most prevalent species found in HIV-infected patients in France, Switzerland, Portugal, and United Kingdom (12,14-17). On the other hand, *C. hominis* shows the highest prevalence in Thailand, Japan, Peru and Kenya (18-21). However, several factors could be responsible for the occurrence of different patterns of species distribution such as host immunological status, the prevalence of animal reservoirs in each region, sources of infections being an outbreak or a sporadic infection including the method for manipulating water resources for domestic use. Therefore, further studies are required to address this issue.

Sequence analysis of the Cpg60/45/15 locus demonstrates a remarkable degree of genetic heterogeneity within species of *Cryptosporidium* in Thailand. The present study also confirms and extends similar observation by others (5,12). Although several Cpg60/45/15 alleles of Thai isolates belong to the same subgenotypes as those previously reported, microheterogeneity of sequence exists, rendering it an attractive marker for subgenotyping or strain differentiation of *C. hominis*, *C. parvum* and *C. meleagridis*. Subgenotypic characterization of *Cryptosporidium* could be useful for tracing parasite strain during outbreak investigation and evaluation of therapeutic responses to certain inconclusive efficacy of anti-cryptosporidial agents.

Meanwhile, we observe no significant correlation between species or subgenotypes of *Cryptosporidium* and clinical symptoms of the patients in this analysis. Human acquisition of *Cryptosporidium* can plausibly be from diverse sources and the zoonotic transmission cycles of this coccidian protozoan in Thailand are common among HIV-infected patients. Species and strain differentiations of *Cryptosporidium* undoubtedly form a fundamental basis for disease control and prevention.

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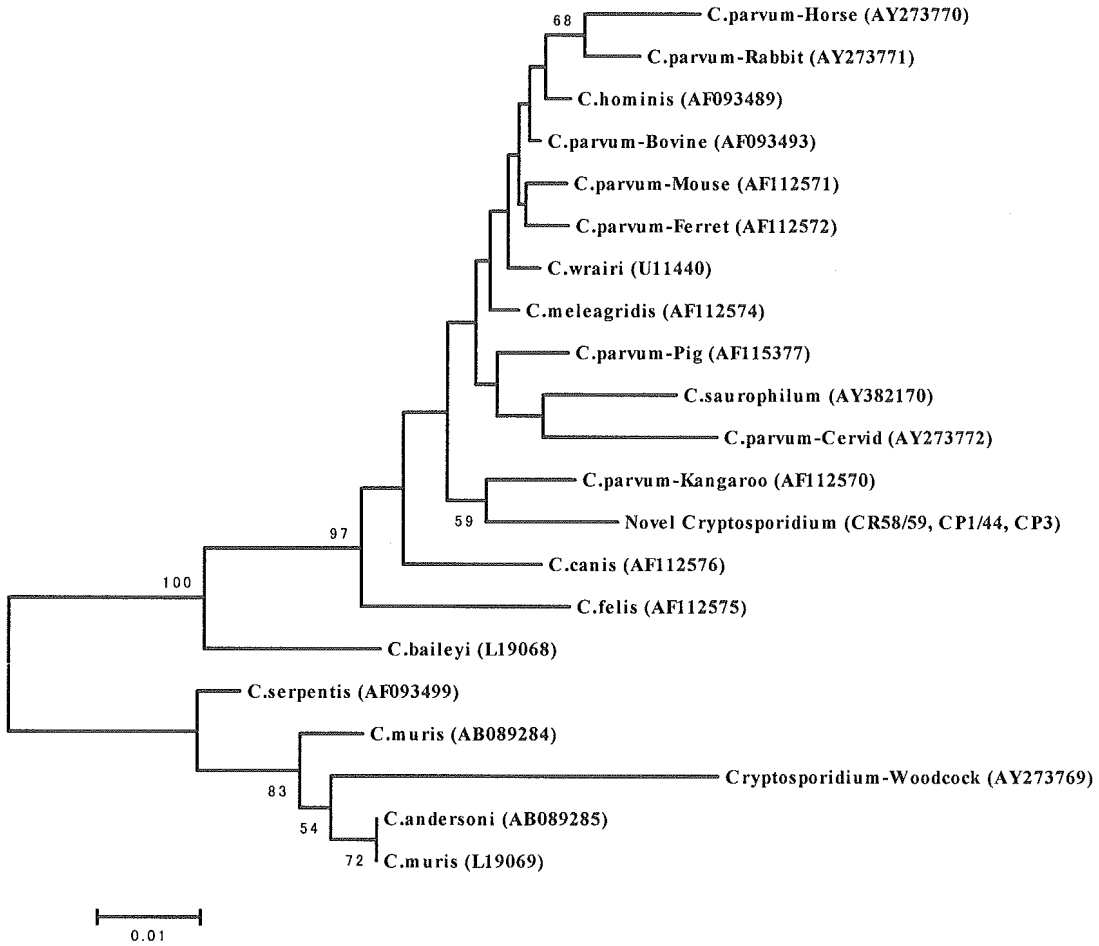

Figure 1 Phylogenetic tree illustrating the relationship between the novel *Cryptosporidium* found in this study and other genetically distinct species.

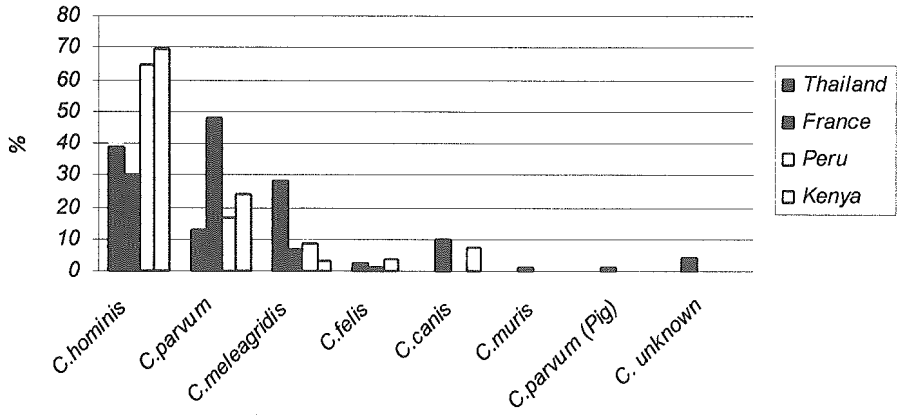

Figure 2 Geographic distribution of *Cryptosporidium* species in Thailand, France, Peru and Kenya

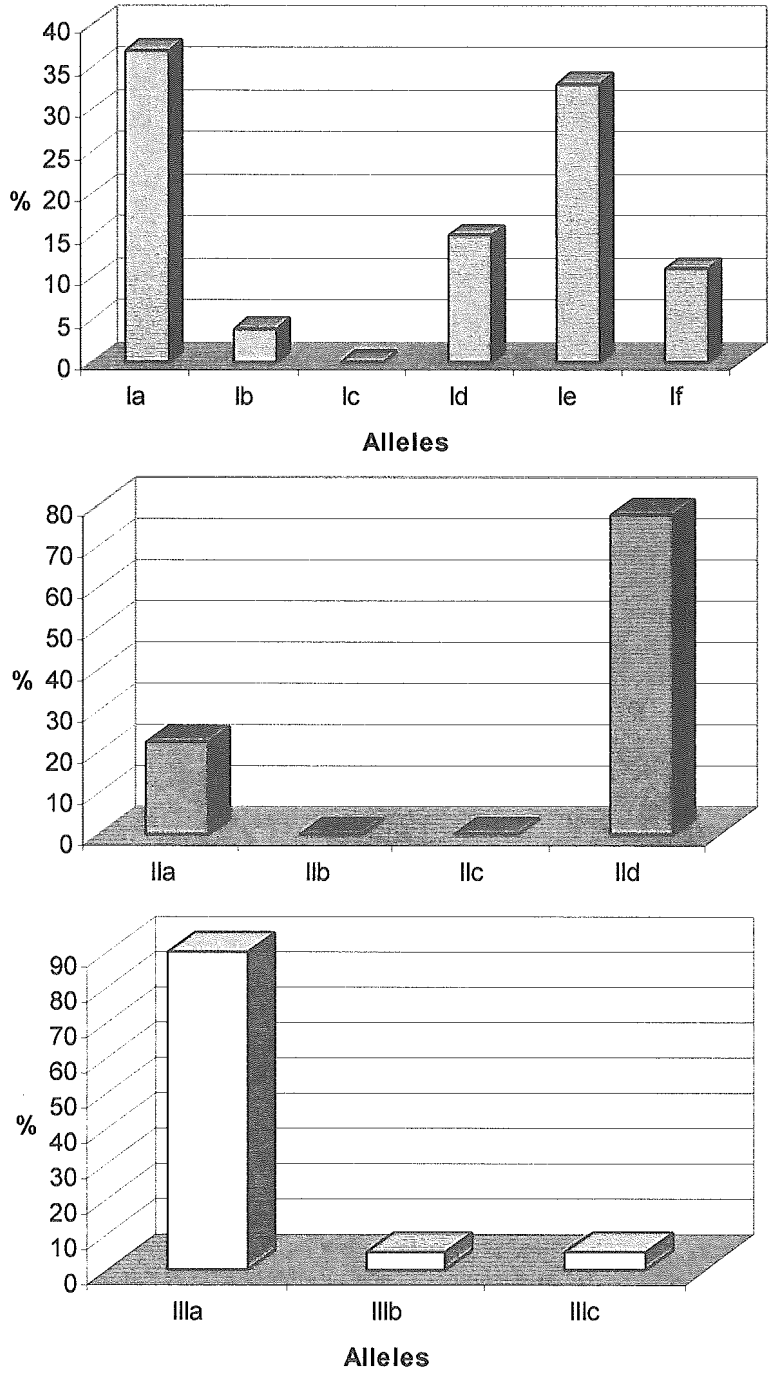

Figure 3 Distribution of Cpg60/45/15 alleles of *Cryptosporidium* found in Thailand

Main Project: Molecular characterization of *Cryptosporidium* spp, *Isospora belli*, *Giardia intestinalis* and *Blastocystis hominis* among Thai patients

Project 2: First Year Report

Title: Isosporiasis in Thailand: Morphometric and molecular analysis

Name of Researchers: Somchai Jongwutiwes MD, PhD [1]
Chaturong Putaporntip PhD [1]
Takuya Iwasaki MD, PhD [2]
Hiroji Kanbara MD, PhD [3]
Takuro Endo PhD [4]

Affiliations:

1. Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Thailand
2. Department of Laboratory Investigation, Institute of Tropical Medicine, Nagasaki University
3. Department of Protozoology, Institute of Tropical Medicine, Nagasaki University
4. Department of Parasitology, National Institute of Infectious Diseases

Summary:

Isosporiasis is an important enteric coccidiosis found in both immunocompetent and immunocompromised patients in Thailand, especially HIV-1 infected patients with low CD4+ lymphocyte counts. We recruited 38 patients infected with *Isospora belli* identified by examination of 78,743 stool specimens submitted to parasitology laboratory of King Chulalongkorn Memorial Hospital in Bangkok during 2002-2004. Isosporiasis patients comprised 35 Thai from diverse regions of the country and 3 recent immigrants from Cambodia, Laos and Pakistan. Of these, 30 were HIV-positive, 3 received prolonged corticosteroid therapy for other diseases and 5 immunocompetent individuals. To address if genetic heterogeneity or cryptic species occurring in *Isospora* infecting humans, we determined the morphometry of oocysts from all patients and performed sequencing analysis of the small subunit ribosomal RNA gene (*SSU rDNA, 18S RNA*) spanning 1,680 base pairs from 26 isolates. Morphometric study of oocysts revealed that the oocyst

dimension in this study varied from 17 to 37 (28.3±3.0) micrometers in length, 8 to 21 (13.5±1.9) micrometer in width and the shape index (length by width) 1.3 to 3.3 (2.1±0.3). Although the oocysts exhibited shape and size variations both within and among isolates in this study, the shape indices of all oocysts observed were consistent with that of *I. belli* being more than 1.2 (range=1.3-3.3), which were distinct from those for *I. natanlensis* and other species infecting nonhuman mammals (<1.2). Additionally, we observed oocyst maturation after passage from intestine from 3 patients who had not yet taken anti-coccidial drugs: 2 HIV-infected patients, one of them had relapse, and an immunocompetent patient presented with chronic diarrhea. In total 100 oocysts for each isolate were examined for sporulation every 6 -8 hours under light microscope using 400x magnification for 20 days. Results revealed that 27% of oocysts (range=20-33%) underwent complete sporulation and the duration for generating 2 sporocysts each of which contained 4 sporozoites was variable ranging from 24 hours to 10 days (3.9±3.4 days)(n= 66). More interestingly, fully sporulated oocysts having a single sporocyst covering 8 sporozoites, designated *Caryospora*-like oocysts, were found in all 3 isolates representing 9-29% of all mature oocysts (n=15). The SSU rDNA sequences revealed minimal sequence variation containing 2 sequence types. Thus, no correlation between distinct *I. belli* strain and disease severity was observed. Phylogenetic tree showed that *I. belli* in this study was within the same clade as *I. ohioensis, I. suis, I. orlovi, I. felis, Toxoplasma gondii* and *Sarcocystis* sp. In conclusion, unlike *Cryptosporidium* infecting humans that comprises both zoonotic and anthroponotic species, our study demonstrated that human isosporiasis is caused by a single species belonging to *I. belli* based on morphometric and molecular evidences.

Introduction

Isospora belli is a coccidian protozoan in phylum Apicomplexa that parasitizes epithelium of upper small intestine of human causing diarrhea. Since the pandemic of human immunodeficiency virus-1 (HIV-1) infection, human isosporiasis has been commonly identified; especially patients who had low CD4+ lymphocyte counts. *I. belli* infection usually produces more aggressive and prolonged period of symptoms in immunocompromised hosts than immunocompetent individuals (1,2). In general, the patients respond well to combination of dihydrofolate reductase/thymidylate synthase inhibitors and sulfonamides. However, relapses have been observed that require repeat treatment or prophylaxis (3,4). Despite the increasing significance of enteric coccidiosis, comparatively little has been known about *Isospora* infecting humans. To address if genetic heterogeneity or cryptic species exists, we performed molecular analysis and morphometric study of oocysts of *I. belli* from patients

who had normal immune status and those with compromised immunity.

Purposes:

1. To determine species and intraspecific variation of *Isospora belli* among Thai patients.
2. To correlate clinical severity among strains of *Isospora belli.*
3. To study the sporulation of *Isospora belli.*

Methods:

During January 2002-December 2004, 38 patients with isosporiasis were identified by examination of 78,743 stool specimens submitted to parasitology laboratory of King Chulalongkorn Memorial Hospital in Bangkok. Isosporiasis patients comprising 35 Thai from diverse regions of the country and 3 recent immigrants from Cambodia, Laos and Pakistan, were diagnosed by the presence of *I. belli* oocysts in stool samples using direct unstained smear and/or formalin-ethylacetate sedimentation method. Aliquots of each *I. belli*-positive stool sample were preserved by adding approximately 4 volumes of absolute ethanol and stored at ambient temperature. Three freshly passed stool samples were observed for sporulation of *I. belli* oocysts every 6 hours under light microscope using 400x magnification for 20 days.

DNA of *I. belli* was extracted from either fresh stools or ethanol preserved specimens by the method described previously except that the QIAamp DNA Stool Mini Kit (QIAGEN, Germany) was used. The small subunit ribosomal RNA gene (*SSU rDNA*) of *I. belli* was amplified by nested PCR. We used the same thermal cycling profiles for both primary and secondary PCR: denaturation at 94°C, 40 s; annealing at 64°C, 40 s; extension at 74°C, 2 min, and 30 cycles of amplification. Sequences of the outer pair of primers were Iso-18SF0, 5'-CTGGTTGATCCTGCCAGTA-3' and Iso-18SR0, 5'-TCCGTAGGTGAACCTGCC-3', and the inner pair, Iso-18SF1, 5'-GATCCTGCCAGTAGTCAT-3' and Iso-18SR1, 5'-TCCGGTGAATTATTCGGACC-3'. To minimize the error introduced in the sequences during PCR amplification, we used ExTaq DNA polymerase that possesses efficient 5'→3' exonuclease activity for increased fidelity and no strand displacement (Takara, Japan). Sequences were determined directly from both directions for each isolate using purified PCR products from two independent amplifications and the Big Dye Terminator v3.1 Cycle Sequencing Kit in an ABI310 Genetic Analyzer (Applied Biosystems, USA).

Results

Of 38 *I. belli*–infected patients, 30 were HIV-positive, 3 received prolonged corticosteroid therapy because of systemic lupus erythematosus or idiopathic thrombocytopenic purpura, and 5 immunocompetent individuals. More than half of isosporiasis patients were 30-39 years old and the ratio of male to female 1.38:1. Laboratory tests showed relative eosinophilia (>4%) in half of HIV-positive and all immunocompetent patients (Table 1). The absolute CD4+lymphocyte counts in HIV-positive patients were 8-484 (mean±S.D.=67.5±57.4) cells/μl

All HIV-infected individuals developed watery diarrhea ranging from 1 week to 1 year (3.3 ±3.4 months) while 2 patients who received corticosteroids presented with chronic watery diarrhea and the other without symptom. The clinical profiles of isosporiasis in immunocompetent patients were asymptomatic, chronic watery diarrhea, and dyspepsia without diarrhea, occurring in 2, 2 and 1 case, respectively. Diarrhea and dyspepsia resolved within a few days after initiation of treatment with combination of trimethoprim and sulfamethoxazole. Relapse occurred once in 2 HIV-infected patients that could be controlled by repeat treatment. Meanwhile, multiple recurrent episodes of diarrheal symptoms over a decade due to isosporiasis occurred in an immunocompetent patient who required prolonged treatment with pyrimethamine.

To date, *I. belli* is considered to be the main species that causes disease in humans. However, *I. natanlensis* was incidentally detected in stool of a patient in 1953; however, since then no subsequent report has confirmed the role of this species in human infection (5). Oocysts of *I. belli* can be structurally differentiated from *I. natanlensis*: the former measures 23-36x12-17 μm, the latter 24-30x21-25 μm (1). Therefore, we carried out a morphometric study of oocysts of all isolates by measuring the length and width of each oocyst under 400x magnification. Results revealed that the oocyst dimension in this study varied from 17 to 37 (28.3±3.0) μm in length, 8 to 21 (13.5±1.9) μm in width and the shape index (length by width) 1.3 to 3.3 (2.1±0.3). Although the oocysts exhibited shape and size variations both within and among isolates in this study, the shape indices of all oocysts observed were consistent with that of *I. belli* being more than 1.2 (range=1.3-3.3), which were distinct from those for *I. natanlensis* and other species infecting nonhuman mammals (<1.2)(1).

To address if genetic heterogeneity or cryptic species occurring in *Isospora* infecting humans, we amplified the small subunit ribosomal RNA gene (*SSU rDNA*) by nested PCR spanning 1,680 base pairs from 26 isolates. All isolates examined yielded single PCR fragments at expected size (data not shown). Results showed that *SSU rDNA* of 23 isolates from HIV-infected patients and 2

isolates from asymptomatic and symptomatic immunocompetent subjects (DQ060659-83) were identical with those of strains CI1 and CJLPHD2 (GenBank accessions U94787 and AF441289) but differed from the isolate reported by Franzen et al. (AF106935) at A679T and A682C (positions after U97487)(6). Meanwhile, 3 additional nucleotide substitutions occurred at T583C, C638A and G1240T of an sequence of the isolate that caused multiple relapses in this study (DQ060658). Phylogenetic tree showed that *I. belli* in this study was within the same clade as *I. ohioensis, I. suis, I. orlovi, I. felis, Toxoplasma gondii* and *Sarcocystis* sp. but not *I. robini* and *Caryospora begenetica* (Figure 1).

Additionally, we observed oocyst maturation after passage from intestine using freshly passed watery stool samples from 3 patients who had not yet taken anti-coccidial drugs: 2 HIV-infected patients, one of them had relapse, and an immunocompetent patient presented with chronic diarrhea. These stool samples were directly diluted with equal volume of sterile water. The stool suspension was applied onto clean glass-slides, covered with 22x22 mm cover-slips and the edges of cover-slips were tightly sealed and incubated at ambient temperature (25-30°C) in humidifier boxes to minimize evaporation of fluid from the samples. In total 100 oocysts for each isolate were examined for sporulation every 6 hours under light microscope using 400x magnification for 20 days. Results revealed that 27% of oocysts (range=20-33%) underwent complete sporulation and the duration for generating 2 sporocysts each of which contained 4 sporozoites was variable ranging from 24 hours to 10 days (3.9±3.4 days)(n= 66). More interestingly, fully sporulated oocysts having a single sporocyst covering 8 sporozoites, designated *Caryospora*-like oocysts, were found in all 3 isolates representing 9-29% of all mature oocysts (n=15) with the earliest appearance on day 5 after incubation (range=5-14 days).

Discussions

Unlike *Cryptosporidium* infecting humans that comprises both zoonotic and anthroponotic species, our study demonstrated that human isosporiasis is caused by a single species belonging to *I. belli* based on morphometric and molecular evidences. Although isosporiasis is more common among immunocompromised patients than immunocompetent hosts, it seems likely that the severity of infections does not simply depend on the immune status of infected individuals because some immunocompetent patients exhibit chronic debilitating illness with multiple recurrent prolonged diarrheal episodes (1,7). On the other hand, minimal sequence variation in *SSU rDNA* of *I. belli* has suggested that the possibility of cryptic species of *Isospora* responsible for varying degree of

morbidity is also unlikely. In this study we identified a novel sequence of *SSU rDNA* in an isolate from a patient with multiple episodes of relapses. Whether strain difference in *I. belli* contributes to disease severity required further investigation.

The life cycle of *I. belli* is known to be momoxenous in human without known intermediate or paratenic hosts. In general, sporogony of *Isospora* occurs outside the host. The stages of oocysts that are freshly passed from large intestine mostly contain a single sporont, but oocysts with 2 sporoblasts can be occasionally encountered. The duration for complete sporulation of *I. belli* described herein is highly variable. Although we used different experimental conditions from other studies, the earliest appearance of fully sporulated oocysts is similar, i.e. within 24 hours (8,9). It is well recognized that mature oocysts of *I. belli* produce 2 sporocysts, each with 4 sporozoites. Interestingly, a small percentage around less than 2% of mature oocysts containing 8 sporozoites in a single sporocyst, known as *Caryospora*-like, have been reported in other species of *Isospora* infecting mammals such as *I. canis, I. suis* and *I. rivolta* (10-12). The presence of *Caryospora*-like oocysts of *I. belli* was first described in 1968 by Zaman who studied sporogonic development of *I. belli* from patients (8). However, no additional studies have confirmed the presence of this stage. In this study we demonstrated the presence of *Caryospora*-like oocysts in all 3 isolates examined after an extended period of incubation, suggesting that this stage is a normal exogenous development of *I. belli*. Factors such as temperature, moisture, the level of oxygen and other unknown conditions could influence the capability and duration required for complete sporulation of the oocysts (1). If viable period of *I. belli* after complete sporulation was limited, unsynchronized sporulation could extend the period of transmission of this important enteric coccidian.

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Publication list for this work:

Manuscript in preparation.

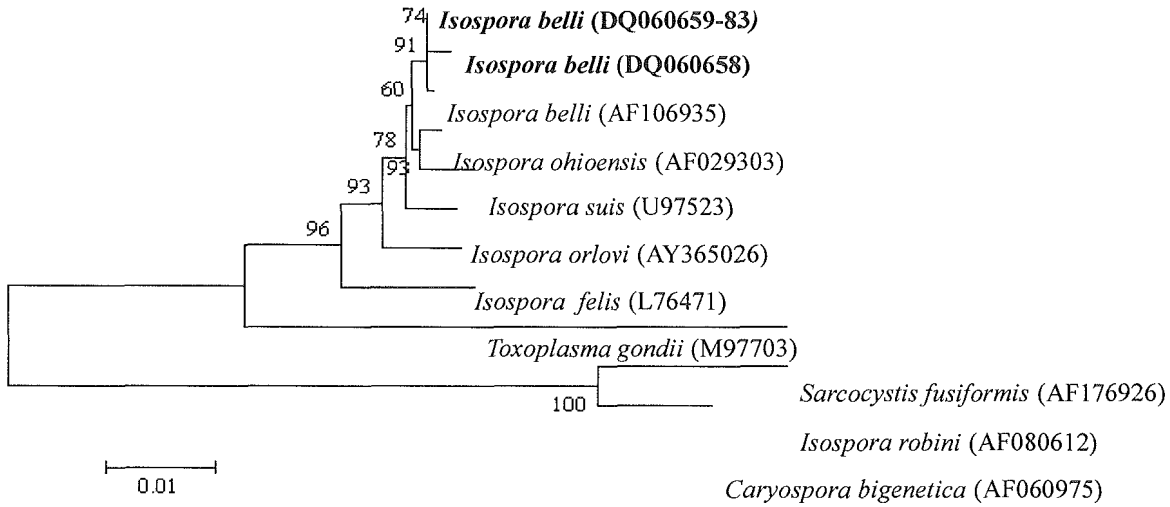

Figure 1. Phylogenetic relationships among isolates of *Isospora belli* in this study (DQ0606 and reported by others (U94787 and AF106935) in relation to other nonhuman species of *Isos ohioensis*, *I. suis*, *I. orlovi*, *I. felis*, and *I. robini)*, *Sarcocystis fusiformis.*, *Toxoplasma gon Caryospora bigenetica* as inferred from the SSU rDNA sequences and the neighbor-joining n Bootstrap percentages more than 50% based on 1,000 replicates are shown on the branches.

Table 1 Clinical profiles of isosporiasis and morphometry of *Isospora belli* oocysts

Patient profiles	n	age range (mean±SD)	CD4+ cell/μl range (mean±SD)	Eosinophil(%) range (mean±SD)	Oocyst dimension*		
					Length(μm) range (mean±SD)	Width(μm) range (mean±SD)	Shape Index range (mean±SD)
HIV infection (18 males, 12 females)							
Diarrhea							
?3 weeks	5	23-52 (35.6±11.0)	89-134 (104.3±25.7)	0.4-9.1 (5.4±3.6)	18-33 (27.2±2.6)	8-19 (12.8±1.8)	1.3-3.0 (2.2±0.3)
>3 weeks - <1 year	22	25-50 (36.7±6.9)	8-480 (60.4±52.9)	0.1-14.0 (4.5±3.3)	17-35 (28.4±2.9)	8-21 (13.7±1.9)	1.3-3.3 (2.1±0.3)
?1 year	3	21-37 (27.7±8.3)	25-484 (80.3±53.6)	2.0-5.5 (3.5±1.8)	18-34 (28.4±3.8)	10-19 (13.8±2.2)	1.4-3.0 (2.1±0.4)
Corticosteroid treatment (1 male, 2 females)							
No symptom	1	37	ND	0.8	30-37 (33.7±2.0)	12-18 (14.0±1.6)	1.9-2.7 (2.4±0.2)
Diarrhea							
?3 weeks	1	51	ND	0	23-32 (28.3±2.6)	10-15 (12.8±1.2)	1.8-3.1 (2.2±0.3)
>3 weeks	1	23	ND	1.0	23-30 (27.4±2.1)	11-16 (13.4±1.3)	1.4-2.6 (2.1±0.3)
Immunocompetence (3 males, 2 females)							
No symptom	1	37	ND	12.0	25-31 (28.0±1.9)	11-17 (14.0±1.9)	1.5-2.5 (2.0±0.3)
Dyspepsia	1	31	ND	11.1	25-35 (30.3±2.6)	12-16 (13.7±1.2)	1.6-2.5 (2.2±0.3)
Diarrhea							
<1 year	2	29-32 (30.5±2.1)	ND	8.0-9.0 (8.5±0.7)	20-32 (27.0±2.4)	9-18 (12.6±2.2)	1.5-3.1 (2.2±0.4)
?1 year	1	57	730	16.0	26-30 (28.4±1.4)	12-17 (15.6±1.7)	1.6-2.3 (1.8±0.2)

* Measurement under 400x magnification from 20 oocysts from each isolate.

RAPPORTEUR'S REPORT

Malaria and Enteric Protozoa Meeting, Japan
31 January – 01 February 2006

The meeting started at exactly 9:50 in the morning with **Dr. Takuro Endo** giving the **Opening Remarks** to welcome the participants coming from different Asian countries. This was followed by the appropriate introduction on the goals of the **"Network on Control of Infectious Diseases in Asia"** by **Dr. Haruo Watanabe**, Vice-Director of the National Institute of Infectious Diseases.

There were **3 sessions** on the first day, January 31st. The first 2 sessions were devoted to paper presentations on malaria: 4 papers in the morning (**Dr. Bell** of WHO, **Dr. Espino** from the Philippines, **Dr. Wibisono** from Indonesia and **Dr. Kanbara** from Japan) and 4 papers in the afternoon (**Dr. Prachumsri** from Thailand, **Dr. Sinuon** from Cambodia, **Dr. Kawamoto** from Japan, and **Dr. Lee** from Korea). The third session consisted of 3 paper presentations on enteric protozoa (**Dr. Natividad** from the Philippines, **Dr. Jongwutives** from Thailand, and **Dr. Izumiyama** from Japan).

At the end of the last session, the Rapporteur gave the summary of the day's presentations and discussions.

The second day (February 1st) was a half-day meeting, which consisted of one session for paper presentations and one session for discussion. There were 3 paper presentations on malaria (**Drs. Ishikawa, Tsuboi** and **Ohmae** from Japan). After the last presentation, Dr.Takuro Endo opened an *en banc* discussion on the "Future plan of research".

Other highlights of the meeting were 2 social activities. A small dinner party was held on January 31st. This served as a venue for more informal interaction among the participants in a more relaxed atmosphere. The topics on malaria and enteric protozoa were set aside momentarily, and other less serious matters were discussed. On February 1st, the participants enjoyed the half-day bus tour of Tokyo despite the rain and cold weather.

All in all, this initial meeting of the Malaria and Enteric Protozoa Group of the Asian Laboratory Network on Infectious Diseases achieved more than its goal of bringing together the network members in a forum. The meeting has served to encourage each member to be involved in gathering accurate data through high quality research. In this way, information that is shared from every country will be accurate and will lead to effective control and treatment strategies for infectious diseases, which is the main goal of the network.

The group's slogan, **"We need to make it happen!"**, reflects the feeling of enthusiasm in very participant after the meeting.

The pages that follow contain the summary of the discussions that ensued on the "Future Plan of Research".

Malaria and enteric protozoan infections in Southeast Asia

(Current situation and future control plan)

January 31 – February 1, 2006

National Institute of Infectious Diseases(NIID)

1-23-1 Toyama, Shinjuku, Tokyo, Japan

January 31 (Tuesday)

9 :50			Opening
9 :50	～	10:10	Research project:Construction of laboratory-based net work in Asia and Pan-Pacific Rim (Watanabe H. Vice Director, NIID.)
			Chairpersons: Drs Ohmae H & Sinuon M.
10:10	～	10:40	Asian Vivax Malaria Network: Addressing the re-emerging of vivax malaria (Bell D. Scientist, Malaria, Other Vectorborne and Other Parasitic Diseases, WPRO)
10:40	～	11:10	Current situation of malaria in the Philippines: research needs to improve control (Espino F.E. RITM, DOH, the Philippines)
11:10	～	11:40	Strengthening Malaria Surveillance System in Central Java Province (Wibisono H., Tobing C. Vectorborne Diseases Control Programme, MOH, Indonesia)
11:40	～	12:00	How to use chemotherapy for malaria control (Kambara H. Inst. Trop. Med., Nagasaki Univ.)
12:00	～	13:00	Lunch
			Chairpersons: Drs Kambara H & Eibisono H.
13:00	～	13:30	Research on Malaria Transmission in Thailand (Prachumsri J.S. Dept. Entomol., AFRIMS. Thailand)
13:30	～	14:00	Malaria situation and control programme in Cambodia (Sinuon M. Malaria Centre, MOH, Cambodia)
14:00	～	14:20	Epidemiology of G6PD deficiency in Southeast Asia (Kawamoto F. Oita Univ.)
14:20	～	14:50	Border malaria characters of Korea *Plasmodium vivax* and its Control (Lee H.W. Div. Malaria & Parasitic Dis., NIH, Korea)

14:50 ～ 15:20 Coffee break

Chairpersons: Drs Furuya K & Jongwutiwes S.

15:20 ～ 15:50 Current situation of enteric protozoan infections in the Philippines and research needed for better control
(Natividad F.F. St. Luke's Med. Center, the Philippines)

15:50 ～ 16:20 Cryptosroridiosis and isosproriasis in Thailand: Morphometric and morecular analysis
(Jongwutiwes S. et al. Dept. Parasitol., Chulalongkorn Univ., Thailand)

16:20 ～ 16:40 Molecular epidemiology of cryptosporidiosis in Japan
(Endo T, Izumiyama S, Yagita K. Dept. Parasitol., NIID)

16:40 ～ 17:00 Dynamic changes of malaria epidemiology in Southeast Asia and South Pacific areas and researches for next steps of control
(Ohmae H, Endo T. Dept. Parasitol., NIID)

Chairpersons: Drs Endo T & Ohmae H

17:00 ～ 17:30 Discussion

17:30 Closing

18:30 ～ A small party

February 1 (Wednesday)

Chairpersons: Drs Endo T & Ohmae H

9 :30 ～ 9 :50 Mathematical model of malaria transmission and control
—Re-emerging of vivax malaria in Korea—
(Ishikawa H., Fujii K. Okayama Univ.)

9 :50 ～ 10:10 Development of transmission blocking vaccine of *Plasmodium falciparum*
(Tsuboi T. Cell-free Sci. & Tech. Res. Center, Ehime Univ.)

10:10 ～ 11:10 Discussion (Future plan of research)

11:10 Closing

Laboratory Network on Control of Infectious Diseases in Asia

Tokyo, Japan
31 January-01 February 2006

Overview of the Network: Dr. Watanabe

- Goals of the Network
- PulseNet Asia (Pacific (PNAP)
- Planned Researches of network members

Asian Vivax Network: Dr. Bell

- Joint research activities
- Inter-country projects
- Training activities
- Clinical trials for potential replacements of primaquine

Reports on Malaria

- Philippines
- Indonesia
- Thailand
- Cambodia
- Korea

Research Needed for Better Control of Malaria

- Evaluation of detection tools
- Epidemiology
- Vector biology and control
- Parasite:
  - Molecular characterization
  - Antigenic variation
  - Relapse studies
- Drug trials:
  - Efficacy studies
  - Drug resistance
- Vaccine development
- Malaria Control Programs

Reports on Enteric Protozoa

- Philippines
- Thailand
- Japan

Research Needed for better control of Enteric Protozoa

- Molecular Epidemiology
- Database development
- New/improved methods of detection
- Standardization of protocols
- Clinical studies and drug trials

SUMMARY

- Where are we now?
- Where are we going?
- Where do we want to go?
- How do we get there?

Let's make it happen!

Reminder!

- Project reports are due at the end of next month.
- Meeting tomorrow starts at 9:30
- For those joining the bus tour tomorrow: Please get information from Ms. Reiko
- Tonight's party: Assembly at the entrance at of this building at 5:50PM

Research project:
Construction of laboratory-based net work in Asia and Pan-Pacific Rim

Consist of three groups ,three-year project
(project leader; H.Watanabe)

1)Enteric pathogens surveillance (Dr. Watanabe)
2)Virus surveillance (JE, Dengue fever; Dr.Kurane)
3)Parasite surveillance (Malaria etc. ; Dr.Endo)

Projects for collaboration

Project title:

Construction of laboratory-based net work on the characterization of pathogens prevalent in Asia and Pan-pacific area

Purpose:

1) Understanding the epidemiological situation of the infectious diseases prevalent in Asia and pacific rim
2) The phenotypic and genotypic characterization of causative pathogens
3) The creation of new strategies for the control and prevention of the diseases and the stop of further spreading.
4) Promote communication and exchange of the information on the pathogens and their-derived diseases
5) Construction of laboratory net work among Asia and Pacific Rim

Support

- **Research fund:**
  A research grant-in-aid of the Ministry of Health, Labor and Welfare (A grant on "Research for emerging and re-emerging infections").
- It will be supported for three years in principle but the project will be reviewed by the review board every year.

Usage of the fund

Trustee can use the money;

1) for reagents and equipments of the research
2) for travel and accommodation fares of the meeting and research investigation

Research contract:

- A representative participant of each country will make a research plan for the year and send it to the project leader (Dr. Haruo Watanabe, NIID Japan)
- In each year, project leader will make a contract with an authorized person of the institute (director-general or director of the department of the institute) where the researcher belongs.

Responsibility

- Each participant must write a report of the research result, which should be sent to the project leader until the end of February, 2006.