



Molecular Characterization of *Cryptosporidium* from Stools of Filipino Children with Diarrhea



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INTRODUCTION



The Philippines



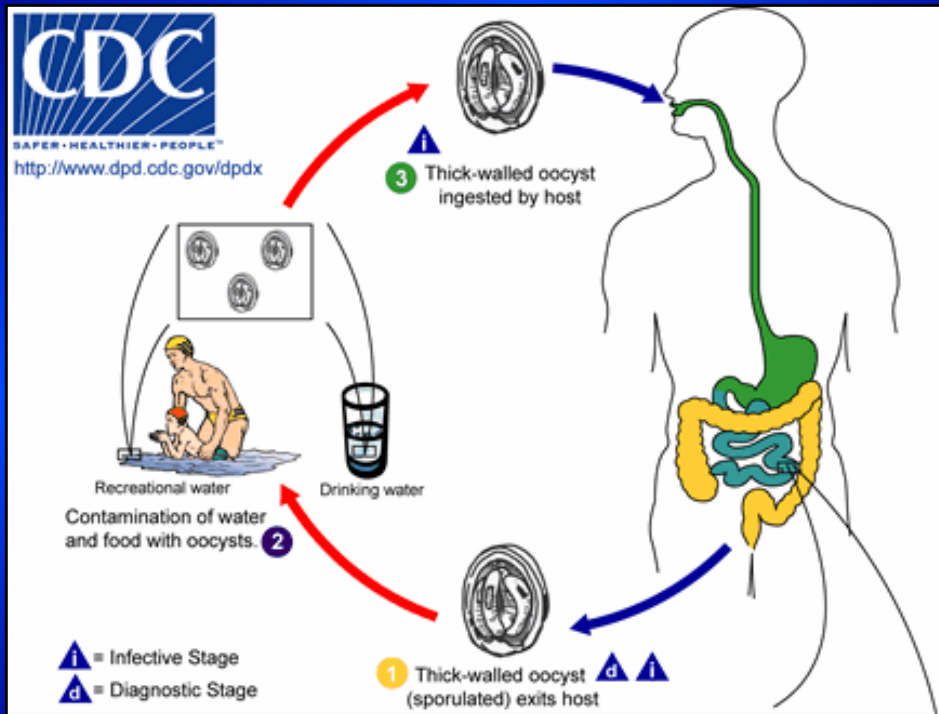
- 300,000 km² land area
- 7,100 islands
- 3 major islands
- 90M population in 2007
- wet and dry seasons



Historical Background of *Cryptosporidium*

- first described from a mouse in 1907 by Clarke and Tyzer
- first reported as human pathogen in 1976 by Nime
- recognized as a cause of diarrhea in 1982 by Current
- first reported in Filipino children in 1985 by Cross *et al.*

Life Cycle



- 5-28, mean 7.2 days incubation
- various transmission modes
- requires single host



The Parasite

- found in over 150 species of mammals
- oocyst is chlorine-resistant
- causes cholera-like diarrhea
- no effective chemotherapy

Human-pathogenic Species

Species	Major hosts	Minor hosts
<i>C. parvum</i>	cattle, sheep, goats, humans,	deer, mice, pigs
<i>C. hominis</i>	humans, monkeys	dugongs, sheep
<i>C. meleagridis</i>	turkeys, humans	parrots
<i>C. canis</i>	dogs	humans
<i>C. felis</i>	cats	humans, cattle
<i>C. muris</i>	rodents, bactrian camels	humans, rock hyrax, mountain goats
<i>C. suis</i>	pigs	humans



Recent Molecular Tools

- **PCR-RFLP: SSU rRNA, cowp, GP60 genes**
- **PCR-SSCP: SSU rRNA, its, HSP70, GP60 genes**
- **PCR-heteroduplex analysis of double-stranded RNA**
- **DNA sequencing**



Objectives

- to characterize *Cryptosporidium* isolates by PCR-RFLP and sequence analyses of poly-threonine and 18S rRNA genes
- to determine major pattern of transmission

MATERIALS and METHODS





General Flow Chart

Stool sample collection



Modified FEA concentration



Immunofluorescence microscopy



Molecular characterization

Molecular Characterization

Cryptosporidium isolates



Oocyst disruption using glass beads

McLauchlin *et al.* 2000



DNA extraction

Yagita *et al.* 2001

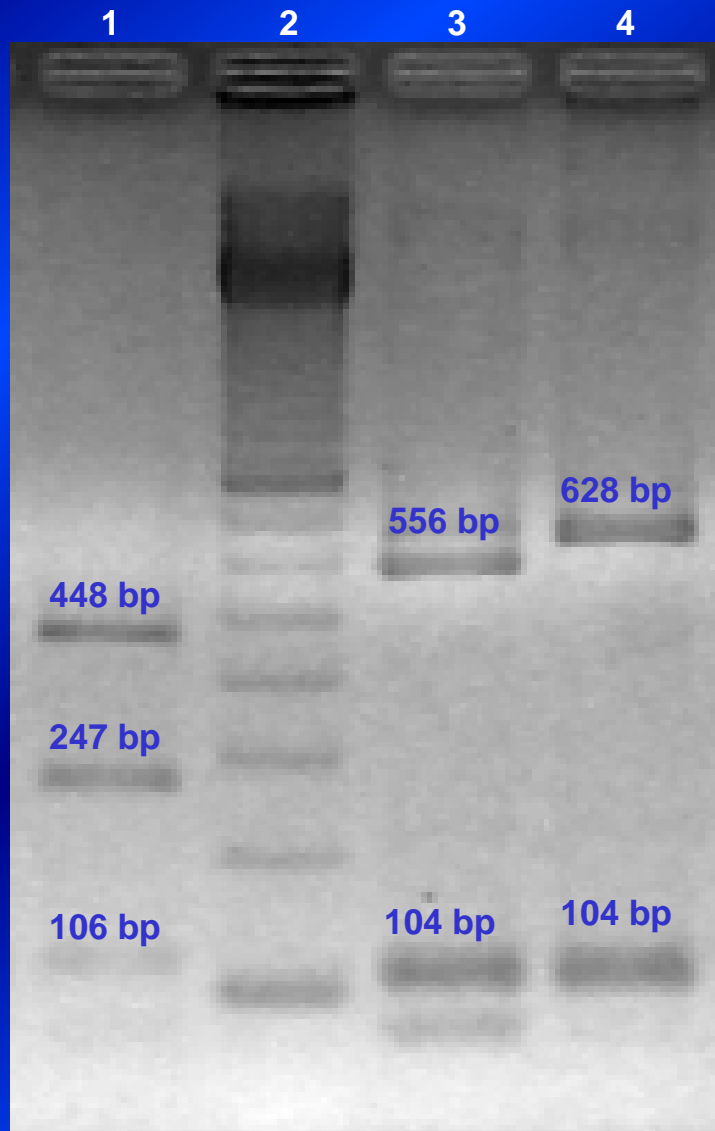


PCR-RFLP of polythreonine gene (Yagita *et al.* 2001) **or**
PCR-RFLP and sequencing of 18S rRNA gene (Xiao *et al.* 1999)

RESULTS



Fig. 1. RFLP of *Cryptosporidium* 18S rRNA gene.



1 - *Sspl* of *C. hominis*/*C. parvum*

2 - 1kb+ ladder

3 - *Vspl* of *C. hominis*

4 - *Vspl* of *C. parvum*

Table 1. Isolates of *Cryptosporidium* identified from pediatric patients in the Philippines.

Isolate code	Age (mos.)	Sex	Location	Polythreonine gene		18S rRNA gene	
				RFLP	Sequencing	RFLP	Sequencing
NCR 044	3	M	Luzon	<i>C. hominis</i>	<i>C. hominis</i>		
NCR 070	12	F	Luzon	<i>C. hominis</i>	<i>C. hominis</i>		
NCR 111	31	M	Luzon	<i>C. hominis</i>	<i>C. hominis</i>		
NCR 134	19	F	Luzon	<i>C. hominis</i>	<i>C. hominis</i>		
NCR 192	3	F	Luzon	<i>C. hominis</i> & <i>C. parvum</i>	<i>C. hominis</i> & <i>C. parvum</i>		
NCR 234	9	F	Luzon	<i>C. hominis</i>	<i>C. hominis</i>		
EAMC 005	NI	NI	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 026	12	M	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 030	24	F	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 048	10	M	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 217	72	M	Luzon			<i>C. hominis</i>	<i>C. hominis</i>
EAMC 220	24	M	Luzon			<i>C. parvum</i>	<i>C. hominis</i>
EAMC 233	3	M	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 277	84	F	Luzon			<i>C. parvum</i>	<i>C. hominis</i>
EAMC 296	8	M	Luzon			<i>C. hominis</i>	<i>C. hominis</i>
EAMC 440	10	M	Luzon			<i>C. parvum</i>	<i>C. parvum</i>
EAMC 484	12	F	Luzon			<i>C. hominis</i>	<i>C. hominis</i>
VIS 082	40	M	Visayas			<i>C. hominis</i>	<i>C. hominis</i>
VIS 120	33	F	Visayas			<i>C. hominis</i>	<i>C. hominis</i>

Table 2. Summary of *Cryptosporidium* species identified.

<i>Cryptosporidium</i> species	No. of isolates
<i>C. hominis</i>	10
<i>C. parvum</i>	6
co-infection	3
Total	19



Discussion

- **Co-infection of *C. hominis* and *C. parvum* (15.8 %) is relatively higher compared to other studies**
 - 0.4 % in diarrheic patients in UK
 - 4.1 % in diarrheic children in Uganda
 - 8.1 % in patients in Switzerland
- **there are few reports on characterization of *Cryposporidium* from diarrheic children in other countries**

Table 3. Molecular characterization of *Cryptosporidium* in other countries

Authors	Sources of isolates	Molecular techniques	Results	Transmission pattern
Xiao <i>et al.</i> 2001	diarrheic children in Peru	nested PCR-RFLP of 18S rRNA gene	67 <i>C. hominis</i> 8 <i>C. parvum</i> 7 <i>C. meleagridis</i> 2 <i>C. canis</i> 1 <i>C. felis</i>	anthroponotic
Tumwine <i>et al.</i> 2003	diarrheic children in Uganda	PCR-RFLP of <i>cowp</i> gene	72.8% <i>C. hominis</i> 18.4% <i>C. parvum</i> 4.1% with both <i>C. hominis</i> and <i>C. parvum</i> 4.1% unclassified or <i>C. meleagridis</i>	anthroponotic
Glaeser <i>et al.</i> 2004	diarrheic children in Switzerland	not indicated	11 <i>C. hominis</i> 3 <i>C. parvum</i>	anthroponotic
Sulaiman <i>et al.</i> 2005	children in Kuwait with gastrointestinal symptoms	PCR-RFLP of SSU rRNA and sequencing of GP60	58 (94%) <i>C. parvum</i> 3 (5%) <i>C. hominis</i>	zoonotic



CONCLUSION

- *C. hominis* was the most prevalent species infecting diarrheic children in this study
- the major pattern of transmission is anthroponotic



Acknowledgments

- **Department of Parasitology, National Institute of Infectious Diseases, Japan**
- **Collaborating clinics and hospitals, Department of Health, Philippines**
- **Supported by grants from National Institute of Health of Japan, Japan Health Sciences Foundation, and St. Luke's Medical Center, Philippines**



Thank you!

