THE EPIDEMIOLOGY OF *CAMPYLOBACTER JEJUNI* IN COMMERCIAL BROILER FLOCKS IN NEW ZEALAND

A thesis submitted in partial fulfilment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

at Massey University, Palmerston North,
New Zealand

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2005

Abstract

When written in Chinese, the word 'crisis' is composed of two characters - one represents danger, and the other represents opportunity.

SAUL DAVID ALINSKY

New Zealand maintains the highest incidence rate of human campylobacteriosis of the industrialized countries (334.2 cases per 100,000 in 2002), it accounts for more than 56% of all disease notifications in the country. New Zealand is unique globally, with a 'notification-based surveillance system for notifiable diseases that is complemented by laboratory reporting. In other countries (Australia, US, UK), the notification system is entirely laboratory based. Thus, the high incidence of Campylobacteriosis in humans may be related to the methods of reporting rather than the reality of the disease situation. However, the reason for such high incidence has not yet been fully elucidated, and several studies conducted in New Zealand and overseas have implicated the consumption of poultry meat as the main cause of human infections.

The reduction or elimination of *Campylobacter jejuni* in the food chain, particularly from poultry meat products, is a major strategy in efforts to control campylobacteriosis. One approach to this is to prevent *C. jejuni* colonization of broiler chickens. This approach has been used to control *Salmonella* contamination of poultry, but the measures put in place for control of *Salmonella* have not controlled *C. jejuni*. It is generally unknown how frequently *C. jejuni* colonizes commercial broiler chickens in New Zealand, or what could be done to prevent these infections from occurring. The present study was undertaken in order to describe some of the basic epidemiology of *C. jejuni* in commercial broiler flocks in New Zealand.

The thesis is intended to further describe the epidemiology of colonisation of commercial broiler chickens by *C. jejuni* in NZ, and present possible risk factors that could be controlled in future to decrease the number of positive flocks of birds that are processed.

The thesis set out to elucidate first the extent of *C. jejuni* colonisation of birds, flocks and farms while the birds were on the farm, having had minimal risk of exposure to Campylobacter spp., by sampling 15 birds in 80 flocks belonging to two companies prior to the first partial depopulation, an event during which the flock are exposed to potentially contaminated fomites and biosecurity levels are dropped, doors opened and personnel movements are extensive. The resulting prevalence estimates are 25.6% of farms, and

12.5% of sheds, are likely to be used to rear broiler chickens colonised with *C. jejuni*. When a positive flock is discovered, 76.9% of the birds are likely to be colonised with *C. jejuni*. These figures are results across the whole study population of farms and sheds, as there were no significant differences between prevalence estimates between companies.

Following this prevalence estimation, a longitudinal study was conducted involving 12 sheds, to determine whether the environment or the birds were colonised with *C. jejuni* first. Although 12 sheds were observed every other day from day 14 to the end of the rearing period, it was determined that the birds were positive either first, or at the same time as the environment. Having said that, the sensitivity of the testing method for the environment was dubious, as there were instances where a shed that had positive samples collected on one occasion appeared negative the next, before returning a positive result on the third consecutive sampling occasion.

A cross-sectional study of 810 flocks was undertaken to determine the most relevant risk factors for colonisation of the broiler chickens with *C. jejuni*. Because of the vertically integrated structure of the poultry industry, these 810 flocks corresponded to data collected from 77 farmers about their farms and the 219 sheds on those farms. The caeca from ten birds from each flock processed were pooled and examined for the presence of *C. jejuni*. These results were used to create a case definition, such that the flocks could be analysed with the questionnaire data, and different risk factors were seen in each season. More flocks reared for Company One were colonised by *C. jejuni* than for Company Two. Protective factors included having hard (i.e. gravel, asphalt or concrete) pathways to the growout houses, being near to another broiler farm, using the reticulated town water supply for the birds drinking water, using tunnel or crossflow shaped growout houses, using a Chore-Time™ feed delivery system within the growout house and chlorinating the water supply to the birds (only in winter).The odds of raising flocks colonised with *C. jejuni* increased if rodents were seen on the farm, if the growout houses were constructed with a concrete nib wall, if gas heaters were used during brooding, if cattle were farmed on the property, or if workers were employed on the farm. Sanitising the annex at least as frequently as once per run decreased the odds during summer, and tended to have a similar effect in other seasons.

Chlorinating the water supply appeared to have a protective effect in only one season, though the trend appeared towards protection in the other seasons. The risk factor was validated by sampling the drinking water that broilers chickens had access to for the FAC to see whether the levels that were present in the drinking water could have an effect on *C. jejuni*. 11 sheds that were known to chlorinate the water were sampled to determine

whether they met the drinking water standards for humans in NZ, or met the requirements presented by one of the companies involved. Only three sheds met the human drinking water standards for FAC, and two of these (one from each company) met Company Two's requirements.

This thesis is for both regulatory and industry stakeholders to assist with developing risk management approaches to diminishing the number of *C. jejuni* positive flocks. Where management practices are altered, it is hoped that the efficacy of such practices be measured by examining the changes in the rates of *C. jejuni* colonization within the industry.

Acknowledgements

I would maintain that thanks are the highest form of thought, and that gratitude is happiness doubled by wonder.

GILBERT CHESTERTON

Firstly, I'd like to acknowledge the farmers who tend the growout houses utilised in this study, for their willing acceptance of the intrusion of study personnel and for also helping tremendously during the questionnaire process. Several staff in the processing plants who collected the viscera and caecal samples for long periods of time, without slowing production aided and abetted this project.

This work could not have been undertaken without the support of Tegel (NZ) Foods Ltd, and Inghams Enterprises (Pty) Ltd, notably the broiler management teams and the laboratory staff. The Poultry Industry Association of New Zealand (PIANZ) are acknowledged and commended for providing the funds necessary to research this area. This study would not have been possible without TechNZ funding of the author.

It is widely acknowledged within academia that the undertaking of a Ph.D. is to indicate slight madness. Either that, or a reluctance to step outside the cosseting warmth of the academic environment. This is not the reason I decided to attempt this project: no, the supervisors for my Masters of Veterinary Studies prompted me. So saying, I'd like to extend a wary thank you to Stan Fenwick (aka. The Mad Scotsman) and Dirk Pfeiffer. Both of these gentlemen were instrumental in my taking on this Ph.D., and both left the country soon after they had persuaded me! Appendix B (only available in the 'paperback version'!) is included especially for Stan.

Peter Davies subsequently stepped into the breach to supervise me, and with the help of Nigel Perkins, they forced me to the other side. There were times when I didn't do nearly enough work: and they had no hesitation in letting me know this. I think I'm appreciative! Cord Heuer has managed to maintain his esteemable cool, even when I was next to tears during the examination and amendment-making process. The numbers are all his fault...I tried to do without them. Roger Morris made sure that I finished it all, despite my (high) heels dragging, so this really wouldn't be here were it not for him.

So many people have influenced, nurtured and supported me during this exhausting and often all-encompassing task; to list them all would push me over my page limit (ha ha) but I

hope the following people, in particular, realise how important they are to me. Vivienne Boxall (my mother), the most elegant and strong-willed person I know: I hope I grow up to be like her one day! Thanks go also to the ultimate iron-fist-in-kid-glove combination Yvonne Hilder (my grandmother). And if it weren't for Ron Boxall (my father), I never would have pursued a career in science. Thanks Dad, I think. 'Job satisfaction' and 'employability' all sounded quite reasonable when I was 14!

Liorah Atkinson has called me "fffd" for years now; so thanks for the continued support doll. I'm looking forward to being across the road with the pink rinse and the 24 cats, fingers twitching the curtains in a semaphoric language only she will understand. David Woods has been marvellously good company, he is a friend and editor par excellence, with whom I hope I remain friends for many years. My flatmates and the extended 'family' thereof (Joseph Glancy, Chris Farnsworth, Emma Thompson, Emily Maire, Paul Martin - and Ana Puke too!) must be sick and tired of me celebrating 'thesis-related' milestones ("you have finished the book – CHAMPAGNE!") yet are (too?) often prepared with a sympathetic bottle of bubbles.

Joanna McKenzie, Carola Sauter-Louis, Renè Corner, Nina Kung, Dinusha Fernando, Kay O'Rourke and Lou Gallagher have each shared an office with me (I know...they do deserve medals!), and have thus mentored me in many different ways; tempering my enthusiasm with good sense and bolstering me in moments of crippling insecurity.

Mike O'Hara and Brenden Print, my two karate senseis: one has shed insight along with punches, while the other encouraged me to better myself. Kylie Walker has been an absolute rock, never wavering in her belief that I could do it. Leigh Burling (nee Vandy) and Janine Print have fed, watered and encouraged me on numerous occasions, and I'm very grateful for them bringing me back to earth. The altos (Michelle Burrell, Helena Muldoon and Nicci Tong) are my girls - and certainly all stars in my eyes. Brett Childs, David Bond and John Goddard have each ensured my self-esteem did not drop below the horizon - an alto hug (from one) in your direction. Midge and Ross, who understood how truly important it was to me, you guys are two in ten million (exponential).

Thanks, snuggles, treats and laser pointer games are much deserved by the black and white moggies Memnoch, Mefitis and Lestat (RIP my lovely) who have witnessed many a dawn curled up on my lap in the way of the keyboard. And a quick 'shout-out' to the other cats too.

Now if I could just get a little golden statuette....? <*queue music*>

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AFLP	Amplified fragment length polymorphism
AIDS	Acquired Immunodeficiency Syndrome
Breeder	From age 0 to 24 weeks, birds are considered pullets. From 24 weeks on they are full-fledged breeder hens.
Broiler	Young (normally six weeks old) male or female birds weighing (1.36kg - 1.59kgs)
CDT	Cytolethal distending toxin
CE	Competitive Exclusion
CFU	Colony forming units (i.e. viable cells)
Chick	Newly hatched broiler chickens
DNA	Deoxyribonucleic Acid
DNases	Enzymes that degrade DNA in a non-specific manner
Fla	Flagellin (gene or protein)
GBS	Guillain-Barre syndrome
GC	Guanine & Cytosine (i.e. two of the four components of DNA)
GP	General Practitioner
H_2S/TSI	Hydrogen sulphide production on triple sugar iron agar
HeLa	Cells of the first continuously cultured human carcinoma strain (from cancerous cervical tissue of *He(nrietta) La(cks)*)

HLA B7	Human histocompatibility (HLA) surface antigen encoded by the b locus on chromosome 7
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Kbps	Kilo (1000) base pairs
Layer	These specialized birds have been bred to be finely honed egg producing animals and are very different from the breeder lines. They produce the table eggs sold in stores.
mCCDA	Modified *Campylobacter* blood free selective agar
MEE	Multi-locus enzyme electrophoresis
MLST	Multi-locus sequence typing
MPN	Most Probable Number
NaCl	Sodium chloride
NCTC	National Collection of Type Cultures
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pH	p(otential of) H(ydrogen); the logarithm of the reciprocal of hydrogen-ion concentration in gram atoms per litre
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
Rpm	Revolutions per minute
TSA	Trypticase Soy Agar
UK	United Kingdom
UPGMA	Unweighted pair-group method

USA	United States of America
UV	Ultraviolet (light)
Vero	African Green Monkey (*Cercopithecus aethiops*) kidney cells