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Campylobacter species in dogs and cats and significance to public health in New Zealand

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

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Abstract

Campylobacter spp. are a major cause of bacterial gastroenteritis in people in the developed world, including New Zealand. Many sources and transmission routes exist, as these bacteria are common in animals and the environment. *C. jejuni* is most frequently associated with poultry whereas *C. upsaliensis* and *C. helveticus* with dogs and cats, respectively. Published data on *Campylobacter* in dogs and cats in New Zealand and on the pathogenic potential of *C. upsaliensis* and *C. helveticus* are very limited. This thesis investigated the prevalence of *Campylobacter* spp. in household dogs and cats in Manawatu region, New Zealand, and in raw meat pet food commercially available in Palmerston North, New Zealand. Five *Campylobacter* spp. were isolated and the prevalence rates were significantly influenced by the culture methods used. *C. upsaliensis* and *C. helveticus* were most frequently detected from dogs and cats, respectively and *C. jejuni* in pet food samples. An expanded panel of culture methods was used to screen working farm dogs and their home-kill raw meat diet in Manawatu. This study reported three *Campylobacter* spp. and *Helicobacter winghamensis* as being isolated from dogs for the first time. The culture methods were again shown to bias the prevalence estimates. The isolates of *C. upsaliensis* and *C. helveticus* from the household pets study and *C. hyointestinalis* from locally farmed deer were used in a study to investigate the analytical sensitivity in spiked human clinical faecal samples using the ProSpecT^{TM} *Campylobacter* Microplate Assay test that was developed for detection of *C. jejuni/coli*. The results showed the ability of the test to detect all three species and showed the influence of bacterial dose, faecal consistency and of the individual faecal samples on the test results. Further studies investigated the pathogenic potential of *C. upsaliensis* and *C. helveticus* in comparison to *C. jejuni* using an insect model of disease, *Galleria mellonella*, and whole-genome analyses, respectively. The results of the survival analysis in the *G. mellonella* study indicated that *C. upsaliensis* and *C. helveticus* have pathogenic potential, but to a lesser extent than *C. jejuni.* Additionally, several variables of experimental design were shown to significantly influence estimates of hazard rates in survival analysis. Whole genome analyses also showed indications of the pathogenic potential of *C. upsaliensis* and *C. helveticus* relative to *C. jejuni,* and how it varies between and within species in association with the core and accessory genomes, functional gene content profiles, and documented and predicted pathogenic proteins. This thesis has furthered our understanding of the epidemiology, detection, and pathogenicity of *Campylobacter* spp. in dogs, cats and humans, and confirmed raw meat animal food as a potential source of *Campylobacter* spp. for both people and animals.

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This work is dedicated to dogs and cats.

Publications

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- **Journal of Microbiological Methods (2016 Vol. 127, p236-241)** 'Analytical sensitivity of the ProSpecT® Campylobacter Microplate Assay for detection of *C. upsaliensis*, *C. helveticus* and *C. hyointestinalis* in spiked human clinical faecal samples' K Bojanić, AC Midwinter, JC Marshall, LE Rogers, PJ Biggs, E Acke
- **CHRO conference 2015** (Rotorua, New Zealand) Research Abstract 'Pathogenicity of *Campylobacter jejuni*, *C. upsaliensis* and *C. helveticus* in the invertebrate disease model *Galleria mellonella*' K Bojanić, AC Midwinter, PJ Biggs, JC Marshall, E Acke
- **CHRO conference 2015** (Rotorua, New Zealand) Poster Abstract 'Diagnosis of campylobacteriosis in humans from the Manawatu, New Zealand' K Bojanić, A Nohra, LE Rogers, J Delores-Main, JC Marshall, PJ Biggs, AC Midwinter, E Acke
- **ECVIM 2015** (Lisbon, Portugal) Poster abstract 'Pathogenicity investigation of *Campylobacter jejuni*, *C. upsaliensis* and *C. helveticus* isolated from dogs and cats using *Galleria mellonella* larvae' K Bojanić, AC Midwinter, PJ Biggs, J Marshall, E Acke

- **ECVIM 2014** (Mainz, Germany) Poster abstract 'Whole-genome analyses of *Campylobacter upsaliensis* and *C. helveticus* isolated from dogs and cats and *in silico* investigation of pathogenic potential' K Bojanić, AC Midwinter, PJ Biggs, NP French, E Acke
- **NZVA conference 2014** (Hamilton, NZ) Invited Research lecture 'Canine diarrhoea – *Campylobacter* conundrum' K Bojanic, AC Midwinter, PJ Biggs, E Acke
- **CHRO conference 2013** (Aberdeen, UK) Research abstract 'Comparison of six culture protocols for isolation of *Campylobacter* spp. from faecal and meat samples' K Bojanic, AC Midwinter, L Rogers, PJ Biggs, E Acke
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- **ECVIM 2012** (Maastricht, The Netherlands) Research Abstract ‘ProSpecT Elisa in the diagnosis of *Campylobacter* spp. infections’ K Bojanic, AC Midwinter, L Rogers, PJ Biggs, E Acke

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List of abbreviations

General

CC	Clonal Complex
COG	Cluster of Orthologous Groups
CoxPH	Cox proportional hazard
Ctrl	Control larvae
DALY	Disability-adjusted life year
DNA	Deoxyribonucleic acid
EIA	antigen test / ProSpecT® Campylobacter Microplate Assay
Fig.	Figure
HL	High bacterial inoculum load
ID	Identity number
KM	Kaplan-Meier
LL	Low bacterial inoculum load
LOD	Limit of detection
ML	Medium bacterial inoculum load
MLST	Multilocus sequence typing
NAAT	Nucleic acid-based test
PBS	Phosphate buffered saline
PBS-ctrl	Phosphate buffered saline-inoculated larvae control
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
rMLST	Ribosomal multilocus sequence typing
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SNP	Single nucleotide polymorphism
spp.	Species
SSF	Semi-solid faeces
ST	Sequence type
VBNC	Viable but non culturable
WF	Watery faeces

COG-specific functional groups

A	RNA processing and modification
B	Chromatin structure and dynamics
C	Energy production and conversion
D	Cell cycle control, cell division, chromosome partitioning
E	Amino acid transport and metabolism
F	Nucleotide transport and metabolism
G	Carbohydrate transport and metabolism
H	Coenzyme transport and metabolism
I	Lipid transport and metabolism
J	Translation, ribosomal structure and biogenesis
K	Transcription
L	Replication, recombination and repair
M	Cell wall/membrane/envelope biogenesis
N	Cell motility
O	Posttranslational modification, protein turnover, chaperones
P	Inorganic ion transport and metabolism
Q	Secondary metabolites biosynthesis, transport and catabolism
R	General function prediction only
S	Function unknown
T	Signal transduction mechanisms
U	Intracellular trafficking, secretion, and vesicular transport
V	Defense mechanisms
W	Extracellular structures
X	Mobilome: prophages, transposons
Y	Nuclear structure
Z	Cytoskeleton

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