling food and after handling raw food and poultry. In NZ, only 39% of consumers washed their hands before food preparation, and 22% washed hands between food preparation steps. Only 5% washed their hands after food preparation. Moreover, only 26.4% washed their hands correctly (using hot water, soap, and drying) and only 18.8% of the surveyed consumers dried their hands with the same towel that is used for drying dishes (Gilbert et al., 2007). The NZFSA educational film for under school-age children asks kids to wash their hands before eating, but only to wipe their hands with a cloth towel after eating.

12.3.3 Observational consumers practice

Griffith and Redmond (2001) compared observed consumer practice in the U.K with previous knowledge, behavioural intentions, and self-reported practice with regard to hand washing and cross-contamination. It was concluded that in spite of the participants' knowledge (100% of the participants knew the extreme importance of handwashing), attitude and positive intention, only 85% indicated that they would "very likely wash hands" the next time the food is prepared. However, no participants washed their hands adequately all the time during the observed food preparation session. From the findings of the same study, with regard to preventing cross-contamination, although 100% knew the importance of preventing cross-contamination and 80% had a positive attitude, intention and self-reported practice, only 48% used a different utensil for raw or cooked food and to adequately clean surfaces between the preparation of raw food and cooked or ready-to-eat food during that session.

There are few observational studies that compared consumers' knowledge with actual behaviours, such as one by Jay et al. (1999b). This report indicated that half of the study participants did not use detergent or cleaner to clean kitchen surfaces. It concluded that there is a significant difference between consumer knowledge and their actual behaviour and practice. Anderson et al. (2004) and Redmond and Griffith (2003) reported a similar conclusion that 98% of consumers cross-contaminate ready-to-eat or cooked food with raw meat or raw egg. (Anderson et al., 2004) indicated that only 45% of the participants in a U.S. study attempted to wash their hands before beginning to prepare food, although 87% claimed that they wash their hands at all or most of the time. In the U.S., 5-57% of those surveyed neglected to wash their hands or did not wash their hands correctly, and 13-71% used improper-cross contamination procedures (Kendall et al., 2004, Redmond and Griffith, 2003, DeDonder et al., 2009). Only 45% washed their hands before

preparing a meal, and only 84% of those used soap (Anderson et al., 2004). In Australia, approximately 75% failed to wash their hands, 47% failed to wash their hands after handling raw meat, and 44% of those failed to use soap (Jay et al., 1999b). The same study also reported that approximately 35% failed to wash utensils between preparing raw foods and ready-to-eat food, and 30% failed to clean the preparation surfaces before preparing ready-to-eat food. Similar discrepancies among U.S. adults were reported (Abbot et al., 2009), and among U.S. Latinos (Dharod et al., 2007). There appear to be no studies on food preparation observational studies have been conducted in NZ.

12.3.4 Bacterial transfer

Perez-Rodriguez et al. (2008) reviewed and critically analysed published research on bacterial transfer, and they discussed bacterial recovery methods, environmental and intrinsic factors influencing bacterial transfer, and bacterial transfer models used in microbial risk assessment studies (Kusumaningrum et al., 2004, Perez-Rodriguez et al., 2006, Rosenquest et al., 2003, Schaffner, 2004a). Perez-Rodriguez et al. (2008) stated that moisture, pressure and contact time are the main factors that increase bacterial transfer. *L. monocytogenes* which was attached to polyethylene and wooden cutting boards and recovered after holding time up to 1 h. Transmissions of *L. monocytogenes* to cooled cooked chicken meat from both types of cutting boards were relatively higher than hot cooked chicken meat (Goh et al., 2014). The currently available bacterial transfer models need more experimental data. Stochastic models (probability distribution) can be utilised as a transient solution for the apparently random and imprecise nature of the current experimental data. One limited study of bacterial transfer in food preparation (Gilbert et al., 2006) and a consumer home practice survey (Gilbert et al., 2007) were conducted after the highest numbers of campylobacteriosis outbreak in NZ history (426 cases per 100,000 persons) reported in 2006. New Zealanders had been lulled into a false sense of security for many years. Gilbert et al. (2006) summarised several bacterial transfer studies in the literature (see Appendix 3; Gilbert et al. study). These transfer rates reported by Kusumaningrum et al. (2003), Luber et al. (2006) and Montville and Schaffner (2003). Gorman et al. (2002) evaluated cross-contamination resulting from naturally contaminated chicken and reported that four pathogens (*Salmonella*, *Campylobacter*, *Escherichia coli*, *Staphylococcus aureus)* could easily spread from contaminated raw chicken to hands and kitchen surfaces and contact food. Other similar studies evaluated the ease of how different microorganisms can be transferred to food by different consumer handling scenarios (Barker et al., 2003, Haysom and Sharp, 2005, Redmond et al., 2004). A limited number of studies about bacterial transfer between surfaces (Humphrey et al., 1994, Deboer and Hahne, 1990) have also been

conducted. There is a significant discrepancy in the reported values of transfer rate from a donor to recipient and this confirms that identifying the transfer rate is a massive challenge. For example, Chen et al. (2001) reported a transfer rate of 8.7% of the surrogate bacterial indicator (*Enterobacter aerogenes*) from chicken to hands and Luber et al. (2006) reported a *Campylobacter* transfer rate of 2.9 - 3.8%. Montville et al. (2001) indicated a transfer rate of 0.71% (of surrogate bacteria). Montville and Schaffner (2003) reported a similar transfer rate of 0.59% in a later study (surrogate bacteria). A NZ study (Gilbert et al., 2006) reported a *Campylobacter* transfer rate of 0.4 - 6.2%, and Kusumaningrum et al. (2004) 2.4 %. A study by Chen et al. (2001) indicated that the transfer rate could reach 30% from raw poultry to the cutting board and 50% from the cutting board to lettuce, and up to 100% to hands. The transfer rate of *Campylobacter* at 42.5% was significantly higher than for *Salmonella* (4.8%) and *E. aerogenes* (12.6%) from a steel surface to food (Kusumaningrum et al., 2004). This confirms the capacity of *Campylobacter* to spread quickly and easily to and from surfaces and to cause contamination and consequently infection if ingested by consumers, more than by any other pathogen (Redmond et al., 2004). Transfer data studies have facilitated the understanding of the microbial risks associated with food handling practices. Many observational studies were conducted with actual bacterial contamination of prepared meals (van Asselt et al., 2009, de Jong et al., 2008, Nauta et al., 2008, Redmond et al., 2004, Luber et al., 2006). Christensen et al. (2005) developed a model for hygiene practice and consumption patterns, and Mylius et al.(2007) built a mechanistic model for cross-contamination during domestic food preparation. Van Asselt et al. (2008) succeeded in developing a model for transfer and validated the model with actual consumer observational data (van Asselt et al., 2009). Although the accuracy of these models is not high, the model prediction can be used to quantify cross-contamination in the home and can be incorporated in a QMRA model.

It has been stated that there is a lack of data in NZ with regards to studies on consumer's knowledge and handling practices. The few studies (Bloomfield and Neal, 1997, Kerslake, 1995, Gilbert et al., 2007) conducted in NZ were not comprehensive and have increased the ambiguity around food handling practices in NZ. Thus, the data extracted from these limited studies should be treated with caution. It is difficult to compare NZ surveys and international surveys due to differences in sample size, objectives, targeted population groups, methodology and the interpretation of results. The limited amount of consumer-based research, which is of significant importance for the safety of New Zealanders, has declined due to a lack of motivation and/or necessary research funds.

It appears, from the findings of this study, that New Zealanders' knowledge of basic food hygiene is less in comparison to people of other developed countries. For example, in preventing the dissemination of *Campylobacter* in New Zealanders' kitchens during preparation such as

handwashing, use of different chopping boards, knives and utensils for cooked or ready to eat and raw food, or proper storage, in the kitchen, New Zealanders have scored very low in their knowledge about food safety or hygiene and reported self-practices.

In spite of the limitations of the self-reported practice surveys such as social desirability bias (reported frequently) with questionnaire techniques and telephone interviews rather than with a face to face interviews (Redmond and Griffith, 2003), these surveys can be useful for the evaluation of health promotion initiatives and/or to provide data about awareness and knowledge.

The most critically researched practices that prevent cross-contamination are hand washing and the use of clean and separate utensils for raw and cooked or ready-to-eat food during food preparation. Hand washing is considered the most important practice for preventing gastroenteritis (Mutters and Warnes, 2019). Hand hygiene has been demonstrated to result in reductions in gastrointestinal disease by 30-50% (Aiello et al., 2008, Curtis and Cairncross, 2003, Ejemot et al., 2008). The actual percentage of New Zealanders that cross-contaminate could be higher than reported in NZ studies, as the respondents mostly claimed that they performed the perceived correct practice. This is due to the Hawthorne effect. One observational study observed New Zealanders in the washrooms after using the toilets (Garbutt et al., 2007). This study was an attempt to determine how New Zealanders wash and dry their hands and for how long. It was reported that 13.3% of New Zealanders did not wash their hands after using the toilet (Garbutt et al.) This is a low observed percentage. The actual percentage could be lower as in this study, and all the participants were aware that someone was standing and observing their handwashing practice after using the toilet. The percentage of people who did not wash their hands after using the toilet may be due to the design of many NZ houses especially the older houses where the washing facilities are separate from the room containing the toilet. With this unique constructional and regulatory fault, New Zealanders have to walk to the bathroom or shower-room to access washing facilities for washing their hands after using the toilet. This unique design of older homes in NZ may have contributed to the increased number of participants who did not wash their hands after using the toilet in Garbutt et al. (2007) study. Generally, the lack of a washing facility in toilets may also contribute to the general habit of washing hands in the other five critical instances of washing hands. Thus, the number of patients with *Campylobacter,* introduced to homes via purchase of chicken, has possibly increased dramatically in the last 20 years in NZ.

In 2007, the NZ Food Safety chief executive considered the level of hygiene practice of New Zealanders to be the reason for the high rate of NZ campylobacteriosis (McKenzie, 2007). In 2008, during an interview with a NZ Radio Station, a prominent NZ and International *Campylobacter* expert (Nigel French) indicated that if New Zealanders improved their practice of washing their

hands, the rate of campylobacteriosis would decline dramatically and would consequently solve the campylobacteriosis problem in NZ. In 2007, a member of the NZ parliament indicated that New Zealanders were too lazy to wash their hands, preferring to wring their hands more often than they washed them, and warned New Zealanders that if they did not wash their hands, they would become ill (Coddington, 2007). Thus, more research on the behaviour of New Zealanders is needed for NZ researchers to understand and address these issues comprehensively in the future.

However, most of the evidence collected in this study appears to confirm the hypothesis that New Zealanders are not as good in food hygiene compared to people in other developed countries. This may have possibly contributed to NZ having the highest rate of campylobacteriosis amongst developed countries. The other reasons which may contribute to the higher rate in NZ are the prevalence of and contamination level of carcasses produced in NZ, leaky packaging of chicken sold in NZ, and inadequacies in the reporting system.

If the present large number of campylobacteriosis increase further, this would result in an increased cost to the health care system, could affect the NZ tourism industry and also agricultural produce since both champion the New Zealand's "clean green" image when advertising abroad.

12.4 Conclusions

Cross-contamination is considered both internationally and in NZ to be the main factor in *Campylobacter* transmission of. A lack of knowledge concerning food safety during domestic food preparation is prevalent amongst NZ consumers. Most of the evidence collected in this study confirmed to some extent the hypothesis that New Zealanders have poor food hygiene practices and food safety knowledge than those in other developed countries, and this is possibly is the reason for the higher rate of campylobacteriosis in NZ than in other developed countries. The practice of handwashing and the separation of raw and cooked or ready-to-eat food during preparation and storage needs to be addressed in any new health promotion initiative. These practices are also crucial to exploring why New Zealanders may deviate from the best microbiological food safety practices and for improving NZ consumers' home practice to control campylobacteriosis. Further research is required to investigate the extent of these unsafe practices and to review the legislation or codes for building homes in NZ so that the habit of handwashing can be encouraged.

13 Consumer poultry handling practice survey

13.1 Introduction

The acknowledgement of the importance of adequate consumer food-handling practices has been discussed in the previous chapter(s), and it is widely recognized as the last line of defence against foodborne illness (Nesbitt et al., 2014, Redmond and Griffith, 2003). The prevention of foodborne illness requires the collaboration of all members of the food continuum, from farm producers to consumers. Therefore, research and consumer education regarding the risk of food safety malpractices is an important element in precluding food-borne diseases. It is rational to expect that a fraction of the human exposure to *Campylobacter* spp., specifically, originates from cross-contamination that occurs in home kitchens during food handling (Brynestad et al., 2008, Hartnett et al., 2001, Lindqvist and Lindblad, 2008, Mylius et al., 2007, Nauta et al., 2007). Therefore, the number of human campylobacteriosis cases can be reduced by reducing the degree of *Campylobacter* spp. cross-contamination by improving hygiene in kitchens (Kusumaningrum et al., 2004, Jones et al., 2016).

Bruhn (2014) reported that personal hygiene is insufficient if 65% of the participants do not wash their hand before the meal preparation, and 40 % not wash their hands after handling raw chicken. (Maughan et al., 2016) reported that about 40% wash their hands after handling the chicken breast. They also reported that only 37% of consumers used a thermometer to check the 'doneness' of the chicken breast. Evans and Redmond (2018) observed the handling practice of raw chicken by consumers who are over 60 years old in a model kitchen and reported that older adults did not obey safe food handling practices with 90% failing to implement adequate hand decontamination immediately after handling raw chicken. Diplock et al (2018) investigated the effectiveness of food handler training programs to improve safe food handling behaviour among high school students. This study concluded that despite a significant increase in the correct behaviour, students continued to use risky practices post-intervention, suggesting that the risk of foodborne disease remained. Several studies (Kosa et al., 2015, Sterniša et al., 2018, Koppel et al., 2016, Koppel et al., 2015, Bearth et al., 2013) have addressed consumer reported handling practices of poultry products. All these studies indicated that consumers are not good at safe food handling leading to cross-contamination, caused by improper refrigerated food storage and thawing, undercooking, and general hygiene practices. Recently, (Katiyo et al., 2019) assessed the practice and the knowledge of South African consumers with respect to the safety risks associated with

handling raw chicken meat. This study also confirmed unsafe practices during chicken purchasing, thawing, and handwashing by consumers with 62 % not following safe practices during the handling of raw chicken. (Bearth et al., 2014) researched on consumer risk perception and knowledge related to campylobacteriosis and revealed that lack of food safety knowledge and personal risk perception were the central reasons for food safety violations during poultry preparation. (Donelan et al., 2016) investigated the consumer poultry handling behaviour in the grocery store and in-home storage and found that there was no hand sanitizer or wipes available in 71% of grocery store meat sections of the stores visited. Plastic bags were available in the meat section, 85% of the time, but only 25% of the shoppers used the bag for their raw poultry purchases.

13.2 New Zealand consumer studies

In New Zealand, there is a lack of research and also data related to studies on consumer knowledge and handling practices. The few studies conducted in New Zealand (Hodges, 1993, Kerslake, 1995, Gilbert et al., 2007) have indicated that there is lack of knowledge of food safety with 28-41% of New Zealanders re-using knives and food surfaces without washing between preparation of raw and cooked food. However, these studies are not that comprehensive. Thus, the data extracted from these limited studies should be treated with caution. There is no previous study in New Zealand which targeted consumers self-reported practice of handling poultry purchase, transport, storage, and food preparation to provide an insight into campylobacteriosis risk factors. Due to the large economic and health consequences of campylobacteriosis, which has been estimated to be ~ NZ $ 50 million (Duncan, 2014) urged introducing intervention programmes to reduce the disease rates. Thus, it is clear that there is a need for a more in-depth investigation of safe food preparation at home. Moreover, the chief executive of the -NZ Food Safety Authority revealed that cross-contamination during chicken preparation at New Zealand home as a possible hypothesis to explain the high reported rate of campylobacteriosis (McKenzie, 2007). Thus, it is necessary to test whether the high rate of campylobacteriosis in New Zealand has a significant link to malpractices by New Zealanders in their home kitchens.

Laws and regulations cannot enforce controls and measures to prevent unsafe practises at home, although such controls can be applied to commercial food processors and retailers. The only possible way to change consumer behaviour is through education. To change behaviour, it is important to make people aware of the consequences of their behaviour. A lack of food safety knowledge and gaps in applying knowledge into practice are major obstacles food handlers have to overcome to reduce food contamination effectively. To educate food handlers, it is important

to conduct formative research to determine basic knowledge, perceptions and information of the target audience, formulate a clear food safety message and use diverse platforms to convey the information.

The objective of this research was to fill the data gap in NZ regarding poultry handling practices by consumers. The study will provide an insight into the relationship between consumer knowledge and poultry handling practices. This study will investigate the best hygiene measures that can be used to provide public health professionals, risk managers and regulatory authorities with scientific support for future health promotion plans that will discourage consumers from unsafe poultry preparation practices. It is aimed to determine what consumer groups have inadequate food safety knowledge and hence most likely to practice unsafe poultry preparation at home. Thus, the study goal was two-fold. 1) Assess which information poultry consumers lack and hence should be included in future food safety communications, and to 2) Identify which groups are at more risk so that future food safety education programme can be targeted towards them. The handling of poultry meat preparation data will also be used as an input for further optimisation of a risk assessment strategy for campylobacteriosis in NZ from consumption of poultry meat. This study will contribute to the existing knowledge to tackle the reasoning behind the increasing of campylobacteriosis incidents by giving an updated insight on the assessment of knowledge of people on food safety and their practices on preparing poultry at home.

13.3 Methods

Several research designs are available to gather information on consumers' food preparation practices. The main design, which is used in this study, is described briefly below:

13.3.1 The quantitative design

This method tests objective theories by examining the relationship among variables which are measurable (Creswell, 2018). Quantitative questions will result in data that is easy to convert into objective, numbers-based analysis. Survey research (non-experimental) and experimental research are the main strategies for conducting the research design. Survey research provides a numeric or quantitative description of opinions, attitudes, trends of the population by studying a sample of that population. It uses a questionnaire or structured interviews for data collections. The answers to the questions can be assigned numeric values. These numbered data can be analysed using statistical methods. Quantitative techniques include cross-sectional studies which collect data only on time and longitudinal studies which collect data over an extended period of time. A questionnaire, with closed-ended questions, structured interview methodologies are appropriate

when the issue is known, relatively explicit and straightforward, and can be validated with reliable measurement.

13.3.2 The study approaches

The study will use a quantitative survey design, a strategy that has been commonly used for consumer poultry handling investigations involving large populations (Kosa et al., 2015, Sterniša et al., 2018, Koppel et al., 2016, Koppel et al., 2015, Bearth et al., 2013).

A self-reporting questionnaire on consumer behaviour can be administered on a large sample in a short period of time. Thus, interpretations can be made about the behaviours, opinions and attitudes of a population. Moreover, the self-reported questionnaire is a more economical choice than observational study approach. However, a self- reporting questionnaire can show a disparity between observed and reported behaviour (Evans and Redmond, 2018, Redmond and Griffith, 2003). A self-completed survey can provide information on how consumers may apply their knowledge during food handling (Cody and Hogue, 2003). However, specific consideration needs to be given to the structure of the questions to overcome the problem of disparity between the consumer reported practice and actual practice. For example, questions need to be asked the participants on what their practical behaviour is in the kitchen rather than statements that deal with safe food handling. Many of the previously reported consumer food handling studies had used the Likert scale option statements in their questionnaire, such as strongly agree, agree, undecided, disagree, strongly disagree. Moreover, the participant in this study was not directly confronted with the notion of general hygiene, cross-contamination, storage and temperature control but indirectly through questions about their actions and deeds at the last occasion they purchased raw poultry or prepared raw poultry, what utensils were used, what was the status of the utensils they used during preparation of raw poultry or what they did immediately after handling or cooking raw poultry.

13.3.3 Questionnaire development

The biggest challenge to obtain accurate and reliable information about consumer practices and behaviour concerning food safety from studies using self–completed questionnaires, is the design of the questions to guarantee aimed and accurate responses. To do so, it is essential to use language that is understandable to the type of respondents whom you are targeting.

A structured questionnaire is designed based on suitable and relevant questions from previously validated questionnaires applied in similar studies (Byrd-Bredbenner et al., 2007b, Mazengia et al., 2015, Bearth et al., 2014, Kosa et al., 2015, Sampers et al., 2012). The respondents need to be requested to be sincere and encouraged to report their actual behaviour. The innovative method

of a street-intercept survey of main public places such supermarkets (Pak and Save, New World, Countdown) and major public libraries in main district and suburbs of Canterbury region were combined with the distribution of the survey via email (containing an embedded electronic link to the survey) to the Lincoln University academic and non-academic staff. The questionnaire was administered as an online survey using Qualtrics (Qualtrics, Utah). When a print version was used in the survey at supermarkets and public libraries, the respondents were requested to return it on completion on the spot. In addition, the participants who wanted additional time were a postage-paid pre-addressed envelope or to use the electronic link and complete the questionnaire at their convenience.

13.3.4 The Questionnaire

The questionnaire comprised of 31 multiple-choice questions designed to collect information on self-reported practice during the last time they purchased and prepared raw poultry at home. The first four questions in the first section consisted of respondents' food purchasing habit, such as how/where/when they purchased poultry products during their shopping. This was followed by ten questions in the second section, about the respondents' food preparation practices. These questions were designed to address the sequence of events that occurred the last time poultry food was prepared and cooked at home. This was to encourage respondents to report their actual practice rather than their perceived practice and to help minimize biases associated with self-reporting and social desirability. For all questions, respondents selected an answer from a list of five to six answers to avoid signalling respondents to the "correct" response. The questions collected information on respondents' compliance to the recommended food safety practices of the WHO's five keys for food safety manual (Fontannaz-Aujoulat et al., 2019), U.S. Department of Agriculture (USDA) guidelines for handling, storing, and preparing raw poultry at home or following the 3 Cs (clean, cook and chill) food safety tips recommended by New Zealand Food Safety Authority (NZFSA) (Anonymous, 2019). Specifically, there were four questions related to personal food hygiene practices, five questions on prevention of cross-contamination practices, and another five questions related to food temperature control and proper food storage practices. The third section consisted of seven questions related to the respondents' basic food safety awareness, such as awareness of clean, separate, cook, chill cover rules, or the knowledge of whether NZ chicken carries bacteria etc. Each question answered correctly would award the respondent one point and zero for incorrect answer. The maximum score was 21.

The fourth section consisted of 10 questions to obtain information about how frequently poultry was cooked, their source of food safety information, demographic information including gender,

age, education level, occupation and household income etc.

Consent was obtained from the respondents, and they were informed of the research topic and the anonymity of data. The survey was pilot tested with 20 participants with different education level and background for time estimation, question comprehension, and clarity. Face validity and content validity was established by NZ and international experts who agreed that the questionnaire was a valid measure of the 'poultry handling practices and food safety awareness' concept following modification and refinement of the questionnaire. Data collected from the pilot testing was not included in the final data set. A copy of the questionnaire is in appendix G.

13.3.5 Target participants

The participants of the study were people over the age of 18 who lived in Christchurch and its suburbs which has a high incidence of campylobacteriosis notification rate in NZ with restrictions on that they can understand the English questionnaire and handle and cook poultry at home.

13.3.6 Data collection

The participants were selected at random and were approached both in-person with a print version of the survey or an electronic link to the survey which was sent out for participation and completion of the survey. The print version was to be returned on the spot after it was completed, or the respondents were given a postage-paid pre-addressed envelope who preferred because of time constraints. Participants who preferred to fill the electronic version through a tablet/laptop at the site and sometimes or later at their convenience at home were given a link to the survey. The survey was conducted between November 2019 and January 2020.

13.3.7 Data analysis

Data collected via Qualtrics was downloaded into SPSS software for analysis. The survey data were analysed using descriptive statistics (e.g. frequencies,) percentages of correct and incorrect answers for each question. Additionally, each sub-section mean scores of the survey and standard error were calculated. Several normality tests of the data were performed for the data. The z values test of data skewness and kurtosis, Shapiro-Wilk test, Kolmogorov–Smirnov test, histograms, normal Q-Q plot and box plots were the tests performed to analyse the data normality. One-way ANOVA test was used to compare the mean scores between the respondents with a different type of socio-demographics. For all analyses, a p-value < 0.05 was considered statistically significant. A correlation between different sections of the questionnaire was also assessed by the Pearson test.

13.4 Results and discussion

The questionnaire answers were recorded as 1 for a correct answer and 0 for an incorrect answer so that the data can be analysed quantitively as binary data. The reliability test for dichotomous data set Kuder Richardson 20 was used for this study data set. (Vaucher et al., 2012). This calculations used in this test was similar to that used in the Cronbach alfa test, and the mean value for the 21 questions was 0.707. This value is acceptable to confirm the internal consistency, that is, how closely related the tested questions were. All data normality tests confirmed that the data was normally distributed. Therefore, a parametric analysis was considered as the most appropriate method for analysing the data (Sullivan, 2016). Specifically, one way ANOVA was used to determine the impact of the respondents' detail and demographic characteristics on the response scores. For all analyses, a p-value < 0.05 was considered as statistically significant. The number of valid responses was 301, and the mean score was 9.8306 with a SD value of 3.49779 and a SE of 0.20161. The maximum score was 19, and the minimum 2. Since the respondents were randomly selected, some demographic percentages of groups participation were very low due to the small number of the respondents in those groups. So, the data obtained from those groups may not truly reflect the poultry handling practices of these very underrepresented groups in the study sample. Therefore, their results are represented only in the tables for future research and excluded from the detailed analysis and conclusions of the study. Outputs of SPSS analysis are in appendix G.

13.4.1 Association between respondents' socio-demographic characteristics and mean score

All the respondent details, demographic characteristics, descriptive analysis, and mean score for each group are summarised in Table 13.1. Approximately 40% of the respondents had the post-graduate qualification, and another about 40% had a university degree or students at a university. The majority of respondents were NZ European (69%). More than 80% of the respondents cooked poultry at home 1 – 3 times per week. Approximately 30% of the respondents had experienced food poisoning 1 – 4 times during the last year. Previous research has shown that demographic factors influence domestic food safety behaviour (Fischer and Frewer, 2008, Al-Sakkaf, 2015).

Table 13-1 Respondents' details and demographic characteristics

Respondents' Characteristics	Category	Respondents (n.)	Percentage	Mean	SE
Gender *	Male w/o children	53	17.60	8.76	0.46
	Female w/o children	105	34.90	10.15	0.35
	Male with children	23	7.60	8.61	0.73
	Female with children	117	38.90	10.20	0.31
	Prefer not to answer	3	1	12.67	1.20
Age	<20 years	3	1	7.33	0.89
	20-29 years	42	14	8.69[a]	0.44
	30-39 years	67	22.30	9.50[ca]	0.43
	40-49 years	60	19.90	10.20 [cb]	0.45
	50-59 years	55	18.30	10.20 [cb]	0.46
	60 or older	74	24.60	10.30 [cb]	0.44
Which of the following categories describe your ethnic identity? (Please select all that apply)	NZ Maori	19	5.90	9.79	0.83
	NZ European	208	65	9.96	0.24
	Other European inc Australian	35	10.90	10	0.58
	Pasifika	6	1.90	8.5	1.65
	Asian	32	10	9.47	0.69
	Latin American	4	1.30	7.75	1.44
	North American	9	2.80	9	1.27
	Middle Eastern	5	1.60	9.2	1.24
	African	2	0.60	8	2
How many occasions in the past year have you or anyone in your family experienced food poisoning symptoms such as diarrhoea,	1-2 times	69	22.90	9.52	0.42
	3-4 times	16	5.30	8.50	0.84

nausea/vomiting, sudden onset of fever, chills/muscle aches, lack of energy, dry mouth and tongue?	5-6 times	3	1	7.33	0.67
	No experience in past 12 months	87	28.90	10.25	0.39
	No experience in past two years	126	41.90	9.94	0.30
Based on your practices and your behaviour, who or what has most influenced your personal hygiene habits? *	Partner	28	9.30	8.07	0.70
	School/church/leisure centre/educational campaigns	11	3.70	9.54	0.82
	Doctor, counsellor or health clinic	2	0.70	11.50	1.50
	Parents/grandparents, other relatives or friends	67	22.30	8.79	0.39
	TV, newspaper, radio, media in general	32	10.60	8.84	0.51
	Work colleagues	5	1.70	11.40	0.60
	My knowledge	156	51.80	10.74	0.28
How often do you cook poultry at home?	Six to seven times a week	1	0.30	9	0.00
		13	4.30	10.00	1.26
	Four to five times a week	112	37.20	10.08	0.34
	Twice to three times a week	110	36.50	9.75	0.35
	Once a week or in the weekend	65	21.60	9.52	0.34
	Once to twice times per month				
In general, how did you learn to cook? (Please select all that apply)	By myself (from internet; videos, article, recipes)	144	25	9.39	0.29
		101	17.50	10.12	0.34
	From cookery books/TV	82	14.20	9.52	0.41
	From partner/friends	201	34.90	10.26	0.24

	From other relatives (parents, grandparents)	48	8.30	10.44	0.55
	From a training course (School, church, community centre, etc.)				
What is your highest education level?	No formal schooling	2	0.70	6	0.00
	Kura Kaupapa/Primary school (including intermediate)	2	0.70	9	1
	Secondary school (high school)	53	17.60	9.55	0.52
	University student or completed university, Wānanga, polytechnic or another tertiary	130	43.20	10.32	0.31
	Postgraduate or higher qualification	114	37.90	9.49	0.31
Which of these categories best describe your occupational status?*	Employed — full time (+ 30 hours week)	145	48.20	9.78	0.31
	Employed – part-time (15-30 hours week)	49	16.30	10.61	0.43
	Retired	51	16.90	10.57	0.51
	Unemployed	10	3.30	7.80	1.11
	Housewife/husband /home duties	14	4.70	9.71	0.85
	Student	32	10.60	8.38	0.46
Which one of the following categories best describes the total yearly income of everyone in your household from all sources before tax?	Lower than $20,000/yr.	29	9.60	8.38 [a]	0.46
	$20,001 – 40,000/yr.	40	13.30	9.93 [b]	0.54
	$40,001 – 60,000/yr.	55	18.30	9.91 [b]	0.48
	$60,001 – 80,000/yr.	35	11.60	9.31 [b]	0.63
	$80,001 – 100,000/yr.	40	13.30	9.78 [b]	0.59

$100,001 or more	102	33.90	10.42 [b]	0.33

* Denotes significant difference in the mean scores within all groups, p <0.05 by one-way ANOVA test.

Means with different superscripts within columns, for each score category, were significantly different based on the bootstrap method.

Gender

There were significant differences in the mean scores observed between the respondents' gender groups (p = 0.017). The mean score of the female with children (10.20) was marginally higher than the mean score of females without children at home (10.15). Those who lived in a family prepared food more safely than those living in a single-person household or the presence of children in the house increased the food hazards concerns (Anonymous, 2012, Ruby et al., 2019). About 25% of the female respondents were > 50 years old, and it was assumed that their children might have grown up and left their home. Therefore, in general, female food safety practices at home and their awareness about food safety were better than males without children at home or with children at home with values of 8.75 and 8.61, respectively. Females tend to judge health risks as having a higher potential of danger than men because females are socialised to be mothers and nurturers (Dosman et al., 2001, Ruby et al., 2019). This finding is in agreement with other studies (Fein et al., 2011, Moreb et al., 2017). New Zealand surveillance data for the Canterbury region revealed that males were more at risk than females. The notification rates for the years 2016 and 2017 were 177 and 152 per 100,000 males respectively, and 141 and 119 females respectively.

Age

There were no significant differences between the mean score of the different age groups of the respondents. However, the mean scores of the younger groups, between 20 to 29 or years 30 to 39 years old were 8.69 and 9.50 respectively, lower than the mean score of the 40-year-old groups (10.25). This was expected as the older people were more concerned about food safety practices and hazards. The older age groups were more aware of the food safety issues and also better experienced at safe handling of poultry products compared to the younger groups. This study finding is in agreement with other studies (Katiyo et al., 2019, Kosa et al., 2019). Older people cooked more safely than younger people (Kennedy et al., 2005b). Older people probably learned cooking in school as part of the educational curriculum in the past (Fischer and Frewer, 2008).

Young consumers have less food safety knowledge, and their practices need urgent improvement (Sanlier, 2009). It was reported that age is one of the most important factors in determining public understanding of food safety practices (Mahon et al., 2006). In NZ, the most campylobacteriosis affected population groups were adults between 20 and 39 years old and children < five years (Figure 2.1) (EpiSurv, 2019).

Ethnicity

The mean score of European New Zealanders is 9.96. This mark is below 50% of the total score marks, which is 21 marks. The European New Zealanders represented the majority of the study respondents, with 69% of all respondents. The European New Zealanders represents 66% of the population. The is the majority of the New Zealand population. This indicates that poultry handling practices and general food safety awareness of the European New Zealanders were below the average level. This finding is in agreement with the result of previous chapters in this study, (Al-Sakkaf, 2012) and a previously conducted survey in NZ (Gilbert et al., 2007). It is interesting to note that the mean score of other Europeans and Australians (10.00) was higher than the NZ European mean score. This finding is in agreement with the findings in the previous chapters and the previous study (Al-Sakkaf, 2012, McKenzie, 2007). The other Europeans and Australians respondents' of this study proportion is 11.6%. This is slightly higher than the actual representation of the other European and Australian population in NZ which is only 6.8%. However, the other Europeans and Australians proportion in Canterbury region is 7.6% .

Similarly, the European New Zealander respondents' proportion in this study was a slightly higher than the percentage New Zeeland population (65%) but less than the proportion in the Canterbury region (76.4%). However, The Maori respondents' proportion (6.3%) was below the national proportion population (16%) but closer to their Canterbury region proportion value (9.4%). The Asian respondents' proportion was (10.6%) close to the percentage in the Canterbury region (11.1%). The 2018 NZ surveillance data showed that New Zealanders of European origin (4,977) were the most affected by campylobacteriosis then the Maori (577) and the Asians (367). This is probably due to the overall higher representation of New Zealand with Europeans origin in NZ. Another reason for the higher rate of reported campylobacteriosis cases among Europeans maybe because they access the public health system for food poisoning than other ethnicities.

History of food poisoning

There was no significant difference in the mean score of the number of occasions the respondents' experienced symptoms of food poisoning in the past year or the last two years. Only 1 % (3 respondents) of the respondents reported that they had experienced food poisoning 5 - 6 times in

the previous year and > 5% (16 respondents) reported that they had experienced food poisoning 3 - 4 times in the previous year. However, the NZFSA data reports about 200,000 food poisoning cases in New Zealanders each year (Anonymous, 2019). Over-reporting of "good" and socially desirable behaviour is a well-known phenomenon in self-reported food safety surveys (Dharod et al., 2007, Redmond and Griffith, 2003, Wills et al., 2015). Evidentially, up to 80% of food poisoning cases are acquired at home (van Asselt et al., 2009).

Hygiene habits

There were significant differences in the mean scores observed between the respondents' answers about who or what had most influenced their personal hygiene habits (p. 0.000). More than 50% of respondents reported that their knowledge had the most effect on food hygiene habits, and the highest mean score in the group was 10.74. This is a relatively high score for this group and could be interpreted as those respondents of this group mostly sought information about food hygiene and food safety. Another explanation might be that repeated food safety incidences in recent decades in NZ and internationally had raised the respondents' concern of food hygiene and food safety. Either way, the consumer interest in food hygiene and food safety signifies a prospect for public health professionals to target the consumers with new food safety messages or campaigns about risk prevention at the domestic level. The lower mean score groups than the 'My knowledge' group were the respondents who indicated that media (TV, newspapers, radio) and their parents/grandparents or other relatives or friends influenced their food hygiene habits which achieved (8.84) and (8.79) respectively. The lowest mean score group was the group who selected that their partner influenced their food hygiene habits with a score of 8.07. As far as it is concerned, the level of the score and data show that those who identified themselves as their knowledge has influenced their food hygiene habits with the highest mean score within all groups, their score was at the average or the mean score level (10.5). This is a crucial finding, as this target group and the other groups might be unaware of the good food hygiene practices or appropriate food safety knowledge. However, the group with the group of media would benefit from food safety messages or awareness communication campaign used the media channel. For example, messages targeting consumers should include information and education and aim at increasing consumer awareness of good hygiene practices and food safety knowledge, and these must be translated into behavioural changes (Maia et al., 2019). An idea of what might influence the selection of food safety information sources by consumers would be crucial to design a food safety message and also to enhance information provision by a public health professional and health promoters (Tiozzo et al., 2019). The findings of this study will assist in determining the channels which are likely to be the most effective in educating the consumers.

Frequency of poultry preparation

People who prepare food least frequently are the most dangerous cooks (Byrd-Bredbenner et al., 2013). Conversely, the people who prepare chicken more often at home would be better at cooking food safely. The results of this study indicated that there was no significant difference in the mean score of all groups. However, the score of those who reported that they prepare poultry more frequently, in general, was marginally better than the score of the groups who prepared poultry less frequently across all the main groups included in the analysis. It is noteworthy to reiterate that the highest score of the respondents in this group with 10.08 was still below the average score (10.5).

Learning to cook

It is known that learning to cook also involves learning to prepare food safely with the proper handling, preparation and storage of food. For many people, cooking can be described as a habitual behaviour because it is a frequently repeated process (Fischer and De Vries, 2008). Many cooks, parents and individuals may exhibit bad food-handling habits. Thus, people who learn to cook from those with bad habits will also exhibit similar behaviour. It is sometimes difficult to change such behaviours. The results of this study revealed there was no significant difference in the mean score of each of the above-mentioned groups. However, the highest mean score was achieved by the group who learned cooking through a training course (school, church, community centre). This group was under-represented in this study, just 8.3% of the total number of respondents. The majority of the respondents (35%) learned to cook from parents and grandparents. The home has always been the main place to learn about cooking and food safety mostly by watching others. However, it was apparent from this study that the knowledge of parents and grandparents were not adequate as the mean score was below the average score (10.5).

Similarly, the group who learned cooking by following cookery books/TV shows did not achieve the average mean score either. It appears that TV cooking shows or cookery books rarely stress on food safety practices. The lowest mean score was for those who learned cooking from partner/friends or by themselves from the internet (videos, article, recipes). It is important to teach food safety principles, in-school cooking classes since the behaviour of individuals is mostly influenced by teachers during school years. It has been reported that the basic food safety principles learned before the age of 16 years have an impact on individuals' food safety behaviour (Lange et al., 2018).

Education level

The majority of respondents (81%) in this survey had a university degree or was studying at a university. Such a high proportion was expected since the survey was distributed to university staff

and academics (117 respondents from a university out of 301). Moreover, high-level education may have been a reason for wanting to participate in a survey that could contribute to the improvement of consumer food safety in NZ. In general, there were no significant differences between the mean score of all the respondent groups and their education. Surprisingly, the mean scores of the groups with a tertiary qualification or postgraduate qualification were below the average score.

Interestingly, the mean score (9.49) of the group with post-graduate qualifications was lower than the mean score of the group with a university degree or studying at university (10.31). Several studies have reported that individuals with a higher level of education were less worried about food hazards or practised less food safety measures at cooking (Al-Sakkaf, 2015, Katiyo et al., 2019, Fischer and Frewer, 2008). The reason for the lower mean score of the higher education level group was probably because they failed to use their knowledge into practice due to psychological factors such as optimistic bias and habit (Al-Sakkaf, 2015). Another study indicated the higher educated group might habitually cook less because someone else prepared the food for them in restaurants, takeaways, delicatessens or departmental stores etc. and therefore they lack the knowledge of food safety during food preparation (Fischer and De Vries, 2008). In contrast, several studies have reported that individuals with a high level of education were more concerned about food hazards or safe poultry handling practices(Nesbitt et al., 2009, Sterniša et al., 2018, Moreb et al., 2017).

Occupation

There is a significant difference in the mean scores of the groups of the respondents based on their occupation status. The mean score of the part-time working group and the retired group were similar 10.61 and 10.57, respectively. These are the only groups which gained the average mark threshold (10.5). The full-time working group score was 9.78, which is below the mean score mark threshold. Previous studies found that work status played a significant role and added that time investment or current lifestyle prevents consumers from following best practice (Brennan et al., 2007, Gkana and Nychas, 2018). Generally, increased working hours or the increased number of women working reduced significantly the time spent at home and in food preparation. The student groups were with the lowest mean score in the group, with a mean score of 8.38. This result is in agreement with other studies which have confirmed that students have low perceptions of health risks, lack the knowledge in food safety and safe food preparation practices (Courtney et al., 2016, Her et al., 2019, Lazou et al., 2012).

Income

There is a significant difference observed between the mean score of the lowest income group (<

NZ $ 20,000) and the higher income groups. This finding is in agreement with the reports of (Gong et al., 2016, Kwon et al., 2008) who reported income as an important factor in an individual's food safety knowledge and proper poultry handling practice. However, surveillance data has confirmed that incidence of campylobacteriosis is higher among the high-income groups than those in earning a low-income (Nichols et al., 2012, Spencer et al., 2012, Taylor et al., 2012, Newman et al., 2015). Income is not reported in NZ surveillance data for the notified cases of campylobacteriosis, and there are only limited studies which have addressed the spatial distribution based on social deprivation area notification. (Spencer et al., 2012) explained that the lower notification rate from deprived urban areas in adults and in children > 6 years as could be explained as only <6 years children would not be charged a fee for visiting a general medical practitioner (GP) which is regarded as the main route of notification. Campylobacteriosis incidence in deprived urban areas for the children <6 years old was higher than in those from the higher-income areas (Newman et al., 2015, Nichols et al., 2012). However, other studies (Nesbitt et al., 2009, Tonsor et al., 2009) have shown that individuals with higher incomes are less worried about food safety, engage in more unsafe practices or have lower risk perception or attitudes than those with a lower income. Proper food-handling skills may be obtained through practice and experience with handling food. For example, if individuals with higher income levels did not prepare meals as regularly as individuals with a lower income level, it is possible that more risky behaviours would be observed among the higher-income individuals (Nesbitt et al., 2014) A few studies found no association between income and the consumers' food safety knowledge and safe preparation practices (Moreb et al., 2017, Pearl et al., 2009)

13.4.2 Consumer food hygiene practices

The mean score of food hygiene practice for respondents is summarised in Table 13.2. It is noted that the respondents scored the lowest value of the mean score in this section (0.25) than any other section of the questionnaire. The majority of the respondents have only been able to answer only one question correctly out of the four food hygiene questions. This result confirmed previous study findings (Al-Sakkaf, 2012, Gilbert et al., 2007)of poor hygiene level of New Zealanders in general. Surprisingly, only < 17% of the respondents selected the cleanness of the premises in their decision about where to shop for their food. Despite that, the majority of the respondents (80%) earned > NZ$ 40,000 / annum and a similar percentage (80%) had tertiary education level or higher. Only 13% mentioned that they washed and dried their hands correctly. However, Gilbert et al. (2007) study indicated that about 26.4% washed their hands and dried them properly. However, the format of the questions in this study and Gilbert et al. study (2007) was different. For example, the

washing and the drying period for the Gilbert et al. study were only 10 seconds. The question posed in this survey was 20 seconds or more for washing and drying. In both studies, the percentage of the respondents who knew how to wash and dry their hands properly was low compared with other developed countries (Al-Sakkaf, 2012).

Table 13-2 Score on reported practices of personal food hygiene

Reported practices of personal food hygiene	Correct answer	Respondents (n.)	Percentage (%)
Which of the following factors are most important to you when deciding where to shop for food?	The cleanliness of premises	51	16.90
For each hand washing occasion, how long do you spend washing your hands?	More than 20 seconds	41	13.60
If you wash your hands, how do you usually dry your hands after hand washing in the kitchen?	With a disposable paper kitchen towel	39	13
How often do you clean (using soap or cleaning product) your kitchen sink and bench-top?	After every meal	167	55.50
Mean score		24.75	
SE		1.36	

13.4.3 Consumer cross-contamination prevention practices

The mean score of cross-contamination prevention practices is summarised in Table 13.3. The mean score (0.55) marginally improved in this section than in the previous hygiene section as the majority of the respondents were able to select the correct answer for more than two questions but still less than three questions out of a total of five questions. It is only 25% of the respondents who mentioned that they separated raw poultry from other food items they purchased at the supermarket in their carry-on bags and their refrigerators. There was no similar question in Gilbert et al. (2007) study to this question. However, 35% of the respondents reported that they follow safe practice after handling raw poultry to prevent cross-contamination. However, Gilbert et al. (2007) reported that about 60 % of their respondents handled the knife used for poultry and cleaned the surfaces in a way to prevent cross-contamination. In this study, 78% of the respondents correctly answered the specific question about their handling of the cutting board and knife. The content and the structure of the questions in both surveys were not similar. This makes the comparison between the respondents' answers in the two surveys difficult to compare. It is evident

that despite the improvement in the respondents' answer to some of the questions of this section, the overall score of this section was only marginally above the average score. Cross-contamination is considered to be the main factor in the transmission of campylobacteriosis (Mylius et al., 2007).

Table 13-3 Score on reported practices in cross contamination prevention

Reported practices in cross contamination prevention	**Correct answer**	**Respondents (n.)**	**Percentage (%)**
Was the poultry bagged separately from other items?	Yes, in the trolley, carry-on bag and freezer/chiller	75	24.90
For each raw poultry handling occasion, which of the following do you usually do immediately after handling poultry?	I use hand sanitiser and water or soap with hot water OR change my gloves	106	35.20
There are many times that raw poultry may be handled or came into direct contact with utensils during preparing or grilling. Please indicate how the raw poultry was handled/came into contact with utensils?	In contact with a previously unused fork/ unused spoon/unused utensil OR handled while wearing previously unworn/gloves OR handled using clean hands.	137	45.50
After cutting or preparing the raw poultry, what was the next thing you did with the cutting board/utensil and knife?	Wash the cutting board/knife with soap and hot water before reuse OR use a different cutting board and knife for preparing the other food	235	78.10
Do you ever place cooked poultry on the same plate/surface where raw poultry meat has been?	No, not at all OR only after washing the plate/surface with hot water and detergent.	278	92.40
Mean		55.22	
SE		1.30	

13.4.4 Consumer food temperature control and proper storage practices

The mean score of food temperature control and proper storage practices and the descriptive analysis are summarised in Table 13.4. The mean score (0.49) in this section with five questions is slightly lower than the consumer mean score of the cross-contamination practices section. Only about 5% of the respondents purchased poultry products after purchasing other non-perishable items. The majority followed the flow of the supermarkets. Only a few of the supermarkets in NZ have the appropriate flow design to help consumers to choose perishable food items in the last section. Regarding the question of how long it takes the respondents to reach home after purchasing the poultry products, in this study for 88 % it took < 40 mins which were somewhat similar percentage (88.9%) which reported by Gilbert et al. (2007) study. In terms of thawing the frozen poultry products, 34.2% of the respondents of this study followed the best practice. The respondents of Gilbert et al. study (2007) reported of 25.8% in the refrigerator for up to 12 hours and 4.1% in the sink with cold water. It is not possible to thaw a whole chicken within 12 hours. Therefore, the respondents' of both studies confirmed that they do not thaw safely and thoroughly before cooking. Proper thawing of frozen raw poultry products can minimize the growth of bacterial pathogens and reduce the risk of cross-contamination (Mazengia et al., 2015). Only 47% of the respondents knew when the poultry was properly cooked and safe to eat. 27.3% of respondents of Gilbert et al. (2007) study reported that they preferred medium cooked, roasted chicken and 22.1% of the respondents preferred medium cooked chicken liver. The consumption of undercooked chicken or chicken liver is considered a significant risk factor for campylobacteriosis (Butzler, 2004, Wensley and Coole, 2013).

Table 13-4 Score on reported practices in food temperature control and food storage

Reported practices in food temperature control and food storage	Correct answer	Respondents (n.)	Percentage (%)
During your last grocery shopping occasion when you purchased raw poultry, at what stage did you select raw poultry?	After, I had chosen all other non-perishable items	16	5.30
Usually, how long do you leave the raw poultry at room temperature before storing it in a refrigerator or freezer at home (including the time you take to travel from the shop to your home)?	0 minutes– 40 minutes	265	88
When thawing frozen raw poultry for cooking, how do you usually do it?	Thaw it in the bottom shelf of the refrigerator OR in/under running cold water in the sink	103	34.20
How do you check to see if a poultry product you have cooked is safe to eat?	I observe when the exterior colour is golden brown, and the interior juices run clear OR measure the internal temperature of the poultry meat.	142	47.20
Do you cover your food during storage?	Yes, all the time	217	72.10
Mean		49.37	
SE		1.21	

13.4.5 Consumer general food safety awareness

The mean score of general food safety awareness and descriptive analysis are summarised in Table 13.5. The majority of the respondents were able to select the correct answer for > two questions but <three out of seven questions. It is important to note that the mean score for this section is 0.52, which is at the average food safety awareness level. There were 67% of the respondents who indicated that they think about the basic food safety rules such as clean, separate, cook, chill and cover when they prepare the food at home. However, only 24% of the respondents indicated that

they always follow these rules. It is a common phenomenon that only a low percentage of respondents who always follow the basic food safety rules and that some respondents' knowledge or awareness' of proper chicken handling was not fully translated into practice (Mazengia et al., 2015, Katiyo et al., 2019). Surprisingly, only 50% of the respondents knew that chicken carries *Campylobacter* despite the intensive coverage in the media that NZ has the highest campylobacteriosis notification rate in the developed world and the association of chicken consumption to this highest rate of notification in New Zeeland for more than a decade. This finding confirmed that the information channels governmental institutions and health communication practitioners used to deliver risk/benefit on food safety issues targeting the NZ population should be revised and it is time to rethink about new channels and type of messages especially via social media so that it reaches nearly all of the New Zealand population.

The average level of food safety awareness in this section confirmed the findings of the previous chapters and the finding of another study (Al-Sakkaf, 2012). The respondent scores in each poultry handling practice section provided decisive evidence about the respondents lack food safety awareness as there were statistically significant correlations between all the questionnaire sections analysed by Pearson test at $p<0.01$ (Table 13.6). Another NZ study (Allan et al., 2018) partially tested the food safety awareness of consumers, and the primary objective of that study was the introduction of comprehensive, high-quality, chicken safety labelling with all chicken products in NZ as an intervention which may lead to changes in consumer behaviour and reduce the incidence of campylobacteriosis in NZ. This study indicated that there are several gaps in consumer knowledge of proper handling of poultry products. It also confirmed the need to introduce a new chicken label containing all the information about safe handling of poultry products.

Table 13-5 Score on general food safety awareness

General food safety awareness	Correct answer	Respondents (n.)	Percentage (%)
What temperature should refrigerators operate at for optimum safe food storage?	Between 4 ℃ and 7 ℃	157	52.20
How do you decide whether or not to consume food stored in the fridge?	By the best before and use-by date	145	48.20
When preparing a meal, do you think about the basic food safety rules such as clean, separate, cook, chill and cover?	Yes, I am aware of all of them or most of them	202	67.10
How likely are you to follow these rules clean, separate, cook, chill, cover?	Always	74	24.60
Does raw poultry carry bacteria (bugs) that can make you sick?	Yes, I know that it carries *Campylobacter* bug	152	50.50
Cross-contamination can be defined as the transfer of harmful bugs to food from other foods, hands and other food contact surfaces. Please indicate your awareness and self-reported practice of this concept below	I have heard about the concept of cross-contamination, and I always take the necessary steps to prevent it	200	66.40
How important do you consider it to follow/implement all good hygiene practices all of the time to protect yourself and your family from foodborne illness?	I consider it as extremely important	157	52.20
Mean		51.59	
SE		1.39	

Table 13-6 Correlation between questionnaire sections by Pearson test

	Food Safety Awareness	Temperature Control and Proper storage	Hygiene	Cross Contamination Prevention
Food safety awareness	1	0.373** 0.000	0.378** 0.000	0.417** 0.000
Temperature control and Proper storage	0.373** 0.000	1	0.329** 0.000	0.321** 0.000
Hygiene	0.378** 0.000	0.329** 0.000	1	0.237** 0.000
Cross-contamination Prevention	0.417** 0.000	0.321** 0.000	0.237** 0.000	1

**. Correlation is significant at the 0.01 level (2-tailed).

13.5 Conclusions

The survey results identified many areas of low adherence to currently recommended food safety practices, based on self-reported data, personal food hygiene practices, prevention of cross-contamination not only at home during poultry preparation, proper storage and temperature control such as determining 'doneness', and proper thawing of raw poultry, shopping and transportation. Additionally, there are several gaps in consumer basic food safety awareness and information about risks and the pathogens related to poultry products. It is noteworthy that about 80 % of the respondents were with a tertiary qualification or postgraduate qualification but still their food safety practices, and food safety awareness was below the average score. It is important to target the less qualified New Zealanders in a future survey as they were an under-represented group in this study sample. It is important to find better ways to convince the less qualified and low-income groups to participate in future research surveys. However, from the score of the higher qualified New Zealander score which can be considered as another decisive evidence that the less qualified New Zealanders food safety practices and food safety awareness could be worse than in those with higher qualifications. Therefore, the evidence collected in this study confirmed the hypothesis that New Zealanders are less educated in food hygiene practices and food safety knowledge than those in other developed countries, and this is possibly a reason for the higher rate of campylobacteriosis in NZ. More research is necessary to recognise and understand the reasons for poor consumer practices in order to develop a more effective food safety message and transmit

that through new information channels including social media so that it reaches a larger audience. This could lead to a reduction in the number of campylobacteriosis cases in NZ.

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14 General discussion

14.1 Background

The main objective of this thesis was to understand why NZ has a higher rate of reported cases of campylobacteriosis than other developed countries. Thus, it was important to investigate the reasons for the unexplained campylobacteriosis increase, which causes significant economical and health consequences in NZ. The problem is caused by a bacterium, which naturally occurs in the gastrointestinal tract of chickens. Because it is present in the faeces of production-animals, the bacterium can be transferred from the intestine to the fresh meat during slaughtering (Keener et al., 2004). Thus, the investigation of this study targeted the pathogen behaviour and followed the survival of the pathogen under the environmental conditions prevalent in poultry processing plants. In addition to the comprehensive investigation of campylobacteriosis, this study identified the causes for the unusual high endemic rate in NZ and also describes the pathogen fate at the consumer handling phase which contributes to the high incidence (12 -100%) of food poisoning cases occurring at homes (Redmond and Griffith, 2009, van Asselt et al., 2008). Approximately 500 New Zealanders contract food poisoning each day, of which about 40% of the cases believed to be caused by unsafe food handling at home (Anonymous, 2016). Recently, the NZFSA confirmed that about 200,000 New Zealanders contract food poisoning each year (Anonymous, 2019). Moreover, the prevention and control methods at the poultry processing and consumer levels are highlighted in the context of this study. The quantitative microbial risk assessment was conducted using the Bayesian method to determine and evaluate new interventions. Finally, the examination of the time-series models to predict campylobacteriosis risk was also tested.

14.2 The pathogen

14.2.1 Heat resistance

The thermal inactivation kinetic parameters (D and z) for the tested NZ strains of *C. jejuni* isolated from both humans and poultry were obtained by a broth experiment and did not indicate that the most implicated strains in human food poisoning cases were more heat resistant than the international strains recorded in published data overseas. Fitting the survival curves of the tested NZ strains in broth and in food matrix to different models following one-step regression analysis supported the fact that NZ tested strains were heat sensitive. Moreover, NZ strains were, to some

extent, more heat-sensitive than most of the international *C. jejuni* strains. The *D* values for all NZ isolates at 60 °C (1.3 - 4.2 s) as shown in Table 4.1 were slightly lower than the minimum *D* value of the published international isolates, which ranged from 7 - 30 s (Li et al., 2002, Ray, 2014, Juffs and Deeth, 2007). Similarly, the *z* values calculated for all isolates and listed in Table 4 -1 and 4 - 2 as ranging from 4.0 ° to 5.2 °C were within the range of the published *z* values of 2.8 - 5.8 °C (Jackowska et al., 2008, Li et al., 2002, Sorqvist, 2003, Juffs and Deeth, 2007). The results obtained for the tested NZ strains using chicken skin under isothermal conditions are in agreement with most of the published international data. The *D* and z values obtained by a skin experiment at 60 °C (Table 5.2) were 2 to 5 times higher than the values obtained from the broth experiment; 11 to 18 s and 8 to 11 °C, respectively. It is evident that the presence of a food matrix altered the kinetic parameters for *Campylobacter* heat resistance (Yang et al., 2001). However, a Wageningen University group (Bergsma et al., 2007, de Jong et al., 2012) reported that the D values were not significantly increased. Sampers et al. (2010) with a similar experimental set-up as Bergesma's study and using frying chicken fillet found that 4.5 log cfu/g of *C. jejuni* inoculated into chicken burgers were reduced to below the detectable level (<10 cfu/g) after 4 minutes of frying to an internal temperature of 57.5 °C, and that 2.5 log cfu/g of the naturally contaminated chicken burger was reduced to below the detectable level after 4 minutes of frying to an internal temperature of 52.1 °C. Although the objective of Samper et al. (2010) was not to determine the kinetic parameters (*D* and z) in their relatively complex experimental design, the results obtained were useful to determine the minimum time and the internal cooking temperature required to eliminate the contamination. This is in agreement with the conclusions of this study regarding the heat sensitivity of *C. jejuni* in general. It is assumed that the results reported by the Wageningen University were artefactual in the temperature measurement of the chicken fillet and in the enumeration and identification method used by them. Moreover, the data of de Jong's was presented only at a conference in 2008 and is yet to be published in a peer review journal (Personal communication in 2010 with the co-author Dr.van Asselt). It was published in 2012 in an open access journal (International Journal of Microbiology with an impact factor in 2019/2020 of 1) by the Hindawai publisher. The heat sensitivity of *Campylobacter* remains a characteristic of this pathogen in NZ and internationally. Moreover, a study by Gunsen (2008) found that baking a chicken drumstick for three or five min at a core temperature of 80 °C or 70 °C killed all *Campylobacter* cells, which is not in agreement with the findings of the Wageningen University group. Moreover, *C. jejuni* was not detected following simulated home pan-frying of artificial inoculation of 4 log cfu/g *C. jejuni* into steaks, fillets, hamburgers and meat strips (Lahou et al., 2015). These meat samples were from a range of animal species, pig, cattle, chicken, sheep, turkey, horse, crocodile and kangaroo. Only after a further

enrichment step, *C. jejuni* was detected in few samples of pork hamburger, horse steak and crocodile steak. Additionally, the *D* and *z* results obtained under dynamic conditions for NZ strains are broadly in agreement with the published international isothermal data and with isothermal data of this study obtained for the same isolates. None of these indicate that the NZ *Campylobacter* strains are more heat resistant than other strains. The parameters obtained under dynamic conditions are more robust than the isothermal parameters and can be obtained with less experimental effort. The *D* and the *z* values generated under dynamic conditions for *C. jejuni* in this study is the first reported values in the scientific literature. These have not been reported before, probably because of the mathematical complexity of the calculations involved and the instrumentation required to generate such data.

This study results confirm that the tested NZ *C. jejuni* strains do not have unusual heat resistance, and the high rate of campylobacteriosis in NZ is not associated with the emergence of more heat resistant strains in the country. This finding has a significant impact for the poultry industry, regulators, consumers and researchers. There is now ample scientific evidence that shows the importance to maintain the standards for heat treatment practices at the food processing plants, food services and in homes. Any relaxation to the heat treatment protocols can cause unpredictable loss to the poultry industry. It is a challenge for the regulatory bodies to repeatedly convey the message of the importance of cooking temperatures to all consumers, given the reluctance of NZ consumers to use thermometers to verify the cooking temperatures at homes (Gilbert et al., 2007).

14.2.2 Air tolerance

The investigation of NZ strains tested in this study using three methods revealed that the air tolerance of NZ strains was not different to the international strains (Chen et al., 2001, Garenaux et al., 2005, Oh et al., 2017) and the previously tested NZ strains (Chynoweth et al., 1998). The results of this study did not support the hypothesis that the survival of *C. jejuni* was not influenced by the storage atmosphere aerobically or microaerobically, as the *C. jejuni* survival was shorter in both the agar and broth experiments under aerobic conditions than in the microaerobic conditions at all temperatures tested, except in a broth experiment at 10 °C where after the fifth day, the survival rate was similar under both aerobic and microaerobic conditions. This finding confirms the fundamental and best-known hypothesis that *Campylobacter* is a microaerophilic microorganism that requires a specific gas composition for its growth and longer survival.

Biological aspects and molecular mechanisms responsible for microaerobic growth or better survival from a physiological and genetic perspective are not fully understood. It is assumed that due to the inhibition of enzymes at high O_2 concentrations (> maximum level 10 %), the *C.jejuni*

cells are vulnerable to reactive oxygen species (ROS) and free radicals resulting in changes to molecular components of cells, their function in addition to the production of highly toxic components (Kaakoush et al., 2009). The *C.jejuni* poultry isolates survived longer than the human isolates, especially at the incubation temperatures of 10 and 20 °C. However, there was no significant difference between the survival of the poultry and human isolates when the differences in the initial concentrations of both isolates are accounted for at 4 °C incubation.

This study revealed that the survival of *C. jejuni* under oxidative stress is more influenced by the temperature rather than the incubation atmosphere. A low temperature of 4 °C had less effect on *C. jejuni* and was able to survive more than four weeks in both agar and the broth. Garenaux et al. (2008) assumed this to be due to a less active metabolism, a decrease in catalytic activity, or the O_2 was less toxic to cells at 4 °C.

Despite the fact that this study was not conducted with chicken meat, the combination of three methods to address the oxidative stress on *C. jejuni* increased the validity of the results reported by this study and saved time, resources and cost.

Practically, most international data on *C. jejuni* survival has been for short periods (1 – 2 days) (Solow et al., 2003) or 1- 2 weeks (Bhaduri and Cottrell, 2004, Davis and Conner, 2007, Garenaux et al., 2008, Oh et al., 2017). This study results are in agreement with the results obtained in NZ using chicken mince and chicken nuggets (Chynoweth et al., 1998). Internationally, the *C. jejuni* survival data obtained from 4 biological materials; water (incubation for four weeks), human urine, (five weeks), human faeces and cow milk (three weeks) were similar in spite of the differences between the four biological materials (Blaser et al., 1980). Similar results have been generated by (Garenaux et al., 2008) using Colombia agar plates and by (Bhaduri and Cottrell, 2004) using chicken mince or chicken skin at 4 °C. The implications of the spoilage of chicken meat or chicken skin during sample storage above 4°C hinders the use of a food matrix in long survival studies. Moreover, the irradiation of skin or chicken meat eliminates the microflora (pseudomonads, micrococci, Staphylococci) which are found naturally on poultry, and also inhibits the growth of *Campylobacter* (Mai, 2003). The conclusion derived from irradiation studies of chicken meat must be treated with caution. Thus, the results of this study reject the hypothesis that the high rate of campylobacteriosis in NZ may be due to the emergence of unusual new strains with more O_2 tolerance or unique survival ability under a range of storage or handling temperatures (4, 10, 20, 25 °C).

Given that the *C. jejuni* infection dose that causes illness is about 500 cells (Robinson, 1981) and the length of time that *Campylobacter* can survive as revealed by this study for NZ strains is a

significant finding from a health perspective as the shelf life of a fresh chicken is eight days at 4 °C whereas *Campylobacter* can survive for > 4 weeks at that temperature. Hence it is important for the poultry processing plants to apply the necessary intervention to assure that chicken carcasses and poultry products are released to the retail market with as low a contamination level as practically possible, and theoretically, the counts should not exceed 500 cells per a chicken portion serving.

14.3 Poultry Processing Plant

It is important to investigate initially the poultry processing practices which influence the prevalence of *Campylobacter* in the end product, as the prevalence of *Campylobacter* in chicken products in NZ is among the highest of the countries in the world. Thus, the focus should be on the processing practices, which may lead to an increase in the prevalence of *Campylobacter* contamination in chicken carcasses and poultry products. An overview of the current intervention strategies used in poultry plants to reduce contamination and their implications for consumers is discussed below, along with suggestions for alternative more effective interventions.

14.3.1 Marination or tenderisation by needle injection technology:

Traditionally, it has been proven that needle injection technology introduces pathogenic bacteria from the carcass surface into the interior muscle tissue (Gill and McGinnis, 2005). Moreover, since the marination solution is usually recirculated, there is also a risk of spreading contamination to products previously free of pathogens (Ray et al., 2010). In addition, pathogens introduced into the internal tissue may be better able to survive cooking processes and cause illness (Gill et al., 2008).

Generally, marination is mainly used to improve palatability, yield and shelf life by inhibiting spoilage bacteria in treated meat. It is considered that the marination currently used in most poultry processing plants does not inhibit the survival of *Campylobacter,* whereas a new marinade, produced from a mixture of organic acids and different food ingredients that can reduce *Campylobacter*'s contamination level by one log (Ray, (2010). It appears that there is no evidence that the latter has been used commercially in the poultry industry. Others have used organic acids as a *Campylobacter* reduction hurdle (2015).

This study investigated two main marinades used by the largest NZ poultry producer, and it was apparent that the needle injection process could pose a high risk to consumers if marinade "A " (with5% salt) (used for products such as drumsticks) before freezing the products contaminated with *C. jejuni* up to 3.5 log cfu/g. However, after freezing, when the drumsticks were sampled from the retail market, the prevalence of contaminated samples was reduced to 20% (an 80% reduction), and also the contamination was only on the surface with 450 to 900 cfu/portion (= 2.6 to 2.9 log).

The reduction in *C.jejuni* prevalence was possibly due to the freezing process which can cause a reduction of 1.3 - 2.2 log (Boysen and Rosenquist, 2009, El-Shibiny et al., 2009, Georgsson et al., 2006, Hofshagen and Kruse, 2005).

The results of poultry products injected with marinade " B " showed only 30% contamination with *Campylobacter,* and the contamination level was about 450 cfu/portion and only the surface of the drumsticks when tested at the processing plant. The sampling of the injected drumsticks at the retail which sold fresh products revealed that the contamination level of *Campylobacter* was reduced to 20 %, but the sampling of whole chicken carcases injected with marinade or solution " B " and analysed for whole surface contamination, showed 70% prevalence with about 3.9 log cfu/carcass. The Bayesian analysis is convenient to implement when the microbial data contains results which are below the detection limit as it presents the best way to mine all the data available to generate acceptable and unbiased results. However, it was noted that the mean of external or internal samples by Bayesian analysis was similar to the mean obtained by the classical mean calculation by Excel. Moreover, the use of Bayesian analysis was hindered by its complexity and the lack of its general applicability as it is a more useful tool for generating data for the quantitative risk assessment. In spite of the free software available for Bayesian analysis, the method is not that used in the field of food microbiology data and should be encouraged to be used only when the microbiological data are below the detection limit.
The detection of *Campylobacter* in the poultry processing plant and retail chicken samples injected with marinade “B” is questionable. It is because the marinade “B” has shown inhibition capability against *Campylobacter* in a liquid medium, as observed in the initial experiments conducted in a flask at the laboratory (section 8.3.1), as the salt content of marinade “B” exceeded the maximum growth tolerable salt content (>2%) for *Campylobacter)* (Sampers et al., 2010). This may be due to the buffering capacity of the chicken meat which permits *Campylobacter* to survive for a longer time than its survival period in a liquid medium (Perko-Makela et al., 2000), or by the increased bacterial resistance by attachment to poultry skin (Zhang et al., 2013).

The information provided from this study indicated that the contamination level of the fresh products sampled at the market varies and can exceed (at 3.9 log cfu/carcass) which is above the limit of *Campylobacter* contamination level set by the NZFSA (3.78 log cfu/carcass). Thus, it is apparent that *Campylobacter in* un-marinated fresh products exceeds the NZFSA limit significantly. However, the current contamination level is far better than the level reported by a study conducted in 2008, which found up to 6.7 log cfu/carcass in fresh, non-marinated products (Chrystal et al., 2008). A more comprehensive survey covering the entire chicken processing chain in NZ would be useful. This study (see section 8.3.2) also indicated that a ‘reduced salt’ marinade was not as

effective in reducing the *Campylobacter* contamination level compared to a 'high salt' marinade. However, all products injected with the low salt content solution were sold frozen, which, as reported in section 8.3.3 reduced the initial contamination *Campylobacter* level.

Cumulatively, the processing practices tested in this study using two marinades did not indicate that the marination process could be the reason for increased contamination level of chicken or chicken products available in the NZ retail market. The results of this study are the first study that investigated the *Campylobacter* contamination via needle injection practices in poultry processing plants, as most of the previous studies were focussed on beef or pork processing plants and *E. Coli* (Gill et al., 2005a, Gill and McGinnis, 2005, Gill et al., 2005b, Gill et al., 2009, Gill et al., 2008, Chancey et al., 2013). In addition, all previous marinated poultry studies investigated only the surface contamination of injected or marinated product or the prevalence of contamination (Perko-Makela et al., 2000, Sampers et al., 2008), or explored the best method for detection of *Campylobacter* in marinated products (Katzav et al., 2008) or investigated the most effective marinade against *Campylobacter;* seasoning dry mixture and wine (Ray et al., 2010). So, this study could be classed as pioneering comprehensive research that investigated the effect of marinated practices at poultry processing plants in NZ to gauge the contribution of such practices to *Campylobacter* contamination of chicken meat sold in NZ.

14.3.2 Evaluation of *Campylobacter* mitigation strategies

Chemical intervention (organic acids, chlorine-based and phosphate-based) of broiler carcasses has been used in the US for many years (Oyarzabal, 2005, Lu et al., 2019) and in some other countries, to decontaminate poultry products but has not been that successful (Boysen and Rosenquist, 2009). In NZ poultry processing operations, acidified sodium chlorite (ASC) has been used mainly as a major intervention bacterial reduction step along with the chlorinated washes at many stations in the processing line and also chlorinated spin chillier dipping for about 70 min. Such chemical treatments reduced the bacterial contamination level in chicken especially after NZFSA set a performance target (3.78 log cfu/carcass) in April 2008 with the interim aim of a 90 % contamination reduction level (~1 log cfu/carcass) from the levels observed in 2007 without any restrictions on the type of equipment or materials that could be used to achieve the mandatory target. However, due to adverse health effects caused by chemical to consumers due to the formation of toxic compounds, it was reported that the use of chemicals should not replace the good hygienic practice at all poultry processing plants (Hugas and Tsigarida, 2008, EFSA, 2011b, Schraer and Edgington, 2019). Trihalomethanes (THM), a by-product of chlorine cannot be detected in poultry tissues when exposed to chlorine at 50 ppm or less using either the AOAC or

EPA analytical methods. However, the NZ chicken was prior to 2010 were exposed to a higher chlorine concentration than the safe-limit.

A new regulation on the strict use of chemicals in EU (EC) No. 853/2004 was implemented in 2006 which permitted the use of chemicals but only after the European Food Safety Authority (EFSA) has been provided with a risk assessment by the manufacturer or by the poultry processing plant detailing the possible negative impact of the suggested chemical to be used in the poultry processing plant (Hugas and Tsigarida, 2008, EFSA, 2011b, Schraer and Edgington, 2019). To date, no such chemicals have actually been authorised by the EC because of insufficient documentation (Boysen and Rosenquist, 2009, Schraer and Edgington, 2019).

A published survey of 26 EU countries in 2008 indicated that the prevalence of *Campylobacter* was very high in chicken ranging from 5 – 100% (EFSA, 2010). New Zealand government agencies, poultry industry, farmers and growers face a huge challenge with the European policy on the use of chemicals. The consumer groups in NZ have over the years initiated campaigns against the use of chemicals in poultry processing.

To replace the use of chemicals, many other safer methods have been tried. Hot water immersion method reported in section 9.3 provided evidence as a promising, simple, cost-effective alternative which can be used by the poultry industry if a ban on the use of chemicals is implemented in NZ in the future. This study results emphasise the need to conduct commercial trials to better evaluate the feasibility of the hot water immersion intervention to reduce the naturally contaminated chicken in poultry processing environments. In addition, the sensory quality of the treated carcasses needs to be evaluated by poultry industry experts to gauge the best time-temperature combination with the least adverse sensory effect.

Moreover, this study suggested the replacement of the current tap water spray washers with hot water spray washer, which can serve as an additional hurdle to reduce *Campylobacter* contamination level in chicken. Also, in this study, the importance of dry air chilling was highlighted (in section 9.3) as it reduced 0.3 to 1.4 log of *Campylobacter* contamination (Allen et al., 2007, Boysen and Rosenquist, 2009, Huezo et al., 2007, Lu et al., 2019, Rosenquest et al., 2007). Hence, dry air chilling should be considered as an alternative chilling method to the current spin chillier method which has the potential to cross-contaminate (Sánchez et al., 2002, Whyte et al., 2002, Lu et al., 2019) as a comprehensive solution for poultry processors. It is important to encourage research on the physical interventions which would be more acceptable to consumers and also practically applicable to the poultry industry.

14.4 The consumer

It is believed that consumers play a major role in preventing campylobacteriosis. Definitely, the broad and complex consumer-aspect cannot be covered in a chapter or two in a thesis but has been addressed in a thesis internationally (Redmond and Griffith, 2003). The main objectives were to investigate the causes of the high rate of campylobacteriosis and then to advise about the possible best remediation and intervention methods that could reduce the high rate in NZ.

14.4.1 The consumer kitchen

Many consumers are unaware that home practices may be responsible for food-borne disease incidence. Investigations on the microbial level and type of microbes harboured in the domestic kitchen ((Davis and Conner, 2007, Kennedy et al., 2005b, Evans and Redmond, 2019), domestic environment (Beumer et al., 2002, Kagan et al., 2002, Ojima et al., 2002) have been conducted. Other studies have concentrated on the kitchen after food preparation (Gorman et al., 2002, Evans and Redmond, 2019). All these studies have reported that most surfaces in the kitchen were heavily contaminated with pathogenic and non-pathogenic bacteria.

It was surprising that a few studies reported the kitchen to be more heavily contaminated than the toilet or bathroom (Ojima et al., 2002, Todd et al., 2009). The most heavily contaminated locations or items in the kitchen were dishcloths, cleaning cloths, sponges, the sink environment, towels and drain areas (Beumer et al., 2002, Doyle et al., 2000, Evans and Redmond, 2019). *Campylobacter,* in particular, has been isolated from most of those places and from chopping boards, work surfaces, floors, refrigerator and door, waste and pedal bin (Redmond and Griffith, 2009). Generally, most bacterial contamination was detected in wet and moist areas of the kitchen with only a few bacteria on dry surfaces (Aiello et al., 2008). The kitchens of NZ consumers will be similar. There is no data in NZ regarding consumer kitchens, and more research is required.

14.4.2 The consumer's knowledge and practices

There is a lack of data and studies in New Zealand with respect to consumer knowledge and handling practices. There are a few studies (Gilbert et al., 2007, Bloomfield and Neal, 1997) but not comprehensive. So, the data from these limited studies should be treated with caution. It is difficult to compare NZ surveys with international surveys because of the differences in sample size, objectives, targeted population groups, methodology and results interpretation. The survey conducted by this study identified that many people did not adhere to currently recommended food safety practices. This conclusion was based on self-reported data based on personal food hygiene practices, cross-contamination prevention at home during poultry preparation, adherence

to proper storage and temperature control including determining 'doneness', proper thawing of poultry, and shopping and transportation. Additionally, there were several gaps in consumer basic food safety awareness and information about risks and the pathogens that can be present in poultry products. It is noteworthy that in spite of about 80 % of the respondents were with a tertiary qualification or postgraduate qualification, their food safety practices, and food safety awareness was below average. In future surveys, it is important to target the less qualified New Zealanders as they were underrepresented groups in this study sample. It is also important to find better ways to convince the less qualified and low-income groups to participate in future research surveys. The evidence collected in this study survey confirmed the hypothesis that New Zealanders are less knowledgeable in food hygiene practices and food safety than people in other developed countries, and this could possibly be the reason for the higher rate of campylobacteriosis in NZ than in other developed countries. More research is required to recognise and understand the reasons for poor consumer practices in order to help develop a more effective food safety message and promote it through new information channels such as through social network sites to improve their practices in the domestic environment. This could, in turn, lead to a reduction in the number of campylobacteriosis cases. In 2007, the New Zealand Food Safety Authority chief executive considered that the level of hygiene practice of New Zealanders could be the reason for the high rate of campylobacteriosis in the country (McKenzie, 2007). In 2008, a prominent NZ and International *Campylobacter* expert, Professor Nigel French indicated during an interview with a NZ Radio that if New Zealanders could improve their practice of washing their hands, the campylobacteriosis rate would drop dramatically In 2007 a member of the NZ parliament indicated that many New Zealanders are too lazy to wash their hands, just wringing their hands instead of washing them, and warned that if they do not wash their hands, they will become ill (Coddington, 2007).

A large number of campylobacteriosis cases present a high cost on the health care system and could also affect the NZ tourist industry and agricultural produce both of which champion New Zealand's "clean green" image.

14.5 The government

The aim of health professionals, risk managers, policymakers, and regulators is to encourage consumers to practice desirable food safety practices and to discourage improper or unsafe ones. It is not possible to enforce by law consumer handling practices at home. The only possibility is to combat the burden of disease and to optimise consumer risk management by designing an effective communication initiative regarding food safety hazards and proper food handling practices in the

domestic environment (Farias et al., 2019).

A reduction of the disease burden and the incidence of foodborne diseases are possible through improvements in consumer food safety practices (Redmond and Griffith, 2009, Farias et al., 2019). Researchers have identified a gap or a decline in consumers' knowledge which can increase the risk of food poisoning (Kennedy et al., 2005a, Gkana and Nychas, 2018). This indicates that consumer knowledge is correlated with current practices, and this can affect willingness to change from the current practices.

It is clear that the process of communicating the domestic food safety risk is a complex and challenging task. In NZ, it appears that there is a significant deficit in f good hygiene practice and food safety knowledge amongst a majority of the population. The responsibility appears to be for food safety experts and risk communication community to understand the variety of socioeconomic/demographic and psychological factors that prevent the public from changing its behaviour.

Given the cost of campylobacteriosis to NZ economy which is > NZ $ $77 Million per year (NZFSA, 2007) and the potential decline in NZ reputation as a safe food-producing country, financial loss in the tourism industry, it may be more cost-effective to utilise social marketing for a future educational or communication strategy aimed at educating New Zealanders to improve their domestic food handling practices. The social marketing approach was used in NZ during a nutritional initiative called the "5 + a day programme" to encourage New Zealanders to eat five or more fresh fruits or vegetables per day. The more expensive social marketing approach has been proven to possibly change consumers' behaviour (Redmond and Griffith, 2006). In order to ensure effective domestic food safety risk communication, a full appreciation of the target audience is required, as each audience brings unique challenges to the development, conveyance and delivery of food safety and safe practice information. The efforts of health professionals are well-coordinated in NZ and are based on a more scientific approach, which can help to educate New Zealanders on food hygiene practices to reduce the high rate of campylobacteriosis cases. The findings of this study have specified which poor hygiene practices need to be improved.

A cost-effective option is to utilise social marketing in future educational and/or communication strategy aimed at educating New Zealanders to improve their domestic food handling practices, which will possibly later help to reduce the number of campylobacteriosis cases in NZ (NZPHO, 2010). NZFSA has conducted a few educational campaigns at shopping malls. However, a complete approach of social marketing as a holistic and long term or permanent strategy, and/or the inclusion and improvement of food safety education at childcares and schools could be

implemented in NZ. In addition to social marketing, publicising foodborne disease outbreaks by the "scare tactics" could also motivate consumers to better understand the severity of food poisoning and persuade them to change their practices and habits and adopt safe food handling practices (Katiyo et al., 2019).

The key findings of this study contributed to exploring the reasons for the high rate of campylobacteriosis in NZ. The results of this study provided some answers to the important and complicated questions regarding the campylobacteriosis crises in NZ. This study results have rejected the hypothesis of a more heat resistance phenotype of *C.jejuni* and its longer survival during storage or handling under the aerobic atmosphere. This would help the scientific community to investigate poultry processing practices to learn about the contribution of the current poultry processors practices to the campylobacteriosis problem and develop interventions at the poultry processing plants. This study investigation at poultry processing plants of one of the critical practices (marination by needle injection) revealed that the current practices could contribute to the increase in *Campylobacter* contamination of carcasses by NZ consumers.

Moreover, the finding from the poultry plant intervention study identified alternate, cost-effective physical interventions with fewer health implications or consequences compared to the current chemical intervention procedures. The use of chemicals in poultry processing plants has been banned in the EU, and consumer groups in NZ have been pressing the NZFSA also ban the use of chemicals in NZ. The physical interventions highlighted in this study provide another solution for a possible chemical ban in New Zealand.

The complete food chain has been modelled by the Bayesian approach, which provides better insight into the food chain and is more informative as it incorporates all the factors that impact the final risk estimation. Thus, it can easily determine the impact of any intervention in the food chain. Therefore, the effect of a new intervention (for example, a consumer education plan or other intervention at poultry plants and farms) is clear for the policymakers, risk managers and health professionals before the implementation of the new intervention, by altering the priors of consumers' hygiene practice for example, and the priors of farms or the poultry plants. The association between the reduction in flock/ poultry prevalence, improvement in hygiene practices and the calculated reduction in risk of human disease was estimated to be 1:1 which is in agreement with international findings (Rosenquest et al., 2007).

A simplified Bayesian model used in this study is able to identify the expected outcomes and provide a fast response and more evidence about the possible main risk drivers in the NZ campylobacteriosis epidemic. The model used the Bayesian methodology but requires refinement

in the future. It is also recommended that another more sophisticated software package be used in the future to facilitate the convergence of the developed model. The procedure followed in this study applied Gellman Robin statistics to determine the model convergence after running three channels (Gelman and Rubin, 1992). However, this was time-consuming as the model convergence occurred after 240, 000 iterations. In addition, to give more confidence and consistency to the model convergence, the model has to complete 700,000 iterations in total before extracting the statistical results (coda and Kernel density plots). There are new software packages which may perform better than WinBUGS, and they conduct a complete sensitivity analysis as an automated routine (Smid et al., 2010). This study showed that the ARIMA intervention models have the lowest forecast error with only 657 cases more than the actual notified cases (see Table 14.1.) The Holt-Winters method, being local in nature, quickly adapts to the new regime post-intervention and gives good predictions which are comparable with the ARIMA intervention models. The Holt-Winters method has an additional advantage in that it is a simple method, whereas the ARIMA intervention model requires extra statistical expertise to conduct it. It is confirmed that classical time-series techniques such as the ARIMA with intervention and the Holt-Winters method can provide a good prediction performance to predict campylobacteriosis risk.

Table 14-1 Comparison of models in predicting campylobacteriosis cases in New Zealand for the year 2010

2010	*TRUE	AR_Ia	HWm	AR_Im	Bayesian	MC
Cases	7333	7990	8442	8666	9250	846451

*True: actual number of notified cases, HWm: Holt-Winters multiplicative, AR_Ia: ARIMA intervention additive, AR_Im: ARIMA intervention multiplicative

Cumulatively, the findings of this PhD study was not limited to identifying solutions to the campylobacteriosis problem in NZ and providing some direction for prevention and control, but they are also extending it to present promising alternative physical interventions for a possible new crisis in the poultry industry if a ban on the use of chemicals is imposed in NZ in the future, as it is the norm in EU countries. The final validation of the proposed method needs to be implemented at the poultry processing plant level.

Additionally, the approaches of investigating the heat resistance and oxygen tolerance of *C.jejuni* in this study were novel and developed new and fairly advanced methodologies in this study investigations.

Finally, the provision of the Bayesian model to assess all the factors which may contribute to the campylobacteriosis risk in NZ, and the time-series models such as the ARIMA intervention model, are considered as significant tools for risk managers, policymakers, and health professionals to enhance food safety in NZ.

It is important to indicate that this study has provided insights into the possible factors for the high rate of campylobacteriosis in NZ compared to other developed countries. However, this study has not explored the factors for the remarkable decline of the campylobacteriosis rate in 2007 and 2008 (see Figure 14.1). This rapid decline is considered one of the largest decline in disease incidence ever recorded for a national epidemic of this type (Baker, 2011) and it remains unclear as to how it was possible for this rapid decline to have been achieved since even the *Campylobacter* risk management strategy set by the NZFSA in 2007 set only a 50% reduction after five years (Slorach, 2008) but the reduction happened within one year. Moreover, the lack of a reliable and scientific method which can measure the contribution of each intervention to this unexpected decline accurately makes it more questionable for the scientific community in NZ (Nelson and Harris, 2011) and internationally. The source attribution model has not performed well or is not stable, as on one occasion it indicated in a Manawatu survey that poultry attributed to 80% of the campylobacteriosis cases (Mullner et al., 2009a), and another indicated in the same survey that only 67% of cases were attributed to poultry (French, 2008a), and in 2011 a report about the same study showed that >50 % was attributed to poultry (Sears et al., 2011). Furthermore, with changes in the reporting system at the end of 2007 to improve the surveillance system, it was expected that the number of notified cases would have increased. However, questionably, there was a 60% reduction in the number of notified cases in 2008 from 2006, and about a 50% reduction from 2007.

The prohibition of NZ poultry exports to the U.S and E.U for a long time raises more questions about the quality and safety standards implemented in NZ poultry processing plants.

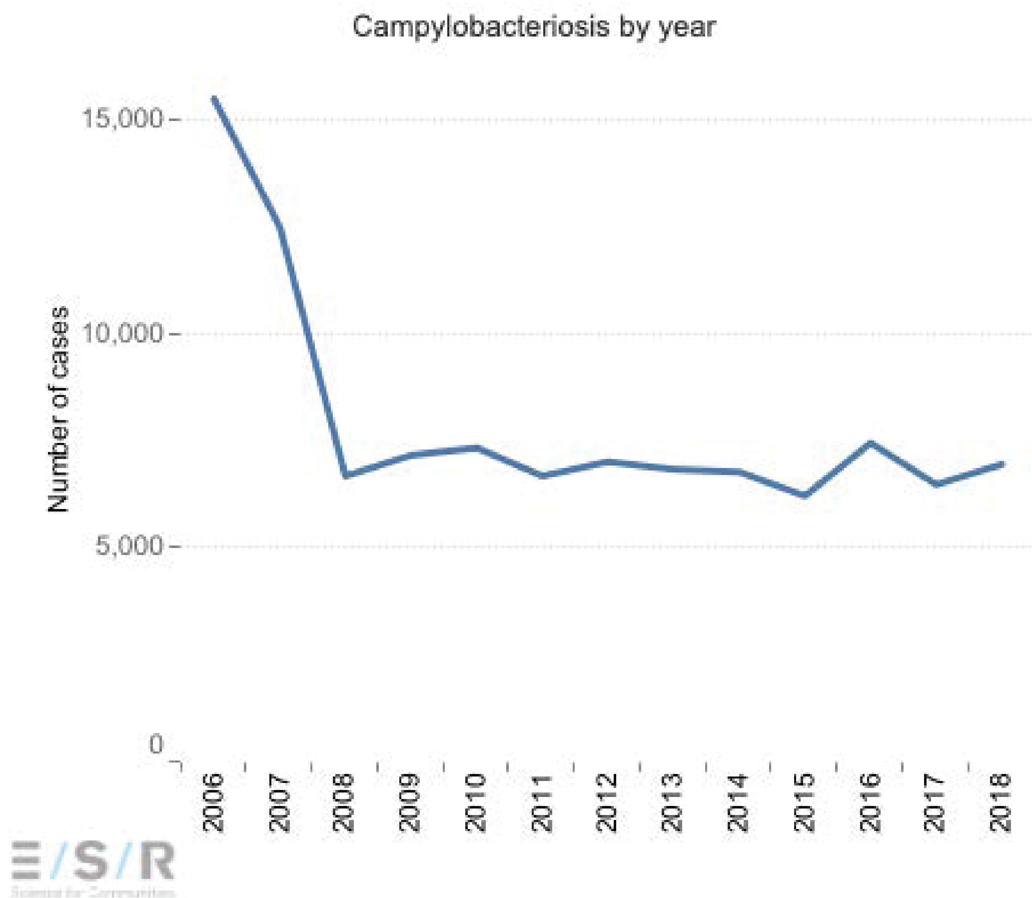


Figure 14-1 Campylobacteriosis Number of cases in New Zealand (EpiSurv, 2019)

Evidentially, the priority for the scientific community, risk managers, policymakers and health professionals is not to celebrate the rapid decline in campylobacteriosis, but to accurately investigate further the proportion of each contributor to the rapid decline and to think about how more can be done to reduce the current rate as NZ still has the highest rate among the developed countries.

Therefore, it is advisable for the NZ government to invest more into prevention and control research of campylobacteriosis. Based on the findings, take appropriate actions and implement interventions that would reduce the disease incidence. New Zealand is a food exporter and also promotes tourism, both of which generate significant revenue. There is a concern that NZ might lose the trust of food importers and tourists if the government does not tackle the food poisoning related to campylobacteriosis. In addition, the government will also save by increased productivity indirectly by reducing the number of New Zealanders who take days off from work due to

associated illnesses. There will also be a saving if the number of hospitalisation which currently cost about 100 millions of NZ $ a year could be reduced.

14.6 Conclusions and opportunities for future research

14.6.1 Conclusions

The D_{60} and *z* values in the broth medium (for the most important strain *C. jejuni* implicated in human cases of campylobacteriosis in NZ) lie within the range from two to five s and 4 to 5.2 °C, respectively. In food, the values ranged from 12 to 18 s and 8 to 11 °C under isothermal conditions, and 13 to 16 s and 8.7 to 10.2 °C under dynamic conditions. These values are broadly in agreement with the published literature and do not indicate that the NZ *Campylobacter* strains are more heat resistant than others. The Weibull model better fitted the survival data than the log-linear model. However, the Weibull shape factor varied significantly between the data sets. When the mean shape factor was considered and the scale factor obtained by re-fitting the model, the goodness of fit was poorer but equivalent to the log-linear model. The scale factor of the Weibull model showed a similar dependency on temperature as the *D* value in the log-linear model, and the time required to achieve a given process extent (e.g. a 7D reduction) was similar for both models. Therefore, the likelihood of a systematic error in using the log-linear model in quantitative risk models appears to be low.

Investigations of the specific *Campylobacter* strains for their ability to survive under other environmental conditions such as atmospheric gas composition at processing plants, handling and storage temperatures in the three methods (liquid media, agar, hydrogen peroxide) revealed that the NZ strains did not differ from most other internationally reported strains in O_2 tolerance or their survival at the investigated temperatures. At 20 or 25°C New Zealand selected strains survived in liquid media (broth) up to only one- week aerobically, but at 4°C, the organisms survived aerobically for > three weeks.

The evaluation of poultry processing practices contributing to the possible increase of *Campylobacter* contamination in NZ chicken and chicken products revealed that the processing practices tested in this study (with two marinades used in the poultry industry) does not support the hypothesis that the marination process is the reason for the significantly high chicken or chicken product contamination in NZ. This study also indicated that a low-salt brine solution was not effective in reducing *C. jejuni* contamination. However, this brine was used for products that would be sold only after freezing, and it reduced the contamination level by at least 2 log of cfu. Higher concentrations of the brine solution did not inhibit the *C. jejuni* survival, and the pathogen could

be detected over the period of product shelf life at the retail outlet up to 10 days. There is no information about other brine solutions or ingredients used in other poultry plants in NZ.

In NZ, government agencies, the poultry industry and the farmers face an immense challenge from the policy on the use of chemicals in poultry production enacted by the EU in 2006. It is most likely the consumers in NZ will also initiate a campaign against the use of chemicals in poultry processing. In this regard, the hot water immersion technique investigated in this study provides a promising, simple, cost-effective alternative which can be used by the poultry industry if a ban on chemicals is implemented in NZ in the future. However, the study emphasised the need for commercial trials to be carried out to evaluate the feasibility of the hot water immersion intervention as a successful intervention to reduce *Campylobacter* in naturally contaminated chicken in a processing environment. In addition, the sensory quality of the treated carcasses also needs to be evaluated by poultry industry experts for the best time-temperature combination with the least adverse sensory effects if this to be universally approved.

Moreover, this study also suggests the replacement of the current tap water spray washers with hot water spray washers, which can serve as an additional aid to reduce the *Campylobacter* contamination level in chicken. The importance of dry air chilling is also highlighted since it reduces the *Campylobacter* level by 0.3 to 1.4 log, as a replacement for the current spine-chiller, which has the potential to cross-contaminate (Sánchez et al., 2002, Whyte et al., 2002). This could be a comprehensive solution with multiple control measures for poultry processors and government agencies.

This study QMRA model was able to identify the consumer hygiene practices, the initial contamination prevalence at the farm and the practices at the processing plant, as the significant factors influencing the final risk estimate. The association between the reduction in flock/ poultry prevalence and improvement in home hygiene practice and the calculated reduction in risk of human disease was estimated to be 1:1, which agrees with international findings. Thus, QMRA model can easily determine the impact of any intervention in the food chain. Therefore, the effect of a new planned intervention such as consumer education on the final risk estimate should be clear for the policymakers, risk managers and health professionals. This can be achieved by altering the priors of the consumer hygiene practices in QMRA model. Similarly, other interventions at poultry plants and farms also be assessed by altering the priors of farms and poultry plants. This study highlighted the importance of the Bayesian model to assess all the factors which may contribute to the campylobacteriosis risk and confirmed that it can provide better conclusions for QMRA than the MC technique because of its interactive link between the data and the parameter

(backward inference).

It is confirmed from this study findings that classical time series techniques such as the ARIMA with intervention and the Holt-Winters method can provide a better prediction performance for the risk of campylobacteriosis than the QMRA by Bayesian approach. Moreover, the general strength of the Holt-Winters forecast method is that it managed to predict accurately the monthly campylobacteriosis cases in New Zealand and it can also be used easily with a minimum of statistical expertise required with the currently available software. The time series models, such as the ARIMA intervention model which has the lowest forecast error and the Holt-Winters method, are considered as significant models for risk managers, policy makers, and health professionals in order to enhance food safety in New Zealand.

This study was aimed to test if the high campylobacteriosis rate in NZ has a significant link with any negligent food handling practices in NZ domestic kitchens. There appears to be a lack of good hygiene practice and cross-contamination prevention at home during poultry preparation, proper storage, temperature control, determination of 'doneness', proper thawing of raw poultry, food shopping and transportation. It is noteworthy that about 80 % of the respondents in the food consumer survey had tertiary qualification or postgraduate qualification but still their food safety practices, and food safety awareness was below the average knowledge.

The lack knowledge concerning food safety during domestic food preparation was prevalent among New Zealand consumers. Most of the evidence collected from this study survey confirmed the hypothesis that New Zealanders are poorer in food hygiene and food safety awareness than the citizens of other developed countries, and this is possibly the major reason for the high rate of campylobacteriosis in NZ compared to the rates in the other developed countries. Other reasons may be the prevalence and contamination level of chicken carcasses in New Zealand. Consumers have a responsibility to handle, store and prepare food in a safe way to avoid the risk of foodborne diseases.

The aim of health professionals and risk managers is to encourage consumers to practice desirable and safe practices and to discourage improper or unsafe ones, by designing an effective communication initiative regarding food safety hazards and proper food handling practices in the domestic environment. It is clear from the literature that the process of communicating domestic food safety risk is a complex and greatly challenging task.

The responsibility is on the experts and risk communication community to understand the variety of socioeconomic/demographic and psychological factors indicated in this study.

The cost of campylobacteriosis to NZ economy is about 100$ million. Given the potential the loss

of NZ reputation in the world and the financial loss from the tourism industry, this study has recommended using of innovative and effective food safety messages in future educational or communication strategies aimed to educate New Zealanders and improve their domestic food handling practices, which will reduce the number of campylobacteriosis cases that occur in NZ homes.

It is important to state that the efforts of the NZFSA in requesting all poultry processors to collect samples for monitoring the prevalence and contamination level in flocks and carcasses on each day in March 2007 and in February 2008 imposed a performance target limit for the contamination level on chicken carcasses. Later in 2013 imposed the detection limit as well. This forced poultry processors in NZ to increase their efforts to implement interventions throughout the poultry processing lines to further reduce the contamination level in chicken carcasses. Poultry processors achieve the target by using harmful available chemical interventions which may be banned by the authority in the future.

14.6.2 Future research

As the consumer demand increases for more chemical-free products, and with the ban on chemicals used in slaughterhouses in Europe, the poultry industry and government agencies are turning to more consumer acceptable interventions. It is likely that a ban on chemical use will also be enforced in NZ. Thus, further research is required to evaluate the results of this study as a potential, cost-effective, consumer and environment-friendly solution for reducing *Campylobacter* in poultry processing plants in NZ.

It is important to investigate the effect of NZ poultry practices at poultry processing plants in order to gauge the contribution of such practices to *Campylobacter* contamination in NZ chicken meat processing in a more comprehensive survey. Moreover, the model used for the QMRA by the Bayesian approach needs further improvement and investigation.

More research is needed on consumer's food safety knowledge, perception, practice and communication in NZ, as food safety is a complex issue, and requires constant attention, dedication, and persuasive communications. All relevant parties in NZ (the government, researchers, the poultry industry, farmers, growers and consumers) need to collaborate to tackle the complex nature of the campylobacteriosis problem. The success of NZ scientific research in addressing this problem is being watched with interest worldwide. Therefore, it is important to improve disease surveillance and its autonomy and to fund useful research.

Despite the dramatic drop in the campylobacteriosis notification rate in 2007 and 2008, it remains

higher than the rate in other developed countries and some New Zealand *Campylobacter* strains develop antimicrobial drug resistance which threatens the effective treatment of campylobacteriosis (French et al., 2019). The rate 142 cases per 100,000 is seven times higher than the rate in the USA and three times higher than the rate in the Netherlands, France, Ireland and Iceland (ECDC, 2017). Further efforts are required to decrease the incidence rate to at least at a comparable level with other developed countries. Such a reduction could save about NZ $100 million a year in addition to preserving NZ reputation in the world as a 'clean-green' food exporter which would also indirectly assist NZ as a tourist destination.

Appendix A

A.1 Code for the programme to estimate *D* and *z* values by one step regression technique performed by R Software (version 2.9.1)

```
#P474
Dref<-42
z<-4.7
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_P474.csv")
Start<-read.csv("Start_P474.csv")
#n0<-Start$n0
n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6],n0.7=n0[7],n0.8=n0[8],n0.9=n0[9],n0.10=n0[10],n0.11=n0[11])
Fit_P474.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6+n0.7*s7+n0.8*s8
+n0.9*s9+n0.10*s10+n0.11*s11 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_P474.nls)

#P190
Dref<-22
z<-4.3
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_P190.csv")
Start<-read.csv("Start_P190.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6],n0.7=n0[7],n0.8=n0[8])

Fit_P190.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6+n0.7*s7+n0.8*s8
  -K1*K2^T.d*t,trace=TRUE,start=Startlist,data=Data)
```

```
summary(Fit_P190.nls)

#P45
Dref<-30
z<-4
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_P45.csv")
Start<-read.csv("Start_P45.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6])
Fit_P45.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_P45.nls)

#H474
Dref<-38
z<-4.6
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_H474.csv")
Start<-read.csv("Start_H474.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6])
Fit_H474.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_H474.nls)

#H474
Dref<-38
```

```
z<-4.6
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_H474.csv")
Start<-read.csv("Start_H474.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6])
Fit_H474.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_H474.nls)

#H190
Dref<-24
z<-5.6
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_H190.csv")
Start<-read.csv("Start_H190.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6])
Fit_H190.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_H190.nls)

#H45
Dref<-30
z<-4
K1<-1/Dref
K2<-10^(1/z)
Data<-read.csv("Data_H45.csv")
```

```
Start<-read.csv("Start_H45.csv")
n0<-Start$n0
#n0<-c(7.7,8.1,7.9,8,8.2,7.7,8.9,7.7,8.4,8.1,7.8)
Startlist<-list(K1=K1,K2=K2,n0.1=n0[1],n0.2=n0[2],n0.3=n0[3],n0.4=n0[4],
n0.5=n0[5],n0.6=n0[6])
Fit_H45.nls<-nls(n~n0.1*s1+n0.2*s2+n0.3*s3+n0.4*s4+n0.5*s5+n0.6*s6 -K1*K2^T.d*t,
trace=TRUE,start=Startlist,data=Data)
summary(Fit_H45.nls)
```

A.2 Output (Results of Chapter 4)

P474

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.021676	0.00037	58.54	<2e-16
K2	1.552101	0.017322	89.6	<2e-16
n0.1	7.781425	0.142206	54.72	<2e-16
n0.2	8.803506	0.126776	69.44	<2e-16
n0.3	7.71231	0.100909	76.43	<2e-16
n0.4	7.869977	0.100909	77.99	<2e-16
n0.5	8.224296	0.098875	83.18	<2e-16
n0.6	7.562352	0.125908	60.06	<2e-16
n0.7	9.438821	0.106435	88.68	<2e-16
n0.8	8.471587	0.11869	71.38	<2e-16
n0.9	8.357541	0.106882	78.19	<2e-16
n0.10	7.276851	0.138857	52.41	<2e-16
n0.11	7.760851	0.138857	55.89	<2e-16

Residual standard error: 0.5188 on 276 degrees of freedom

	Estimate	Std. Error
Dref	46.13504	0.787949
z	5.2378	0.132973

H474

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.024513	0.000634	38.65	<2e-16
K2	1.685991	0.018725	90.04	<2e-16
n0.1	7.710397	0.157679	48.9	<2e-16

n0.2	8.683295	0.145631	59.62	<2e-16
n0.3	7.927546	0.136132	58.23	<2e-16
n0.4	7.608491	0.147141	51.71	<2e-16
n0.5	9.034026	0.155443	58.12	<2e-16
n0.6	8.20164	0.164047	50	<2e-16

Residual standard error: 0.6232 on 159 degrees of freedom

	Estimate	Std. Error
Dref	40.79468	1.055439
z	4.408096	0.093723

P45

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.067991	0.001448	46.94	<2e-16
K2	1.772768	0.011702	151.5	<2e-16
n0.1	8.384732	0.094766	88.48	<2e-16
n0.2	7.988861	0.087466	91.34	<2e-16
n0.3	7.114367	0.09764	72.86	<2e-16
n0.4	7.77277	0.079494	97.78	<2e-16
n0.5	7.41625	0.089446	82.91	<2e-16
n0.6	7.827225	0.165366	47.33	<2e-16

Residual standard error: 0.3484 on 115 degrees of freedom

	Estimate	Std. Error
Dref	14.70783	0.313232
z	4.021686	0.046367

H45

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.063248	0.001819	34.76	<2e-16
K2	1.786784	0.016919	105.61	<2e-16
n0.1	8.292211	0.132925	62.38	<2e-16
n0.2	7.42766	0.124872	59.48	<2e-16
n0.3	7.038507	0.129579	54.32	<2e-16
n0.4	6.663056	0.113924	58.49	<2e-16
n0.5	7.400597	0.117341	63.07	<2e-16
n0.6	7.912529	0.227721	34.75	<2e-16

Residual standard error: 0.4932 on 124 degrees of freedom

	Estimate	Std. Error
Dref	15.81078	0.454715
z	3.96712	0.06472

P190

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.058477	0.001602	36.49	<2e-16
K2	1.67249	0.020709	80.76	<2e-16
n0.1	7.935879	0.102763	77.22	<2e-16
n0.2	8.209294	0.112955	72.68	<2e-16
n0.3	7.471911	0.150304	49.71	<2e-16

n0.4	8.943868	0.088995	100.5	<2e-16
n0.5	7.282378	0.106337	68.48	<2e-16
n0.6	8.550544	0.100022	85.49	<2e-16
n0.7	7.013655	0.16138	43.46	<2e-16
n0.8	6.526296	0.212263	30.75	<2e-16

Residual standard error: 0.4190 on 140 degrees of freedom

	Estimate	Std. Error
Dref	17.10074	0.468481
z	4.477007	0.107784

H190

Parameters:

	Estimate	Std. Error	t value	Pr(>|t|)
K1	0.044428	0.000678	65.57	<2e-16
K2	1.683517	0.009732	172.99	<2e-16
n0.1	7.922236	0.062066	127.64	<2e-16
n0.2	8.133696	0.077803	104.54	<2e-16
n0.3	7.841556	0.069883	112.21	<2e-16
n0.4	8.234689	0.064786	127.11	<2e-16
n0.5	7.851038	0.090938	86.33	<2e-16
n0.6	7.379568	0.091492	80.66	<2e-16

Residual standard

error:
0.2774 on
127 degrees
of freedom

	Estimate	Std. Error
Dref	22.50838	0.343291
z	4.420526	0.049058

A.3 An example of the software output from *GInaFit*.(Chapter 4)

Time	Measured LOG10(N)	Identified LOG10(N)	Squared difference
0.00	8.04	7.99	0.00
0.00	8.04	7.99	0.00
0.00	7.98	7.99	0.00
5.00	8.02	7.99	0.00
5.00	8.03	7.99	0.00
5.00	8.03	7.99	0.00
50.00	7.83	7.87	0.00
50.00	7.90	7.87	0.00
50.00	8.01	7.87	0.02
100.00	7.31	7.53	0.05
100.00	7.32	7.53	0.04
100.00	7.25	7.53	0.08
150.00	6.77	6.96	0.04
150.00	6.79	6.96	0.03
150.00	6.81	6.96	0.02
200.00	6.30	6.28	0.00
200.00	6.29	6.28	0.00
200.00	6.26	6.28	0.00
250.00	5.81	5.59	0.05
250.00	5.83	5.59	0.06
250.00	5.88	5.59	0.08
300.00	4.62	4.88	0.07
300.00	4.88	4.88	0.00
300.00	5.04	4.88	0.02
350.00	4.80	4.18	0.39
350.00	4.51	4.18	0.11
350.00	4.63	4.18	0.21
400.00	3.87	3.47	0.16
400.00	3.70	3.47	0.05
400.00	3.04	3.47	0.19
450.00	2.00	2.77	0.59
450.00	2.48	2.77	0.08
450.00	2.48	2.77	0.08
Least Sum of Squared Error			2.42

Parameters	Parameter	Standard Error
Sl (Shoulder length)	79.49	13.61
kmax	0.03	0.00
LOG10(N0)	7.99	0.10

Mean Sum of Squared Error	0.0808
Root Mean Sum of Squared Error	0.2843
R-Square	0.9783
R-Square adjusted	0.9769

Inactivation model identified

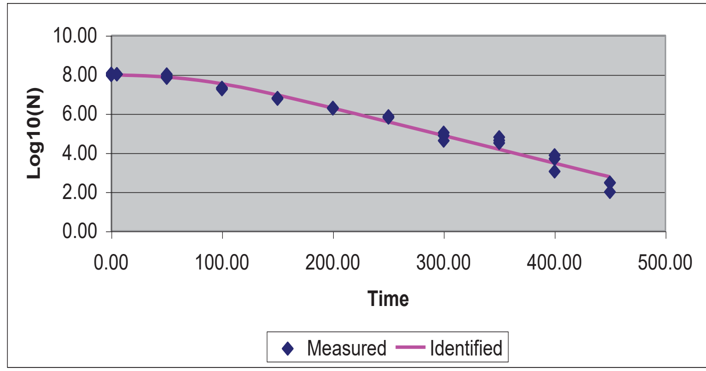
N= N0 * exp(-kmax * t) * (exp(kmax * Sl))/(1+(exp(kmax * Sl) - 1) *exp(-kmax*t)))

For identification purposes reformulated as

Log10(N) = Log10(N0) - kmax * t / Ln(10) + Log10(Exp(kmax * Sl) / (1 + (Exp(kmax * Sl) - 1) * Exp(-kmax * t)))

as can be derived from

A.H. Geeraerd, C.H. Herremans and J.F. Van Impe 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. International Journal of Food Microbiology, 59(3), 185-209



Appendix B

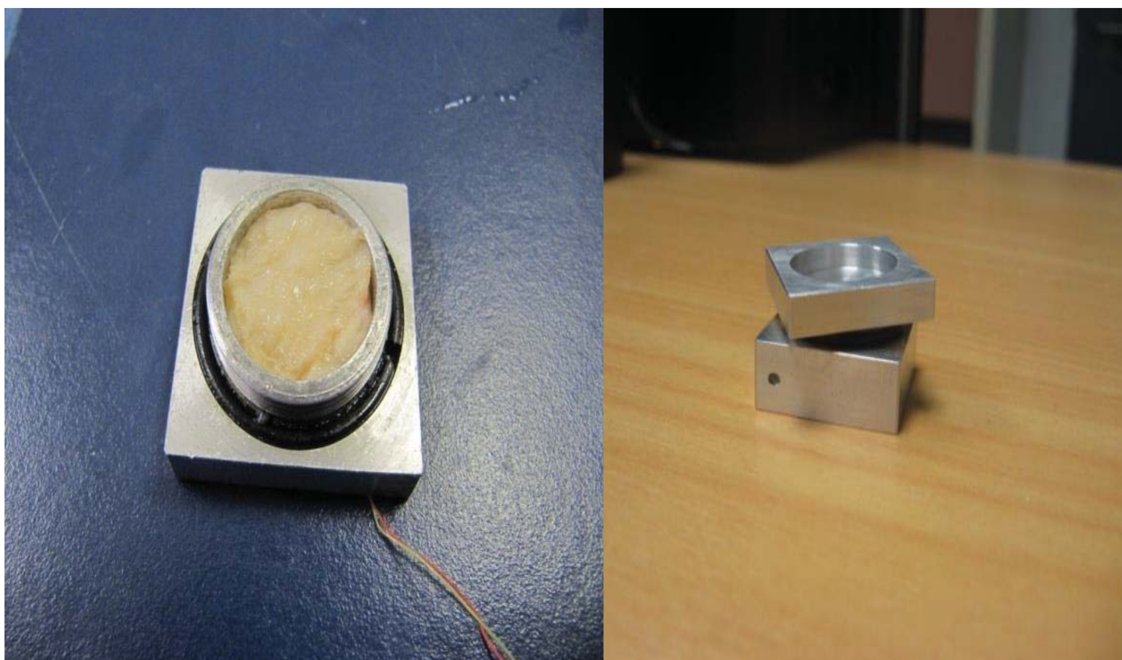


Figure B1. The aluminium cell used in experiments (Chapters 5 and 6).

Figure B2. The aluminium cells during heating inside the water bath (Chapter 5)

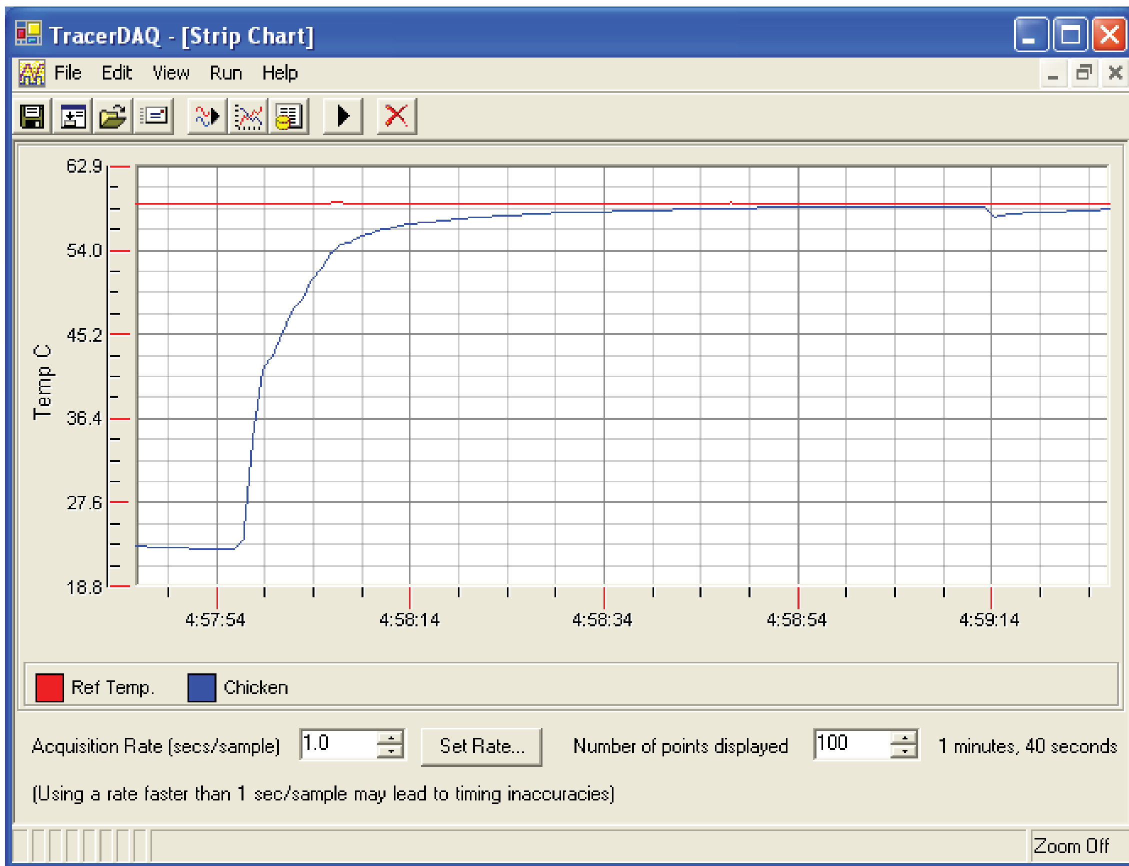


Figure B3. Temperature measured on the surface of chicken skin during heating to 60 °C in the programmable heating water bath (Chapter 5).

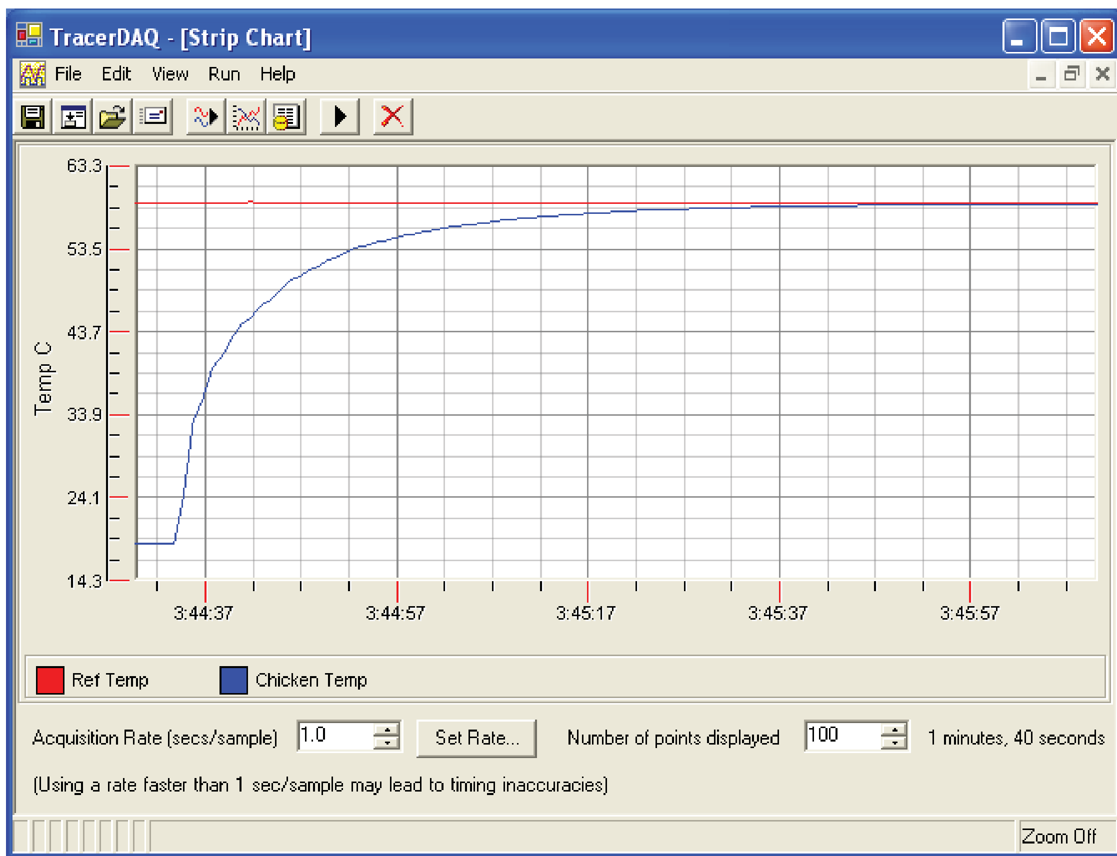


Figure B4. Temperature measured on the surface of chicken skin during heating to 60 °C in the programmable heating water bath (Chapter 5)

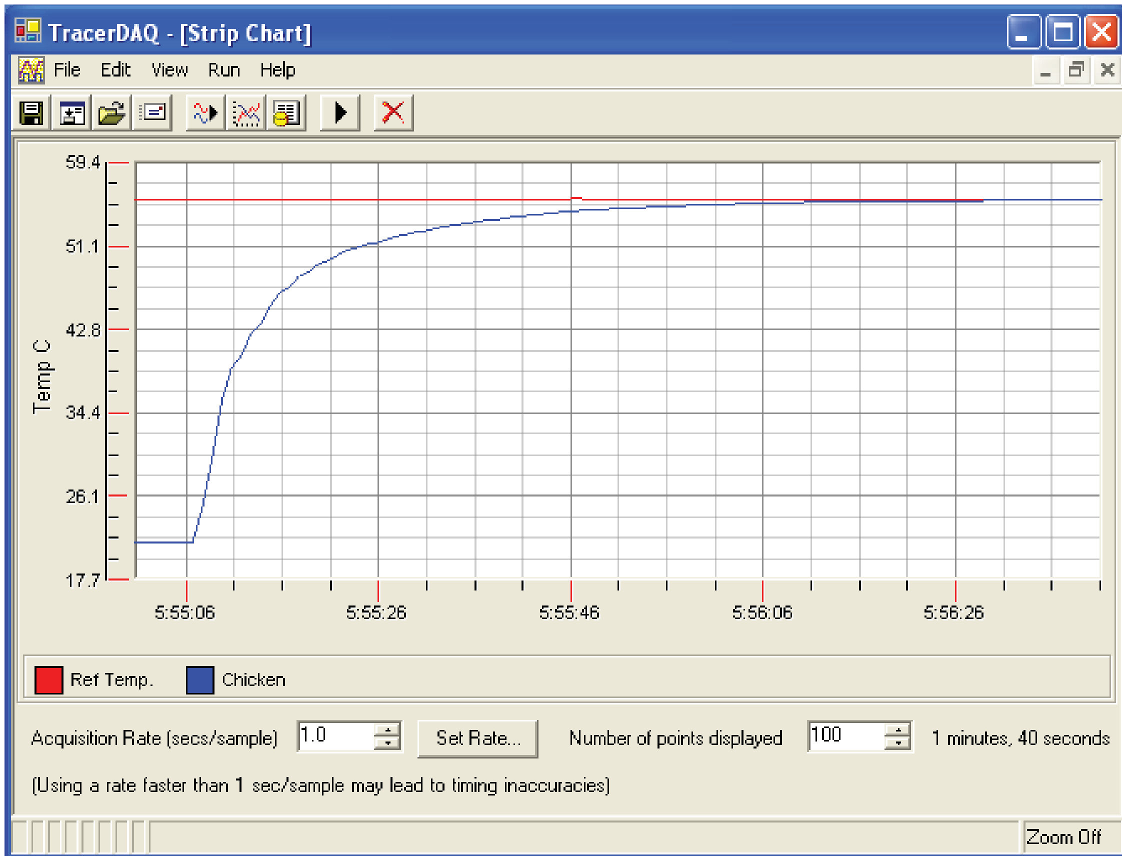


Figure B5. Temperature measured on the surface of chicken skin during heating to 56.5 °C in the programmable heating water bath (Chapter 5).

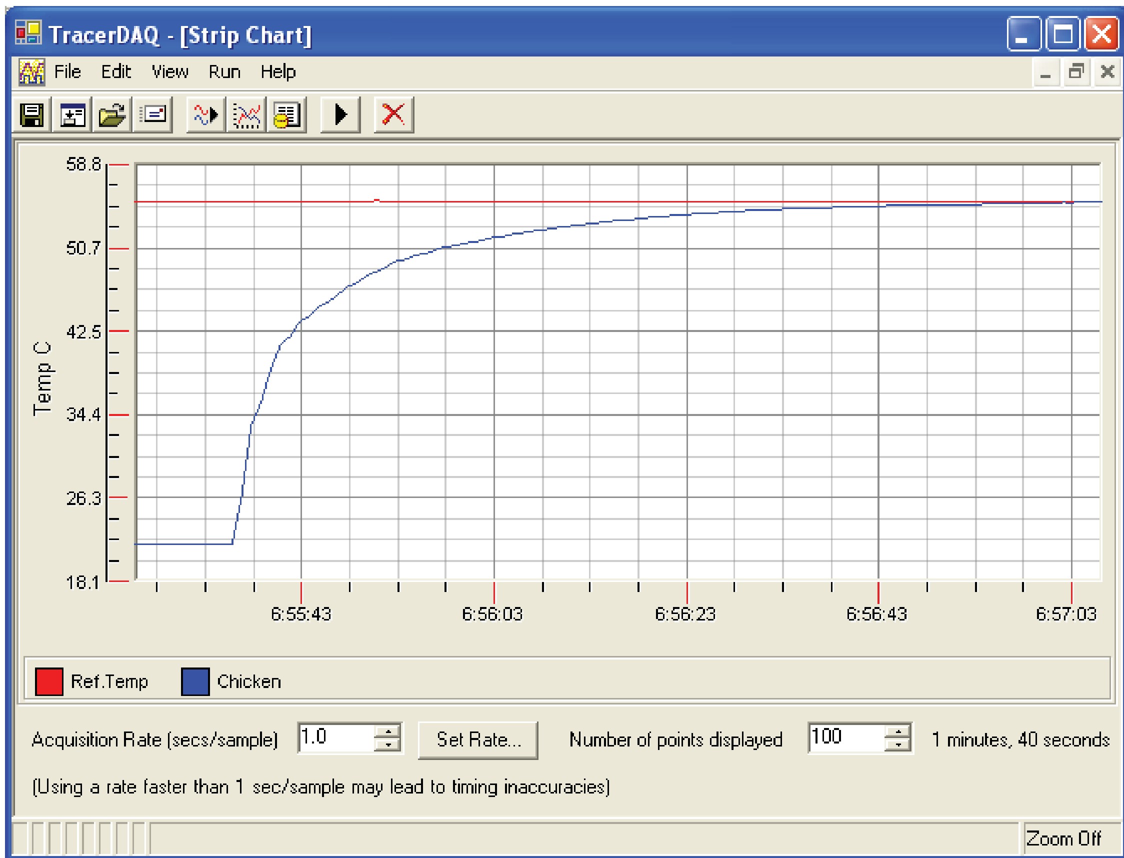


Figure B6. Temperature measured on the surface of chicken skin during heating to 56.5 °C in the programmable heating water bath (Chapter 5).

Appendix C

Figure C1. Programmable Water Bath used (chapter 6)

A stainless steel electrical 1.8 l kettle (Fast boil 2400-watt concealed element, Breville) was used as a water bath. Eurostar electric stirrer (model ST PB, 300 RPM) was used to assure the temperature uniformity inside the kettle during the heating of the samples. The kettle was connected to a PID controller to input the desired heating temperature and heating time period for each step. (nine steps per pattern are available for program control). The PID has enhanced Communication functions, and it was fitted with a RS 485 interface card to allow communication via a Novus (USB-i485) isolated converter to a PC to allow control, display and acquisition of the temperature profile for each run.

C1. The code for kinetic parameters calculation by MATLAB Optimisation Toolbox (MathWorks Inc. Natick, MA, USA). (Chapter 6)

```
global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18
nobs_18;

Tref = 60.75;
Tact = 47;
K1 = 0.0637;
%K1 = 0.077688;
K2 = 1.1275;
%K2 = 1.1;
c0 = 0;
%c0 = -6;
n0_8 = 8.8222;
n0_18 = 10.1634;

% 18min expt:
P474_18min_in;          % load the data
t = t-1;         % times are measured in seconds starting at 0
b=0:30:max(t);        % knotpoints for the spline fit to the temperature data; every 30 seconds
Tspline=spline(b,T/spline(b,eye(length(b)),t)); % construct the best spline fit to data
Tsp = ppval(Tspline,t);
t_0_18 = max(t(Tsp<Tact))+1; %t_0 is time to reach activation temp
t_i_18 = 0:(t_0_18-1);      %t_i before reaching activation temp
t_a_18 = t_0_18:max(t);      %t_a after reaching activation temp
Tspline_18 = Tspline;
t_18 = t;

% 8min expt:
P474_8min_in;          % load the data
t = t-1;         % times are measured in seconds starting at 0
b=0:30:max(t);        % knotpoints for the spline fit to the temperature data; every 30 seconds
Tspline=spline(b,T/spline(b,eye(length(b)),t)); % construct the best spline fit to data
```

```
Tsp = ppval(Tspline,t);
t_0_8 = max(t(Tsp<Tact))+1; %t_0 is time to reach activation temp
t_i_8 = 0:(t_0_8-1);      %t_i before reaching activation temp
t_a_8 = t_0_8:max(t);      %t_a after reaching activation temp
Tspline_8 = Tspline;
t_8 = t;

x0 = [K1 K2 c0 n0_8 n0_18];
sse = ssefun(x0)

[x,resnorm,residual,exitflag,output,lambda,jacobian] = lsqnonlin(@resfun,x0);

x
sse = resnorm
df = length(tobs_8)+length(tobs_18)-5
mse = sse/df
Sigma = mse*inv (jacobian'*jacobian)
SE = sqrt(diag(Sigma))


function d = model(t,y)

global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18 nobs_18;

% Evaluate the cubic spline fit to the temperature data at time t
T = ppval(Tspline,t);
A = -K1*K2^(T-Tref);
d=[A/(1+10^y(2)); A];

return

function d = model_18(t,y)
```

```
global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18
nobs_18;

% Evaluate the cubic spline fit to the temperature data at time t
T = ppval(Tspline_18,t);
A = -K1*K2^(T-Tref);
d=[A/(1+10^y(2)); A];

return

function d = model_8(t,y)

global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18
nobs_18;

% Evaluate the cubic spline fit to the temperature data at time t
T = ppval(Tspline_8,t);
A = -K1*K2^(T-Tref);
d=[A/(1+10^y(2)); A];

return



%Times and temperatures:
t = 1:655;
T = [20.5 ………];

%Observed data:
tobs_8 = [360 380 400 420 450 460];
nobs_8 = [7.837272703 7.745270024 6.515211304 5.161368002 3.844321821 2.640978057];

function res = resfun(x)

global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18
```

```
nobs_18;

K1 = x(1);
K2 = x(2);
cs = x(3);
ns_8 = x(4);
ns_18 = x(5);

[t_8,y_8]=ode45('model_8',t_a_8,[ns_8,cs]);
y_8 = [repmat([ns_8 cs],length(t_i_8),1) ; y_8];
nmod = y_8(tobs_8,1)';
res_8 = (nmod-nobs_8)';

[t_18,y_18]=ode45('model_18',t_a_18,[ns_18,cs]);
y_18 = [repmat([ns_18 cs],length(t_i_18),1) ; y_18];
nmod = y_18(tobs_18,1)';
res_18 = (nmod-nobs_18)';

res = [res_8; res_18];
return

function sse = ssefun(x)

global Tref K1 K2 Tspline_8 t_i_8 t_a_8 tobs_8 nobs_8 Tspline_18 t_i_18 t_a_18 tobs_18
nobs_18;

K1 = x(1);
K2 = x(2);
cs = x(3);
ns_8 = x(4);
ns_18 = x(5);

[t_8,y_8]=ode45('model_8',t_a_8,[ns_8,cs]);
y_8 = [repmat([ns_8 cs],length(t_i_8),1) ; y_8];
```

```
nmod = y_8(tobs_8,1)';
sse_8 = (nmod-nobs_8)*(nmod-nobs_8)';

[t_18,y_18]=ode45('model_18',t_a_18,[ns_18,cs]);
y_18 = [repmat([ns_18 cs],length(t_i_18),1) ; y_18];
nmod = y_18(tobs_18,1)';
sse_18 = (nmod-nobs_18)*(nmod-nobs_18)';

sse = sse_8 + sse_18;
return
```

Appendix D

D.1 R software code (Chapter 7).

```
Data<-read.csv("Data.csv")
Data$Temp<-factor(Data$Temp)
Data$Time<-factor(Data$Time)
Data$Rep<-paste(Data$Strain,Data$Atm,Data$Temp,Data$Time,Data$Replicate)
Data$Rep<-factor(Data$Rep)
Data$Exp<-paste(Data$Strain,Data$Atm,Data$Temp,Data$Time)
Data$Exp<-factor(Data$Exp)

write.csv(Data,"Ndata.csv")

library(nlme)
library(lme4)

cuniq<-function(v) length(unique(v))

#Temp=4
Data4<-Data[Data$Temp=="4",]
Data4$Time<-factor(Data4$Time)
Data4$Rep<-factor(Data4$Rep)
Data4$Exp<-factor(Data4$Exp)
write.csv(Data4,"Data4.csv")
Model4.Exp<-lm(LogN~Strain*Atm*Time,data=Data4)
Model4.Rep<-lm(LogN~Rep,data=Data4)
anova(Model4.Exp,Model4.Rep)
anova(Model4.Exp)
Model4.aov<-aov(LogN~Strain*Atm*Time+Error(Rep),data=Data4)
summary(Model4.aov)

head(Data4)
Emean<-tapply(Data4$LogN,Data4$Exp,mean)
```

```
cPlate<-tapply(Data4$Plate,Data4$Exp,cuniq)
cRep<-tapply(Data4$Replicate,Data4$Exp,cuniq)
cbind(Emean,cRep,cPlate)
sig2e<-summary(Model4.aov)[[2]][[1]][1,3]
sig2r<-summary(Model4.aov)[[1]][[1]][8,3]
SE<-sqrt(sig2e/cPlate+sig2r/cRep)
Res4<-cbind(Emean,cRep,cPlate,SE)

#Temp=10
Data10<-Data[Data$Temp=="10",]
Data10$Time<-factor(Data10$Time)
Data10$Rep<-factor(Data10$Rep)
Data10$Exp<-factor(Data10$Exp)
write.csv(Data10,"Data10.csv")
Model10.Exp<-lm(LogN~Strain*Atm*Time,data=Data10)
Model10.Rep<-lm(LogN~Rep,data=Data10)
anova(Model10.Exp,Model10.Rep)
anova(Model10.Exp)
Model10.aov<-aov(LogN~Strain*Atm*Time+Error(Rep),data=Data10)
summary(Model10.aov)

head(Data10)
Emean<-tapply(Data10$LogN,Data10$Exp,mean)
cPlate<-tapply(Data10$Plate,Data10$Exp,cuniq)
cRep<-tapply(Data10$Replicate,Data10$Exp,cuniq)
sig2e<-summary(Model10.aov)[[2]][[1]][1,3]
sig2r<-summary(Model10.aov)[[1]][[1]][8,3]
SE<-sqrt(sig2e/cPlate+sig2r/cRep)
Res10<-cbind(Emean,cRep,cPlate,SE)


#Temp=20
Data20<-Data[Data$Temp=="20",]
Data20$Time<-factor(Data20$Time)
```

```
Data20$Rep<-factor(Data20$Rep)
Data20$Exp<-factor(Data20$Exp)
write.csv(Data20,"Data20.csv")
Model20.Exp<-lm(LogN~Strain*Atm*Time,data=Data20)
Model20.Rep<-lm(LogN~Rep,data=Data20)
anova(Model20.Exp,Model20.Rep)
anova(Model20.Exp)
Model20.aov<-aov(LogN~Strain*Atm*Time+Error(Rep),data=Data20)
summary(Model20.aov)

head(Data20)
Emean<-tapply(Data20$LogN,Data20$Exp,mean)
cPlate<-tapply(Data20$Plate,Data20$Exp,cuniq)
cRep<-tapply(Data20$Replicate,Data20$Exp,cuniq)
sig2e<-summary(Model20.aov)[[2]][[1]][1,3]
sig2r<-summary(Model20.aov)[[1]][[1]][8,3]
SE<-sqrt(sig2e/cPlate+sig2r/cRep)
Res20<-cbind(Emean,cRep,cPlate,SE)

#Using lme:
Data4$RT<-paste(Data4$Rep,Data4$Time)
Data4$RT<-factor(Data4$RT)
Data4.lme<-lme(LogN~Strain*Atm*Time,random=~1|RT,data=Data4)
```

Appendix E

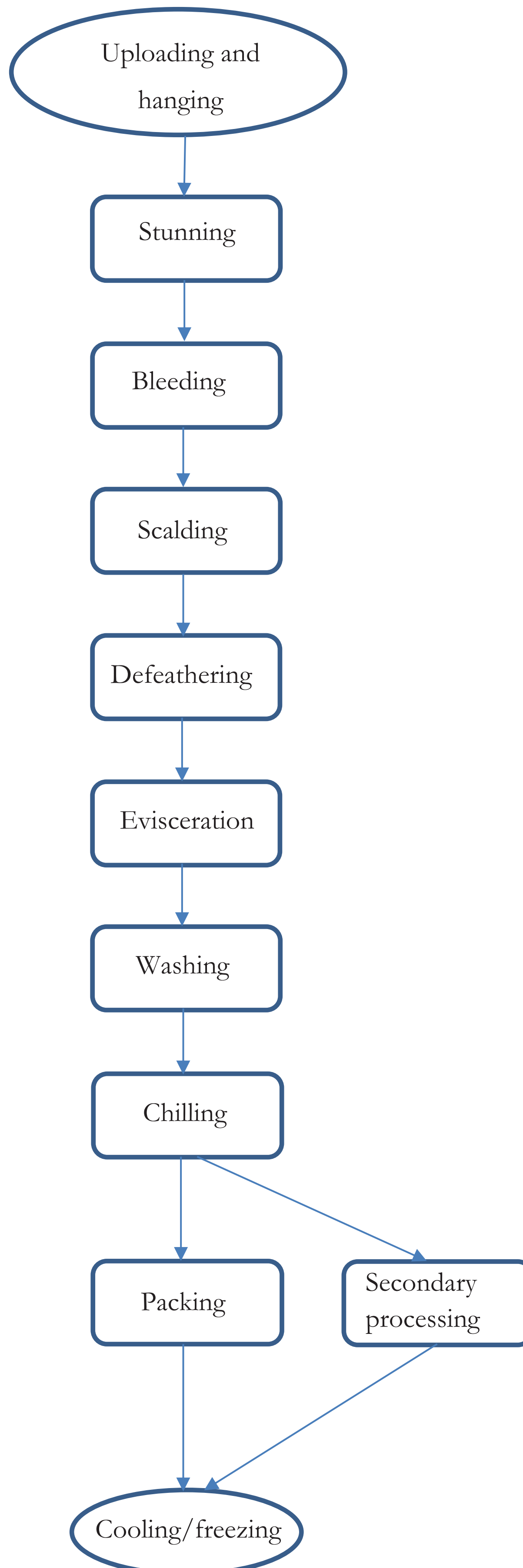
E.1 CORE MODEL used for risk assessment by the Bayesian approach (Chapter 10).

```
model # starting the model ###
############################################
### CHICKEN FARM MODULE ......................................
p.f <- exp(lp.f)/(1+exp(lp.f)); #vi
lp.f ~ dnorm(m.f, tau.f); #..
m.f ~ dnorm(0.0,20.66); #pa
s.f ~ dunif(0,0.2); tau.f <- 1/(s.f*s.f); #pa
### BROILER PRODUCTION MODULE .............................
p.b <- exp(lp.b)/(1+exp(lp.b)); #vi
lp.b ~ dnorm(m.b, tau.b); #..
m.b <- m.f + d.bf; #cv
d.bf ~ dnorm(0.1,206.6)I(0,); #pa
s.b ~ dunif(0,0.2); tau.b <- 1/(s.b*s.b); #pa
### HYGIENE MODULE ......................................
p.h <- p.hc * p.hh; #vi
p.hc ~ dbeta(8,8); #pa
p.hh ~ dbeta(166,355); #pa
#p.hh ~ dbeta(22,20);# pa
### CONSUMPTION MODULE ..................................
lambda.cs ~ dgamma(8,2); #vi
### EXPOSURE MODULE .....................................
p.ey <- 1 - pow((1-p.e),13); #vi
p.e <- 1 - exp(-lambda.e); #cv
lambda.e <- p.b * p.h * lambda.cs; #cv
### ILLNESS MODULE ......................................
p.ib <- p.ey * p.ie; #vi
p.it <- p.ib/(1 - (1-p.iq)*(1-p.ey)); #vi
p.ie <- (1-pow(1-p.ne,d)) * p.in; #cv
d <- vd[c.d]; #cv
p.ne ~ dbeta(0.024,0.011)I(0.00001,0.999999); #pa
p.in <- 0.33; #pa
```

```
p.iq ~ dbeta(39,12); #pa
c.d ~ dcat(c.di[]); #..
### AUGMENTED MODEL FOR DATA INCORPORATION #############
### CHICKEN FARM MODULE ......................................
for (i.fs in 1:n.fst) #..
g.fs[i.fs] ~ dbin(p.fs[i.fs],n.fs[i.fs]); #da
p.fs[i.fs] <- exp(lp.fs[i.fs])/ #cv
(1 + exp(lp.fs[i.fs])); #..
lp.fs[i.fs] ~ dnorm(m.f,tau.f); #..
} #..
### BROILER PRODUCTION MODULE .............................
for (i.bs in 1:n.bst) #..
g.bs[i.bs] ~ dbin(p.bs[i.bs],n.bs[i.bs]); #da
p.bs[i.bs] <- exp(lp.bs[i.bs])/ #cv
(1 + exp(lp.bs[i.bs])); #..
lp.bs[i.bs] ~ dnorm(m.b,tau.b); #..
} #..
### HYGIENE MODULE .....................................
### CONSUMPTION MODULE .................................
n.csb ~ dpois(lambda.csb); #da
lambda.csb <- n.cs*lambda.cs;#cv
### EXPOSURE MODULE ....................................
### ILLNESS MODULE .....................................
g.its ~ dbin(p.its,n.its); #da
logit(p.its) <- logit(p.it) + err; #cv
s.s <- 1; tau.s <- 1/(s.s*s.s); #pa
err ~ dnorm(0,tau.s); #..
} # ending the model ########################################
list(# starting the doses .................................
c.di=c(0.500,0.163,0.222,0.097,0.018), vd=c(1,2,10,100,300))
list(# starting the data incorporated ......................
n.fst=50,
n.fs=c(188,150,148,195,170,309,211,144,145,158,107,153,150,181,160,204,57,52,62,84,87,40,83,5
4,84,181,160,172,229,141,168,181,176,159,211,163,157,140,176,146,151,67,61,61,70,64,65,60,66,7
```

```
5),
n.bst=44,
n.bs=c(75,60,60,72,60,60,60,57,60,72,60,57,57,72,60,72,60,60,60,60,72,57,72,60,72,60,54,60,69,42,
57,60,72,60,72,60,60,57,72,60,75,60,55,75),
n.cs=21037900,
n.its=4292350,
g.fs=c(133,108,102,128,119,131,142,107,102,117,95,117,125,148,122,
137,20,12,17,34,24,31,50,50,63,66,140,106,133,107,114,121,119,142,154,126,137,132,153,98,83,17
,15,20,19,16,27,23,34,55),
g.bs=c(34,31,37,31,32,26,35,31,32,45,34,26,34,52,31,41,37,36,
37,46,60,36,43,27,21,30,29,23,29,15,27,24,32,36,39,34,46,39,47,40,55,36,39,32),
n.csb=78634000,
g.its=6926,
```

E.2 A simplified poultry processing flow diagram.



Appendix F

F1. The code of R programme used for ARIMA with intervention model used in the time-series Chapter (11).

```
Inc<-read.csv("Incidence .csv")
head(Inc)
I.ts<-ts(Inc$Inc.10E5, frequency=12, start=c(1997, 1))
plot(I.ts, ylab="Cases per 100,000",type="b")

x<-c(rep(0,122),rep(1,34))

par(mfrow=c(2,1))
acf(I.ts,lag.max=48); pacf(I.ts,lag.max=48)
acf(diff(I.ts,12),lag.max=48); pacf(diff(I.ts,12),lag.max=48)

I.tr<-ts(Inc$Inc.10E5[1:144], frequency=12, start=c(1997, 1))
x.tr<-x[1:144]

I.ar<-arima(I.ts, order=c(1,0,0),seasonal=c(0,1,1),xreg=x)
print(I.ar)
par(mfrow=c(2,1))
I.res<-residuals(I.ar)
acf(I.res,lag.max=48); pacf(I.res,lag.max=48)
Box.test(I.res,12)

I.f<-predict(I.ar,n.ahead=12,newxreg=rep(1,12))
as.numeric(I.f$pred)

I.fit<-I.tr-I.res
cat(I.fit, sep=" ")

I.ar<-arima(I.tr, order=c(1,0,0),seasonal=c(0,1,1),xreg=x.tr)
print(I.ar)
par(mfrow=c(2,1))
I.res<-residuals(I.ar)
```

```
acf(I.res,lag.max=48); pacf(I.res,lag.max=48)
BP<-c(Box.test(I.res,1)$p.val,Box.test(I.res,12)$p.val,Box.test(I.res,24)$p.val)
names(BP)<-c("1","12","24")
BP

I.f<-predict(I.ar,n.ahead=12,newxreg=rep(1,12))
as.numeric(I.f$pred)

#Multiplicative - log transform
lnI.tr<-ts(log(Inc$Inc.10E5[1:144]), frequency=12, start=c(1997, 1))
I.ar<-arima(lnI.tr, order=c(1,0,0),seasonal=c(0,1,1),xreg=x.tr)
print(I.ar)
par(mfrow=c(2,1))
I.res<-residuals(I.ar)
acf(I.res,lag.max=48); pacf(I.res,lag.max=48)
Box.test(I.res,12)

I.fit<-lnI.tr-I.res
cat(exp(I.fit), sep=" ")

I.f<-predict(I.ar,n.ahead=12,newxreg=rep(1,12))
as.numeric(exp(I.f$pred))

#Full data
lnI.ts<-ts(log(Inc$Inc.10E5), frequency=12, start=c(1997, 1))
I.ar<-arima(lnI.ts, order=c(1,0,0),seasonal=c(0,1,1),xreg=x)
print(I.ar)
par(mfrow=c(2,1))
I.res<-residuals(I.ar)
acf(I.res,lag.max=48); pacf(I.res,lag.max=48)
Box.test(I.res,12)

I.f<-predict(I.ar,n.ahead=12,newxreg=rep(1,12))
as.numeric(exp(I.f$pred))
```

Appendix G

G1. Research Information Sheet, Ethics Approval and the questionnaire used for the survey study (Chapter 13)

Lincoln University

[Faculty of Agriculture and Life Sciences. Department of Wine, Food and Molecular Biosciences]

Research Information Sheet

Introduction and invitation
I would like to invite you to participate in a project about poultry preparation practice at home. This will be a part of my PhD research program at Lincoln University. My research has been reviewed and approved by the Lincoln University Human Ethics Committee.

What is the aim of the project?

The project aims to investigate the handling practices of consumers during chicken preparation at home. The study would assess which poultry safe handling information poultry consumers lack and hence should be included in food safety campaigns, consumer education programmes and health promotion strategy in the future.

What types of participants are being sought?

I am inviting all participants who are 18 and above and cook poultry products at home to take part in the project. The participation in this research is voluntary, and there is no obligation to take part.

What will I be asked to do?

Your participation will involve completing a questionnaire, which I estimate will take 5 to 10 minutes to complete. The survey will ask you various questions about the approach you take to preparing the poultry products and about your food safety basic knowledge. You are kindly requested to assist in providing sincere responses to the questions contained in this questionnaire. All questions need to be completed. You may decline to answer any question in the survey. However, any data collated via incomplete surveys will be excluded from the analysis. Submission of the completed survey is deemed consent.

What use will be made of my data?

The results of the project will be included in my PhD thesis and submitted for publication in academic journals and officially reported to food safety professionals and health promotion professionals. The survey is anonymous. Your identity would not be recorded, and data will remain private. No one will have access to this information, other than the Human Ethics Committee in the event of an audit. Individual survey data will be stored in an electronic form with secure password protection. Only aggregated data will be presented in any publications.

Can I withdraw from the project?
We will not be able to withdraw your answers once submitted as the survey is anonymous.

What if I have any questions?

- *Contact Information*
  Mr Ali Al-Sakkaf, Lincoln University
  Department of Wine, Food and Molecular Biosciences
  Faculty of Agriculture and Life Sciences

RFH Building, P O Box 85084
Lincoln 7647
Christchurch
Mobile: 0211030175
Email: *Ali.al-sakkaf@lincolnuni.ac.nz*

- *Supervisor Contact Information*

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Research Management Office

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New Zealand
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23 September 2019

Application No: 2019-53

Title: Survey of NZ consumer poultry handling practices at home to provide an insight into risk factors for campylobacteriosis in New Zealand.

Applicant: A Al-Sakkaf

The Lincoln University Human Ethics Committee has reviewed the above noted application. Thank you for your response to the questions which were forwarded to you on the Committee's behalf.

I am satisfied on the Committee's behalf that the issues of concern have been satisfactorily addressed. I am pleased to give final approval to your project.

Please note that this approval is valid for three years from today's date at which time you will need to reapply for renewal.

Once your field work has finished can you please advise the Human Ethics Secretary, Alison Hind, and confirm that you have complied with the terms of the ethical approval.

May I, on behalf of the Committee, wish you success in your research.

Yours sincerely

Grant Tavinor
Chair, Human Ethics Committee

PLEASE NOTE: The Human Ethics Committee has an audit process in place for applications. Please see 7.3 of the Human Ethics Committee Operating Procedures (ACHE) in the Lincoln University Policies and Procedures Manual for more information.

Please fill in this questionnaire if you are aged 18 or above and cook any raw poultry products at home.
For each of the following questions, please tick one response only.

Section A: Food purchasing habits

1) Which of the following is most important to you when deciding where to shop for food?

- ○ The diversity of food items for sale.
- ○ The cleanliness of the premises.
- ○ I know the origin of the food items.
- ○ Price.
- ○ Close to home.
- ○ None of the above.

2) During your last grocery shopping occasion when you purchased raw poultry, at what stage did you select raw poultry?

- ○ Soon after I entered the shop.
- ○ I followed the supermarket/grocery shop layout.
- ○ After I had chosen all other non-perishable items.
- ○ I have no routine for this.
- ○ I do not know.

3) During your last grocery shopping occasion when you purchased raw poultry, was the raw poultry bagged separately from other items?

- ○ No.
- ○ Yes, only at my freezer/chiller after I returned home.
- ○ Yes, in my carry-on bags.
- ○ Yes, only in the trolley.
- ○ Yes, in the trolley, carry-on bag and freezer/chiller.

4) Usually, how long do you leave the raw poultry at room temperature before storing it in a refrigerator or freezer at home (including the time you take to travel from the shop to your home)?

- ○ 0 minutes – 40 minutes.
- ○ 41 minutes – One hour.
- ○ More than an hour to one and a half hours.
- ○ More than one hour and a half hours.
- ○ I do not know.

<u>Section B: Preparation of raw poultry in the home</u>

5) When thawing frozen raw poultry for cooking, how do you usually do it?

- ○ Thaw it using the microwave.
- ○ Thaw it on the kitchen countertop.
- ○ Thaw it in/under running hot water in the sink.
- ○ Thaw it in the kitchen sink.
- ○ Thaw it in the bottom shelf of the refrigerator OR under running cold water in the sink.
- ○ 'Never' thaw raw poultry

6) For each raw poultry handling occasion, which of the following do you usually do immediately after handling the poultry?

- ○ I wipe my hands on a disposable towel.
- ○ I wipe my hands on a reusable towel.
- ○ I wash with soap and cold water.
- ○ I use hand sanitiser and water or soap with hot water OR change my gloves.
- ○ I do not clean my hands or change my gloves and continue to handle other utensils/items or foods in the kitchen.

7) For each handwashing occasion, how long do you spend washing your hands?

- ○ 10 seconds or less.
- ○ 11 – 20 seconds.
- ○ More than 20 seconds.

O It varies, depending on how rushed I am.

O I do not know.

8)	**If you wash your hands, how do you usually dry your hands after handwashing in the kitchen?**

O With the apron or on my clothes I am wearing.

O With an in-use' tea towel.

O With a disposable paper kitchen towel.

O With a towel used only for drying hands.

O I do not dry my hands.

9)	**There are many times that raw poultry may be handled or came into direct contact with utensils during preparation or grilling. Please indicate how raw poultry was handled/came into contact with utensils?**

O Handled using bare hands.

O In contact with a previously unused fork/ unused spoon/unused utensil or handled while wearing previously unworn/gloves or handled using clean hands.

O In contact with a previously used fork/ used spoon/used utensil.

O Handled while wearing gloves.

O I do not remember.

10)	**After cutting or preparing the raw poultry, what was the next thing you do with the cutting board/utensil and/or knife?**

O Continue to use the cutting board/utensil and knife for preparation of other food.

O Rinse the cutting board/utensil and knife with water before reuse or storage.

O Wash the cutting board/knife with soap and hot water before reuse or use a different cutting board and knife for preparing the other food.

O Wipe the cutting board/knife with a dishcloth before reuse or storage.

O Use a different cutting board/ utensil for other food but continue using the same knife.

11) Do you ever place cooked poultry on the same plate/surface where raw poultry meat has been?

- O Yes, sometimes, especially when I do not have much time.
- O Yes, especially when I barbecue poultry.
- O Yes, after wiping it with a dishcloth.
- O No, not at all OR only after washing the plate/surface with hot water and detergent.
- O Yes, after rinsing it with water.

12) How do you check to see if a poultry product you have cooked is safe to eat?

- O When it looks cooked.
- O After an appropriate cooking time.
- O From observing the texture and firmness (by inserting a knife to check uniform meat tenderness).
- O I never check to see if the poultry has been cooked adequately.
- O I observe when the exterior colour is golden brown, and the interior juices run clear OR measure the internal temperature of the poultry meat.

13) How often do you clean (using soap/water and cleaning products) your kitchen sink and benchtop?

- O After every meal.
- O Once a day.
- O Once a week.
- O When they look dirty (food debris visible).
- O I do not clean the kitchen benchtop.

14) Do you cover your food during storage?

- O Yes, only if I keep the food for several hours.
- O No, not at all.

○ Yes, all the time.

○ Yes, but only if I leave it overnight.

○ Yes, sometimes, especially when I store it in the fridge.

Section C: Food safety awareness

15)	**What temperature should refrigerators operate at for optimum safe food storage?**

○ I do not know.

○ Less than 3°C.

○ 8°C.

○ Between 4°C and 7°C.

○ More than 8°C.

16)	**How do you decide whether or not to consume food stored in the fridge?**

○ By the best before and the use-by date.

○ I practice – 'first in – first out' techniques for food storage based on my memory.

○ I do not know.

○ I think I know when food is spoiled by its appearance and or smell.

○ I do not pay attention to the expiry or best before dates.

○ I do not pay attention to the use-by dates.

17)	**When preparing a meal, do you think about the basic food safety rules such as clean, separate, cook, chill and cover?**

○ Yes, I am aware of all them, or most of them.

○ Yes, I have heard about them, but I do not understand them clearly.

- No, I have not heard about them.
- I have heard about a few of them clean, cover and chill.
- I have heard about two of these rules like clean and cover.

18)	**How likely are you to follow these rules clean, separate, cook, chill and cover?**

- Always.
- Almost always.
- Sometimes.
- Rarely.
- Never.

19)	**Does raw poultry carry bacteria (bugs) that can make you sick?**

- I do not know.
- Yes, I know that it carries bugs.
- Yes, I know, but I do not know the name of that bug.
- Yes, I know that it carries *Campylobacter* bug.
- No, I am not concerned about the bugs.

20)	**Cross-contamination can be defined as the transfer of harmful bugs to food from other foods, hands and other food contact surfaces. Please indicate your awareness and self-reported practice of this concept below:**

- I am not familiar with the concept of cross-contamination.
- I have heard about the concept of cross-contamination, but I do not understand it.
- I have heard about the concept of cross-contamination, but I do not always take the necessary steps to prevent it.
- I have heard about the concept cross-contamination, and I always take the necessary steps to prevent it.
- I am not concerned.

21) How important do you consider it to follow/implement all good hygiene practices all of the time to protect yourself and your family from foodborne illness?

- ○ I consider it as extremely important.
- ○ I consider it as important.
- ○ I consider it not important.
- ○ I consider it as just important.
- ○ I am not concerned.

Section D: Participant details

22) How many occasions in the past year have you or anyone in your family experienced food poisoning symptoms such as diarrhoea, nausea/vomiting, sudden onset of fever, chills/muscle aches, lack of energy, dry mouth and tongue?

- ○ 1-2 times.
- ○ 3-4 times.
- ○ 5-6 times.
- ○ 7-8 times.
- ○ No experience in the past 12 months.
- ○ No experience in the past 2 years.

23) Based on your practices and your behaviour, who or what has most influenced your food safety practices during food preparations?

- ○ Partner.
- ○ School/church/leisure centre/educational campaigns.
- ○ Doctor, counsellor or health clinic.
- ○ Parents/grandparents, other relatives or friends.
- ○ TV, newspaper, radio, media in general.
- ○ Colleagues at work.
- ○ My knowledge.

24) How often do you cook poultry at home?

- ○ Once to twice per month.
- ○ Once a week or in the weekend.

- ○ 2 to 3 times a week.
- ○ 4 to 5 times a week.
- ○ 6 to 7 times a week.

25) In general, how did you learn to cook? (tick all that apply)

- ○ By myself (from internet: videos, recipes, articles).
- ○ From cookery books/TV.
- ○ From partner/friends.
- ○ From other relatives (mum, grandparents).
- ○ From a training course (school, church, community centre, etc.).

26) What is your highest education level?

- ○ No formal schooling.
- ○ Kura Kaupapa/Primary school (including intermediate).
- ○ Secondary school (high school).
- ○ University student or completed university, Wānanga, polytechnic or another tertiary.
- ○ Postgraduate or higher qualification.

27) Which of these categories best describe your occupational status?

- ○ Employed – full time (+ 30 hours week).
- ○ Employed – part-time (15-30 hours week).
- ○ Retired.
- ○ Unemployed.
- ○ Housewife/husband – home duties.
- ○ Student.

28) Which one of the following categories best describes the total yearly income of everyone in your household from all sources before tax?

- ○ Lower than $20,000/yr.
- ○ $20,001 – 40,000/yr.
- ○ $40,001 – 60,000/yr.
- ○ $60,001 – 80,000/yr.
- ○ $80,001 – 100,000/yr.
- ○ $100,001 or more.

29) Which of the following categories describe your ethnic identity? Tick all that apply

- O NZ Maori.
- O NZ European.
- O Other European, including Australians.
- O Pasifika.
- O Asian.
- O Latin American.
- O North American.
- O Middle Eastern.
- O African.
- O Other, please specify:____________

30) What is your gender and which statement best applies?

- O Female without children at home.
- O Female with children at home.
- O Male with children at home.
- O Male without children at home.
- O Prefer not to answer.

31) What age category do you fall under?

- O Under 20 years.
- O 20 – 29 years.
- O 30 – 39 years.
- O 40 – 49 years.
- O 50 – 59 years
- O 60 years or older.

Thank you for completing this questionnaire.

G2. Examples of SPSS analysis results' outputs for the consumer survey(Chapter 13).

Descriptive Statistics (13.4)

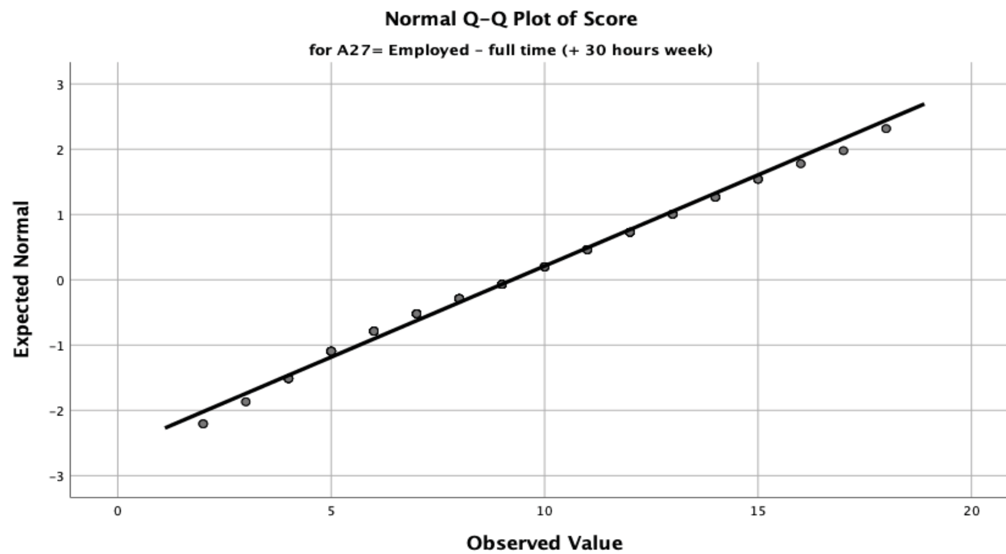
	N	Mean		Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Std. Error	Statistic	Statistic	Std. Error	Statistic	Std. Error
The total score of all sections	301	9.8306	0.20161	3.49779	0.256	0.14	-0.134	0.28
Valid N (listwise)	301							

Tests of Normality (13.3.7)

	Which of these categories best describe your occupational status?	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Score	Employed – full time (+ 30 hours week)	.086	145	.011	.982	145	.059
	Employed – part-time (15-30 hours week)	.156	49	.004	.947	49	.028
	Retired	.147	51	.008	.964	51	.126
	Unemployed	.212	10	.200*	.942	10	.581
	Housewife/husband – home duties	.109	14	.200*	.965	14	.804
	Student	.109	32	.200*	.972	32	.559

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction



Association between respondents' socio-demographic characteristics and mean score

(13.4.1)

Score * How many occasions in the past year have you or anyone in your family experienced food poisoning symptoms such as diarrhoea, nausea/vomiting, sudden onset of fever, chills/muscle aches, lack of energy, dry mouth and tongue?

Score

How many occasions in the past year have you or anyone in your family experienced food poisoning symptoms such as diarrhoea, nausea/vomiting, sudden onset of fever, chills/muscle aches, lack of energy, dry mouth and tongue?	Mean	Std. Error of Mean	Std. Deviation	N
1-2 times	9.5217	.41887	3.47939	69
3-4 times	8.5000	.84163	3.36650	16
5-6 times	7.3333	.66667	1.15470	3
No experience in the past 12 months	10.2529	.39280	3.66383	87
No experience in the past two years	9.9365	.30337	3.40528	126

Total	9.8306	.20161	3.49779	301

Score * Based on your practices and your behaviour, who or what has most influenced your personal hygiene habits?

Score

Based on your practices and your behaviour, who or what has most influenced your personal hygiene habits?	Mean	Std. Error of the Mean	Std. Deviation	N
Partner	8.0714	.69566	3.68107	28
School/church/leisure centre/educational campaigns	9.5455	.82422	2.73363	11
Doctor, counsellor or health clinic	11.5000	1.50000	2.12132	2
Parents/grandparents, other relatives or friends	8.7910	.39188	3.20765	67
TV, newspaper, radio, media in general	8.8438	.51217	2.89727	32
Colleagues at work	11.4000	.60000	1.34164	5
My knowledge	10.7436	.28323	3.53758	156
Total	9.8306	.20161	3.49779	301

Score * How often do you cook poultry at home?

Score

How often do you cook poultry at home?	Mean	Std. Error of Mean	Std. Deviation	N
Six to seven times a week	9.0000	.	.	1
Four to five times a week	10.0000	1.25576	4.52769	13
Twice to three times a week	10.0804	.33752	3.57201	112
Once a week or in the weekend	9.7455	.35404	3.71320	110

Once to twice times per month	9.5231	.34328	2.76760	65
Total	9.8306	.20161	3.49779	301

Score * What is your highest education level?

Score

What is your highest education level?	Mean	Std. Error of Mean	Std. Deviation	N
No formal schooling	6.0000	.00000	.00000	2
Kura Kaupapa/Primary school (including intermediate)	9.0000	1.00000	1.41421	2
Secondary school (high school)	9.5472	.52414	3.81579	53
University student or completed university, Wānanga, polytechnic or other tertiary	10.3154	.31139	3.55038	130
Postgraduate or higher qualification	9.4912	.30603	3.26755	114
Total	9.8306	.20161	3.49779	301

Score * Which of these categories best describe your occupational status?

Score

Which of these categories best describe your occupational status?	Mean	Std. Error of Mean	Std. Deviation	N
Employed – full time (+ 30 hours week)	9.7793	.30583	3.68267	145
Employed – part-time (15-30 hours week)	10.6122	.43430	3.04012	49
Retired	10.5686	.50971	3.64008	51
Unemployed	7.8000	1.06249	3.35989	10

Housewife/husband – home duties	9.7143	.84794	3.17269	14
Student	8.3750	.46174	2.61201	32
Total	9.8306	.20161	3.49779	301

Score * Which one of the following categories best describes the total yearly income of everyone in your household from all sources before tax?

Score

Which one of the following categories best describes the total yearly income of everyone in your household from all sources before tax?	Mean	Std. Error of Mean	Std. Deviation	N
Lower than $20,000/yr.	8.1724	.60999	3.28491	29
$20,001 – 40,000/yr.	9.9250	.53993	3.41481	40
$40,001 – 60,000/yr.	9.9091	.47869	3.55003	55
$60,001 – 80,000/yr.	9.3143	.62681	3.70827	35
$80,001 – 100,000/yr.	9.7750	.58888	3.72440	40
$100,001 or more	10.4216	.32660	3.29847	102
Total	9.8306	.20161	3.49779	301

Score * What is your gender and which statement best applies?

Score

What is your gender and which statement best applies?	Mean	Std. Error of Mean	Std. Deviation	N
Male without children at home	8.7547	.45709	3.32766	53
Female without children at home	10.1524	.35207	3.60763	105
Prefer not to answer	12.6667	1.20185	2.08167	3
Male with children at home	8.6087	.72977	3.49986	23

Female with children at home	10.1966	.31145	3.36882	117
Total	9.8306	.20161	3.49779	301

Score * What age category do you fall under?

Score

What age category do you fall under?	Mean	Std. Error of Mean	Std. Deviation	N
Under 20 years	7.3333	.88192	1.52753	3
20 – 29 years	8.6905	.44368	2.87536	42
30 – 39 years	9.5075	.43291	3.54355	67
40 – 49 years	10.2000	.45024	3.48751	60
50 – 59 years	10.2000	.45866	3.40152	55
60 or older	10.2973	.43950	3.78074	74
Total	9.8306	.20161	3.49779	301

Descriptive Statistics (13.4.2-5)

	N	Mean		Std. Deviation		
	Statistic	Statistic	Std. Error	Statistic	Skewness	Kurtosis
Which of the following factors are most important to you when deciding where to shop for food?	301	.17	.022	.376	1.771	1.145
During your last grocery shopping occasion when you purchased raw poultry, at what stage did you select raw poultry?	301	.05	.013	.225	4.004	14.122
Was the poultry bagged separately from other items?	301	.25	.025	.433	1.166	-.646

Usually, how long do you leave the raw poultry at room temperature before storing it in a refrigerator or freezer at home (including the time you take to travel from the shop to your home)?	01	.88	.019	.325	-2.356	3.576
When thawing frozen raw poultry for cooking, how do you usually do it?	301	.34	.027	.475	.669	-1.563
For each raw poultry handling occasion, which of the following do you usually do immediately after handling the poultry?	301	.35	.028	.478	.622	-1.624
For each hand washing occasion, how long do you spend washing your hands?	301	.14	.020	.344	2.132	2.561
If you wash your hands, how do you usually dry your hands after hand washing in the kitchen?	301	.13	.019	.336	2.217	2.935
There are many times that raw poultry may be handled or came into direct contact with utensils during preparing or grilling. Please indicate how the raw poultry was handled/came into contact with utensils?	301	.46	.029	.499	.181	-1.980
After cutting or preparing the raw poultry wh, at was the next thing you did with the cutting board/utensil and knife?	301	.78	.024	.414	-1.364	-.141
Do you ever place cooked poultry on the same plate/surface where raw poultry meat has been?	301	.92	.015	.266	-3.205	8.327

How do you check to see if a poultry product you have cooked is safe to eat?	301	.47	.029	.500	.114	-2.000
How often do you clean (using soap or cleaning product) your kitchen sink and benchtop?	301	.55	.029	.498	-.222	-1.964
Do you cover your food during storage?	301	.72	.026	.449	-.990	-1.027
What temperature should refrigerators operate at for optimum safe food storage?	301	.52	.029	.500	-.087	-2.006
How do you decide whether or not to consume food stored in the fridge?	301	.48	.029	.500	.074	-2.008
When preparing a meal, do you think about the basic food safety rules such as clean, separate, cook, chill and cover?	301	.67	.027	.471	-.732	-1.474
How likely are you to follow these rules clean, separate, cook, chill, cover?	301	.25	.025	.431	1.186	-.596
Does raw poultry carry bacteria (bugs) that can make you sick?	301	.50	.029	.501	-.020	-2.013
CroCrosscontaminationn is defined as the transfer of harmful bugs to food from other foods, hands and other food contact surfaces. Please indicate your awareness and self-reported action of this concept below	301	.66	.027	.473	-.700	-1.520

How important do you consider it to follow/implement all good hygiene practices all of the time to protect yourself and your family from foodborne illness?	301	.52	.029	.500	-.087	-2.006
Score	301	9.8306	.20161	3.49779	.256	-.134
Valid N (listwise)	301					

Score (13.4.2-5)

		Frequency	Per cent	Valid Percent	Cumulative Percent
Valid	2.00	3	1.0	1.0	1.0
	3.00	5	1.7	1.7	2.7
	4.00	6	2.0	2.0	4.7
	5.00	19	6.3	6.3	11.0
	6.00	23	7.6	7.6	18.6
	7.00	24	8.0	8.0	26.6
	8.00	31	10.3	10.3	36.9
	9.00	31	10.3	10.3	47.2
	10.00	32	10.6	10.6	57.8
	11.00	29	9.6	9.6	67.4
	12.00	39	13.0	13.0	80.4
	13.00	18	6.0	6.0	86.4
	14.00	14	4.7	4.7	91.0
	15.00	6	2.0	2.0	93.0
	16.00	11	3.7	3.7	96.7
	17.00	2	.7	.7	97.3
	18.00	3	1.0	1.0	98.3
	19.00	5	1.7	1.7	100.0
	Total	301	100.0	100.0	

Descriptive Statistics

	Mean	Std. Deviation	N
The score Food Safety Awareness section	3.6113	1.69462	301
The score Temperature Control and Proper Storage section	2.4684	1.05349	301
The score of Hygiene section	.9900	.94687	301
The score of Cross-Contamination Prevention section	2.7608	1.13252	301

Correlations (13.4.6)

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations					
		The score Food Safety Awareness section	The core temperature Control and Proper Storage section	The score of Hygiene section	The score of Cross Contamination Prevention section
The score of Food Safety Awareness section	Pearson Correlation	1	.373**	.378**	.417**
	Sig. (2-tailed)		0.000	0.000	0.000
	N	301	301	301	301
The score of Temperature Control and Proper Storage section	Pearson Correlation	.373**	1	.329**	.321**
	Sig. (2-tailed)	0.000		0.000	0.000
	N	301	301	301	301
The score Hygiene section	Pearson Correlation	.378**	.329**	1	.237**
	Sig. (2-tailed)	0.000	0.000		0.000
	N	301	301	301	301
The score of Cross Contamination Prevention section	Pearson Correlation	.417**	.321**	.237**	1
	Sig. (2-tailed)	0.000	0.000	0.000	
	N	301	301	301	301

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