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# **Epidemiological Studies of Cryptosporidiosis**

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

**Doctor of Philosophy**

in Veterinary Pathology

**Massey University, Palmerston North, New Zealand**

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## ABSTRACT

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An interpretive overview of the literature on intestinal cryptosporidiosis in humans and domestic mammals (Chapter 1) is followed by two studies of the population genetic structure of the protozoan parasites *Cryptosporidium parvum* and *Cryptosporidium hominis* (Chapter 2), five epidemiological studies of cryptosporidiosis in foals, calves and humans in New Zealand (Chapter 3), and an investigation of a serendipitous outbreak of cryptosporidiosis among a class of veterinary students, which occurred at the end of 2006 (Chapter 4).

The analysis of the population genetic structure of *C. parvum* and *C. hominis* indicates the existence of a significant genetic segregation of geographically separated parasite populations, consistent with allopatry. The results do not conform to a simplistic model that considers all *C. parvum* as multi-host anthrozoönotic agents, and provide statistical support to the idea of the occurrence of anthroponotic cycles that do not involve cattle. Rather than conforming to a rigid paradigm of either a clonal or a panmictic species, data are consistent with the co-occurrence of clonal and recombinatorial diversification in *C. hominis*, and perhaps *C. parvum*.

The results of the epidemiological studies in New Zealand suggest cryptosporidiosis caused by *C. parvum* is relatively common in young foals and calves. In the time and space frames underlying these studies, humans, calves, and foals were infected with a genetically homogeneous *C. parvum* population. This feature is in accordance with previous reports that have indicated *C. parvum* as the dominant species in humans during the peaks of incidence of cryptosporidiosis in winter and spring, and support the view that the peaks are in large part attributable to direct and/or indirect zoonotic transmission of *C. parvum*.

Finally, the outbreak of cryptosporidiosis among a class of veterinary students highlighted the potential hazard for explosive large-scale outbreaks in New Zealand. The results of the investigation were consistent with point-source exposure and zoonotic transmission of a rare *C. parvum* subtype through direct contact with calves during a practicum.

## PREFACE

With the advent of HIV-AIDS and the re-emergence of the importance of infectious diseases at the end of the 20<sup>th</sup> century, several microorganisms not previously known to cause disease, including members of the genus *Cryptosporidium*, were recognised as novel aetiological agents in humans and animals. In the inaugural issue of the journal *Emerging Infectious Diseases* in 1995, Dr. David Satcher, Director of the US Centers for Disease Control and Prevention, included *Cryptosporidium* in a group of microorganisms which in his opinion were the “major etiologic agents” identified since 1973.

*Cryptosporidium* is a genus of protozoan parasites first described in animals in 1907. Despite the early description of these parasites, cryptosporidiosis, that is, the disease caused by members of the genus, was initially described in 1971 in a heifer and then in 1976 in a 3-year old girl from a farm, both in the US. The most common clinical presentation of cryptosporidiosis in developed countries is of self-limiting diarrhoeal illness. However, in HIV-positive or otherwise immunocompromised patients, cryptosporidiosis may manifest as a chronic debilitating or fulminant disease. Conversely, in many developing regions of the world the infections with *Cryptosporidium* are associated with persistent childhood diarrhoea, high mortality, malnourishment, and stunted growth.

Cryptosporidiosis is a notifiable disease in New Zealand. According to a recent Public Health Surveillance Report, the rate of notifications of cryptosporidiosis in the trimester October-December 2008 was of >2 cases per 10,000 population, similar to the rate of salmonellosis and giardiasis over the same period ([http://www.surv.esr.cri.nz/PDF\\_surveillance/](http://www.surv.esr.cri.nz/PDF_surveillance/), accessed April 2009). In addition to the endemism of cryptosporidiosis, *Cryptosporidium* parasites pose a significant hazard due to the resistance of the oocysts to chlorination and the potential to cause massive water-borne disease outbreaks, such as the epidemic in Milwaukee, Wisconsin in 1993, when an estimate of 400,000 people acquired an infection through the ingestion of contaminated municipal water supply. Thus, ensuring preparedness against outbreaks of cryptosporidiosis is critical.

Many *Cryptosporidium* taxa can infect both humans and animals, and infections in humans are often acquired zoonotically, through direct or indirect contact with the faeces of infected animals. Thus, understanding the biology and epidemiology of cryptosporidiosis in different host species is important, to both enhance the health of animals, and also to devise strategies for the control of the zoonotic spread of the parasites.

This PhD thesis includes an interpretive overview of the relevant literature, and accounts of eight epidemiological studies of *Cryptosporidium* infections in humans and animals undertaken between 2002 and 2007 by the author. A simple search in Medline between 1995-2008 using the keyword '*Cryptosporidium*' returned 2343 citations. Therefore, the overview of the literature

reports only those articles that in the author's view shaped the thinking in each particular area; however, further articles are cited in the sections for the individual studies. Six of the eight studies have been published in peer-reviewed journals. To integrate the individual studies into the whole picture and avoid corrupting the published manuscripts, each study is preceded by an Introduction. Some successive studies have been published in different years. Thus, the literature cited may differ between studies, mainly as a reflection of the progressive accumulation of publications on the same topics. The published manuscripts have been slightly modified. Modifications included the addition of cross-references and material and methods that - for conciseness - could not be included in the published manuscripts. In addition, the bibliography was re-formatted according to the requirements of the *New Zealand Veterinary Journal* and the relevant raw data were included in Appendices.

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The names of friends and colleagues who helped me to perform the studies have been included in the list of authors of the relevant published papers. Prof. Andy Tait, Wellcome Centre for Molecular Parasitology, Glasgow, United Kingdom, generously donated the raw data used in the study presented in Section 2.1. Prof. Anne Chao, National Tsing Hua University, Taiwan, calculated the 95% confidence intervals of the Chao1 and ACE1 richness estimates in the same study. The molecular characterisation of the *Cryptosporidium* isolates used in the study presented in Section 2.2 was performed by Dr. Sultan Tanriverdi, Division of Infectious Diseases, Cummings School of Veterinary Medicine, Tufts University, MA, USA. The studies performed in Chapters 3 and 4 utilised several molecular methods developed over the years at the Protozoa Research Unit, Massey University, Palmerston North, and compiled in the *Manual of Methods for Genotyping Cryptosporidium Oocysts from Faecal Specimens*, written in November 2002 by Mrs Kim Ebbett. Mr. Errol Kwan, Anthony Pita and the late Jim Learmonth performed some laboratory analyses reported in Sections 3.1-3.4. Assistance in the laboratory was also provided by Mr. Yi Shi, Animal Health Monitoring and Disease Prevention Unit, Urumqi City, Xinjiang, People's Republic of China, during his stay in New Zealand as a visiting scholar under the author's supervision in 2007-2008. Other people to whom I am very grateful are Mr. Graham Young, former bacteriologist at Gribbles Diagnostic Laboratories, Hamilton, Jan Bird, Microbiology Section Leader of Path Lab Waikato, and Chris Pickett, Hamilton Medical Laboratory, for providing *Cryptosporidium*-positive specimens from cattle and humans. Dr. Isobel Gibson, New Zealand Veterinary Pathology Ltd., kindly provided *Cryptosporidium*-positive specimens from foals. The sympathetic New Zealand farmers who allowed me to take samples from animals under their care and the veterinary students who provided faecal specimens and responded to the questionnaires reported in Chapter 4 are also thanked. Finally, I thank Prof. Saul Tzipori and the staff of the Division of Infectious Diseases, Cummings School of Veterinary Medicine, Tufts University, for hosting me for two months in 2008.

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*For my beloved wife, Vicky*

*not by bread alone does man survive*

*Deuteronomy 8:3*



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## LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
BLST	Bilocus sequence type
bp	base-pairs
CI	confidence interval/confidence limit
CIN	cefsulodin, irgasan and novobiocin agar
COWP	<i>Cryptosporidium</i> oocyst wall protein
GP60	<i>Cryptosporidium</i> surface GP45/15 glycoprotein (or 60-kDA glycoprotein)
HAART	Highly Active Antiretroviral Therapy
HIV	human immunodeficiency virus
HSP70	70 kDa Heat Shock Protein gene
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
LATU	Large Animal Teaching Unit
MLG	multilocus genotype
MU	Massey University
OPG	oocysts per gram of faeces
PAS	periodic acid-Schiff stain
PCR	polymerase chain reaction
poly T	polythreonine repeat
RFLP	restriction fragment length polymorphism
RNR	<i>ribonuclease reductase</i>
SIA	standardized index of association
SNP	single nucleotide polymorphism
UPGMA	Unweighted Pair Group Method with Arithmetic mean
UV	ultra violet
XLD	Xylose Lysine-dehydrocolate
ZN	Ziehl Neelsen
18S rRNA	small subunit 18S ribosomal RNA