ANIMAL SOURCES OF HUMAN CAMPYLOBACTERIOSIS

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

Doctor of Philosophy

in

Veterinary Public Health

At Massey University, Palmerston North,

New Zealand.

FASIUDDIN AHMED

1999

This thesis is dedicated to the memory
Of
My Beloved Father

ABSTRACT

New Zealand has one of the highest reported rates of *Campylobacter* infections in humans in the developed world. It is the single largest notifiable disease in all regions of the country. Consumption of poultry meat has been widely implicated both overseas and in New Zealand as the main cause of human infections. The potential contribution of other animals especially cattle and sheep is less well known. The present study was undertaken to fill this gap in knowledge.

Faecal samples from 300 cattle and 158 sheep were collected from local abattoirs and farms plus 50 samples from the sheep slaughterhouse environment and examined for the presence of thermophilic *Campylobacter* spp. *Campylobacter* spp. were isolated from 45% of the cattle, 44% of the sheep and 56% of the environmental samples. *C. jejuni* and *C. hyointestinalis* were the predominating species isolated from cattle followed by *C. coli* and *C. lari*. In sheep and in environmental samples from the sheep abattoir *C. jejuni* was the only species isolated. The isolation rate and the species of *Campylobacter* varied between beef and dairy cattle, bull and heifer calves, age of the heifer calves, and time of the year. The high isolation rate of *Campylobacter* from the cattle, sheep and their environment strongly suggests the possibility of these micro-organisms finding their way into milk and meat, as faecal contaminants at the farm and slaughter level. There is also the potential to contaminate the environment and water following disposal of abattoir effluents and run off from farms.

The species of the isolates from human diarrhoeal cases were found to be predominantly *C. jejuni* (95%) and *C. coli* (5%). Molecular typing of *C. jejuni* using *Sma* I generated pulsed-field gel electrophoresis (PFGE) profiles yielded 13 to 16 different patterns in the cattle, sheep and human isolates showing a large inter-species variation in the isolates even from the same sources. However, indistinguishable as well as closely related profiles (pulsotypes) were found across the isolates from cattle, sheep and humans. The results obtained from the PFGE typing strongly indicate that cattle and sheep may be important reservoirs of human campylobacter infections. It was also observed that a few closely related types mostly dominate the *C. jejuni* populations in

the host animal species. The possibility of faecal contamination from these animals at slaughter and thus *C. jejuni* entering the meat was studied.

Retail packs of beef (25), lamb (25) and chicken (50) mince purchased from local supermarkets were examined. A combined selective enrichment and PCR based method was evaluated to offer a rapid, sensitive and specific detection method for the identification of *C. jejuni* from meats. *C. jejuni* was detected by culture and PCR in 44% of the chicken, 16% of the lamb and 12% of the beef mince samples. These results lend credibility to our contention that faecal contamination of sheep and beef carcasses at slaughter has significant implications for food safety. The much higher rate of detection in chicken mince may be related to a higher prevalence of infection in chickens or to the method of processing which may facilitate spread between birds and / or between product.

The *C. jejuni* isolates from the animal and human sources were also examined for antibiotic resistance by the disc diffusion method to antibiotics commonly used for the treatment of campylobacter infections in humans. No resistance was detected in the cattle and sheep isolates. Two human isolates exhibited resistance to tetracycline with MICs of >128 μg/ml. All other human isolates were found susceptible to the antibiotics tested. The nil to negligible resistance detected in the animal and human isolates of *C. jejuni* suggest that it is not a major problem in New Zealand at the present time however, further work is required to examine the situation in more intensively farmed species and monitor any changes in human isolates over time.

Acknowledgements

I consider it a privilege to record my deepest sense of gratitude to Professor Colin Wilks for his generous support, constant encouragement and constructive counsel all along the course of investigation and preparation of this manuscript. I would also like to thank him for his genuine concern for my welfare.

I am indeed thankful to my co-supervisor Stan Fenwick for his help and guidance throughout my studies as well as to my other supervisors Per Madie and Alan Murray who have been so generous with their time.

I have been associated with several people during this long period and every one has contributed something towards this investigation. I would like to thank Jane Hunter, Eammon Gormley, Kevin Stafford, Jacek Gwozdz and Hassan Hussein for their help.

Thanks are also due to the team of microbiologists at the Palmerston North Medical laboratory who have provided the human isolates of Campylobacter and to Ayad Alkaissi at the Meat works in collection of samples from animals.

Assistance in the laboratory provided by Magda Gwozdz, Kylie Walker, Jan Schrama and Laurie Sandall is highly appreciated. Special mention should be made of Peter Wildbore who had been most helpful in procuring the most important to the trivial with equal zeal.

I would like to specially thank Allain Scott for her readiness to help and advise in matters of academic complexities and also to the other secretarial staff at the institute for their help.

A big thanks to all my friends in room 2.01 and in the university for their enjoyable company and support.

A special thanks to my wife Kavita for her constant encouragement and support in times of great stress and in giving two lovely children Shireen and Rehan during this period who have been the source of infinite joy.

The completion of this thesis is also due to the support, encouragement, and sound advice I received from my family especially my beloved parents to all of them I am deeply indebted.

And last but not the least, I remember the ***Almighty*** *who gave me strength, courage and perseverance to achieve this goal.*

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PFGE	Pulsed-field gel electrophoresis
PCR	Polymerase chain reaction
NASBA	Nucleic acid sequence based amplification
CCDA	Charcoal-cefoperazone-desoxycholate agar
CCVA	Campylobacter-cefoperazone-vancomycin amphotericin
CSM	Charcoal selective media
HS	Heat stable
HL	Heat labile
FBP	Ferrous metabisulphite pyruvate medium
OMP	Outer membrane protein
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
DNA	Deoxyribonucleic acid
MEE	Multilocus enzyme electrophoresis
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
HACCP	Hazard analysis critical control point
RFLP	Restriction fragment length polymorphism
ERIC	Enterobacterial repetitive intergenic consensus
RAPD	Random amplified polymorphic DNA
REP	Repetitive extragenic palindrome
GBS	Guillain-Barre syndrome
ELGA	Enzyme linked gel assay
CBF	Campylobacter blood free agar
TSI	Triple sugar iron agar
EDTA	Ethylene diamine tetra acetic acid
BSA	Bovine serum albumin
CHEF	Contour clamped homogenous electric field
TBE	Tris borate EDTA
MIC	Minimal inhibitory concentration
DIG	Digoxigenin
bp	Base pair