SPARROWS, FLIES, AND RODENTS AS RESERVOIRS OF *CAMPYLOBACTER* SPP. ON A DAIRY FARM

A thesis presented in partial fulfilment of the requirements for the degree of Master of Veterinary Science in Veterinary Public Health and Meat Hygiene

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ERRATA

Page v, line 13:	This study investigated (rather than investigates)
Page v, line 20:	a sample size of 52 was taken
Page V, line 23:	collected (rather than calculated)
Page xi, 1.1 & 3.2:	*Campylobacter* in italics
Page 16, line 18:	Table 2 should be Table 1.2
Page 17, Table 1.2:	*C. jejuni* subsp *doylei* should be hippurate V *C. nitrofigillis* should be catalase +, nitrate + and hippurate - *C. upsaliensis* should be catalase W
Page 28, line 7 & 8:	detection (rather than isolation)
Page 31, line 5:	contaminated (rather than contamination)
Page 34, line 16:	calves (rather than cattle)
Page 39, line 9:	remove since from the sentence beginning "Since sheep and goats..."
Page 49, line 4:	40-fold (rather than 40 times)
Page 56, line 7:	remove "(Fig 2.8)"
Page 61, line 5:	A 30μg nalidixic acid (NA30) and a 30μg cephalothin (C30) antibiotic disc
Page 67, last line:	remove "and Table 3.2"
Page 73, line 7:	*Campylobacter jejuni* isolates were then classified into patterns A to V on the basis of a one or more band difference.
Page 80, line 1:	shows (rather than showing)
Page 80, line 3:	Letters (rather than Alphabetes...)
Page 81, line 1:	*C. jejuni* in italics
Page 82, Figure 3.7:	proportion (rather than percentage)
Page 85, line 1:	there was a presence of *Campylobacter jejuni* in some milking cows at Massey No. 4 dairy farm over a 24 month period.
Page 88, line 13:	restriction pattern X and XIV (Table 3.6 and Fig. 3.8)
Page 89 and 90	revision of conclusions as below:

Campylobacter jejuni has been isolated from most animal species worldwide. Despite its importance as a human and animal pathogen, relatively little is understood of the mechanisms of *C. jejuni*-associated disease in animals and humans.

This study suggested that dairy cows, rodents, sparrows and flies could be potential reservoirs of *Campylobacter* on a dairy farm. The PFGE analysis of *C. jejuni* isolates from the dairy farm showed a high degree of diversity of the organisms within a limited geographical area. Isolates with common restriction patterns (identical clones) infecting cattle, sparrows, flies and rodents suggested a common source of infection.

The high prevalence of asymptomatic carriage of *C. jejuni* found in cows could be sufficient to maintain infections within the dairy farm ecology via environmental contamination. The number of campylobacters shed by cattle defaecating 25 kg of fresh faeces per animal per day (Matsuzaki, 1975) would exceed that shed by sparrows or rodents, and as such cattle would be expected to constitute a more significant source of environmental contamination. To determine the most likely and significant routes of transmission, further studies of the epidemiology of *Campylobacter* in the farm ecology are needed.

This thesis is dedicated to my beloved parents

Shree Chiranjibi Adhikari

&

Tulasa D. Adhikari

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My chain of gratitude would be incomplete if I were to forget to acknowledge the first cause of this chain, the Lord Shree Pashupatinath.

ABSTRACT

The reported numbers of human *Campylobacter jejuni* infections have increased considerably in many countries during the last few years. In New Zealand, the current annual incidence rate (302.5 cases/100 000) of human campylobacteriosis is higher than that of any other notifiable disease, and surpasses the incidence of campylobacteriosis reported by other developed countries. Although *Campylobacter jejuni* has been isolated from poultry at high prevalence rates worldwide, poultry are probably not the only important source of human campylobacteriosis as it is well documented that many other animal species (sheep, pigs, cattle and free-living birds and mammals) can be carriers of zoonotic campylobacters. The high incidence of the disease in people could be related to the consumption of poorly cooked meat, drinking contaminated water, overseas travel and animal contact.

This study investigates the potential role of free-living animals (sparrows, rodents and flies) as potential reservoirs of *Campylobacter* spp. and was carried out at Massey University No. 4 dairy farm. We isolated *Campylobacter* from the faeces of cattle and from other samples, and used pulsed-field gel electrophoresis (PFGE) typing of the organisms to determine the similarity between isolates. This study also includes a comparision of the prevalence and genetic diversity of *Campylobacter* isolated from sparrow populations on the farm and from an urban environment.

Based on the results of a previous study on the same farm, sample size of 52 were taken for the dairy cows in order to obtain results at the 90% confidence level within 10% accuracy. Faecal samples from 53 farm sparrows, 65 rodents and 56 flies were calculated and examined for the presence of thermophilic *Campylobacter* spp. Faecal samples were also collected from 53 urban sparrows from "The Square" in the central urban area of Palmerston North city about 7 km from the dairy farm. A convenient number of samples of five of grass silage and two from each of water, worker's boots and aprons were collected with the aim to determine the presence of campylobacters in these samples.

All samples were collected between the 5th April 2002 and 25th May 2002. Random samples of rectal contents from 52 Friesian dairy cows were collected during milking time. Rodents were trapped in the feed storage premises approximately 15m from the milking shed using standard spring loaded, baited traps. Flies were captured around the milking shed using standard fly-traps. Bird samples were collected from an 8×10 feet tarpaulin placed on the ground under a tree where sparrows were roosting about 50m from the milking shed. Feed was provided to attract the birds. The same method was used to collect sparrow droppings in the urban area about 7 km from the farm.

Campylobacter jejuni was the only *Campylobacter* species isolated from the 290 samples collected at the dairy farm and from sparrows in the urban area. The highest isolation rate was found in dairy cows (54%), followed by urban sparrows (40%), farm sparrows (38%), rodents (11%) and flies (9%). Other samples from the farm environment such as grass silage, water, worker's apron and boots were also found to be positive for *C. jejuni.* Most of the rodents caught during the study period were mice. The high isolation rate in this study of *Campylobacter* from dairy cows (54%) and sparrows (40%) supports the notation that these species are important reservoirs of infection. Overall the results of the present and previous study show that at least some dairy cows from the same farm can be asymptomatic carriers (intermittent or persistant) of *Campylobacter jejuni* for at least 24 months.

Molecular charecterisation of genomic DNA from 61 *C. jejuni* isolates from farm and urban sources obtained during the study was performed by PFGE after digestion with the enzyme *Sma* I. Of the 22 restriction patterns obtained seven were common to more than one source. The PFGE typing yielded seven, six, nine, six and three restriction patterns from dairy cows, farm sparrows, urban sparrows, rodents and flies respectively. PFGE analysis of the *C. jejuni* isolates shows a high degree of diversity of the organisms within a limited geographical area. But the finding of some common restriction patterns provides evidence of identical clones infecting cattle, sparrows, flies and rodents.

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LIST OF ABBREVIATIONS

BHI	Brain heart infusion
BRENDA	Bacterial restriction endonuclease DNA analysis
BS	Butzler selective
BU	Butzler
Campy-BAP	Campy brucella agar
CBFS	*Campylobacter* blood-free selective
CCD	Charcoal-cefazolin-sodium deoxycholate
CS	Charcoal-based selective
CVA	Campylobacter-cefoperazone-vancomycin-amphotericin
EDTA	Ethylenediamine tetra-acetic acid
GB	Guillain-Barré
Kb	Kilobase
Mb	Megabase
MBU	Modified Butzler
mCCD	modified charcoal-cefoperazone-deoxycholate
mCCDA	modified charcoal-cefoperazone-deoxycholate agar
MF	Miller-Fisher
MPN	Most probable number
MQ	Milli – Q
NARTC	Nalidixic-acid-resistant thermophilic *Campylobacter*
OD	Optical density
PR	Preston
REA	Restriction endonuclease analysis
rpm	Revolutions per minute
SK	Skirrow
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
WHO	World Health Organisation