Number of malaria cases in 2002

No.	Province/city	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1	Pyeongyang	1	1	1	53	954	2,791	2,811	4,650	2,924	824	15	1	15,024
2	Pyeongan S				26	1,047	4,982	7,908	9,330	6,829	2,784	143	6	33,135
3	Pyeonan N				55	848	4,680	7,014	9,156	6,447	2,746	124	2	31,072
4	Hanghae S	2	4	4	209	3,070	10,680	6,825	10,854	7,830	3,886	235	34	43,621
5	Hanghae N		1	1	138	2,822	4,165	2,934	7,431	4,774	2,924	56	7	25,314
6	Gangwon	51	30	69	38	1,398	5,781	4,033	8,193	5,856	2,636	105	21	27,711
7	Hamgyeong N		2	4	30	592	2,860	4,783	7,635	4,758	1,822	12	1	22,548
8	Hamgyeong N		3	5	29	318	1,114	1,602	3,112	2,073	359	1		8,616
9	Gaeseong	13	6	59	244	2,153	5,349	3,043	9,027	6,217	3,818	46	31	29,206
10	Nampo				44	173	723	1,169	1,854	837	141		1	4,942
Total	10 areas	67	47	143	866	13,375	43,124	43,182	71,320	48,583	20,660	747	104	241,190

Number of malaria cases in 2003

No.	Province/city	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
1	Pyeongyang				13	94	216	249	89	54			[illegible]
2	Pyeonan S				19	282	1,827	2,533	1,184	1,031	812	305	[illegible]
3	Pyeonan N			2	20	335	1,576	2,347	1,307	976	485	149	[illegible]
4	Hwanghae S					734	2,235	2,212	1,083	483	597	359	[illegible]
5	Hwanghae N				24	151	855	856	438	1,132	629	483	[illegible]
6	Gangwon	4	5	30	10	214	898	808	789	221	246	154	[illegible]
7	Hamgyeong S			1		187	794	925	589	321	254	125	[illegible]
8	Hamgyeong N				11	9	128	618	241	53	173	54	[illegible]
9	Gaesoeng	13	17	68	115	1,189	1,735	908	622	744	348	287	[illegible]
10	Nampo					52	542	1,541	1,049	315	364	219	[illegible]
Total	10 areas	17	22	101	212	3,217	10,866	13,095	7,391	6,309	3,899	2,131	[illegible]

Primaquine mass chemoprophylaxis in North Korea

No.	County/city	No. of population	No. of contraindications/ long-term travelers	No. of administrated with primaquine	No. of population in the control area
1	Gangnam	33,030	5,194	27,774	27,581
2	Seokcheon	86,000	13,832	72,178	87,000
3	Soncheon	54,570	8,913	45,557	74,250
4	Sincheon	72,857	12,422	60,435	56,455
5	Hwangju	74,030	9,710	64,330	83,670
6	Anbyeon	40,970	5,240	35,730	51,964
7	Panmun	30,000	2,100	27,670	40,955
	Total	391,357	57,411	328,679	421,875

5,267 population have been excluded during the course of 14 days regimen.

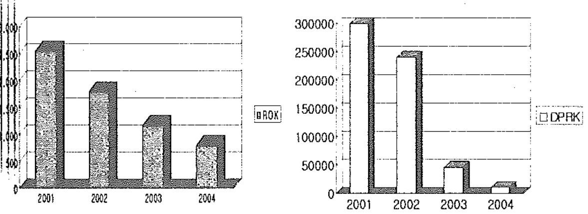
Support North Korea

◎ To reduce the malaria cases in both countries

	In Kind	Education fee
2001	$ 500,000	$ 40,000
2002	$ 600,000	$ 30,000
2003	$ 700,000	$ 30,000
2004	$ 700,000	$ 30,000

Center for Disease Control and Prevention, Korea

Significance of cooperation for the control of border malaria



Center for Disease Control and Prevention, Korea

Conclusions

Goal; Complete eradication of malaria in ROK by 2010

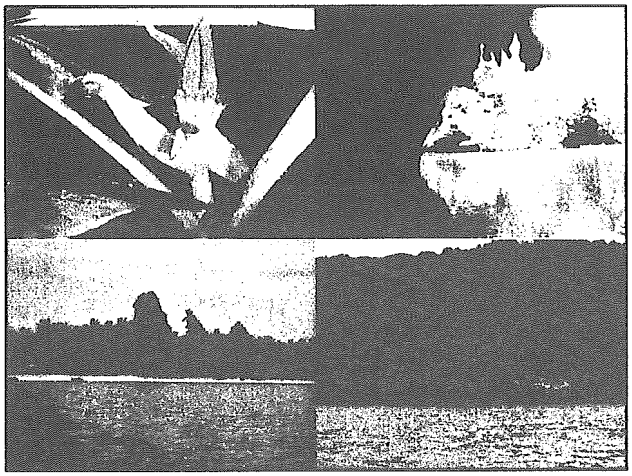
Strategy; Reduce the malaria cases to more than 30% off every year

◎ Concentrate the power of management in risk areas
◎ Enforce the cooperation between civilian and military authorities
◎ Enhance the control abilities of Public Health Centers
◎ Continue the support programs for the control of North Korea
◎ Keep continue the basic researches

Center for Disease Control and Prevention, Korea

Acknowledgement

- Public Health Centers
  - Paju, Gimpo, Gangwha, Yeoncheon, Cheorwon, Pocheon, Uijeongbu, Koyang, Yangju, Tongducheon
- Division of Epidemic intelligence Service, KCDC
- Division of Infectious Disease Surveillance, KCDC



Current Situation of Enteric Protozoan Infections in the Philippines and Research Needed for Better Control

Filipinas F. Natividad, PhD
St. Luke's Medical Center

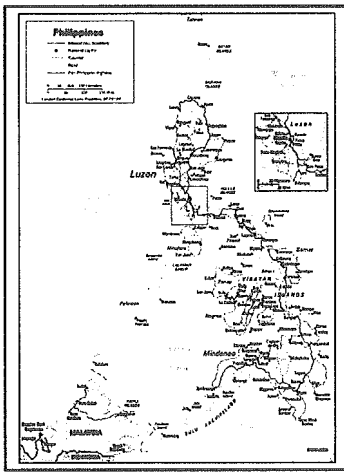
Meeting on Malaria and Enteric Protozoan Infections in Southeast Asia
Tokyo, Japan
31 January- 01 February 2006

Pathogenic Enteric Protozoa

- *Entamoeba histolytica*
- *Giardia lamblia*
- *Cryptosporidium* spp.
- *Blastocystis hominis*
- *Isospora belli*
- *Cyclospora cayetenensis*
- *Balantidium coli*

Outline of Presentation

1. Diarrhea in the Philippines
   - Facts and Figures
   - Diarrheal Control Program
2. Current situation of enteric protozoan infections in the Philippines
   - Recent diarrhea outbreaks
   - Researches on enteric protozoans
3. Collaborative research with National Institute of Infectious Diseases
4. Future perspectives
   - The Asian Laboratory Network on Enteric Protozoa
   - Future research



The Philippines

- 7100 islands
- 300,000 km^2 total land area
- 85M population in 2005
- wet and dry seasons

The tropical temperature in the Philippines favors the existence of parasites.

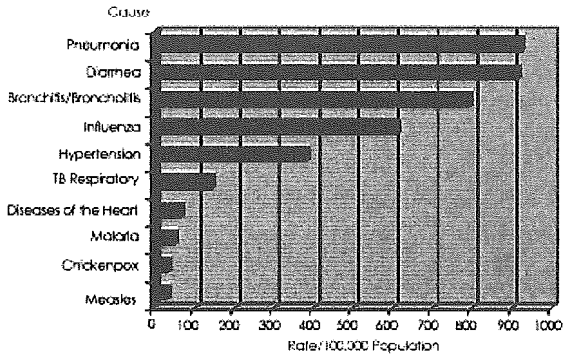
Health Situation

- constraints in basic social services in urban areas
- difficult access to health services in rural areas
- parasitism common in children
- communicable diseases - four leading causes of morbidity

Diarrhea in the Philippines

- second leading cause of morbidity in 2002
- first leading cause of morbidity from 1990 to 2001
- third leading cause of child (1-4 years old) mortality in 2000
- fifth leading cause of child (5-9 yrs old) mortality in 2000

In 2002, diarrhea was the second leading cause of morbidity.



Ten Leading Causes of Child Mortality in 2000

Cause	1-4 years old children			
	Male	Female	Both Sexes	Rate*
1. Pneumonia	1,540	1,341	2,881	37.76
2. Accidents	839	506	1,345	17.63
3. Diarrheas and gastroenteritis of presumed infectious origin	**685**	**546**	**1,231**	**16.14**
4. Measles	452	425	877	11.50
5. Congenital anomalies	350	337	687	9.01
6. Malignant Neoplasm	219	153	372	4.88
7. Meningitis	201	155	356	4.67
8. Septicemia	173	173	346	4.54
9. Chronic obstructive pulmonary disease and allied conditions	174	164	338	4.43
10. Other protein-calorie malnutrition	175	159	334	4.38

** Rate per 100,000 population of corresponding age-group (Philippine Health Statistics 2000, DOH)*

Ten Leading Causes of Child Mortality in 2000

Cause	5-9 years old children			
	Male	Female	Both Sexes	Rate*
1. Accidents	1,044	618	1,662	17.82
2. Pneumonia	368	288	656	7.03
3. Malignant Neoplasm	201	169	370	3.97
4. Congenital Anomalies	135	131	266	2.85
5. Diarrheas and gastroenteritis of presumed infectious origin	**112**	**92**	**204**	**2.19**
6. Other diseases of the nervous system	118	83	201	2.15
7. Meningitis	105	95	200	2.14
8. Diseases of the heart	99	75	174	1.87
9. Tuberculosis, all forms	83	62	145	1.55
10. Septicemia	79	53	132	1.41

** Rate per 100,000 population of corresponding age-group (Philippine Health Statistics 2000, DOH)*

Diarrheal Disease Control Program in the Philippines: Components

- Dept. of Health; to reduce morbidity & mortality due to acute diarrhea in children <5 years old
- Oral rehydration salt (ORS) production, distribution, and utilization
- Training and health education
- Information dissemination and education campaigns

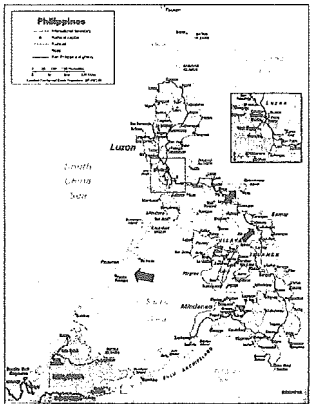
Diarrheal Disease Control Program in the Philippines: Outcomes

- Mortality trends: relative annual decline of 5%
- Morbidity trends: relative annual decline of 2.5%
- Safe water coverage increased from 59% to 86%
- Sanitation facilities increased from 50% to 76%

Current Situation of Enteric Protozoan Infections in the Philippines

Recent diarrhea outbreaks

- Palawan
  - 214 cases reported
  - 32 deaths
- Samar
  - 302 cases reported
  - 3 deaths
- Catanduanes
  - 457 cases
  - 30 deaths



Researches on Enteric Protozoa

Year	# of papers	Journals	Enteric Protozoa identifed
2000-2004	2	Korean J Parasitol	*Giardia, Entamaoeba, Endolimax, Iodamoeba, Blastocystis*
1990-1999	12	Am J Trop Med Hyg Parasitol Res Phil J Pedia Phil J Micro Inf Dis SEAJ Trop Med Ann Trop Med Parasitol Agustinian Acta Medica Phil	*Giardia, Entamoeba, Endolimax, Blastocystis, Cryptosporidium, Trichomonas*
1980-1989	6	Phil J Micro Inf Dis SEAJ Trop Med J Clin Micro	*Giardia, Entamoeba, Endolimax, Iodamoeba, Blastocystis, Cryptosporidium, Trichomonas, Chilomastix*
1977-1979	2	Am J Epid SEAJ Trop Med	*Giardia, Entamoeba, Endolimax, Iodamoeba, Blastocystis, Cryptosporidium, Trichomonas, Chilomastix*

Identification of Enteric Protozoa from Diarrheic Patients in the Philippines

A Joint Project of

St. Luke's Medical Center, Quezon City, Philippines

Research & Biotechnology Division

National Institute of Infectious Diseases Tokyo, Japan

Department of Parasitology

Cooperating Agency

Department of Health, Philippines

Network Hospitals

Brief Project Description:

- Epidemiological study to gather information on the occurrence of enteric protozoa in diarrheic patients
- Establishment of a network for field collection of samples as well as gathering of relevant patient information
- Provision of logistics for transport, storage and archiving of samples
- Creation of an electronic database system
- Setting up of a central laboratory for detection and identification of enteric protozoa

Objectives

- Identification of enteric protozoan pathogens in diarrheic stools collected from various hospitals in the country.
- Determination of the most common types of enteric protozoan pathogens
- Comparison of demographic data of patients infected with various enteric protozoan pathogens.
- Correlation of occurrence of protozoan pathogens with the quality of drinking water.

Significance

- First comprehensive, nation-wide survey of enteric protozoan pathogens isolated from diarrheic patients.
- Data provided basic information on the occurrence of enteric infections caused by these protozoan pathogens.
- Information obtained can be used as basis for recommending appropriate strategies for preventive measures against enteric protozoan pathogens

Methodology

Inclusion Criteria:

✓ Patients seeking medical attention due to diarrhea of any form.

Exclusion criteria:

✗ Patients with irritable bowel syndrome.

Results

Sample collection:

Collection period: May 2004- June 2005
Number of samples collected: 3545
Collection sites:

Luzon	1701
Visayas	1445
Mindanao	399

Patient Database:

Demography
Clinical data
Drinking water source
Health status

Collection Data

Collection area	Frequency	Percent
Luzon	1701	48.0
Visayas	1445	40.8
Mindanao	399	11.3
TOTAL	3545	100.0

Age distribution of samples

Subject classification	Frequency	Percent	Valid Percent
Pediatric (0-18 yrs)	2212	62.4	63.4
Adult (>18 yrs)	1278	36.1	36.6
SubTotal	**3490**	**98.4**	**100.0**
No information	55	1.6	
TOTAL	**3545**	**100.0**	

Data on Microscopy

Analysis	Frequency	Percent
Complete	**3041**	**85.8**
Incomplete	**504**	**14.2**
TOTAL	**3545**	**100.0**

Summary of Results on Microscopy

Protozoa	Collection Site						Philippines	
	Luzon		Visayas		Mindanao			
	Freq	%	Freq	%	Freq	%	Freq	%
Positive	**295**	**17.7**	**214**	**21.9**	**61**	**15.3**	**570**	**18.7**
Negative	**1370**	**82.3**	**763**	**78.1**	**338**	**84.7**	**2471**	**81.3**
TOTAL	**1665**	**100.0**	**977**	**100.0**	**399**	**100.0**	**3041**	**100.0**

Prevalence of Enteric Protozoa

Protozoa	Frequency	Percent
Positive	**570**	**18.7**
Negative	**2471**	**81.3**
TOTAL	**3041***	**100.0**

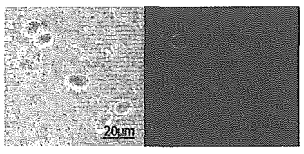
* Samples with incomplete microscopy were excluded from the analysis

Prevalence of Enteric Protozoa

Protozoa	Frequency/Percent **(+) Samples**	Frequency/Percent **(-) Samples**
Giardia lamblia	**71 / 2.3**	**2970 / 97.7**
Cryptosporidium spp.	**64 / 2.1**	**2977 / 97.9**
Entamoeba sp.	**109 / 3.6**	**2932 / 96.4**
Entamoeba dispar	**48 / 1.6**	**2993 /98.4**
Entamoeba histolytica	**48 / 1.6**	**2993 / 98.4**
Entamoeba coli	**43 / 1.4**	**2998 / 98.6**
Cyclospora	**1 /**	**3040 /**
Isospora	**1 /**	**3040 /**

Philippine Isolates of Enteric Protozoa

Giardia lamblia



Sample from a **5**-year old **male.**
Source of drinking water: **artesian well & bottled water**
Type of stool: **watery**
Season of sample collection: **rainy**

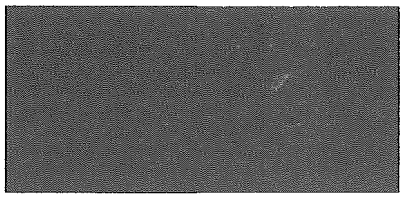
Philippine Isolates of Enteric Protozoa

Cryptosporidium sp.

Sample from a **3**-year old **male**.
Source of drinking water: **MWSS**
Type of stool: **watery**
Season of sample collection: **rainy**

Philippine Isolates of Enteric Protozoa

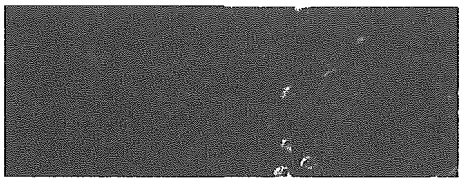
Entamoeba histolytica/dispar



Sample from a **9**-month old **female**
Source of drinking water: **MWSS**
Type of stool: **watery**
Season of sample collection: **rainy**

Philippine Isolates of Enteric Protozoa

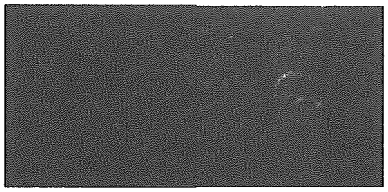
Cyclospora sp.



Sample from a **18**-year old **male**
Source of drinking water: **deep well**
Type of stool: **watery**
Season of sample collection: **rainy**

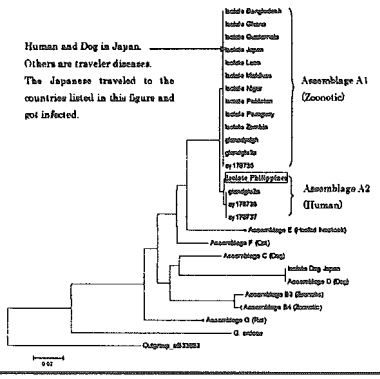
Philippine Isolates of Enteric Protozoa

Isospora sp.

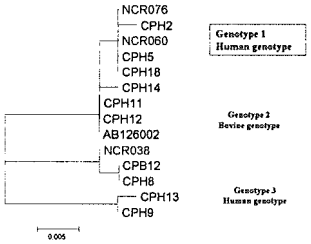


Sample from a **73**-year old **male**
Source of drinking water: **deep well**
Type of stool: **soft**
Season of sample collection: **rainy**

Molecular characterization: *Giardia*



Molecular characterization: *Cryptosporidium*

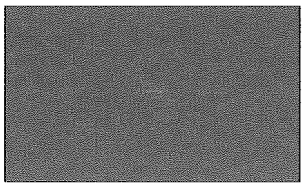


Phylogenetic tree of *Cryptosporidium* based on the sequence of polythreonine gene. Philippine isolates: NCR 076, 060 and 038. Japan Isolates: AB126002, CPH and CPB except for CPH9 from Indonesia.

Molecular characterization: *Cryptosporidium*

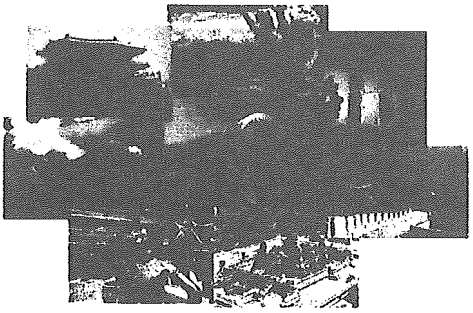
Genotypes of *Cryptosporidium parvum* from the Philippines		
Isolate	RFLP	Sequence
NCR 038		Genotype 2
NCR 044	Genotype 1	Genotype 1
NCR 060		Genotype 1
NCR 070	Genotype 1	Genotype 1
NCR 076		Genotype 1
NCR 111	Genotype 1	Genotype 1
NCR 134	Genotype 1	Genotype 1
NCR 192	Mixed genotype	Mixed genotype
NCR 234	Genotype 1	Genotype 1

Molecular characterization: *Cryptosporidium*



Genotyping of *Cryptosporidium* sp. by RFLP. Lanes 1: 100-bp ladder, 2-7: Philippine Isolates, 8: Positive control- Bovine genotype, 9: Positive control- Human genotype

The Laboratory Network

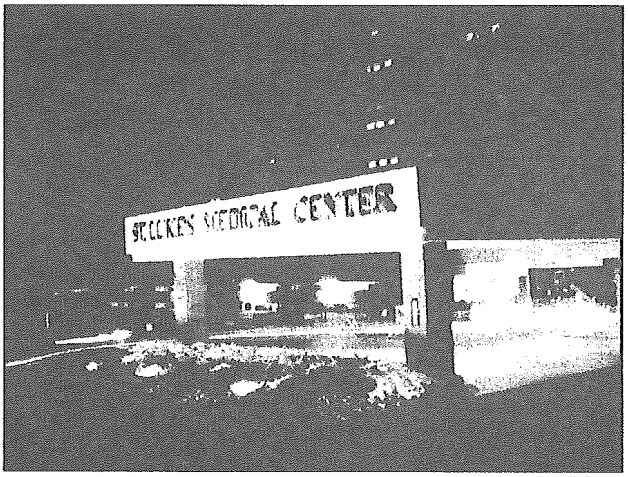


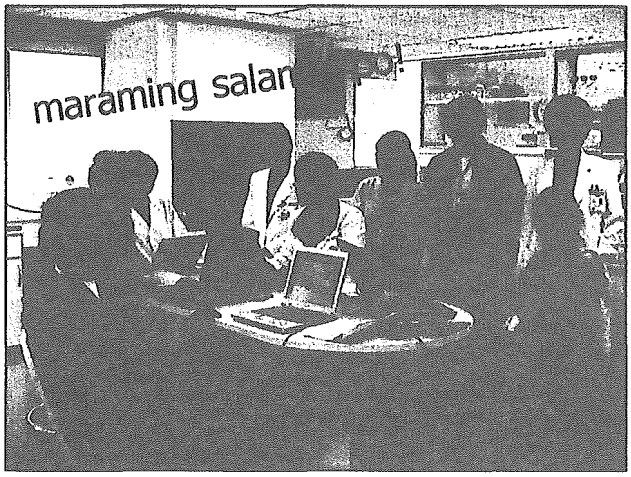
The Laboratory Network: Goals

- Information exchange
  - database development
  - standardized information system
  - accurate data collection
- High quality collaborative research
  - capacity building
  - technical support
  - scientific exchange and interaction
- Improved health and quality of life
  - self-sufficiency of collaborating institutions
  - new/improved methods of prevention, detection & treatment
  - implementation of national programs

Future research on enteric protozoa

- Molecular Epidemiology
  - collection, identification and molecular characterization
  - incidence, prevalence & geographical distribution
  - risk factors, sources of infections
  - susceptible population (immunocompromised, etc)
  - mutual sentinel sites
- Pathogenesis
  - correlation with genotypes
  - animal studies
- Prevention and Control
  - environment and food monitoring
  - health and sanitation
- Clinical studies
  - new drugs (Clinical trials)
  - preventive
  - therapeutic





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2000 Lee K-J, Ahn Y-K, Yong T-S A small-scale survey of intestinal parasite infections among children and adolescents in Legaspi city, the Philippines. *Korean J Parasitol* 38(3):183-5.

2004 Baldo ET, Belizario VY, De Leon WU, Kong HH, Chung DI. Infection status of intestinal parasites in children living in residential institutions in Metro Manila, the Philippines. *Korean J Parasitol* 42(2):67-70.

Cryptosporidiosis and isosporiasis in Thailand: Morphometric and molecular analysis

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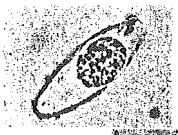
Molecular epidemiology of cryptosporidiosis among human immunodeficiency virus-infected patients in Thailand: analysis of the 18S RNA and the Cpg60/45/15 loci

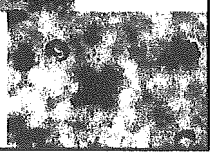
Opportunistic Protozoan Diarrhea in HIV-infected Patients

Isospora belli



Cyclospora cayetanensis

Cryptosporidium spp.



Epidemiology of Cryptosporidiosis

	Diarrhea(%)	No diarrhea(%)
Developed Countries		
HIV-	2.1	0.15
HIV+	13.8	0
Developing Countries		
HIV-	6.1	1.5
HIV+	24.0	5.0

(Xiao et al J Clin Microbiol 2004)

Epidemiology of Human Cryptosporidiosis and Its Medical Significance

The most common cause of protozoan diarrhea in immunocompromised patients.

No effective drugs available for cryptosporidiosis.

A variety of *Cryptosporidium* species circulate among humans and animals: implication for prevention and control.

Identification of *Cryptosporidium* Species

- Host Range
- Oocyst Morphometry
- Site of Infection
- Molecular Characterization

Valid species of *Cryptosporidium*

Species	Major Host	Minor Host
C.hominis	Humans, monkeys	Dugongs, sheep
C.parvum	Humans, cattle, sheep goats	Deer, mice, pigs
C.meleagridis	Humans, turkeys	Parrots
C.felis	Cats	Humans, cattle
C.canis	Dogs	Humans
C.muris	Rodents, camels	Humans, rock hyrax mountain goats
C.andersoni	Cattle, camels	Sheep
C.wrairi	Guinea pigs	
C.baileyi	Chicken, turkeys	Cockatiels, quails ostriches, ducks
C.galli	Finches, chicken capercalles, grosbeaks	
C.serpentis	Snakes, lizards	
C.saurophilum	Lizards	Snakes
C.monari	Fish	

Both anthroponotic and zoonotic species of *Cryptosporidium* are found in humans.

How many species of *Cryptosporidium* occur in HIV-infected patients in Thailand?

The Study

Duration: 1997-2003

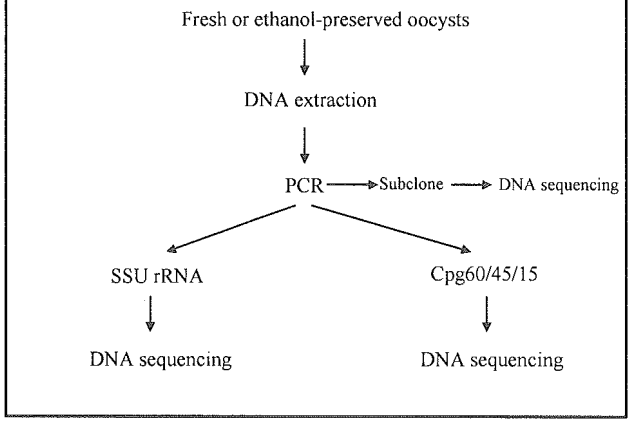
Number of Isolates: 67

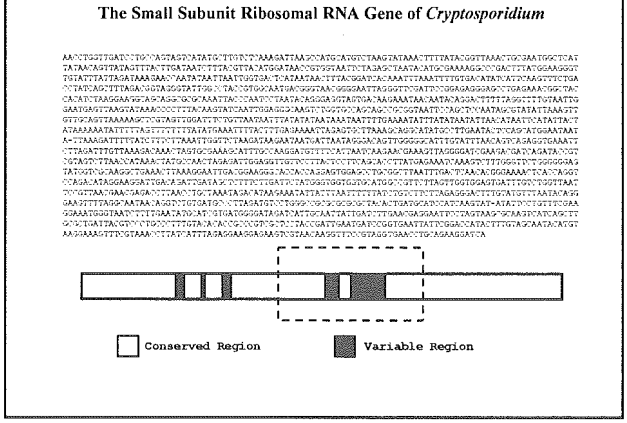
Sex: 55.2% Male, 44.8 % Female

Age: 1-66 years old

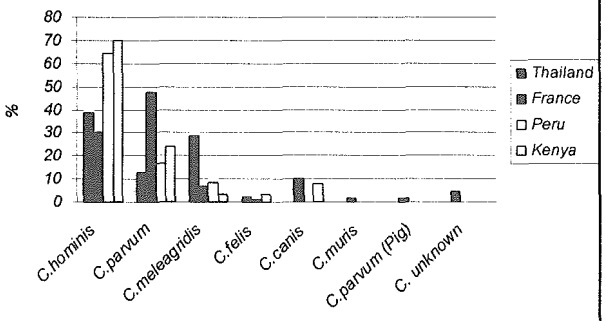
Stool Examinations:

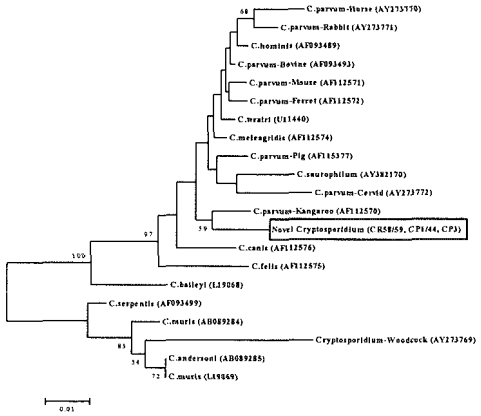
- Direct Smear
- Formalin-Ether Sedimentation
- Acid-fast Staining method





Cryptosporidium species found in HIV-infected patients





Cpg 60/45/15

Mucin-like glycoprotein on the **surface and apical region of invasive stages.**

Possible roles in *Cryptosporidium* **attachment to and invasion** of host cells.

The 60-kDa precursor protein undergoes **proteolytic cleavage** into 15- and 45-kDa fragments.

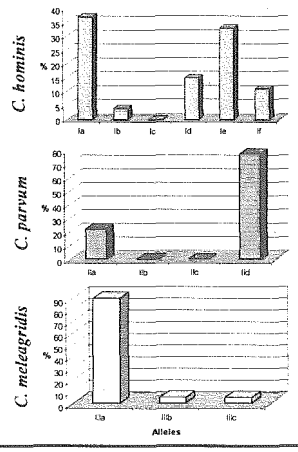
Polymorphism in Cpg60/45/15 can be **a good marker** for strain differentiation.

Sequence of the Cpg60/45/15 of *Cryptosporidium parvum*

TGGAACGTCGTCAAGTAAAAATTGATAACAAATTTTTATACATTTGGCTTGACCCTTCTATAGGTGATAA
TTAGTCAGTCTTTAATAAGTAGGCAACTAAGGACAAAGGAAGATGAGATTGTCGCTCATTATCGTATTAC
TCTCCGTCATAGTCTCCGCTGTATTCTCAGCCCCGGCCGTTCCACTCAGAGGCACCTTGAAGGATGTTTC
TGTTGAGGGATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCA
TCATCATCATCATCAACATCGACCGTCGCACCAGCTCCAAAGAAAGAAAGAACTGTAGAGGGCGGCACGG
AAGGAAAGAACGAAGAAAGCAGTCCAGGTTCTGAAGAACAAGACGGTGGTAAGGAGGACGGTGGTAAGGA
AAACGGTGAAGGAGACACAGTAGACGGGGAACAAACCGGGAGTGGTTCTCAAGTTACTCCATCTGGAAGT
GCCGGCACAGCTACCGAGTCCACAGCAACTACTACTCCAAAGGAAGAATGTGGTACTTCATTTGTCATGT
GGTTCGAGAAAGGCACCCCGGTTGCGACCTTGAAGTGTGGTGATTACACTATCGTCTATGCACCTATAAA
AGATCAAACAGATCCCGCACCAAGATATATCTCTGGTGAAGTTACATCTGTATCCTTTGAAAAGAGTGAA
AGTACAGTTACAATCAAGGTTAATGGTAAAGAGTTCAGCACTCTCTCTGCTAACTCAAGTAGTCCAACTA
AAGATAACGGTGAATCTAGTGACAGTCAGGTTCAATCAAGATCAAGAAGATCACTCGCAGAGGAGAATGG
TGAAACAGTTGCAACAGTTGATTTGTTTGCCTTTACTCTTGATGGTGGTAGAAGAATTGAAGTGGCTGTG
CCAAAGGACGAAAATGCAGACAAAAGAAGCGAGTACAGTTTGGTTGCAGACGATAAGCCTTTCTATACCG
GCGCAAACAGTGGCATCACCAATGGTGTCTACAAATTGGATGAGAATGGAATGGAAACTTGGTTGACAAGGACAA
CAAAGTTCTCTTGAAAGATGCTGGTTCCTCTGCCTTTGGATTCAGATACATCGTTCCTTCCGTTTTTGCA
ATCTTTGCAGCCTTATTCGTGTTGTAAATTTTTTTCAATTAAATTTTAAAAGTTTAAGAGTTTTAAAAGT
AATTACAATGGAAATCTTTCATGCAAATTTGCATTAAAGGTTTTGTTCATTACATTGAATCAGGACGCCA
GTTTTTACAATGCTGAAGAATAATTAATTTTTAATTTCCAGAATTTTCCCACGGGTTCCGCAGGTTTTTT
TCGTATGAATGAGTTGATTATCGTAAACTTATAAGCAAAATAGAGTAAGCAATTTGGTGAGATAATTCGA

(O'Connor et al Mol Biochem Parasitol 2002)

Distribution of Cpg60/45/15 alleles of *Cryptosporidium*



C. hominis (n =27)

Ia	11
Ib	1
Ic	0
Id	4
Ie	8
If	3

C. parvum (n=9)

IIa	2
IIb	0
IIc	0
IId (NEW)	7

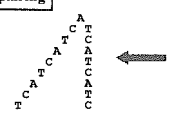
C. meleagridis (n=20)

IIIa	18
IIIb	1
IIIc	1

DNA Replication

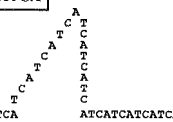
5′....TCATCATCATCATCATCATCATCATCATCATCATCATCATCATCA....3′
3′....AGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGT....5′

Slipped-Strand Mispairing



5′....TCATCATCA ATCATCATCATCATCATCATCATCA....3′
3′....AGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGT....5′

Insertion: TCATCATCA



5′....TCATCATCA ATCATCATCATCATCATCATCATCATCATCATCA....3′
3′....AGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGT....5′

Does cryptosporidiosis cause more morbidity than those caused by other pathogens?

Does any specific species of *Cryptosporidium* cause more severe symptoms than others?

Clinical Features of *Cryptosporidium*-positive and *Cryptosporidium*-negative HIV-infected patients

	Cryptosporidium Positive	*Cryptosporidium* Negative
Numbers	67	75
Age	29.9 ± 12.5	33.3 ± 13.2
CBC		
Hb	33.7 ± 7.8	33.7 ± 7.8
WBC (cells/μl)	6414 ± 409	6646 ± 4297
Neutrophil (%)	59.6 ± 16.1	59.7 ± 16.9
Lymphocyte (%)	26.9 ± 12.8	24.5 ± 14.0
Monocyte (%)	7.5 ± 4.2	8.1 ± 5.0
Eosinophil (%)	3.5 ± 4.5	4.8 ± 6.5
Platelets (/μl)	265750	286520

(mean ± S.D.)

Clinical Features of *Cryptosporidium*-positive and *Cryptosporidium*-negative HIV-infected patients

	Cryptosporidium Positive	*Cryptosporidium* Negative
CD4+lymphocyte (μl)		
Min	4	5
Max	736	702
Mean	124	143
CD8+lymphocyte (μl)		
Min	101	135
Max	2548	1862
Mean	549	797
CD4+/CD8+		
Min	0.01	0.04
Max	1.01	0.7
Mean	0.14	0.14

Clinical Features of *Cryptosporidium*-positive and *Cryptosporidium*-negative HIV-infected patients

	Cryptosporidium Positive	*Cryptosporidium* Negative
No Gastrointestinal Symptom	3	5
Gastrointestinal Symptoms	64	70
Diarrhea		
Watery	61	63
Watery & Mucus	6	8
Bloody Mucus	0	4
Abdominal Pain	15	16
Nausea/Vomiting	14	17
Dyspepsia	21	19
Weight (Kg, mean ± S.D.)	46.7 ± 17.8	45.1± 17.6

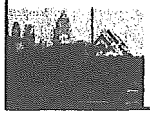
No correlation between species/strains of *Cryptosporidium* and....

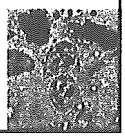
1. Age of the patients
2. Level of CD4+ or CD8+ lymphocytes
3. Characteristics of diarrheal symptoms
4. Weight loss
5. Associated gastrointestinal symptoms

Summary

1. A number of zoonotic species of *Cryptosporidium* circulate among HIV-infected patients including Thailand.
2. Identification of a novel species of *Cryptosporidium* phylogenetically related to *C.parvum* (Kangaroo genotype).
3. Mixed species infection occurs at low prevalence.
4. Extensive polymorphism in the Cpg60/45/15 locus occurs within *C.hominis, C.parvum* and *C.meleagridis.*
5. No specific species or genotype of *Cryptosporidium* defines distinct disease severity.

Molecular characterization of *Isospora belli* and identification of *Caryospora*-like oocysts





Geographic Distribution of *Isospora belli*

Worldwide, especially in tropical and subtropical areas.

Infection occurs frequently in immunodepressed individuals especially those with AIDS.

Outbreaks have been reported in institutionalized groups in the United States in the long past.

Epidemiology of Protozoan Diseases at King Chulalongkorn Memorial Hospital Thailand

Protozoan Infections (6,211 cases)

Protozoa	%	
Giardia lamblia	46.16	
Blastocystis hominis	19.30	
Entamoeba coli	19.21	
Endolimax nana	7.79	
Pentatrichomonas hominis	2.06	
E.histolytica/dispar	2.05	
Isospora belli	1.45	←
Sarcocystis hominis	1.42	
Iodamoeba butschlii	0.48	
Balantidium coli	0.08	

Clinical Manifestations

Asymptomatic Infection (rare)

Acute Watery Diarrhea

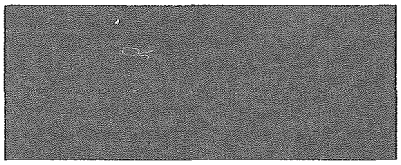
Chronic Watery Diarrhea

Associate symptoms:
anorexia, nausea, vomiting
abdominal pain, malaise
malabsorption, steatorrhea, weight loss
usually no fever

Clinical Manifestations

AIDS Patients

Intestinal infection
Severe diarrhea
Fever, Dehydration
Chronic diarrhea



Relapse in Human Isosporiasis

Mechanism: unknown

Incidence ~ 30% of infected cases. (Pape et al N Eng J Med 1989)

Unizoic cyst in extraintestinal tissues such as lymph nodes in AIDS patients. (Restrepo et al Am J Clin Pathol 1987; Michiels et al Pathol Res Pract 1994; Frenkel et al Rev Soc Bras Med Trop 2003)

Species difference (Cryptic species) ???
→Definite species identification is mandatory.

Research Questions:

1. **Does Genetic Diversity or Cryptic Species Exist?**
   - → Morphometry of Oocyst
   - → Maturation of Oocyst
   - → Sequences of the SSU rRNA
2. **Do Specific Genotypes or Species Confer Disease Severity?**
   - → Clinical Profiles of Isosporisasis Patients and Species/ Genotypes of Isospora.

Patients

38 isosporiasis patients at King Chulalongkorn Memorial Hospital.

Group 1 (HIV-infection): 18 males & 12 females

Group 2 (Corticosteriod): 1 male & 2 females

Group 3 (Immunocompetence): 3 males & 2 females

Methods

Sample Collection: fresh stool or ethanol preserved samples.

Morphometry: measurement of oocyst dimension under 400x magnification from at least 20 oocysts/isolate.

Oocyst Maturation Study: freshly passed stool from 3 patients. Samples were washed with sterile water and kept at ambient temperation. Direct observation under microscope for every 4-6 hours for 20 days.

DNA Sequencing: amplification by PCR encompassing 1.7 kb and direct sequencing of the SSU rRNA gene of *Isospora*.

Isospora species known to infect humans

***Isospora belli*:** cosmopolitan, generally known.

***Isospora natanlensis*:** one case report in Malaysia (Elsdon-Dew J Trop Med Hyg 1953).

No additional reports in humans.

Measurement of oocysts of *Isospora* species from mammals

Species	Host	Dimensions (μm) of:	
		Oocysts	Sporocysts
I. belli	Humans	23-36 x 12-17	12-14 x 7-9
I. natalensis	Humans	24-30 x 21-25	17 x 12
I. arctopitheci	NH primates	21-30 x 21-25	13-21 x 10-16
I. callimico	NH primates	13-21 x 12-17	10-13 x 7-9
I. endocallimici	NH primates	25-31 x 21-27	15-20 x 10-15
I. scorzai	NH primates	23 x 20	14 x 9
I. canis	Dogs	34-40 x 28-32	18-21 x 15-18
I. ohioensis	Dogs	19-27 x 18-23	15-19 x 10-13
I. burrowsi	Dogs	17-22 x 16-19	12-16 x 8-11
I. rivolta	Cats	18-28 x 16-23	14-16 x 10-13
I. felis	Cats	38-51 x 27-39	20-26 x 17-22
I. suis	Pigs	17-25 x 16-21	11-14 x 8-11

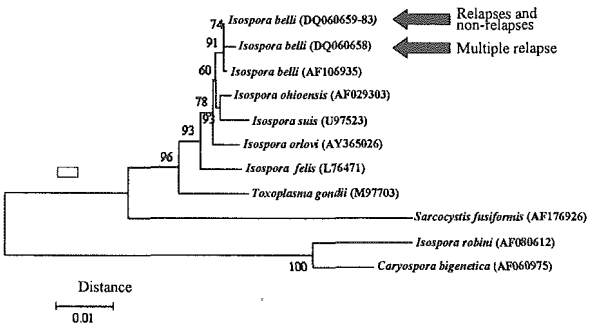
Caryospora-like oocyst of *Isospora*

Less than 2% of *Caryospora*-like oocysts were observed in several species of *Isospora* such as *I. canis, I. suis* and *I.rivolta*.

(Lepp et al Trans Am Micros Soc 1976; Linsay et al J Parasitol 1982; Matsui et al Parasitol Res 1993)

"Observation on human *Isospora*"
(Zaman V. Trans R Soc Trop Med Hyg 1968)

Phylogenetic Relationship among *Isospora* and Related Coccidia



Conclusions

1. First report on genetic diversity in *Isospora belli*.
2. Single species of *Isospora* infecting humans.
3. Heterogenous development of oocyst:
   Unknown significance, need further study.
4. No correlation between specific strain (based on *SSU rRNA*) and disease severity.
5. *Isospora belli* possesses 2 types of oocysts.

Future Plan

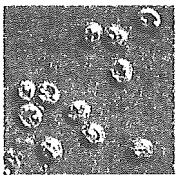
-Giardia intestinalis

-Blastocystis hominis

Molecular epidemiology of cryptosporidiosis in Japan

S. Izumiyama, K. Yagita and T. Endo.
Department of Parasitology,
National Institute of Infectious Diseases, Japan.

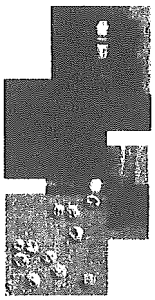
- Protozoan parasite
- 4 ~ 5 x 5 ~ 6 μm
- Chlorine resistance
- Waterborne outbreaks



Cryptosporidium oocysts

We had 4 large-scale outbreaks of cryptosporidiosis cases in Japan

- 1994 Hiratsuka 461 cases
- 1996 Ogose 8,812 cases
- 2002 Hokkaido 129 cases
- 2004 Nagano 283 cases



Loci reported for genotyping of *Cryptosporidium*

- 18S rRNA (small subunit rRNA)
- poly-threonine
- hsp70 (heat shock protein)
- gag-repeat (microsatellite)
- cowp (cryptosporidium oocyst wall protein)
- actin
- cpgp 40/15 (C. parvum glycoprotein)

Genotypes of 4 cryptosporidiosis outbreaks in Japan

- 1994 Hiratsuka *C. parvum* genotype 2
- 1996 Ogose *C. parvum* genotype 1
- 2002 Awaji *C. parvum* genotype 1
- 2004 Nagano *C. parvum* genotype 1

Cryptosporidium isolates in Japan

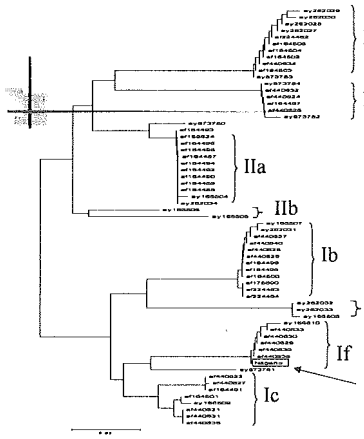
Isolate		Identification	Genotype
Cp/H01-04, 1997	Outbreak in Ogose	*C. parvum*	1
Cp/H05, 1997	AIDS	*C. parvum*	1
Cp/H06, 1997	-*	*C. parvum*	1
Cp/H07, 1997	Overseas traveler; Indonesia	*C. parvum*	1
Cp/H08, 1997	-	*C. parvum*	2
Cp/H09, 1998	Overseas traveler; Indonesia		
Cp/H10, 1998	-		
Cp/H11, 1998	Overseas traveler; Nepal/Thailand	*C. parvum*	1
Cp/H12, 1998	Overseas traveler; India/Pakistan	*C. parvum*	1
Cp/H13, 1998	Overseas traveler; Indonesia		
Cp/H14, 1998	Overseas traveler; Thailand/Indonesia	*C. parvum*	1
Cp/H15, 1998	Overseas traveler; Kenya	*C. parvum*	2
Cp/H16, 1998	Osaka; congenital immunodeficiency	*C. felis*	
Cp/H17, 1998	Osaka; congenital immunodeficiency	*C. parvum*	1
Cp/H18, 1998	AIDS	*C. parvum*	1
Cp/H19, 1998	Outbreak in Hiratsuka	*C. parvum*	2
Cp/H20, 1999	Overseas traveler; India/Nepal	*C. parvum*	1
Cp/H21, 1999	Overseas traveler; India/Pakistan	*C. parvum*	1
Cp/H22, 1999	Overseas traveler; India	*C. parvum*	1
Cp/H23, 1999	Overseas traveler; Thailand	*C. felis*	
Cp/H24, 1999	Overseas traveler; India	*C. parvum*	1
Cp/H25, 2001	Overseas traveler; Singapore/Madagasc	*C. parvum*	1
Cp/H26, 2001	Tokyo	*C. parvum*	1
Cp/H27, 2001	Tokyo	*C. parvum*	2

Isolate		Identification	Genotype
Cp/H28, 2001	Overseas traveler; Zimbabwe/Madagasca	*C. parvum*	1kN14-p
Cp/H29, 2001	Overseas traveler; India/Bangladesh	*C. parvum*	1/2 mix
Cp/H30, 2001	Kanagawa; AIDS	*C. parvum*	1
Cp/H31, 2001	-	*C. parvum*	1
Cp/H32, 2002	AIDS	*C. parvum*	1
Cp/H33, 2002	Overseas traveler	*C. parvum*	1
Cp/H34, 2002	-	*C. parvum*	1
Cp/H35-39, 2002	Outbreak in Hokkaido	*C. parvum*	1
Cp/H40, 2003	-		
Cp/H41, 2003	-	*C. parvum*	2
Cp/H42, 2003	-		
Cp/H43, 2003	-	*C. parvum*	2
Cp/H44, 2003	AIDS	*C. parvum*	1
Cp/H45, 2003	Tokyo	*C. parvum*	
Cp/H46, 2003	Overseas traveler; India	*C. parvum*	1
Cp/H47, 2003	AIDS	*C. parvum*	1
Cp/H48, 2003	AIDS	*C. parvum*	1
Cp/H49, 2003	AIDS	*C. parvum*	1
Cp/H50, 2003	AIDS; Giardia infection	*C. parvum*	1
Cp/H51, 2003	Tokyo; AIDS	*C. parvum*	1
Cp/H52, 2004	Overseas traveler; Madagascar	*C. felis*	
Cp/H53, 2004	-		
Cp/H54-59, 2004	Outbreak in Nagano	*C. parvum*	1

* no information

Sequence homology between the Nagano isolates and the record of the public databases (DDBJ/EMBL/GenBank)

- 18S rRNA 99.8% (1279/1281, AF093489)
- poly-T 99.6% (478/480, AB126002)
- gag-repeat 98.5% (191/194, AJ249587)
- cowp 100% (506/506, AF266272)
- actin 100% (1059/1059, AF382337)
- cpgp 40/15 93.0% (845/909, AY700391)



Phylogenetic analysis of cpgp40/15 nucleotide sequence obtained from the Cryptosporidium outbreak in Nagano

C. parvum genotype 1, cluster If of cpgp 40/15 from phylogenetic analysis

Appropriate procedure for genotyping of cryptosporidiosis

- PCR-Direct sequencing
- 18S rRNA locus for genus level
- cpgp 40/15 locus for sub-species level