Genetic Characterisation and Transmission Cycles of *Cryptosporidium* Species Isolated in New Zealand

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Abstract

Sixty-nine years separated the first observation of *Cryptosporidium* by Tyzzer in 1907 from the realisation in 1976 that this enteric protozoan parasite was pathogenic. It is the third major cause of diarrhoeal disease worldwide causing a self-limiting infection in immuno-competent humans and young vertebrates. As yet there is no antimicrobial agent that combats *Cryptosporidium* so the organism poses a life threatening risk to the immuno-compromised e.g., AIDS patients, patients on immuno-suppressive drugs, chemotherapy or congenital immune deficiencies. By 2000 AD 152 species of mammals had been reported as being infected with *Cryptosporidium* plus 57 reptilian species and many birds and fish.

The advent of AIDS stimulated research into *Cryptosporidium* resulting in the large amount of information now becoming available, however little is known about the genetic characteristics, distribution and transmission cycles of *Cryptosporidium* species that cause human disease in New Zealand. To address these questions 1613 animal faecal samples and 423 human faecal specimens containing *Cryptosporidium* oocysts were collected from throughout New Zealand and examined by the polymerase chain reaction - restriction fragment length polymorphism technique (PCR-RFLP). Indeterminant results were resolved by DNA sequence analysis of the small subunit ribosomal DNA (rDNA).

Only 2.8% of the animal faecal specimens contained oocysts with the vast majority of these being *C. parvum* bovine genotype from calves.

Two regions supplied the majority of human isolates, one rural and one urban. Overall *C. hominis* accounted for 47% of all human isolates with the remaining 53% being *C. parvum* bovine genotype. A difference however, was observed between the *Cryptosporidium* species from rural and urban isolates with *C. hominis* dominant in the urban region while *C. parvum* bovine genotype was prevalent in rural New Zealand. A shift in transmission cycles was detected between seasons with an anthroponotic cycle in autumn and a zoonotic cycle in spring. A novel *Cryptosporidium*, that on DNA sequence analysis showed a close relationship with *C. canis,* was detected in two unrelated children from different regions, illustrating the genetic diversity within this genus.

Acknowledgements

As with any research of this nature and performed over a lengthy period there are many people to acknowledge, most of them inadequately. Those mentioned below played a major part in the completion of this project and hopefully persons not included in these acknowledgements will forgive me for the oversight.

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I well remember going to my first lecture in more years then I am willing to admit to, with Liz Keys. It was a DNA Technology lecture given by the inspiration educator Dr. Stowell, who started the lecture with "we will not have to go over this point as we covered it last year" – it was to become a recurring theme. I really wondered at times at what I was doing there, as must have the ever patient Dr. Stowell.

Collection of the human faecal specimens was carried out by a great many medical laboratory scientists from throughout New Zealand but special thanks go to Jan Bird, who at the time worked for MedLab Hamilton and Vicky McKnight from Medical Laboratory Wellington. Karen Cooper of Gribbles Veterinary Pathology diligently collected animal faecal specimens over many months, allowing me access to many potential *Cryptosporidium* hosts that I could not have otherwise examined. To collect positive faecal specimens and send them to the PRU takes a considerable effort when working in a busy diagnostic laboratory and I thank them all. Without Dr. Padraig Duignan I could not have searched through the faecal specimens from his collection of

marine mammals. Positive horse samples came courtesy of Alex Grinberg also from IVABS. Hopefully all the faecal suppliers will have learnt a little more of the nature of *Cryptosporidium* from this work.

The perpetually busy Ina Te Wiata, who coincidentally was working with *Cryptosporidium* in the mid 1970s when the pathogenicity of the organism was being debated, helpfully proofread the thesis. Her many helpful comments were incorporated into this document.

Finally I must thank my family for their support and words of encouragement; Heather who in the early stages of this project provided me with food, drink, time and space in which to work, Richard for his good humour, Andrew for his understanding (having just handed in his own Master's thesis) and Peter who will finally get the computer back so that he may "clock" whichever game is currently all the rage amongst his age group.

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