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***YERSINIA ENTEROCOLITICA* INFECTIONS
IN PEOPLE AND OTHER ANIMALS**

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A NEW ZEALAND STUDY

STANLEY GORDON FENWICK

**A thesis presented in partial fulfilment of the requirements for the
degree of Doctor of Philosophy in Veterinary Microbiology**

Massey University

July 1997

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ABSTRACT

During the past three decades, *Yersinia enterocolitica* has risen to worldwide prominence from an obscure and taxonomically undefined organism to a common zoonotic pathogen, capable of causing a wide range of clinical syndromes in both animals and people. Prior to this study, however, there was little evidence of the importance of the organism in New Zealand and the overall aim of the thesis was to investigate its role as a human pathogen in this country and the involvement of animals in the epidemiology of yersiniosis.

Initially, a survey of human infections was initiated with the cooperation of medical laboratories throughout the country, and this eventually continued for eight years. In total, epidemiological data pertaining to 2737 cases was obtained for analysis, including age and sex of patients, details of clinical symptoms, duration of infection, seasonality and the distribution of bioserotypes. Results of the survey showed that yersiniosis was a common human enteric pathogen, with peaks of infection in children under five and young adults. The predominant clinical symptoms were diarrhoea and abdominal pain and the course of infection was usually 1 to 2 weeks. The principal bioserotype combination throughout the study was 4/O:3, however, annual differences were recognised in the incidence of infection with two other bioserotypes, 2/O:5,27 and 2/O:9. No obvious seasonality was detected.

Two other surveys were later initiated, one in which the tonsils of slaughter pigs were examined for the presence of human pathogenic strains of *Y. enterocolitica*, and the other to investigate the faecal carriage of the same strains in a range of domestic animals. As in other countries, pigs were found to be infected with *Y. enterocolitica*, with approximately 24% of pigs harbouring strains of the organisms potentially pathogenic for people, including bioserotypes 4/O:3 and 2/O:5,27. Dogs were the only other animal from which 4/O:3 strains were isolated, however, a wide range of domestic animals were found to carry bioserotypes 2/O:5,27 and 2/O:9.

As human infections with *Y. enterocolitica* have been linked to contact with dogs, a study was designed to examine the carriage and transmission of bioserotype 4/O:3 in a group of 14 young dogs. The animals were separated into 5 groups, 2 containing 4 dogs (Groups I and II) and the others 2 dogs each (Groups III-V). Each of the 4 dogs in Group I, and 2 of the dogs in Group II were challenged orally with the test organism. Regular bacteriological examination of faecal samples from these animals showed that dogs can be readily infected and can excrete the organism for up to 23 days. The 2 in-contact dogs in Group II started to shed the test organism after 5 days. Subsequent transfer of these dogs to Group III and those in Group III to Group IV showed that *Y. enterocolitica* bioserotype 4/O:3 can be transmitted between dogs. At no time did any of the dogs show clinical signs of infection. These findings suggest that dogs can carry *Y. enterocolitica* 4/O:3 asymptotically and hence might act as a potential source for human infection.

Standard laboratory procedures for the isolation and identification of *Y. enterocolitica* are both time-consuming and insensitive and the development of a rapid molecular method for identification of the organism was attempted. Initially, a non-radioactive DNA probe based on a cloned fragment of the *Yersinia* virulence plasmid was assessed for its ability to distinguish pathogenic from non-pathogenic strains of the organism. Results using the probe were equivocal, and the polymerase chain reaction (PCR) was adopted as the rapid method of choice. Using the published sequence data from a *Y. enterocolitica* invasion gene (*ail*), present only in pathogenic strains of the organism, primers were designed for use in the PCR. With both extracted DNA and simple broth cultures as the template, the PCR proved to be highly specific and sensitive for pathogenic *Y. enterocolitica*.

The PCR was subsequently adapted for use directly with clinical samples, including tissues and faeces from experimentally infected pigs and dogs. Following initial inhibition of the PCR, two methods were designed that overcame the reduced sensitivity, a nested PCR assay applied to pig tissues and a pre-PCR enrichment step used with faecal samples. The sensitivity of the PCR was comparable to culture, and in some cases was enhanced.

Finally, pulsed field gel electrophoresis was used to examine a total of 602 strains of *Y. enterocolitica* recovered from animals and people during the study, to identify likely reservoirs of infection and to assess the heterogeneity of the organism in New Zealand. Bioserotypes 4/O:3, 2/O:5,27 and 2/O:9 were subdivided into 18, 20 and 40 pulsotypes respectively, with 4/O:3 and 2/O:9 being comparatively homogeneous (approximately 80% of isolates corresponding to one major pulsotype in each) and 2/O:5,27 having a high degree of heterogeneity (approximately 70% of isolates clustered into 6 pulsotypes). The principal pulsotypes in each bioserotype were recovered from a wide range of animal species and from most regions in the country.

ACKNOWLEDGEMENTS

“ I get by with a little help from my friends”

John Lennon and Paul McCartney

Thanks are due to a great many people both inside and outside the Faculty of Veterinary Science for their support, encouragement, assistance and friendship. In particular, the staff of the Department of Veterinary Pathology and Public Health, whose forbearance in the face of all my foibles has been much appreciated. Special thanks go to my longsuffering fellow microbiologists, Jane Hunter and Joanne Meers, for giving me the time and space to create this thesis. I am very grateful to my supervisors Alan Murray and Colin Wilks, whose support, ideas, constructive criticism and scientific acumen I have valued during the project. Others who have assisted me with advice, encouragement and helpful hints over the years include Robert Hickson, Per Madie, Roger Marshall, Joseph O’Keefe, Diana Martin, Roger Morris, Matthew Perrott, Fraser Allan and Mark Collett and I thank them all sincerely.

Mention must be made of the many funding agencies who have supported my work on yersiniosis, without their support much of this research would not have been performed. They include the Palmerston North Medical Research Foundation, Massey University Research Fund, Veterinary Research Fund, Lotteries Health and the Health Research Council.

The surveys that I carried out into *Yersinia* infections in animals and people would not have been possible without the cooperation of medical and veterinary laboratory staff the length and breadth of the country, and I owe them all a debt of gratitude. A special vote of thanks must go to my friend and colleague Mike McCarthy of Diagnostic Laboratory, Auckland, who was there at the beginning and the end and whose continued enthusiasm helped keep the project alive.

Help and support have not been limited to New Zealand, and I am grateful for the advice and assistance I have received from yersiniologists in many parts of the world at various times during my studies. A number of people in this small family have since become my friends and I would especially like to thank Georg Kapperud, Jens Kirk Andersen, Kaisa Granfors and Georges Wauters for all they have done for me.

Many are the pitfalls that await a “young” scientist and I am indebted to Hilary Shaw, Kylie Walker, Magda Gwozdz and James Dickson for their invaluable injection of technical expertise at appropriate moments. Their willingness to assist and their friendly faces have often helped to dispel the gloom that occasionally descends on postgraduate students. I am also grateful to Jan Schrama and Peter Wildbore for their work behind the scenes.

But all work and no play makes Stan a dull boy, and without the ability to laugh, moan and generally unwind with my “coffee and conference” friends I would indeed have ended up a quivering wreck. A big thankyou, therefore, to Magda, Jacek, young Jane, older Jane, Richard, Suzanne, Laurie, Kylie, Colin (remember the pissed gorilla) and last, but by no means least, to my partner-in-crime, Eamonn. Thanks for the memories!

I would like to pay tribute to my parents-in-law, Danny and Grace, who have been my spiritual mentors and who have always believed in me, thanks for everything.

I have been blessed in my life to have been loved, supported, encouraged and generally pampered by two wonderful parents. I cannot possibly repay them for all they have done for me and only hope that I have made them proud. Perhaps the most effective way that I can show my eternal gratitude to them is by carrying on the tradition and ensuring that my own children have all my support and love whenever they need it.

Finally, my family. Words cannot truly express the debt I owe my wife, Anne, and my children, Jennifer, Aidan and Kirsten. They have lived every day of my PhD with me, sharing my highs and lows, putting up with my moods and always giving me shoulders to lean on. Anne’s eternal optimism has constantly encouraged me and her faith in me has helped me believe in myself. Without their love I would not have finished and I hope that I can now relax, put my feet up and rejoin the human race! Asante sana, shukran gazeelan, na penda wewe kabisa x 17!

This thesis is dedicated to the memory of my dear sister-in-law, Grace.

**“Although you are no longer with us,
your light shines on forever.”**

The road goes ever on and on
Down from the door where it began.
Now far ahead the road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

Bilbo Baggins, *The Lord of the Rings*.

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and thinking what nobody has thought”

Albert Szent-Gyorgyi

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**“Research! A mere excuse for idleness, it has never achieved,
and will never achieve, any results of the slightest value”**

Benjamin Jowett

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