In this phase 3 of the project period, 1807 ITNs were distributed and 823 nets were re-impregnated by village volunteers, so the ITN coverage is 100% in the study villages.

Activities of ITN distribution & bed net reimpregnation in study villages

5- The monitoring of the other indicators on changes related with demographic and environmental indicators in the study villages

In order to detect and evaluate the other changes in addition to the malaria data, other data were also monitored from the study villages such as the demographic information, including number of household, total population, population increase, land transformation etc...

The additional indicator monitored in the Stung Keo commune

Month	Total HH	Total pop.	HH increase	Pop increase	Land transformation (in Hectare)	Fever patients	RDT positive	Refer to HCs
Sep/07	976	5395	0	0	194	50	38	0
Oct/07	976	5395	0	0	112	54	36	0
Nov/07	1069	5695	12	49	72	61	46	0
Dec/07	1069	5695	0	0	2	74	52	0
Jan/08	1092	6046	16	46	9	31	21	1
Feb/08	1092	6046	0	0	0	59	24	0
Total	**1092**	**6046**	**28**	**95**	**389**	**329**	**217**	**1**

VII. Discussions and conclusions

1- From the onset of the project so far, 204 households (888 HHs-1092HHs) have been increased in the Stung Keo (23%) and the population also augmented from 4498 to 6046 people (43.41% increase).i.e the population at risk enlarges study areas.

2- From year 1 to year 3 of the study period, 893 hectares of land was transformed for farming, especial raised up in the last year of the project (389 hectares). Therefore, it leads to the increase in the number of people that expose to malaria transmission in the study commune.

3- The malaria cases monitored in the study project have gone up from year to year of the study project (from 101 cases to 217 cases during the similar period of monitoring the last 2 years. So it reflects the strong association with the trend of land transformation in this commune.

4- The positive rate was dropped from 80% to 66% (14%) when compared to the same duration of year 2 and year 3 of the project though the malaria cases in the study period have been lifted up.

5- The malaria patients for RDT positive in the age group 5-14 was decreased from 20% in phase II to only 1% in phase III. However the increasing rate was obviously observed in age group 15-49 year male and female from 67% to 84% and 7% to 15% respectively. So there is the shift of risk age group exposed to malaria transmission since 99% of the malaria cases in year 3 contributed from the age group 15-49.

6- The referral cases were reduced from phase I to phase III of the monitoring because of the active health education conducted, the ITN distribution and net re-impregnation in the project site, especially the early diagnosis and prompt treatment provided by the village volunteer network in the study areas.

7- Since the trends of migration and population movement increase from year to year to the newly developed areas where the malaria transmission is very high and the study period is short, it is important to continue to conduct further studies to get more information on the mobile population for assessing the epidemiological, epidemiological including the drug resistance, the Pv trend, demographical, geographical indicators and other factors. This could help to identify the effective malaria intervention measures in those affected areas and follow up the tendency of the changes in the study areas.

VIII. Acknowledgements

We would like to express our profound sincere thanks and gratitude to the Ministry of Health, Welfare and Labor, Japan, for generous support to the National Center for Parasitology, Entomology and malaria control in Cambodia , especially the heartfelt thanks to the General National Institute of Infectious Disease, Japan for their kind assistance in coordinating and make the project operate successfully.

A special thanks also to the study project staff at national and provincial levels for their effort made in managing, supervise and monitor the study activities during the project operation period.

Last but not least, a profuse thanks the village volunteers for their hard work for not only collect data but also assist the National Malaria Control Program to provide intervention and treatment in the study areas.

The National Malaria Center, Cambodia, strongly hope that the Ministry of Health, Welfare and Labor of Japan will continue to support our Center in the forthcoming future.

Final Report for April 2005-February 2008

Project title: Establish new monitoring tools of malaria

Principle Investigator:
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Research activities:

In Thailand *Plasmodium falciparum* and *P. vivax* are highly prevalent in many malaria endemic areas, whereas *P. malariae* and *P. ovale* have been found only in a small proportion of cases. For 4 years we have studied malaria transmission in a village in western Thailand where malaria is hyperendemic. The majority of parasite carriers show no symptoms with low parasitaemia all year round, which suggests that these individuals have naturally acquired protective immunity. We proposed to identify the correlate(s) of protective immunity to *P. falciparum* and *P. vivax* in this population. Blood was collected by venipuncture from people with malaria positive or negative blood smears. Serum and peripheral blood mononuclear cells (PBMC) were separated for each sample. Humoral immunity to sporozoite, blood and liver stages of *P. falciparum* and *P. vivax* were characterized to identify parasite epitope(s) which will be a vaccine candidate(s) that is (are) able to enhance human protective immunity. This approach will also give us a chance to find out new parasite antigens for the diagnostics and/or epidemiological surveillance.

The Specific Aims:

1. To identify protective immune response in population with symptom and non-symptom by characterize humoral immunity to *P. falciparum* and *P. vivax* parasite using parasite crude extracts and recombinant proteins of both known malaria vaccine candidates and novel candidates.

2. To identify parasite antigen(s) that activates humoral immune response to blood, liver and sporozoite stage parasites.

3. To evaluate if new malaria diagnosis, LAMP, can be performed for malaria diagnosis at malaria clinics in a malaria endemic area.

Approaches:

1. Plasma that showed transmission blocking efficacy was sent to Dr. Takafumi Tsuboi laboratory for screening of malaria antigens that reacted to these plasma. The antigens that react with the collected plasma may be used as candidate for future vaccines or developing of diagnosis for surveillance of malaria transmission.

2. Liver stage antigens have been prepared for screening of pre-erythrocytic stage antigens that will be recognized by naturally acquired immunity.

3. Evaluation of LAMP for diagnosis of malaria at field clinics has been started in May 2007.

Identify protective immune response in naturally acquired immuned population

1. Blood samples were collected from 102 villagers live in Kong Mong Tha, a malaria endemic village in Kanchanburi, who had blood smear positive for either *Plasmodium falciparum* or *P. vivax*. Plasma and blood cells from each donor were separated and frozen in dryice before being transported to laboratories in Bangkok for further experiments. An aliquot of infected blood was fed to *An. dirus* mosquitoes to study the parasite infectivity and host immunity. Standard membrane feeding assay was performed to access natural transmission-blocking immunity by using plasma collected from villagers in Kong Mong Tha mixed with malaria infected blood and fed to An. dirus mosquitoes.

2. Plasma collected from 3 of 70 villagers from Kong Mong Tha who had *P. vivax* positive blood smear show transmission blocking immunity against P. vivax parasites collected from each donor and malaria patients in Maesod. Plasma collected from 3 of 32 villagers from Kong Mong Tha who had P. falciparum positive blood smear show

transmission blocking immunity against P. falciparum parasites collected from each donor and malaria patients in Maesod. In summary, naturally acquired transmission blocking immunity to P. falciparum and P. vivax occur in an endemic population in western Thailand.

3. To prepare malaria blood stage antigens for IFA assay, the parasites were prepared from malaria infected blood collected from patients who came to malaria clinic in Maesod district, Tak province. Sporozoite antigens were prepared from mosquitoes fed on malaria infected blood. Sporozoites were used to inoculate to human liver cells to produce liver stage parasites and the parasites were collected for antigen preparation. The antigen slides will be used for further screening of the new vaccine candidates.

Development of Loop-Mediated Isothermal Amplification (LAMP) for malaria diagnosis:

The conventional diagnostic method for malaria is microscopic examination of thin and/or thick blood smears. Although effective and inexpensive, this method is laborious and time-consuming, and its sensitivity is poor in cases of low parasitaemia. The assays employ lateral flow immunochromatographic method using antibody specific to malaria antigens are rapid but could identify only P. falciparum specific antigens and pan-malarial antigens thus sensitivity and specificity is lower for non-falciparum species. In Thailand malaria disease is caused by P. falciparum, P. vivax, and P. malariae with high prevalence of P. falciparum and P. vivax. Due to different treatment for different malaria parasite species and the requirement of high cost drug (artemisinin-based) for P. falciparum, there is a need for accurate diagnosis that cannot be met by microscopy technique. Nested PCR and real-time quantitative PCR have been developed to achieve higher sensitivity and specificity than microscopic examination. However, their implementation in field clinics has been impeded by the requirement for expensive equipment. The introduction of loop-mediated isothermal amplification (LAMP) has allowed the development of rapid, sensitive, specific, and simple methods for the diagnosis of parasitic diseases. The reaction is performed under one constant temperature. Amplification and detection of genes can be completed in a single step, by incubating mixtures of samples, primers, DNA polymerase and substrate at a constant temperature. The cycling reaction can continue with accumulation of 109 copies of target DNA within less than an hour. Both simple detection and real time detection of the reaction are possible. The template can

be simply detected through the presence of amplified product which can be visualized. The turbidity of magnesium pyrophosphate, a by product of the amplification reaction, is produced in proportion to the amount of amplified products. The sensitivity and specificity of LAMP is as good as nested PCR for malaria diagnosis when performing this assay in our laboratory using blood collected from patients.

After attending the meeting in Shanghai in Jan 2007, a proposal to evaluate LAMP at field clinics has been proposed to WHO office in Thailand. WHO co-sponsored the proof-of-concept research to evaluate if LAMP can be used at field clinics or in malaria endemic areas. This project is the first "Proof-of-Concept" study to demonstrate that LAMP can be performed at malaria clinic and used for malaria diagnosis.

Methods and results:

1. Blood samples collected from patients at Maesod clinics were used to evaluate Loop-Mediated Isothermal Amplification method for malaria diagnosis. Dr. Tsuboi developed the conditions to use LAMP for detection of all four species of malaria parasite in laboratory. Plasma and blood cells from each donor were separated and frozen in dryice before being transported to laboratories in Bangkok and at Ehime University for development of LAMP conditions.

2. DNA preparation from blood samples has been simplified and protocol for performing LAMP assay at the clinics in malaria endemic areas has been established. LAMP has been evaluated at Maesod clinic in Tak province, Thailand. Blood samples were collected from patients as whole blood with or without heparin (anti-coagulant) and spot on filter then used for (1) microscopic examination as a routine diagnosis and (2) LAMP assay and 3) nested PCR. Sensitivity, specificity and feasibility of LAMP for malaria diagnosis were compared with microscopic examination and nested PCR.

3. Evaluation of different DNA extraction methods to simplify this process for blood sample preparation was completed. Protocol for DNA extraction from blood sample collected at filed site has been established. Protocol for LAMP assay performed at filed clinic has been tested. Fifty four blood samples were collected from patients visiting Maesod clinic during September to November 2007.

4. Blood collected using capillary tubes (with heparin) were mixed with equal volume of distill water and boil for 5 minutes then the supernatant required for LAMP reaction

was separated by centrifuge at 10,000 rpm for 2 minutes.

5. Filtered blood was cut into small pieces and put in micro-tube then mixed with distilled water, boiled and processed further as described in step 4.

6. Evaluation of the LAMP assay was performed under different conditions including
a. Blood collection with and without heparin
b. Primer sets specific for Plasmodium genus and 4 human species were used
c. Subset of samples was tested with and without fluorescent dye in the reaction tube
d. LAMP reaction was incubated in water bath or LAMP machine.

7. The results indicated that:

a) When fluorescent dye was used the results were the same as when dye was not added to the reaction tube. But with the dye the result had to be visualized under black light or UV illuminator.

b) We had 5 persons to independently examine the results by naked eyes. All examiners gave the same results with the others for all blood samples.

c) LAMP reaction performed by incubation in water baht or LAMP machine gave the same results.

d) Heparin had no effect on LAMP reaction.

8. Results from microscopic examination showed that 37 cases were as P. vivax , 15 cases were P. falciparum and one case was mixed P. vivax and P. ovale and one negative blood smear.

9. From 37 cases of P. vivax, nested PCR identified 36 P. vivax and 1 mixed P. vivax and P. falciparum. LAMP assay identified 35 P. vivax, 1 mixed P. vivax and P. falciparum, and one sample positive for only genus.

10. For 15 cases of P. falciparum, nested PCR and LAMP assay also identified 15 cases as P. falciparum. All methods identified 2 negative cases. For mixed P.vivax and P. ovale, both nested PCR and LAMP identified this sample as P. vivax.

11. In summary, LAMP is feasible to be performed at field clinic and required only water bath or heat block for sample preparation and LAMP reaction. Total time required to perform LAMP assay after blood collection is about 75 min. When using

microscopic as the gold standard LAMP specificity for this study is about 95% and sensitivity is 100%.

12. From this "proof of concept" study showed that this technique is highly potential to be used for malaria diagnosis at field clinics but further validation for LAMP to identify specificity and sensitivity in larger sample size of patients will be required.

13. Manuscript for the LAMP proof of concept study has been prepared.

Summary of International conference on Vivax malaria

1 Time

International conference on Vivax malaria was held in January 16-18, 2007, Shanghai, PR China, which was organized by National Institute of Parasitic Diseases, China CDC, National Institute of Infectious Diseases(NIID), Ministry of Health and Welfare, Japan and WHO(WPRO and SEARO).

2 Participants

Fourty-one participants from the following countries and organizations: Cambodia, China, Democratic People's Republic of Korea, Indonesia, Japan, Philippines, Republic of Korea, Solomon Islands, Thailand and WHO attended the conference.

3 Aim and Topics

The aim of the conference was to 1) bring together countries in Asia and Pan-Pacifics, namely, Cambodia, China, DPRK, Indonesia, Japan, Philippines, ROK ,Solomon Islands and Thailand to establish the network on epidemiology and control of malaria in this region; 2) review the performance of recent research and control in each country, and 3) strengthen collaborations with international organizations from USA, Australia, WPRO and SEARO. Likewise, this 3 day conference provided updates on Global policies and recommended strategies on malaria control and serve as venue for sharing the experiences of successful Malaria Control Programmes/Projects, highlight innovative approaches and best practices in the prevention and control in the region.

The topics in the conference included the followings 1) reporting the reginal situation 2) reporting the situation of vivax malaria in each country 3) treatment for vivax malaria 4) vector control 5) early detection monitoring system and surveillance 6) advanced technology for control of vivax malaria, and vaccine for prevention as well as new indicators using morecular biology. There were totally 23 presentations in the conference.

4 Output

A multi-country, multi-region network covering vivax endemic countries in Asia and the Pacific has been established to: 1) share information and experiences related to P. vivax epidemiology, diagnosis, treatment, prevention and surveillance; and (2) to carry out collaborative operational research on vivax malaria.

By the end of the conference, delegates had made recommendations in harmonizing effective strategies and identifying future directions for malaria control in the region thereby further strengthening the regional network in contributing to the fight against malaria.

1) To develop evidence based recommendations for the diagnosis and treatment of vivax malaria for all countries and areas.

1.1) To establish a network of sentinel sites for monitoring the efficacy of current drugs and treatment regimens for vivax malaria following the recommended WHO protocol.

1.2) To validate existing treatment regimens being used for vivax malaria including defining an optimum treatment schedule for primaquine treatment.

1.3) To compile and disseminate data on the effectiveness of new drugs and drug combination for the treatment of vivax malaria.

1.4) To review and validate information from the DPRK on use of primaquine prophylaxis as a control strategy for long-incubation vivax malaria.

1.5) To review current policies and practices related to the need for G6PD testing prior to the administration of primaquine for the treatment of vivax malaria in Asia and the Pacific.

1.6) To support the establishment of a QA/QC system for malaria microscopy including both P. falciparum and *P. vivax*.

1.7) To support the development/identification of rapid diagnostic tests that can effectively detect even low density *P. vivax* infections under field conditions.

1.8) To support the further development of a LAMP system for the detection of *P. vivax* in laboratory settings.

1.9) To develop improved algorithms for the clinical diagnosis of vivax malaria in different settings.

1.10) To analyze the economic burden/impact of vivax malaria in various countries.

1.11) To support the development of a test that can effectively detect patients harbouring hypnozoites.

1.12) To establish a case definition of severe vivax malaria.

2) To determine the effectiveness of current vector strategies in areas where vivax is predominant

2.1) To carry out multi-country studies to determine the relative susceptibility of

vectors for the transmission of *P. vivax*.

2.2) To review existing data and identify additional research that will measure the effectives of ITN and LLIN for the control of vivax malaria in difference settings.

2.3) To evaluate the effectiveness of indoor residual spraying in vivax malaria areas.

2.4) To measure the cost effectiveness of ITN/LLIN compared to IRS for malaria control in areas where vivax malaria is predominant.

3) To develop the necessary surveillance tools and create networks for monitoring vivax malaria including the establishment of early warning system

3.1) To Support the development of surveillance guidelines for vivax malaria including use of new diagnostic tools (RDT and LAMP).

3.2) To establish specific surveillance indicators for vivax malaria including those that can be used as an early warning system for detection of outbreaks of vivax malaria.

4) General Recommendations

4.1) An inventory of institutions and other partner organizations working on vivax malaria should be compiled. This should include the identification of focal points and a description of existing research projects and possible areas of collaboration.

4.2) An inventory of training needs related to vivax malaria should be compiled.

4.3) A web site should be established with general information on vivax malaria and information on the network.

4.4) The next meeting of the network should be organized in late 2008 to review the status of the network and progress made towards achieving the goals

4.5) The National Institute for Parasitic Diseases, China CDC (IPD) will be coordinating institution for the Asia Pacific Vivax Network.

4.6) Additional funding partners should be identified for sustaining/expanding activities of the network.

4.7) The following additional institutions should be considered for membership in the Network: National Institute of Infectious Diseases (Japan), Jiangsu Provincial Institute for Parasitic Disease Control,, Research Institute for Tropical Diseases (Philippines), Korea CDC/National Institute for Health (Republic of Korea),Armed Forces Research Institute for Medical Science/Mahidol University (Thailand),Army Malaria Institute (Australia),National Institute for Malaria Research (India),Papua New Guinea Institute of Medical Research (Papua New Guinea),Institute Pasteur (Iran),Indonesia (North Sumatra and Jakarta).

5) Other Partners

It may include ACTMalaria, WHO/SEARO, WHO/WPRO, WHO/EMRO, National malaria control programmes in WPR, SEAR, and EMR.

5 Acknowledgement

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厚生労働科学研究費補助金（新興再興研究事業）
平成17～19年度　総合分担研究報告書

アジアで流行している感染症の我が国への侵入監視の強化に関する研究
マラリア等原虫疾患（プロジェクト3）総括研究報告書

腸管寄生性原虫対応のアジアネットワーク

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研究要旨
当該研究はアジアにおける腸管寄生性原虫の情報の共有を目指し、方法の整備、分離株の解析と収集を行った。今後とも、アジアネットの参加国を増やし、情報の共有が図られれば衛生の向上、施策への反映等が期待される。
腸管寄生性原虫対応のアジアネットワークの構築を行った。具体的にはクリプトスポリジウムの検査法の共通化、すなわち、蛍光抗体染色による高感度検出、遺伝子解析による種別、および遺伝子型の分類を提案し、タイ、フィリピンおよび日本の3カ国で研究協力体制の確立に努めた。具体的な検討項目としてクリプトスポリジウムを選択し、その検査法の祈祷いつを図った。すなわち、免疫染色による鏡検、検出虫体を用いて18S rDNAを標的とした種別、cpgpを標的とした遺伝子型の決定を提案した。その際、遺伝子検査に用いる陽性対照（鋳型DNA）はわが国で設計し、供給した。また、塩基配列の決定は、必要に応じわが国が支援した。
その結果、種別では、わが国およびアジア地域でのクリプトスポリジウム症は *C. hominis* （ヒト型）が主な起因原虫となっていた。また、多様な遺伝子型の存在が明らかとなり、系統樹解析によって汚染源長鎖に利用可能な程度の解像力の向上が果たせた。
個別研究では、過去の集団感染と散発事例ならびに家畜および野生動物由来のクリプトスポリジウムの感染性評価、家畜および野生動物における汚染状況と遺伝子型別が行なわれた（日本）。フィリピンでは本研究事業の支援を得てはじめて若年層における調査が行われ、高い検出率が報告された。また、タイからは AIDS 患者の感染事例の情報が寄せられた。あわせて、免疫不全者において *Isospora belli* の感染が少なからず存在することが示され、フィリピンでは *Cyclospora* 感染が確認された。

A. 研究目的

都市化と農業の近代化による衛生環境の向上に伴い、わが国の寄生虫疾患は戦後60年を経て著しく減少し、他の疾病に比べて目立たなくなった。開発途上国においても同様の傾向が見られるが、紛争地域を抱える国や山間僻地における事情は感染症対策以前の問題である。今日の世界情勢はこのような状況を抱えつつグローバル化が進行しているわけで、寄生虫症を含めわが国への感染症侵入の監視は重要となっている。当該研究の課題とした腸管寄生性の原虫症は寄生蠕虫類に比べてわが国への侵入、および定着がはるかに容易である。

これら病原体の監視と侵入防止に向けては、最も人的・物的交流の盛んなアジア地域との情報ネットワークの構築、すなわち地域で得られた質の良い情報を獲得することが重要と考える。そこで、当該研究ではクリプトスポリジウム等腸管寄生性原虫の分子疫学情報の収集を目指し、タイおよびフィリピンの協力を得てクリプトスポリジウムを主体として検査方法を統一し、得られた分子疫学的情報の共有化を図った。また、*Cyclospora* や *Isospora* などわが国では稀な疾患に関する詳細情報を得ることができた。これらの原虫類は環境抵抗性が高いことから除去や消毒は難しく、水系感染等で集団感染が危惧されている。

なお、アジア地域とのネットワーク構築に際しては、わが国の技術的、経済的支援が前提となるものと考え、当該研究では積極的に遺伝子検査に関する技術支援を行なった。あわせて、アジア地域においては家畜ならびに野生動物由来のクリプトスポリジウム等を対象とした研究が乏しい傾向にある。人畜共通感染症の病原体であることから情報収集は重要で、わが国における研究を紹介すると供に専門家による技術支援のもとで研究促進を促す必要があるものと考える。

B.研究方法

クリプトスポリジウムは蛍光抗体染色による糞便からの顕微鏡下での検出、18S rDNA 遺伝子の塩基配列による種別、cpgp 遺伝子配列による亜型別を行った。鋳型DNAは糞便から精製したオーシストより抽出した。PCR、RFLP、塩基配列決定は定法に従い行った。アライメント作成には pileup(GCG)、系統樹作成は MEGA4 を用いた。

アジア地域での検査法普及に際してはわが国からの支援が必須で、特に遺伝子検査に用いる陽性対照(鋳型DNA等)の提供は経済面のみならず精度管理の観点からも不可欠である。そこで、国際的に提供することを前提とした腸管寄生性原虫類の遺伝子診断用鋳型 DNA(陽性対照)の開発とその普及を図った。具体的には。陽性対照(鋳型DNA)によって得られる増幅産物は真の増幅産物のサイズと異なること、制限酵素切断部位が導入されていること、改変部位に署名となる配列を組み込むことを設計の基本とした。

畜産動物を対象とした汚染実態調査、種の簡易同定、ならびに感染性試験を実施した。

陽性対照(鋳型 DNA)によって得られる増幅産物は真の増幅産物のサイズと異なること、制限酵素切断部位が導入されていること、改変部位に署名となる配列を組み込む

ことを設計の基本とした。

C.研究結果

クリプトスポリジウム検査として、蛍光抗体による免疫染色による検査、18S rDNA 遺伝子による種別、cpgp 遺伝子による亜型別を提案した。わが国においてこれまでに分離されたクリプトスポリジウムより、cpgp 遺伝子の塩基配列から 36 分離株の遺伝子型が決定された(表 1)。フィリピンでは 10 件(表 2)、タイでは 27 件(表 3)の塩基配列データが得られた。また、3 カ国で取得した臨床株の cpgp 解析を基に系統樹を作成した(図 1)。その中で、集団感染に関する報告はわが国のみに限られたが、3 つの集団感染の原因となった原虫は別々のクラスターに分離されており、解像度は疫学解析への適用が可能と考えられた。フィリピンでは当該研究事業の支援を受けてはじめて小児を対象とした疫学調査がなされた。その結果、低年齢層での流行が確認され、cpgp を用いた遺伝子型別から共通の Ia 型の蔓延が示唆された。わが国では低年齢層の情報に乏しく、検討が必要である。タイでは AIDS 患者における感染状況を中心に報告され、世界的に珍しい種類(ex. *C. parvum* swine type)の感染が確認された。また、わが国や英国と同様、*C. meleagridis* の感染が比較的多くみられた。本種は鳥類に寄生する種として知られているが、ヒトへの感染性が高いものと推測される。今後、詳細な感染経路の解明が必要と考える。人畜共通感染症の立場から、経済動物および野生動物を対象とした汚染調査ならびに分子疫学解析を行った。その結果、ベトナムのウシにはわが国と同様 *C.parvum* および *C. andersoni* が蔓延していることが確認され、その遺伝子情報を取得した。また、わが国には米国分離株と同じ 18S rRNA 遺伝子を有するもののマウス感染性が異なる *C. andersoni* が存在することを示した。

PCR 法等の遺伝子診断法の更なる普及に向けて各種腸管寄生性原虫類に特有な陽性対照(鋳型 DNA 等)を開発し、3カ国で共通使用が始まった。基本的な姿勢として、遺伝子診断では陽性対照の汚染による事故(偽陽性)は防ぎ得ないものとし、反応産物の真偽判定を容易に行なえるよう予め陽性対照の鋳型 DNA の設計(配列を改変)を行なった。これにより、増幅産物の電気泳動での真偽の区別(増幅産物の大きさによる区別)を容易にし、制限酵素切断の有無による確認が可能となり、最終的には遺伝子配列を読むことで改変部位の署名となる配列が確認できる。

D.考察

わが国では多分野において遺伝子検査法が採用されている。感度や迅速性に優れ、基本操作や装置の共通により多様な病原体検査が可能である。遺伝子検査法は培養法や鏡検など従来の検査方法と比べ、結果に担当者の主観が入り難いこと(均質なデータ取得)機材や設備あるいは人材といった導入に際しての障壁が少ないものと判断される。したがって、東南アジア地域との検査ネットワークの構築に際しては積極的に導入を図るべき手法と判断した。一方、アジア地域での検査法普及に際してはわが国からの強力な支援が前提で、中でも、陽性対照(鋳型 DNA 等)の提供を保証することとした。その際、遺伝子検査法では陽性対照の汚染による事故(偽陽性)が防ぎ得ないものとし、反応産物の真偽が容易に判定できるよう鋳型 DNA の設計を行なった。

アジア地域におけるクリプトスポリジウム症の蔓延は明らかであり、多様な遺伝子型が確認された。フィリピンでは積極疫学により若年層の感染率が高いことを明らかにした。タイでは AIDS 患者中心の研究がなされ、わが国では見られない稀な種による感染が報告された。また、免疫不全者で多くのイソスポラ感染が新た確認された。一方、わが国同様に健常者事例は少なく、検査体制の不備が背景にあることは否めない。下痢症の原因究明は感染症対策上の重要課題で、腸管原虫症の検査への関心と技術の普及が急がれる。その中で、当該研究の意義は大きく、疫学、検査法の整備と共有を目的とした調査方法が整備され、アジアにおける腸管寄生性原虫による感染症の動向把握の第一歩が踏み出された。また、アジア地域においては人畜感染症の概念が必ずしも浸透しておらず、わが国における研究の進展状況を紹介すると供に専門家による技術支援が緒に就いた。

E. 結論

クリプトスポリジウムの検査法を共通化し、蛍光抗体染色による高感度検出、遺伝子解析による種別、および亜型分類を提案し、タイ、フィリピンおよび日本の3カ国で研究協力体制を確立した。情報の共有と技術協力が進められており、今後の協力体制の維持が課題として残された。

F. 研究発表

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