Genotyping of *Cryptosporidium* Species Found in Stools of Infected Children

Thesis

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By

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Abstract

The present work constitutes a prospective study on a total of 97 pediatric patients; 65 cancerous patients and 32 non-cancerous patients suffering from diarrhea. All faecal specimens were examined parasitologically using the MZN and EIA techniques. Samples testing positive for *Cryptosporidium* were examined using the PCR-RFLP technique which amplified the *Cryptosporidium* oocyst wall protein (COWP) gene. Among 97 diarrheic pediatric cases, 47 (48.5%) were positive by nested PCR. *Rsa* I digestion of nested PCR product of COWP gene revealed the presence of 2 genotypes: genotype 1 in 32 (68%) and genotype 2 in 15 (32%) of cases. In this study, comparable correlations were observed between the clinical parameters associated with the 2 genotypes as there were no statistically significant associations between genotypes and age, sex, vomiting, abdominal pain or fever or dehydration. The fact that Genotype 1 was found to be relatively more prevalent than genotype 2 among all groups of patients examined suggests a relatively greater risk of human source of infection than zoonosis.

Key words: COWP, *Cryptosporidium parvum*, genotyping

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List of Abbreviations

A:	adenine
ABC:	avidin biotin complex
Abd. Pain:	abdominal pain
Approx:	approximately
bp:	base pair
°C:	degree celcius
C.:	*Cryptosporidium*
Caco-2:	human colonic carcinoma cell line
cAMP:	cyclic adenosine monophosphate
CD:	cluster of differentiation
CHEF:	contour-clamped homogeneous electric field
COWP:	*Cryptosporidium* oocyst wall protein
Cox:	cyclooxygenase
Cpgp:	*Cryptosporidium parvum* glycoprotein
CSA:	*Cryptosporidium* specific antigen
DAB:	diaminobenzidine
DFA	direct fluorescent-antibody
DHFR:	dihydro folate reductase
DNA:	deoxyribonucleic acid
dNTP:	deoxynucleotide triphosphate
E:	extended
EB:	ethidium bromide
EDTA:	ethylenediamine tetraacetic acid
EIA:	enzyme immunoassay
ELISA:	enzyme linked immunosorbant assay
EM:	electron microscope
f:	female
FEA:	formalin-ethyl acetate
fg	femtogram
G:	glycine
g:	gravity
gm:	gram
GST:	glutathione S-transferase
h:	hour
HAART:	highly active anti-retroviral therapy
HCl:	hydrochloric acid

HIV:	human immunodeficiency virus
hsp:	heat shock protein gene
$H_2O_{2:}$	Hydrogen peroxide
IF:	immunofluorescence
Ig:	immunoglobulin
IL:	interleukin
IMS:	immunomagnetic bead separation
Inc:	incorporation
ITS:	internal transcribed spacer
Kb:	kilo base
KCl:	potassium chloride
KDa:	kilo dalton
L:	litre
Ltd:	limited
m:	male
M:	mole
mm:	millimetre
mM:	millimole
mAbs:	monoclonal antibodies
Mbp:	million base pair
mg:	milligram
$MgCl_{2:}$	magnesium chloride
Min:	minute
ml:	millilitre
MZN:	modified Ziehl-Neelsen
N:	nested
NaCl:	sodium chloride
Neg:	negative
NF:	nuclear factor
No:	number
*Not*I:	*Nocardia otitidiscaviarum*
nr:	nuclear ribosomal
PBS:	phosphate buffered saline
PCR:	polymerase chain reaction
PFGE:	pulsed field gradient electrophoresis
pH:	hydrogen potential
pmoles:	pico moles
Pos:	positive
P value:	probability value
r:	ribosomal

RAPD:	random amplified polymorphic deoxyribonucleic acid
*Ras*I:	*Rhodobacter sphaeroides* I
RFLP:	restriction fragment length polymorphism
RPH:	reverse passive haemagglutination
rpm:	revolutions per minute
rRNA:	ribosomal ribonucleic acid
SCID:	severe combined immunodeficient
*Sfi*I:	*Streptomyces fimbriatus* I
Spp:	species
SSF:	sheather sucrose flotation
SSrRNA:	small subunit ribosomal ribonucleic acid
S rDNA:	small subunit ribosomal deoxyribonucleic acid
S rRNA:	small subunit ribosomal ribonucleic acid
SSU:	small subunit
Std:	standard
T:	thymine
TAE:	tris-acetate ethylenediamine tetraacetic acid
Taq:	*Thermus aquaticus*
TE:	tris ethylenediamine tetraacetic acid
TNF:	tumour necrosis factor
TRAP-C:	thrombospondin-related adhesive protein of *Cryptosporidium*
tRNA:	transfer ribonucleic acid
UK:	United Kingdom
USA:	United States of America
UV:	ultra violet
v/v:	volume/ volume
w/v:	weight/volume
µ:	micron
µL:	microlitre
µm:	micrometer

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Introduction

Species of *Cryptosporidium* are protozoan parasites (Apicomplexa) which infect a wide range of vertebrate hosts. The clinical signs of cryptosporidiosis in humans are mainly diarrhoea, dehydration, malabsorption, weight loss and/or wasting. Infection is frequently self-limiting, but chronic infections may establish, particularly (but not exclusively) in young and/or immunodeficient or immunosuppressed individuals (Fayer *et al.,* 2000).

Cryptosporidia are intracellular, extracytoplasmic protozoan parasites with a monoxenous life cycle. They invade the microvillus border of the gastrointestinal and respiratory epithelium of a wide range of vertebrate species, and may cause considerable economic losses in livestock (Sreter *et al.,* 2000). At present, no effective therapy is available (Tzipori, 1998). Environmentally resistant oocysts are transmitted by the fecal-oral route, but zoonotic infection and person-to-person transmission are also known (O'Donoghue, 1995). Cryptosporidia have been reported to cause several waterborne and food-borne outbreaks worldwide (Smith and Rose, 1998), the most severe occurred in Milwaukee, Wisconsin, USA in 1993, where more than 400,000 people were infected (Mac Kenzie *et al.,* 1994). *Cryptosporidium* oocysts are resistant to disinfectants commonly used in drinking water treatment, consequently, they have become a major concern to public health and to the drinking water industry (Xiao *et al.,* 2000) and are recognized as a major cause of waterborne diarrheal disease worldwide (Fayer *et al.,* 1997).