

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

**Alejandro Grinberg
2009**

ABSTRACT

A. Grinberg (2009). Doctoral thesis, Massey University, Palmerston North, New Zealand.

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 20th 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/, 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.

ACKNOWLEDGMENTS

I express my frank appreciation to my supervisors Prof. Bill Pomroy, Dr. Nicolas Lopez-Villalobos, and Prof. Giovanni Widmer, for helping me to complete the studies and write this thesis with enjoyment. I hope this has been a gratifying endeavour for them too. I am grateful to Prof. Grant Guilford, former Head of IVABS, Prof. Kevin Stafford, Director of Post-graduate Studies, Prof. Hugh Blair, Director of Research, and all staff at IVABS, for creating a suitable research environment that facilitated my work.

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.

The Lewis Fitch Veterinary Research Fund, McGeorge Research Fund, Graham Chalmers Allen Memorial Veterinary Scholarship and the New Zealand Ministry of Health provided funding for the projects. Additional funding was obtained from unrelated professional consultancy assignments over the years. The studies presented in Section 3.4 and Chapter 4 have been approved by the Animal and Human Ethics Committees of Massey University.

For my beloved wife, Vicky

not by bread alone does man survive

Deuteronomy 8:3

LIST OF CONTENTS

1 INTESTINAL CRYPTOSPORIDIOSIS IN HUMANS AND DOMESTIC MAMMALS: AN INTERPRETIVE OVERVIEW

1.1	Cryptosporidiosis in the historical perspective	1
1.2	Impact of cryptosporidiosis on human and animal health	2
1.3	The taxonomic classification of genus <i>Cryptosporidium</i>	4
1.4	The life cycle of the intestinal <i>Cryptosporidium</i> parasites	8
1.5	Pathogenesis of intestinal cryptosporidiosis	12
1.6	Infections with <i>Cryptosporidium</i> in domestic mammals	13
1.6.1	Infections with <i>Cryptosporidium</i> in cattle	13
1.6.2	Infections with <i>Cryptosporidium</i> in small ruminants	17
1.6.3	Infections with <i>Cryptosporidium</i> in horses	19
1.6.4	Infections with <i>Cryptosporidium</i> in cervids	20
1.6.5	Infections with <i>Cryptosporidium</i> in dogs and cats	21
1.6.6	Infections with <i>Cryptosporidium</i> in pigs	22
1.7	Genetic typing of <i>Cryptosporidium</i>	23
1.8	<i>Cryptosporidium parvum</i> and <i>C. hominis</i> population genetic structure	24
1.9	Zoonotic cryptosporidiosis	25
1.10	Concluding remarks	31
1.11	References	31

2 STUDIES OF THE POPULATION GENETIC STRUCTURE OF *CRYPTOSPORIDIUM PARVUM* AND *CRYPTOSPORIDIUM HOMINIS*

STUDIES OF THE POPULATION GENETIC STRUCTURE OF <i>CRYPTOSPORIDIUM PARVUM</i> AND <i>CRYPTOSPORIDIUM HOMINIS</i>		51
2.1	Host-shaped segregation of the <i>Cryptosporidium parvum</i> multilocus genotype repertoire	52
2.1.1	Summary	52
2.1.2	Introduction	52
2.1.3	Materials and methods	53
2.1.4	Results	54
2.1.5	Discussion	57
2.2	Inferences about the global population structure of <i>Cryptosporidium parvum</i> and <i>Cryptosporidium hominis</i>	60
2.2.1	Summary	60
2.2.2	Introduction	60
2.2.3	Materials and methods	61
2.2.4	Results	64
2.2.5	Discussion	70
2.3	Concluding remarks	72
2.4	References	73

3 EPIDEMIOLOGICAL STUDIES OF CRYPTOSPORIDIOSIS IN DOMESTIC ANIMALS IN NEW ZEALAND

EPIDEMIOLOGICAL STUDIES OF CRYPTOSPORIDIOSIS IN DOMESTIC ANIMALS IN NEW ZEALAND	79
3.1 Identification of <i>Cryptosporidium parvum</i> 'cattle' genotype from a severe outbreak of neonatal foal diarrhoea	80
3.1.1 Summary	80
3.1.2 Introduction	80
3.1.3 Materials and Methods	81
3.1.4 Results	83
3.1.5 Discussion	85
3.2 Genetic diversity and zoonotic potential of <i>Cryptosporidium parvum</i> causing foal diarrhoea	87
3.2.1 Summary	87
3.2.2 Introduction	87
3.2.3 Materials and Methods	88
3.2.4 Results	90
3.2.5 Discussion	92
3.3 A study of neonatal cryptosporidiosis of foals in New Zealand	94
3.3.1 Summary	94
3.3.2 Introduction	94
3.3.3 Materials and Methods	95
3.3.4 Results	97
3.3.5 Discussion	101
3.4 The occurrence of <i>Cryptosporidium parvum</i> , <i>Campylobacter</i> and <i>Salmonella</i> in newborn calves in the Manawatu region of New Zealand	103
3.4.1 Summary	103
3.4.2 Introduction	103
3.4.3 Materials and Methods	105
3.4.4 Results	107
3.4.5 Discussion	108
3.5 Persistent dominance of two GP60 <i>Ila</i> alleles in diarrhoeagenic <i>Cryptosporidium parvum</i> from man and cattle in the Waikato region of New Zealand	112
3.5.1 Summary	112
3.5.2 Introduction	112
3.5.3 Materials and Methods	113
3.5.4 Results	114
3.5.5 Discussion	116
3.6 Concluding remarks	117
3.7 References	118

4 AN OUTBREAK OF CRYPTOSPORIDIOSIS AMONG A COHORT OF YOUNG ADULTS: MOLECULAR AND DESCRIPTIVE EPIDEMIOLOGY AND RISK FACTOR ANALYSIS

4.1 An outbreak of cryptosporidiosis among a cohort of young adults: molecular and descriptive epidemiology and risk factor analysis	133
4.1 Summary	133
4.2 Introduction	133
4.3 Materials and Methods	134
4.4 Results	137
4.5 Discussion	143
4.6 References	146

5 GENERAL DISCUSSION

151

APPENDICES

156

LIST OF TABLES

Table 2.1 Distribution of <i>C. parvum</i> multilocus genotypes (MLGs) in Scotland, stratified by region (Aberdeenshire or Dumfriesshire), and host species (human or bovine <i>C. parvum</i>)	56
Table 2.2 Rarefaction, Chao1 and ACE1 richness estimators, by comparison	56
Table 2.3 <i>C. parvum</i> and <i>C. hominis</i> linkage disequilibrium and double-banded genotype statistics according to country of origin	70
Table 3.1 Restriction fragment length polymorphisms between <i>C. parvum</i> ‘cattle’ and ‘human’ genotypes	84
Table 3.2 Single nucleotide polymorphisms and deduced amino acid changes in the 60-kDa glycoprotein of <i>C. parvum</i> isolates from foals, as compared with the original sequence reported by Strong et al. (2000)	91
Table 3.3 Bilocus sequence types (BLST) of foal, human, and bovine <i>C. parvum</i> in New Zealand	92
Table 3.4 Haematology, biochemistry and venous blood gas results from a hospitalised foal affected with cryptosporidiosis	99
Table 3.5 Prevalence of <i>C. parvum</i> and <i>Campylobacter</i> spp among newborn calves from 24 dairy farms in the Manawatu region of New Zealand	108
Table 4.1 Demographic characteristics of the outbreak of cryptosporidiosis among a class of veterinary students reported in Section 4.1	140
Table 4.2 Risk factor analysis of the outbreak of cryptosporidiosis reported in Section 4.1	143

LIST OF FIGURES

Figure 1.1	The intestinal and extraintestinal life cycle of <i>C.parvum</i>	11
Figure 1.2	Least square means of the \log_{10} of (1+ number of <i>C. parvum</i> oocyst per gram of faeces) in 20 newborn calves affected with cryptosporidiosis in a dairy farm in Israel	17
Figure 1.3	Nucleotide polymorphisms (in yellow) between the 18S rRNA gene sequences of the so called "horse genotype", <i>C. parvum</i> , and <i>C. wrairi</i>	20
Figure 1.4	Upper graph: The number of cases of cryptosporidiosis notified in New Zealand between 1997 and 2007; lower graph: the seasonal shifts between the number of <i>C. parvum</i> and <i>C. hominis</i> isolates identified in New Zealand between 2000 and 2003	28
Figure 1.5	Dendrogram showing "human only sub-groups" of <i>C. parvum</i> multilocus genotypes in Scotland, as reported by Mallon et al. (2003) (above) and single and double locus variant networks (SDLVN) of the same multilocus genotypes (below)	30
Figure 2.1	Rarefaction curves, by comparison	57
Figure 2.2	Single locus variant eBURST networks for <i>C. parvum</i> and <i>C. hominis</i>	66
Figure 2.3	<i>C. parvum</i> and <i>C. hominis</i> MLG rank abundance plots	67
Figure 2.4	<i>C. parvum</i> and <i>C. hominis</i> analytical rarefaction curves	68
Figure 3.1	Fused and atrophic duodenal villi (arrow) and mildly increased numbers of mononuclear inflammatory cells within the lamina propria	84
Figure 3.2	PCR-restriction fragment length polymorphism analysis of <i>Cryptosporidium</i> β -tubulin, poly T, RNR and COWP genes	85
Figure 3.3	Foals with cryptosporidiosis.	100
Figure 4.1	Duration of illness (upper graph) and distribution of symptoms (lower graph) in the outbreak of cryptosporidiosis, as elicited from the responses to questionnaire Q1	141
Figure 4.2	Epidemic curve of the outbreak of cryptosporidiosis as elicited by the responses to Q1	142

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