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

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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™ *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


- **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


- **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 \textit{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

• **NZVA conference 2013** (Palmerston North, New Zealand) Research abstract ‘*Salmonella*, *E. coli* and *Campylobacter* spp. in Working Farm Dogs in New Zealand and their Home-Kill Diets’ K Bojanic, AC Midwinter, PJ Biggs, J Benschop, N Cave, E Acke

• **WSAVA conference 2013** (Auckland, New Zealand) **Winner of WSAVA Global One Health Congress Recognition Prize** Research Abstract ‘*Campylobacter* spp. in dogs and cats in New Zealand’ K Bojanic, AC Midwinter, E Kwan, PJ Biggs, E Acke

• **International Sheep and Beef Veterinary Congress 2013** (Rotorua, New Zealand) Research presentation ‘*Salmonella*, *E. coli* and *Campylobacter* spp. in Working Farm Dogs in New Zealand and their Home-Kill Diets’ K Bojanic, AC Midwinter, PJ Biggs, J Benschop, N Cave, E Acke
• **Infectious Disease Research Centre Science Symposium 2012**
  (Palmerston North, New Zealand) Research Poster ‘ProSpecT *Campylobacter*
  Microplate Assay: first results but second thoughts?’ K Bojanic, AC Midwinter,
  J Marshall, L Rogers, PJ Biggs, E Acke

• **ECVIM 2012** (Maastricht, The Netherlands) Research Abstract ‘ProSpecT
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  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
VBN C  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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