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Molecular Epidemiology of Campylobacteriosis and Evolution of *Campylobacter jejuni* ST-474 in New Zealand

A thesis presented
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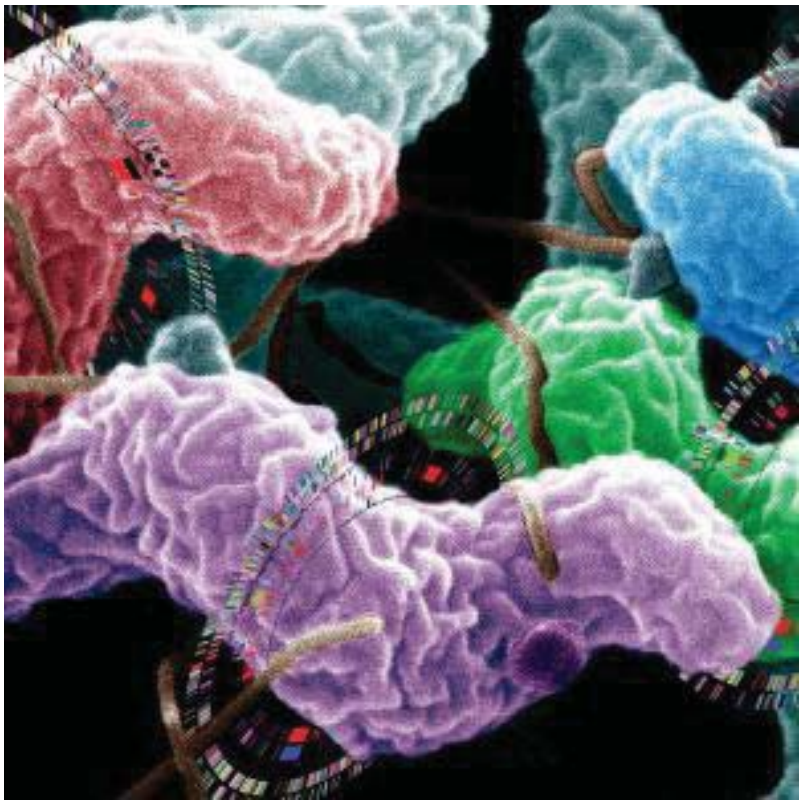

— Abstract —

Population genetics and phylogenetics have the potential to provide enormous insights into the epidemiology and ecology of disease causing pathogens. Molecular datasets are the basis to infer population structure, gene flow (between host populations and between different geographical locations) and to predict the evolutionary dynamics of pathogens. *Campylobacter* colonisation in food producing animals has been extensively studied and the population structure and host association of *C. jejuni*, the most commonly reported gastro-enteric pathogen, has also been well defined. In contrast, host-pathogen relationships and the population structure of *C. jejuni* in urban wild birds and pets have not been well defined on a wide range of spatial and/or temporal scales. A greater understanding of these details should allow disease control authorities to track the transmission of pathogens from one host species to another, identify the origin of pathogens and to better understand environmental factors influencing underlying molecular mechanisms.

In the first study in this thesis the presence of *C. jejuni* in mallard ducks and starlings within five playgrounds in Palmerston North, New Zealand was studied. The prevalence of *Campylobacter* and *C. jejuni* in both species showed a bimodal seasonal pattern. The population structure and population differentiation of *C. jejuni* in these species were examined using multilocus sequence typing (MLST). Rarefaction analyses showed that the *C. jejuni* populations within mallard ducks were more diverse than starlings, particularly during the winter. Pairwise fixation indices showed that the population of *C. jejuni* in ducks was significantly different from that of starlings and that it differed over time. Conspicuous host association was evident with clonal complexes of *C. jejuni* such as ST-1034, ST-692 and ST-1332 specific to ducks and ST-177 and ST-682 specific to starlings. In addition, a larger proportion of *C. jejuni* genotypes that could not be assigned a clonal complex were found in both ducks and starlings, particularly during the winter.

In the second study, *C. jejuni* from domestic pets (dogs and cats) were characterised using MLST and by typing the cell surface antigens, *por*A and *fla*A. The ST-45 complex, a clonal complex predominantly reported in human campylobacteriosis cases, was found to be the predominant clone present in both species. These findings shed some light on the contribution of pets as a putative source of human campylobacteriosis cases in New Zealand.

In the third study, the ST-474 *C. jejuni* genotype, considered to be the endemic strain in New Zealand, was isolated from human cases and poultry carcasses from the Manawatu region from 2005 to 2009. Seven samples of ST-474 were sequenced and a subset of 50 full length genes were studied. These analyses demonstrated molecular differences between full length genes that were identical in the region used for MLST. Further, alleles characteristic of the ST-474 genome within the investigated metabolic housekeeping genes (n = 25) were identified. Our findings were that ST-474 genome is genetically distinct from other *C. jejuni* reference genomes with respect to certain alleles. In addition, MLST alleles were found to be robust predictors of the most recent common ancestors of a genome. The fourth study investigated the genetic stability and vulnerability of the informational genes to various evolutionary forces within the seven ST-474 genomes. Twenty five genes comprised of nucleotide metabolism, repair and ribosomal functions were investigated showing a high level of genetic diversity in the DNA repair as well as nucleotide metabolic genes such as *gid*A, *ogt*, *rec*J, *ssb*, *uvr*A, *uvr*B and *xse*A. In contrast, the ribosomal genes were stable and identical across the seven genomes. The insertion of selenocysteine in three of the 25 genes indicates the presence of horizontal gene transfer within the ST-474 genomes. It is hypothesised that the genetic uniqueness of ST-474 may have arisen due to the geographic isolation of New Zealand, its poultry industry and an absence of exchange of sequence types which might typically occur through international trade of fresh poultry meat.

Collectively, the studies presented in this thesis provide a better understanding of the dynamism of *C. jejuni* as a species and ST-474's adaptational capacity and evolutionary potential (within the investigated set of genes) in response to changing intracellular and extracellular environments. This thesis has introduced the idea of using individual full length gene analysis, demonstrating the molecular differences between genes that contained identical alleles at the MLST loci. The research approaches implemented in this thesis can be readily applied to any pathogenic bacteria, particularly foodborne and emerging pathogens such as *E. coli* and *Salmonella*. This, in turn should provide new opportunities for bacterial drug targets and vaccine candidates.

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Nomenclature

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AT	Adenine – thymine
CBI	Codon usage bias index
CI	Confidence interval
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	De-oxyribonucleic acid
dN	Non-synonymous nucleotide substitution
dS	Synonymous nucleotide substitution
ESR	Environmental Science and Research Ltd
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
GBS	Guillain-Barré syndrome
GC	Guanine – cytosine
GC3	Guanine – cytosine at the third codon position
HGT	Horizontal gene transfer
HL	Heat-labile (antigen)
HS	Heat-stable (antigen)
HK	Housekeeping
Ka	Non-synonymous nucleotide substitution

K*s*	Synonymous nucleotide substitution
mCCDA	Modified cefoperazone charcoal desoxycholate agar
MLEE	Multilocus enzyme electrophoresis
MLSA	Multilocus sequence analysis
MLST	Multilocus sequence typing
MOMP	Major outer membrane protein
MST	Minimum spanning trees
NZ	New Zealand
NCBI	National Center for Biotechnology Information, USA
NZFSA	New Zealand Food Safety Authority
ORFs	Open reading frames
PCR	Polymerase chain reaction
*por*A	porin gene A
PFGE	Pulsed field gel electrophoresis
RAPD	Randomly amplified polymorphic DNA
rRNA	Ribosomal ribonucleic acid
RE	Restriction enzyme
REA	Restriction endonuclease analysis
RFLP	Restriction fragment polymorphism
spp.	Species (multiple)
ST	Sequence type
ST-U/A	Sequence type unassigned
SVR	Short variable region
TD	Tajima D
tRNA	Transfer ribonucleotide

WHO	World Health Organisation

List of Publications

Patrick J Biggs, Paul Fearnhead, Grant Hotter, **Vathsala Mohan**, Julie Collins-Emerson, Errol Kwan, Tom E Besser and Nigel P French., Whole-genome camparison of *Campylobacter jejuni* isolates indistinguishable on the basis of MLST and *fla*A SVR reveals multiple loci of different ancestral lineage. PLoS ONE, 2011. 6:e27121

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tal matrices and humans. Final report: 07-10436. Prepared for the New Zealand Food Safety Authority and Ministry for the Environment, 2010.

Vathsala, M., French, N., Stevenson, M., Marshall, J., and Hotter, G. *Campylobacter jejuni* colonisation and population structure in sympatric urban population of ducks and starlings. In advanced preparation for submission to *Environmental Microbiology*.

Vathsala, M., French, N., Stevenson, M., Marshall, J., and Hotter, G. Characterisation of *Campylobacter jejuni* from pets in New Zealand by combining multilocus sequence typing (MLST), *por*A and *fla*A typing. In advanced preparation for submission to *BMC Infectious Diseases*

Vathsala, M., French, N., Biggs, J. P., Stevenson, M., Marshall, J., and Hotter, G. Molecular phylogeny of seven *Campylobacter jejuni* ST-474 genomes based on an extended subset of housekeeping genes and *por*A/MOMP structural gene. In advanced preparation for submission to *BMC Microbiology*.

Vathsala, M., French, N., Biggs, J. P., Stevenson, M., Marshall, J., and Hotter, G. A genome wide comparison of selected subsets of ribosomal and DNA repair genes among seven *Campylobacter jejuni* ST-474 isolates. In advanced preparation for submission to *Journal of Evolutionary Biology*.

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‘The goal of mankind is knowledge ... knowledge is inherent in man. No knowledge comes from outside: it is all inside. What man ‘learns’ is really what he discovers by taking the cover off his own soul, which is a mine of infinite knowledge.’

Swami Vivekananda

A wise man is superior to any insults which can be put upon him, and the best reply to unseemly behavior is patience and moderation.

Moliere

Patience is the companion of wisdom.

Saint Augustine