FINAL REPORT

Evaluation of Malaria Diagnostic

In North Sumatera Province, Indonesia

MAIN INVESTIGATOR

Lambok Shiahaan, Ph.D.

Department of Parasitology, Faculty of Medicine,

North Sumatera University

BACKGROUND

Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated. Malaria is a complex disease that varies widely in epidemiology and clinical manifestation in different parts of the world. The key strategies has been the promotion of early diagnosis and treatment with effective antimalarials.

In Indonesia malaria is still a major public health problem, especially out of Java and Bali. In North Sumatera Province, malaria is not affecting the entire population, because cases are concentrated in some specific districts. Cases seen in other districts are imported cases, who have visited malaria areas. Since 1997 until 2001, the Department of Health has done a survey of malaria in North Sumatera Province and found that there was two species of plasmodium,

Plasmodium falciparum and *Plasmodium vivax*. But in another study, there was also found *Plasmodium malariae*.

One of the priorities of a malaria elimination programme is to identify and treat malaria patients and all people carrying parasites, including those carrying gametocytes, ensuring that they become non-infectious as soon as possible. Microscopy of Giemsa-stained thick and thin films by a skilled microscopist has remained the standard laboratory method for the diagnosis of malaria both in regions where malaria is endemic and in regions where malaria is nonendemic. In North Sumatera Province, there are problems with microscopic diagnosis, particularly at the periphery of the health care system, Public Health Services (PHC). These include lack of skilled microscopists, maintenance of microscopes, delays in results, and inadequate quality control. Therefore, Department of health in North Sumatera Province usually report Annual Malaria Incidence (AMI) rather than Annual Parasitology Incidences (API). Actually, the API always lower than AMI.

In North Sumatera Province, the Annual Malariae Incidence (AMI) in the year 2000 is 6.03%, and in the year 2001 is 3.42%. Although reliable diagnosis cannot be made on the basis of signs and symptoms alone because of the non-specific nature of clinical malaria, clinical diagnosis of malaria is common in many malarious areas. In much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Clinical diagnosis offers the advantages of ease, speed, and low cost. In areas where malaria is prevalent, clinical diagnosis usually results in all patients with fever and no apparent other cause being treated for malaria. This approach can identify most patients who truly need antimalarial treatment, but it is also likely to misclassify many who do not. Over-diagnosis can be considerable and contributes to misuse of antimalarial drugs. Considerable overlap exists

between the signs and symptoms of malaria and other frequent diseases, especially acute lower respiratory tract infection and can greatly increase the frequency of misdiagnosis and mistreatment. Attempts to improve the specificity of clinical diagnosis for malaria by including signs and symptoms other than fever or history of fever have met with only minimal success. Therefore, diagnostic by Rapid Diagnostic Test, nowadays is used in North Sumatera Province.

Accurate information about the burden of malaria infection at the district or provincial level is required both to plan local malaria control efforts and to measure the impact of such efforts. Although several studies of malaria epidemiology and drug resistance have been conducted at many sites in North Sumatera Province, there is only little published literature describing malaria prevalence at the district and province level. Therefore, prevalence surveys for malaria, designed to estimate malaria prevalence in North Sumatera Province, Indonesia, will be conducted.

OBJECTIVES

This study is cross sectional design and does in 6 districts in North Sumatera Province, such as : Asahan, Serdang Bedagai, Toba, Samosir, Tapanuli Utara and Tapanuli Selatan district. The increasing of the Annual Malaria Incidence in each district is the reason to conduct this study in this area. Eventhough have low malaria incidence than other endemic malaria in North Sumatera Province, but the development and spread of anti-malarial drug resistance, will make it difficult to control malaria.

The objectives of this study are :

1. To evaluate malaria diagnostic tests in North Sumatera Province.

2. To investigate prevalence of malaria cases from wet and dry season in North Sumatera Province.
3. To analyse genes of malaria parasites by PCR
4. To refresh medical worker about malaria diagnostic
5. To cooperate with Department of Health in malaria control in North Sumatera Province
6. As a beginning step for the next study in malaria control, especially to find gene marker for antimalarial resistance in North Sumatera Province.

METHODS

This study conducts in several districts in North Sumatera Province. Once in the wet season and once in the dry season, in order to obtain, district-wide estimates of malaria prevalence. All individuals with fever or history of fever, will be included, except those unwilling to provide informed consent or have a severe or acute illness that require immediate medical attention. Blood from each patient will be taken for diagnostic by Rapid Diagnostic Test (One Step Mal), Microscopic examination and PCR diagnostic. Especially for Microscopic examination and PCR diagnostic, the test will use blood spot on object glass and filter paper. Blood sampling will be collected over the period of the study in the Public Health Center (PHC).

The diagnostic methods that use in this study are :

1. Rapid Diagnostic Test

The diagnostic approach involves the rapid detection of parasite antigens using rapid immunochromatographic techniques. Rapid Diagnostic Tests are able to distinguish between falciparum (detection of the histidine-rich protein

2 (HRP-II) of *P. falciparum*) and non-falciparum infections (detection of a specific parasite enzyme, lactate dehydrogenase or pLDH). Advantages to this technology are that no special equipment is required, minimal training is needed, the test and reagents are stable at ambient temperatures, and no electricity is needed. The principal disadvantages are a currently high per-test cost and an inability to quantify the density of infection. Furthermore, detectable antigen can persist for days after adequate treatment and cure; therefore, the test cannot adequately distinguish a resolving infection from treatment failure due to drug resistance, especially early after treatment.

2. Microscopic examination

Simple light microscopic examination of Giemsa-stained blood films is the most widely practised and useful method for definitive malaria diagnosis. Advantages include differentiation between species, quantification of the parasite density, and ability to distinguish clinically important asexual parasite stages from gametocytes which may persist without causing symptoms. These advantages can be critical for proper case-management and evaluating parasitological response to treatment. Specific disadvantages are that slide collection, staining, and reading can be time-consuming and microscopists need to be trained and supervised to ensure consistent reliability.

3. PCR diagnostic

Detection of parasite genetic material through polymerase-chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis and surveillance of drug resistance in malaria. Specific primers have been developed for each of the four species of human malaria. One important use of this new technology is in detecting mixed infections or differentiating between infecting species when microscopic

examination is inconclusive. Primary disadvantages to these methods are overall high cost, high degree of training required, need for special equipment, absolute requirement for electricity, and potential for cross-contamination between samples.

All blood samples collected from PHC, will be analysed in each center and also confirm to the Parasitology Department, Medical Faculty, North Sumatera University. All malaria-positive samples (by RDT or microscopy), either as a single or co-infection of another *Plasmodium* species will be assayed by PCR to determine specific species.

RESULT

The *accessible population* in this study is people who joined with *the Active Case Detection*, namely Mass Fever Survey (MFS) and Mass Blood Survey (MBS) as mush as 289 participants. The positive samples in Rapid Diagnostic Test (One Step Mal) are fivity six participants, which eighteen are infected with *Plasmodium falciparum* and thirty eight are mixed infected.

Table 1. Distribution of Accesible Population

	District	Acessible Population	RDT (+)
1.	Serdang Bedagai	34	5
2.	Asahan	52	9
3.	Toba	79	15
4.	Tapanuli Utara	51	10
5.	Tapanuli Selatan	26	8
6.	Samosir	47	9
	T O T A L	289	56

But after evaluated with Microscopic examination, apparently it turned out that plasmodium spp was only found in twelve samples that contain about 1000 /µl.of parasites.

Table 2. Distribution of Microscopic Examination

	Diagnosa	Sample	Mean (parasites/µl)	Range (parasites/µl)
1.	P.falciparum	3	373	120 – 560
2.	P.vivax	2	580	320 – 840
3.	P.malariae	1	440	440
4.	P.falciparum + P.vivax (mix)	5	256	120 – 440
5.	P.vivax + P.malariae (mix)	1	240	240
	T O T A L	12		

Where as, in the evaluation of PCR found only five positive samples. Two of them are Plasmodium falciparum, which both among them had been resisted to antimalarial Choloroquine, that moleculer marker *pfcrt* is not broken by APO I enzyme.

Table 3. Distribution of PCR Diagnostic

	Diagnosa	Sample
1.	P.falciparum	1
2.	P.vivax	1
3.	P.malariae	1
4.	P.falciparum + P.vivax (mixed)	1
5.	P.vivax + P.malariae (mixed)	1
	T O T A L	5

DISCUSSION

The sampling was started on May until December 2009. There were not many samples obtained in this study. It since the sampling was equal with Ramadhan and Aidil Fitry that's August and September, so more people were not ready if their bloods taken when they were fasting.

Because of lacking skilled microscopist staff, so for diagnosing malaria still used the Rapid Diagnostic Test (RDT). The RDT basic principle is finding out the existence of antigen as the product of Plasmodium, such as *Plasmodium falciparum* histidine-rich Protein-2 (pfHRP2) that produced by *Plasmodium falciparum* and lactose dehydrogenase (pLDH) produced by *Plasmodium* spp. The Antigen can be survived for a long period in human body,

eventhough the Plasmodium had been died. It may because the RDT will be kept positive while at the microscopic examination no plasmodium found.

The differences between Rapid Diagnostic Test and microcopic examination is a must noticed. The low sensitivity of RDT in this study may be happened because the RDT is out of order as the effect of the uncorret way in storing, in the using procedure and in the reading of the result. The other case RDT is used for patient who had consumed antimalarial and also not recommended for follow-up patients. The problem that's rising up of course will be the matery in re-extension and training for the health staff in that district. But the training of microcopic examination is still be the main priority. The training have been done.

In the meantime, the different result of microscopic examination and PCR also must be inquired. Are the differences of this result have relationship with the different source of DNA from the same patiens with low parasite density? According to the opportunity, it is possible there are different amount of Plasmodium in blood at the glass object (for Microscopic Examinaton) and Whatman paper (for PCR), eventhough come from the same sample. To decrease that imbalance, perhaps it is better the isolated DNA for PCR, are also from the same blood, that's the blood at the glass object for Microscopic Examination.

From this study can be concluded it's needed improvement in Malaria Diagnose. As standard diagnose, the Microscopic Examination still must be done. So that Microscopic training keep hold continously. Where as, it's needed to re-evaluate the sensitivity of RDT used. And also the exact way for storing, the RDT using procedure, and the result reading. Beside that, also needed to activate the cross-check mechanism toward the result of examination of one level to the upper level. So it decreases the error diagnose.

Where as, DNA that will be isolated for PCR, it should taken the blood at that object glass of microscopic examination. It is for avoiding different result, especially in patient with low level of parasites.

CONCLUSION

1. RDT using at Malaria Regular Investigation is not accurate yet. There are some important noticed in using RDT, namely the way in storing, the using procedure and the reading of the result. RDT also may not used for re-checking for patients had been treated before (follow-up patient). The usage of Microscopic Examination is still the first choice in Malaria Diagnose.
2. *Plasmodium falciparum* obtained had been resisted toward anti malarial Chloroquine.
3. It's important to cross check again the result of examination in district area to province, as a quality control of malaria diagnostic.
4. It needs advanced study for obtained *genotyping* from Plasmodium, especially Plasmodium vivax in endemic area, such as Madina and Nias district in North Sumatera Province.

平成２１年度業績

＊研究成果の刊行に関する一覧表

＊学会発表一覧表

研究成果の刊行に関する一覧表　(平成２１年度)

執筆者氏名	刊行書籍又は雑誌名 (雑誌のときは雑誌名、 巻号数、論文名)	刊行書店名	巻名	ページ	刊行年
プロジェクト１：細菌					
Morita-Ishihara T, Terajima J, Watanabe H, Izumiya H	Interaction between enterohemorrhagic *Escherichia coli* O157:H7 EspFu and IRSp53 induces dynamic membrane remodeling in epithelial cells.	Japanese Journal of Infectious Diseases	62	351-5	2009
H. Izumiya, Y. Tada, K. Ito, T. Morita-Ishihara, M. Ohnishi, J. Terajima, and H. Watanabe	Characterization of *Shigella sonnei* isolates from travel-associated cases in Japan.	J. Med. Microbiol	58 (11)	1486-1491	2009
伊豫田淳、寺嶋淳、泉谷秀昌、渡邉治雄	日本国内における腸管出血性大腸菌感染症の現況と分離株の性状	獣医畜産新報	62	801-806	2009
Honda N., Iyoda S., Yamamoto S., Terajima J., and Watanabe H	LrhA positively controls the expression of the locus of enterocyte effacement genes in enterohemorrhagic *Escherichia coli* by differential regulation of their master regulators PchA and PchB.	Mol. Microbiol.	74	1393-1411	2009
Sithivong N, Izumiya H, Munnalath K, Phouthavane T, Chomlasak K, Sisavath L, Vongdouangchanh A, Vongprachanh P, Watanabe H, and Ohinishi M.	Cholera outbreak in 2007, Lao People's Democratic Republic	Emerg. Infect. Dis.		(in press)	

Mitobe J, Morita-Ishihara T, Ishihama A, Watanabe H.	2009 Involvement of RNA-binding protein Hfq in the osmotic-response regulation of *invE* gene expression in *Shigella sonnei.*	BMC Microbiol	9	110	2009
Tokunaga, A., Yamaguchi, H., Morita, M., Arakawa, E., Izumiya, H., Watanabe, H., Osawa, R	Novel PCR-based genotyping method, using genomic variability between repetitive sequences of toxigenic *Vibrio cholerae* O1 El Tor and O139.	Mol. Cell. Probes.		(in press)	
プロジェクト２：ウイルス（デング熱）					
Lim, C.K., Nishibori, T., Watanabe, K., Ito, M., Kotaki, A., Tanaka, K., Kurane, I., Takasaki, T.	Chikungunya virus isolated from a returnee to Japan from Sri Lanka: Isolation of two sub-strains with different characteristics.	*Am. J. Trop. Med. Hyg.*	81(5)	865-868	2009
Lim, C.K., Kurane, I., and Takasaki, T.	Re-emergence of chikungunya virus	In Maeda, A. (ed), Animal Viruses. Transworld Research Network., Kerala, India.		1-22	2010
Aoyama, I., Uno, K., Yumisashi, T., Takasaki, T., Lim, C.K., Kurane, I., Kase, T., Takahashi, K.	A case of chikungunya fever imported from India to Japan, follow-up of specific IgM and IgG antibodies over a 6-month period.	*Jpn. J. Infect. Dis.*	63(1)	65-66	2010
田島茂、高崎智彦	日本脳炎	診断と診療	97（10）	2097-2100	2009
Lim, C.K., Nishibori, T., Watanabe, K., Ito, M., Kotaki, A., Tanaka, K., Kurane, I., Takasaki, T.	Chikungunya virus isolated from a returnee to Japan from Sri Lanka: Isolation of two sub-strains with different characteristics.	*Am. J. Trop. Med. Hyg.*	81(5)	865-868	2009

Aoyama, I., Uno, K., Yumisashi, T., Takasaki, T., Lim, C.K., Kurane, I., Kase, T., Takahashi, K.	A Case of Chikungunya Fever Imported from India to Japan, Follow-Up of Specific IgM and IgG Antibodies over a 6-Month Period.	*Jpn. J. Infect. Dis.*	63(1)	65-66	2010
林　昌宏	チクングニヤウイルス	臨床と微生物	36(3)	211-216	2009
プロジェクト3：ウイルス（高病原体 H5N1 鳥インフルエンザ)					
Kawakami, C., Obuchi, M., Saikusa, M., Noguchi, Y., Ujike, M., Odagiri, T., Tashiro, M.	Outbreaks of oseltamivir-resistant influenza A/H1N1 virus in an elementary school and a family in Yokohama City, Japan during the 2007-2008 season.	Jpn. J. Infect. Dis.	62	83-86	2009
Takahashi, Y., Hasegawa, H., Hara, Y., Ato, N., Ninomiya, A., Takagi, H., Odagiri, T., Sata, T., Tashiro, M., Kobayashi, M.	Protective immunity afforded by H5N1 (NIBRG-14) - inactivated vaccine requires both antibodies against hemagglutinin and neuraminidase in mice.	J. Infect. Dis.	199	1629-1637	2009
Wada, T., Morishima, T., Okumura, A., Tashiro, M., Hosoya, M., Shiomi, M., Okuno, Y	Differences in clinical manifestations of influenza-associated encephalopathy by age.	Microbiol. Immunol	53	83-88	2009
Ikeno, D., Kimachi, K., Kudo, Y., Goto, S., Itamura, S., Odagiri, T., Tashiro, M., Kino, Y.	The prime-boost vaccination of H5N1 heterologous strains in a mouse model	Vaccine	27	3121-3125	2009

Akiyama, M., Kimura, H., Tsukagoshi, H., Taira, K., Mizuta, K., Saitoh, M., Nagano , M., Sutoh, A., Noda, M., Morita, Y., Sakatsume, O., Okabe, N., Tashiro, M.	Development of assay for the detection and quantitation of measles virus nucleoprotein (N) gene using real-time reverse transcription polymerase chain reaction (real-time RT-PCR)	J. Med. Microbiol	58:	638-643	2009
Thongratsaku, S., Songserm, T., Poolkhet, C., Kondo, S., Yagi, H. Hiramatsu, H., Tashiro, M., Okada, H., Kato, K., Suzuki, Y	Determination of *N*-linked sialyl-sugar chains in the lungs of domestic cats and dogs in Thailand susceptible to the highly pathogenic avian influenza virus (H5N1).	Open Glycoscience	2	28-36	2009
Ichinohe, T., Tashiro, M., Sata,T., Hasegawa, H.	PolyI:PolyC_{12}U adjuvant-combined intranasal vaccine protects mice against highly pathogenic H5N1 influenza virus variants.	Vaccine	27	6276-6279	2009
Tashiro, M., McKimm-Breschkin, J., Saito, T., Klimov, A., Macken, C., Zambon, M., Hayden, F.	Surveillance for neuraminidase inhibitor-resistant influenza viruses in Japan, 1996-2007.	Antiviral Therapy	14	751-761	2009

WHO/OIE/FAO H5N1 Evolution Working Group; Brown, I. H., Capua, I., Cattoli, G., Chen, H., Cox, N., Davis, T., Donis, R. O., Fouchier, R. A. M. Garten, R., Guan, Y., Kawaoka, Y., Mackenzie, J., McCauley, J., Mumford, E., Olsen, C., Perdue, M., Russell, C. A., Smith, C., Smith, D., Smith, G. J. D., Shu, Y., Tashiro, M. Vijaykrishna, D., Webster, R	Continuing progress towards a unified nomenclature for the highly pathogenic H5N1 avian influenza viruses: divergence of clade 2.2 viruses.	J. Influenza.Resp. Viral Infect	3	59-62	2009
Hishinuma-Igarashi, I., Mizuta, K., Saito, Y., Ohuchi, Y., Noda, M., Akihama, M., Sato , H., Tsukagoshi, H., Okabe, N., Tashiro , M.,Kimura, H.	Phylogenetic analysis of human bocavirus (HBoV) detected from children with acute respiratory infection in Japan	J. Infection	58	311-313	2009
Sriwilaijaroen, N., Wilairat, P., Hiramatsu, H., Takahashi, T., Suzuki, T., Ito, M., Ito, Y., Tashiro, M., Suzuki, Y.	Mechanisms of the action of povidone-iodine against human and avian I influenza A viruses: its effects on hemagglutination and sialidase activities.	Virology Journal	6	124	2009
Kubota, T., Matsuoka, M., Chang, T.-H., Bray, M., Jones, S., Tashiro, M., Kato, A., Ozato, K	Ebola virus VP35 interacts with the cytoplasmic dynein light chain 8.	J. Virol.	83	6952-6956	2009

Bertozzi, S., Kelso, A., Tashiro, M., Savy, V., Farrar, J., Osterholm, M., Jameel, S., Muller, C.P.	Pandemic flu: from front lines.	Nature	461	20-21	2009
Mizuta, K., Hirata, A., Suto, A., Aoki, Y., Ahiko, T., Itagaki, T., Tsukagoshi, T., Morita,Y., Obuchi, M., Akiyama, M., Okabe, N., Noda, M., Tashiro, M., Kimura, H.	Phylogenetic and cluster analysis of human rhinovirus species A (HRV-A) isolated from children with acute respiratory infections in Yamagata, Japan.	Virus Research	147	265-274	2009
Ichinohe, T., Ainai, A., Nakamura, T., Akiyama, Y., Maeyama, J., Odagiri, T., Tashiro, M., Takahashi, H., Sawa, H., Tamura, S., Chiba, J., Kurata, T., Sata, T., Hasegawa, H.	Induction of cross-protective immunity against influenza A virus H5N1 by an intranasal vaccine with extracts of mushroom mycelia	J. Med. Virol	82	128-137	2010
Nakajima, N., Hata, S., Sato, Y., Tobiume, M., Katano, H., Kaneko, K., Nagata, N., Kataoka, M., Ainai, A., Hasegawa, H., Tashiro, M., Odai, T., Urasawa, N., Ogino, T., Hanaoka, H., Watanabe, M., Sata, T	First autopsy case with pandemic influenza (A/H1N1pdm) virus infection in Japan: Detection of high copy number of the virus in type II alveolar epithelial cells by pathological and virological examination.	Jpn. J. Infect. Dis.	63	67-71	2010

プロジェクト４：ウイルス（風疹と CRS）					
Dong JB, Saito A, Mine Y, Sakuraba Y, Nibe K, Goto Y, Komase K, Nakayama T, Miyata H, Iwata H, Haga T.	Adaptation of wild-type measles virus to cotton rat lung cells: E89K mutation in matrix protein contributes to its fitness. Adaptation of wild-type measles virus to cotton rat lung cells: E89K mutation in matrix protein contributes to its fitness.	Virus Genes	Oct 14	[Epub ahead of print]	2009
Ninomiya K, Kanayama T, Fujieda N, Nakayama T, Komase K, Nagata K, Takeuchi K.	Amino acid substitution at position 464 in the haemagglutinin-neuraminidase protein of a mumps virus Urabe strain enhanced the virus growth in neuroblastoma SH-SY5Y cells.	Vaccine	27	6160-5	2009
Kato S, Ohgimoto S, Sharma LB, Kurazono S, Ayata M, Komase K, Takeda M, Takeuchi K, Ihara T, Ogura H.	Reduced ability of hemagglutinin of the CAM-70 measles virus vaccine strain to use receptors CD46 and SLAM.	Vaccine	27	3838-48	2009
Dey SK, Hayakawa Y, Rhaman M, Islam R, Mizuguchi M, Okitsu S, Ushijima H.	G2 strain of rotavirus among infants and children, Bangladesh.	Emerg Infect Dis,	Jan; 15(1)	91-94	2009
Takanashi S, Hashira S, Matsunaga T, Yoshida A, Shiota T, Phan TG, Khamrin P, Okitsu S, Mizuguchi M, Igarashi T, Ushijima H.	Dectection, genetic characterization, and quantification of Norovirus RNA from sera of children with gastroenteritis.	J Clin Virol	44	161-163	2009

Dey SK, Shimizu H, Phan TG, Hayakawa Y, Islam A, Salim AFM, Khan AR, Mizuguchi M, Okitsu S, Ushijima H.	Molecular epidemiology of adenovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh.	Infect Genet Evol	9(4)	518-522.	2009
Chanit W, Thongprchum A, Khamrin P, Okitsu S, Mizuguchi M, Ushijima H.	Intergenogroup recombinant sapovirus in Japan, 2007-2008.	Emerg Infect Dis	15(7)	1084-1087	2009
Khamrin P, Maneekarn N, Kongkaew A, Kongkaew S, Okitsu S, Ushijima H.	Porcine kobuvirus in piglets, Thailand.	Emerg Infect Dis,	15(12)	2075-2076	2009
Pham NTK, Trinh QD, Khamrin P, Maneekan N, Shimizu H, Okitsu S, Mizuguchi M, Ushijima H.	Diversity of human parechoviruses isolated in stool samples collected from Thai children with acute gastroenteritis.	J Clin Microbiol.	48(1)	115-119	2010
Trinh QD, Pham NT, Lam BQ, Le TP, Truonq KH, Le TQ, Vo HT, Tanq TC, Ha TM, Izumi Y, Mizuguchi M, Hayakawa S, Ushijima H.	Subtypping and env C2/V3 sequence analysis of HIV-1 isolatd from HIV-infected children hospitalized in Children Hospital 1, Vietnam during 2004-2005.	J Trop Peditatr,	55(6)	399-401.	2009
Pham NTK, Trinh QD, Takanashi S, Abeysekera C, Abeygunawardene A, Shimizu H, Khamrin P, Okitsu S, Mizuguchi M, Ushijima H.	Novel human parechovirus from Sri Lanka.	Emerg Infect Dis,	16(1)	130-132	2010

Dey SK, Phan TG, Nishimura S, Muzuguchi M, Okitsu S, Ushijima H.	Molecular and epidemiological trend of sapovirus and astrovirus infection in Japan.	J Trop Pediatr	2009 Sep 11	[Epub ahead of print]	2009
Khamrin P, Chan-it W, Satou K, Nanba Y, Yamashita Y, Okitsu S, Maneekarn N, Ushijima H.	Evaluation of the Newly Developed Immunochromatography Test Kit for Rapid Detection and Differentiation of Norovirus GI and GII.	J Trop Pediatr	2010 Jan 19	[Epub ahead of print]	2010
プロジェクト５：ウイルス（狂犬病ウイルス）					
Boldbaatar B., Inoue S., Sugiura N., Noguchi A., Orbina J.R.C., Demetria C., Miranda M.E., and Yamada A.	Rapid detection of rabies virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP).	Jpn.J.Infect.Dis.	62	187-191	2009
Inoue S., Boldbaatar B., Sugiura N., Noguchi A., and Park C.-H.	Rabies. In: Animal Viruses (Maeda A., ed.). RESEARCH SIGNPOST.		(in press)		2009
Tobiume M., Sato Y., Katano H., Nakajima N., Tanaka K., Noguchi A., Inoue S., Hasegawa H., Iwasa Y., Tanaka J., Hayashi H., Yoshida S., Kurane I., and Sata T.	Rabies virus dissemination in neural tissues of autopsy cases due to rabies imported into Japan from the Philippines: immunohistochemistry.	Pathol.Int	59	555-66	2009
Kojima D, Park CH, Satoh Y, Inoue S, Noguchi A, Oyamada T.	Pathology of the spinal cord of C57BL/6J mice infected with rabies virus (CVS-11 strain).	J Vet Med Sci	71(3)	319-324	2009

プロジェクト６：原虫（マラリア）					
Nakagawa Y, Ueki M, Fueda K, Ohmae H, Ishikawa H.	Risk assessment of re-emerging *Plasmodium falciparum* on Ishigaki Island using a stochastic model.	*Trop Med & Hlth*	37(3)	97-107	2009
Moritoshi Iwagami, Pilarita T. Rivera, Elena A Villacorte, Aleyla S Escueta, Toshimitsu Hatabu, Shin-ichiro Kawazu, Toshiyuki Hayakawa, Kazuyuki Tanabe, Shigeyuki Kano.	Genetic diversity and population structure of *Plasmodium falciparum* in the Philippines.	Malaria J.	8	96	2009
Toshihiro Mita, Kazuyuki Tanabe, Kiyoshi Kita.	Spread and evolution of *Plasmodium falciparum* drug resistance.	Parasitol. Int.	58	201-209	2009
Satoru Kawai, Makoto Hirai, Kosuke Haruki, Kazuyuki Tanabe, Yuichi Chigusa.	Cross-reactivity in rapid diagnostic tests between human malaria and zoonotic simian malaria parasite *Plasmodium knowlesi* infections.	Parasitol. Int.	58	300-302.	2009
Richard Culleton, Mathieu Ndounga, Fadile Yildiz Zeyrek, Cevayir Coban, Prisca Nadine Casimiro, Satoru Takeo, Takafumi Tsuboi, Anjali Yadava, Richard Carter, Kazuyuki Tanabe	Evidence for the transmission of *Plasmodium vivax* in the Republic of Congo, West Central Africa.	J. Infect. Dis.	200 (9)	1465-1469	2009