THE MICROBIAL ECOLOGY OF *CAMPYLOBACTER JEJUNI* IN NEW ZEALAND WITHIN A SPATIAL-TEMPORAL FRAMEWORK

A thesis presented in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy
in
Veterinary Science
at Massey University, Manawatu,
New Zealand

Barbara Mary Binney

2015

The journey is the reward.

Chinese proverb

Look deep into nature, and then you will understand everything better.

Albert Einstein

Abstract

Campylobacter jejuni (*C. jejuni*) is an important cause of gastroenteritis internationally; it is a complex bacterium carried by multiple hosts, showing phenotypic and genotypic variation. This thesis systematically examines the molecular ecology and evolution of *C. jejuni* in New Zealand from the levels of population movement, phenotype, genome and metabolism.

First, the demographic history of cattle, sheep and poultry importations into New Zealand (1860-1979) was quantified. Australia was the most common reported source of cattle sheep and poultry, with large numbers of cattle and sheep being imported in the 1860s, and large numbers of poultry imported from the 1960s onwards. This suggests the population structure of cattle and sheep and the microbial organisms they carried may exhibit a founder effect.

The second level investigated the phenotypes of related sequence types (ST) with generalist and specialist lifestyles and compared them at 42℃ and 22℃ on the basis of carbon source utilisation in Biolog phenotypic microarrays. The isolates utilised a total of 29 carbon sources in a pattern that clustered them together on the basis of ST at 42℃ more than lifestyle and host. At 22℃ they utilised a limited palette of carbon sources (9) related to the tricarboxylic acid cycle (TCA).

The third level, used genomic comparisons to identify a putative new species *C.* sp. nov. 4 spp. in the Australian purple swamphen (*Porphyrio porphyrio melanotus*). Overall, the pattern of relationship between isolates associated with the pukeko (*Porphyrio porphyrio melanotus*), takahe (*Porphyrio hochstetteri*) and the Australian swamphen isolates suggested a recent common ancestor and then divergence after separation. Despite high levels of recombination in *C.jejuni*, the genomes grouped by clonal complex and ST, this suggests there are factors restricting regular recombination between more distant *C. jejuni* STs. The draft genomes for the wild-bird and agricultural-related isolates clustered by lineages in a host(s).

The fourth level involved the comparison of *C. jejuni* metabolic pathways (subsystems) to identify host association. Type VI secretion system, Coenzyme A biosynthesis and *Campylobacter* spp. iron metabolism were identified as important pathways in distinguishing between wild-bird and livestock associated isolates.

We stand on the shoulders of giants.

---

used by Sir Isaac Newton

Acknowledgements

My Supervisors: Professor Nigel French, Dr Patrick Biggs, Associate Professor Barbara Holland, Dr Phil Carter, and Dr Jonathan Marshall.

The mEpiLab team: Angie Reynolds, Dr Anne Midwinter, Dr Julie Collins-Emmerson, Anthony Pita, Neville Hack, Sarah Moore, Rukhshana Nahid Akhter and Lynn Rogers.

Helpers from across the Tasman: Dr Mary Valcanis of the Microbiological Diagnostic Unit (MDU) at Melbourne University and Dr Carl Mulnickel of the ASAP Lab. The volunteers who collected samples: Will Steele, Katrina Lee, Christine Hurring, Sandra Martig, Dorothy Dhaeze,Michael Tarburton, Tiffany Miller-Scott, Peter Dann and James O'Connor of Birdlife Australia.

Massey University Support Staff: Jacque Mackenzie, Wendy Maharey, Christine Cunningham and Debbie Hill, and Noelene White.

Software and IT help: Simon Verschaffelt, Xavier Didelot (ClonalFrame), Marco Galardini (Ductape), Bruno Contreras Moreira (Get Homologues), Markus Goker (opm), Korbinian Strimmer (binda), Vladimir Minin (rbrothers), Michael Woodhams (ADD), Sajia Akhter (Phispy) and Maulik Shukla (PATRIC).

Friends and family: I wish to thank the postgrads of the Hopkirk for their help and comradeship especially Anja Friedrich, Hamid Ishad, Fang Fang and Patricia Jaros. The TTP team including Lesley Stringer, Debbie Leader, Jackie Benshop, Chris Jewell, Becky Frost and many more. Edward George Binney for his warmth and companionship that enriched my life.

Funding: This research was made possible by funding from Massey University, the Marsden fund, and IVABs funding.

I have met and been inspired by many people in my life, which has helped me to and through my journey, too many to list here, so I apologise to those I have not directly mentioned.

Contents

List of Figures

List of Tables

Abbreviations

AFLP: Amplified fragment length polymorphism

AMOVA: Analysis of molecular variance

AU: Australia

blood agar: Columbia horse blood agar

bp: base pair

BIGSdb:Bacterial Isolate Genome Sequence Database.

BRENDA: Bacterial restriction endonuclease DNA analysis

contigs: contiguous reads of DNA sequence

CPS: capsular polysaccharides

CDC: Centers for Disease Control, USA

cfu: colony forming unit

CC: Clonal complex

CRISPR: Clustered regularly interspaced short palindromic repeats

DNA: Deoxyribonucleic acid

D.O.C: New Zealand Department of Conservation

ELISA: Enzyme-linked immunosorbent assay

ESCMID: European Society of Clinical Microbiology and Infectious Diseases

ESGEM: Study Group on Epidemiological Markers

ERIC: Enterobacterial repetitive intergenic consensus

ESR: Environmental Science and Research Ltd.

EU: European Union

FAME: Fatty acid methyl ester analysis

FAFLP: fluorescent AFLP

G+C%: guanine–cytosine content as a percent of nitrogenous bases in a DNA molecule

gDNA: genomic Deoxyribonucleic acid.

HGT: Horizontal gene transfer

HL: Heat labile antigens

hr: hour

HS: Heat stable antigens

IF0a: Omnilog inoculating fluid IF-0a GN/GP

kbp: Kilobase pairs (of DNA)

LOS: lipooligosaccharide mCCDA: Modified Cefoperazone Charcoal Desoxycholate agar

MLEE: Multilocus enzyme electrophoresis

MLST: Multilocus sequence typing

mrp: Macrorestriction profiling

MYA: million years ago

NCTC: National Collection of Type Cultures (UK)

nt: Nucleotides

NGS: Next generation sequencing

NZFSA: New Zealand Food Safety Authority

OIE: World Organisation for Animal Health

Omnilog bags: Biolog Gas Bag (P/N 3032)

PBS: Phosphate Buffer solution

PCR: Polymerase Chain Reaction

peg: protein encoding gene, plural pegs

PFGE: Pulsed Field Gel Electrophoresis

PM plates: Biolog Phenotypic Microarray plates.

PM1: Biolog Phenotypic Microarray plate 1.

PM2A: Biolog Phenotypic Microarray plates 2A.

16S rRNA: 16S ribosomal

RAPD: Randomly amplified polymorphic DNA

RAPD-PCR: Random amplified polymorphic DNA Polymerase Chain Reaction

rDNA: Ribosomal DNA

RE: Restriction enzyme

REA: Restriction endonuclease analysis

RFLP: Restriction fragment length polymorphism

rMLST: ribosomal Multilocus Sequence Typing

RNA: Ribonucleic acid

RNAT: RNA thermometers

rps: ribosomal protein subunit

SD: Standard deviation

SNP: single nucleotide polymorphism

ST: Sequence type, plural STs

sp: Species (single)

spp.: Species (multiple)

subsp.: subspecies

SVR: short variable repeats

VBNC: Viable but Nonculturable State

wgMLST: whole genome MLST

WGS: Whole genome sequencing

WHO: World Health Organisation

WTO: World Trade Organisation

This is dedicated to my parents, Ronald Samuel Binney and Noela Alma Binney (nee Jones), who did not live to see this part of my journey, I love you and I am grateful to have been your daughter. It is because of the greatness within you both that I could live this adventure.