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YERSINIA ENTEROCOLITICA INFECTIONS
IN PEOPLE AND OTHER ANIMALS
A NEW ZEALAND STUDY

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

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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.
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"I get by with a little help from my friends"
John Lennon and Paul McCartney

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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.

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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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Benjamin Jowett
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