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ISID NEWS

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ISID NEWS

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16th International Congress on Infectious Diseases

CAPE TOWN • SOUTH AFRICA
APRIL 2~5, 2014

16th ICID ~ New Plenary Speaker Announced

Peter Piot

Old and New Global Challenges in Infectious Diseases

Professor Baron Peter Piot CMG, FMedSci is the Director of the London School of Hygiene & Tropical Medicine, and Professor of Global Health. In 2009-2010 he was the Director of the Institute for Global Health at Imperial College, London. He was the founding Executive Director of UNAIDS and Under Secretary-General of the United Nations from 1995 until 2008, and was an Associate Director of the Global Programme on AIDS of WHO.

Professor Piot co-discovered the Ebola virus in Zaire in 1976, and led research on AIDS, women's health, and public health in Africa. He was a professor of microbiology at the Institute of Tropical Medicine, Antwerp, the Free University of Brussels, and the University of Nairobi, was a Senior Fellow at the University of Washington, a Scholar in Residence at the Ford Foundation, and a Senior Fellow at the Bill and Melinda Gates Foundation. He held the 2009 chair "Knowledge against poverty" at the College de France in Paris.

He is a member of the Institute of Medicine of the US National Academy of Sciences, of the Académie Nationale de Médicine of France, and of the Royal Academy of Medicine of his native Belgium, and a Fellow of the Academy of Medical Sciences and the Royal College of Physicians. In 2008-11, he was the President of the King Baudouin Foundation and was knighted as a baron in 1995. He has published over 500 scientific articles and 16 books.

The 16th ICID Second Announcement is now available at:

http://www.isid.org/icid/Downloads/16thICID_2ndAnnounce.pdf

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16TH INTERNATIONAL CONGRESS ON INFECTIOUS DISEASES
CAPE TOWN · SOUTH AFRICA · 2014

www.isid.org/icid/

Awards for the 16th ICID:

Emerging African Investigators Symposium and Travel Grant Award: The Clinical Infectious Diseases Research Initiative at the University of of Cape Town (supported by The Wellcome Trust) will generously sponsor approximately 20 young African investigators to attend the 16th ICID and present their work during the 'Emerging African Investigators Symposium' on April 2, 2014.

Novartis Vaccines Awards for Epidemiology of Infectious Diseases, created to identify and reward researchers for their work in furthering the understanding of infectious disease epidemiology. This year, only researchers working in Africa are eligible for the awards.

Sanofi Pasteur Awards for Communicable Disease Epidemiology, designed to stimulate research on and emphasize the importance of applied epidemiology in the field of communicable diseases for the overall improvement of health.

ISID New Investigator Award: The International Society for Infectious Diseases is delighted to continue the ISID New Investigator Award for the 16th ICID.

Detailed information on eligibility and application procedures for all of these awards can be found on the 16th ICID Awards webpage **http://www.isid.org/icid/awards.shtml**

ABSTRACT DEADLINE

16th ICID

December 2, 2013

Planned Symposium Topics

Please note that not all topics are confirmed and that they are subject to change.

Focus on HIV
- Childhood HIV
- ART in Africa
- Opportunistic Infections: Diagnosis, Treatment, Prevention
- Programmatic Challenges and Solutions
- Preventing Transmission

Focus on TB
- Tuberculosis in Children
- Multi-Drug Resistant Tuberculosis: Trends in Low Income Areas
- Utilizing Rapid Diagnostics
- New Drugs on the Horizon
- Implications of HIV Co-infection
- Understanding Transmission
- What Should First Line Therapy Be?
- The Role of Vitamin D

Focus on Tropical Diseases
- Malaria: The Grand Challenge
- The Spread of Artemesinin Resistance
- Dengue Goes Global
- New Strategies for Vector Control
- Neglected Tropical Diseases in Africa: Successes and Challenges

Additional Topics
- Optimizing Tools for Antibiotic Stewardship
- Infection Control: State of the Art and Daily Practice
- Control of Highly Resistant Bacterial Pathogens
- Carbapenemase-Resistant Enterobacteriaceae
- MRSA
- Highly Resistant Gonococcus
- Emerging Infectious Diseases and Disease Surveillance in Africa
- The Human-Animal Interface in Africa
- Laboratory Diagnosis and Surveillance in LMICs
- Gender Differences in Response to Infection
- Genomics and the Microbiome
- Responding to Complex ID Emergencies: Detection, Alerts and Interventions
- Childhood Pneumonia in the Era of Conjugate Vaccines
- Global Controversies in Management of Sepsis
- Upper Respiratory Tract and Allied Infections
- Public Health Challenges of Sexually Transmitted Diseases
- Human Papilloma Virus: Health Impacts and Vaccine Utilization
- Controversies in Vaccination: Pertussis, Polio, Rotavirus, Influenza
- Diarrheal Diseases: Pathogens, Vaccines, Sanitation
- Controlling Typhoid

16th ICID Plenary Speakers Previously Highlighted in the ISID NEWS

RON DAGAN
Otitis Media as an Infectious Disease—The Debate Goes On

SALIM S. ABDOOL KARIM
Preventing HIV

Dr. Jonathan A. McCullers
Influenza: Understanding Pathogenesis to Improve Outcomes

ProMED mail
Program for Monitoring Emerging Diseases

www.promedmail.org

ProMED regrets to announce the passing of Edward J. Prucha, of New Harbor, Maine, USA who had been a dedicated and skillful copy editor since 2007.

Recent Publications by ProMED

ProMED personnel and collaborators at the London School of Tropical Medicine and at HealthMap have published several articles recently. And the work of ProMED has been acknowledged in the press.

Articles by ProMED authors:

Clinical Microbiology and Infection,* 2013** • ***An Overview of Internet biosurveillance.
Hartley D, Nelson N, Arthur R, Barboza P, Collier N, Lightfoot N, Linge J, van der Goot E, Mawudeku A, Madoff L, Vaillant L, Walters R, Yangarber R, Mantero J, Corley C, Brownstein J.
In press.

PLOS ONE,* March 2013** • ***Evaluation of epidemic intelligence systems integrated in the Early Alerting and Reporting project for the detection of A/H5N1 Influenza event.
Barboza P, Vaillant L, Mawudeku A, Nelson NP, Hartley DM, Madoff LC, Linge JP, Collier N, Brownstein JS, Yangarber R, Astagneau P, on behalf of the Early Alerting, Reporting Project of the Global Health Security Initiative.
www.plosone.org,Volume 8,| Issue 3, e57252. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0057252

Lancet,* May 2013** • ***Measuring vaccine confidence: analysis of data obtained by a media surveillance system used to analyse public concerns about vaccines.
Heidi J Larson, David M D Smith, Pauline Paterson, Melissa Cumming, Elisabeth Eckersberger, Clark C Freifeld, Isaac Ghinai, Caitlin Jarrett, Louisa Paushter, John S Brownstein, Lawrence C Madoff
http://www.isid.org/news/downloads/LANCET.ID.FINALMay2013.pdf

International Journal of Infectious Diseases,* 2012** • ***Latest outbreak news from ProMED-mail: Novel coronavirus. S1201-9712(12)01310-0. Pollack MP, Pringle C, Madoff LC, Memish ZA.
http://www.ijidonline.com/article/S1201-9712%2812%2901310-0/abstract

Clin Infect Dis.* 2013** • ***Forecasting high-priority infectious disease surveillance regions: a socioeconomic model. Feb;56(4):517-24.
Chan EH, Scales DA, Brewer TF, Madoff LC, Pollack MP, Hoen AS, Chodin T, Brownstein JS.
http://cid.oxfordjournals.org/content/56/4/517

ProMED in the news:

New York Times,* May 27, 2013** • ***New Tools to Hunt New Viruses. Donald G. McNeil, Jr.
http://www.nytimes.com/2013/05/28/health/new-tools-to-hunt-new-viruses.html?pagewanted=1&_r=2&src=rechp

The Economist,* April 20th 2013** • ***Pre-empting pandemics: An ounce of prevention. As new viruses emerge in China and the Middle East, the world is poorly prepared for a global pandemic, Bangkok and New York.
http://www.economist.com/news/science-and-technology/21576375-new-viruses-emerge-china-and-middle-east-world-poorly-prepared?fsrc=scn/tw/te/pe/anounceofprevention

ProMED-mail Archives

To meet the needs of research and academic institutions for faster, more efficient, more flexible searching of the ProMED archives, we are offering the complete 2012 database of ProMED-mail postings on a memory stick for access on your local system.

Created with FileMaker Pro, this searchable database system is comprised of both a database engine and data records as a royalty-free, self-contained, standalone software application that runs on either Mac or Windows platforms. The fields included in this application are: HealthMap internal ID, time stamp, title, the full text of the post, and meta data fields including diseases and locations. All of the fields are searchable both individually and in combination with standard logical operators.

Your purchase of the archives will support the maintenance of operations, including our regional networks and activities in underserved areas, and the expansion of ProMED services. This program presents an excellent opportunity for state-funded departments of health and research and academic institutions that are restricted from making charitable contributions to non-profits to support ProMED while acquiring a valuable information resource for their libraries and labs.

The Primary Archive, including the entire 2012 global English language library of ProMED posts, can be purchased for $500 (includes up to five users at the same institution.)

The Complete Archive, which comprises the entire 2012 global library of ProMED posts in English, Portuguese (ProMED-PORT), Spanish (ProMED-ESP), Russian (ProMED-RUS), and French (ProMED-FRA), is available for $1,000 (includes up to five users at the same institution.) The user base for either Archive can be enlarged at a fee of $50 per additional user.

ProMED-mail
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ProMED Internet-a-thon

ProMED-mail is a service which ISID provides without cost to ensure that news and information about emerging infectious diseases is disseminated as widely as possible. While many of the clinicians, scientists, and public health practitioners who subscribe to ProMED-mail can afford to support this information resource, many cannot. ISID is committed to making sure that everyone who needs ProMED-mail can receive it without restriction.

ProMED-mail is published 24 hours a day every day. A global network of infectious disease experts vets the information and provides insightful analysis and commentary. Providing this level and quality of service is expensive, however. Fully a third of ProMED-mail's annual budget must be covered through the generosity of its readers. If you are among those who are able to afford to support ProMED-mail, please help keep ProMED-mail unbiased, unfettered, and free.

If you believe that communication and community among the world's infectious disease experts is important, please consider a donation to ProMED-mail.

http://www.isid.org/donate/PMM_donate.shtml

INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

ISID Position Announcement

Vacancy to be filled in 2013

Program Director
International Society for Infectious Diseases
9 Babcock St., Unit 3, Brookline, MA 02446 USA

The Program Director is responsible for overseeing all scientific, training, educational, and professional development programs for this non-profit professional organization with 50,000 members in over 100 countries. Principal responsibilities include program development and management, supervision of personnel, and fund raising in conjunction with the Development Director. This position requires a strong understanding of infectious diseases clinical practice and research, management and communications skills, the ability to work with individuals from diverse background around the world, and solid writing skills. Previous experience working internationally is helpful. Minimum requirements include an MD with training in infectious diseases or a Ph.D. with experience in infectious disease related research. This position is located in Boston, requires a half-time commitment, and involves some travel.

For a full job description please go to this link:
http://www.isid.org/about/201306_ProgramDirector.shtml

New ISID Executive and Council Members

ISID is pleased to announce several additions to the ISID Executive Committe and the International Council.

Dr. Ziad Memish will join the Executive Committee for a term of six years. The Executive Committee is responsible for overseeing all of ISID's activities. It is composed of the officers of the Society and four members-at-large.

The following individuals will join the ISID International Council:

Guillermo Ruiz-Palacios (Mexico)
Claudio Lanata (Peru)
Ursula Theuretzbacher (Austria)
Hanna Nohynek (Finland)
Onder Ergonul (Turkey)
Samir Saha (Bangladesh)
Gagandeep Kang (India)
Paul Newton (Laos)
Paul Anantharajah Tambyah (Singapore)
Philippe Buchy (Cambodia)

The Council assists the Executive Committee in managing ISID's activities through participation on committees and working groups. The Council is distributed according to World Health Organization regions to ensure that all regions of the world are represented in the governing structure of the Society. Council members serve a six-year term.

For a full list of ISID Executive Committee and International Council members, please see our webpage: **http://www.isid.org/about/council_members.shtml**

Beth Mutai

Ms. Mutai holds a Bachelors and Masters Degree in Cell and Molecular Biology from Maseno University, Kenya. She works as a Research Officer at the United State Army Medical Research Unit-KEMRI, Kenya in the Laboratory of Dr. John Waitumbi. Her research interests include molecular epidemiology of emerging and re-emerging Zoonotic diseases and development of high-throughput methods for their diagnosis. Currently, she is in the process of registering for a PhD in infectious diseases at the University of Nairobi, Institute of Tropical and Infectious Disease, Kenya.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Small Grant Report

Developing a multiplex molecular assay for diagnosis of tick borne zoonoses

Beth Mutai • WRP-KEMRI, Kisumu, Kenya

Study Background

Ticks carry microorganisms that are infectious to humans and animals. Some of these pathogens cause acute febrile illness in humans and form the largest proportion of emerging zoonotic infections (Sonenshine, 1991). These include *Anaplasma phagocytophilum, Ehrlichia chaffeensis, Rickettsiae spp, Borrelia spp, Babesia spp, Bartonella spp, CCHF,* and *Coxiella burnetii* among others. Because of the multiplicity of these organisms, individual diagnosis is expensive and time consuming. A rapid multiplex test that allows simultaneous detection of infections would reduce time taken to arrive at a diagnosis without increasing the cost. In Kenya, acute febrile illness is a common presentation in health facilities (Whitty *et al.*, 2008) and are largely and unnecessarily attributed to malaria (Leslie *et al.*, 2012). Various cases of tick borne pathogens are increasingly being reported in Kenya and in neighboring countries (Potasman *et al.*, 2000, Jowi & Gathua, 2005, Richards *et al.*, 2010, Prabhu *et al.*, 2011, Maina *et al.*, 2012). In addition, there is a wide distribution of tick species known to be vectors and reservoirs of various tick borne diseases (Mutai *et al.*, 2013). This study aimed to develop and validate a multiplex molecular assay for diagnosis of tick borne zoonoses.

Specific Objectives

1) Develop a multiplex assay for simultaneous detection of *Anaplasma phagocytophilum, Rickettsiae spp, Ehrlichia chaffeensis, Borrelia burgdorferi (*causes Lyme disease), *Borrelia spp* (causes other tick borne relapsing fever), *Babesia spp, Bartonella spp* and *Coxiella burnetii*.

2) Validate the assay on selected sample sets obtained from patients with acute febrile illness

Main Activities Conducted

We have developed two real time multiplex assays that can simultaneously detect four pathogens each: Assay one comprised primers and probes for detection of *Coxiella, Anaplasma phagocytophilum, Borrelia burgdorferi* that causes Lyme disease and *Ehrlichia chaffeensis* while assay two comprised *Rickettsia, Babesia, Bartonella*, and *Borrelia spp* that cause other tick borne relapsing fever. It was not possible to develop a multiplex assay for all pathogens in one assay because the 7500 Fast Real Time PCR system (Applied Biosystems, Foster City, CA) available at our Lab has five detection channels. Taqman primers and probe sequences were selected from published literature and checked to determine which assays may have potential false positive and/or false negative issues, when compared to genomes that are currently available in the Genbank database. Probes were labeled at the 5' end with a reporter dye and a non fluorescent quencher at the 3' end. Reporter dyes were selected to allow multiplexing of four targets. Each of the primers/probe set was optimized in a singleplex assay for each pathogen and then multiplexed into a one tube assay of four pathogens. Multiplex assay 1 primers and probes were optimized to work under similar PCR condition of: 50˚C for 2 minutes, 95˚C for 10 minutes followed by 45 cycles of 95˚C for 15 seconds and 60˚C for 1 minute. Multiplex assay 2 primers and probes were optimized to work at: 50˚C for 2 minutes, 95˚C for 10 minutes followed by 45 cycles of 95˚C for 15 seconds and 56˚C for 1 minute. PCR was performed in a 10.0 µl reaction volume using PCR master mix sold by Qauntitect Probe RT-PCR kit (QIAGN GmbH, D-40724 Hilden) and 2.0 µl of DNA template.

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ISID Report of Beth Mutai

Developing a multiplex molecular assay for diagnosis of tick borne zoonoses

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

Control DNA for *Anaplasma phagocytophilum*, *Bartonella henselae*, *Babesia microti*, *Coxiella burnetii*, *Ehrlichia Chaffeensis* and *Borrelia burgdorferi* were isolated from bacterial whole cell lysate recovered from IFA slides (Fuller Laboratories, Fullerton CA). Genomic DNA for *Rickettsia* and *Borrelia duttonii* were kindly provided by Dr. Allen Richard of Navy Medical Research Center, Silver Spring Maryland. *Babesia canis* genomic DNA was kindly provided by Øivind Øines of Norwegian Veterinary Institute.

Sensitivity and specificity of the assays was tested using serially diluted control DNA in a singleplex and multiplex formats. The assay dynamics (Ct values at each dilution and limit of detection), are comparable for the singleplex and the multiplex assays. Primers and probes were shown to be specific to targets they were designed for by in silico method against published genomes. By real time PCR, the primers and probes were shown to be specific to their targets both in singleplex and multiplex assay.

Challenges and Way-Forward

We are currently working on defining assay cutoffs, which will be defined as mean signal plus 3standard deviations for 150 negative samples. For unknown samples, assay signal/cutoff (S/CO) greater than 1 will be considered positive, and less than 1 will be considered negative. S/CO values for the 400 positives will be measured. We assume accuracy targets of at least 95% sensitivity and specificity. For the sensitivity analysis with N=400 positive samples, the 95% confidence interval at a 95% sensitivity is 92.9% to 97.1%. Positive predictive value for this 72.7% prevalence collection is 98.2%. For the specificity analysis with N=150 negative samples, the 95% confidence interval at 95.3% specificity is 92.0% to 98.7%. Negative predictive value is 87.7%. Once these elements are defined the assays will be used to test over 3000 human samples available in our bio-bank.

One of the challenges we encountered was obtaining control DNA for some of the pathogens since they are listed as select agents.

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My Phan

Dr. My Phan recently graduated from the Tropical Medicine PhD program at the University of Oxford (Oxford, UK). Her research focuses on different aspects of acute gastroenteritis in young children in Vietnam.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Small Grant Report

The prevalence and phylodynamics of norovirus, an agent of diarrhea, in symptomatic and asymptomatic children in Ho Chi Minh City, Vietnam

Phan Vu Tra My,[1] Ha Minh Lam,[1] Corinne Thompson,[1,2] Hoang Le Phuc,[3] Pham Thi Ngoc Tuyet,[4] Ha Vinh,[5] Nguyen Van Minh Hoang,[1] Pham Van Minh,[1] Nguyen Thanh Vinh,[1] Cao Thu Thuy,[1] Tran Thi Thu Nga,[1] Nguyen Thi Thu Hau,[4] Nguyen Tran Chinh,[5] Tang Chi Thuong,[3] Ha Manh Tuan,[4] James I. Campbell,[1,2] Archie CA Clements,[6] Jeremy Farrar,[1,2] Maciej F. Boni,[1,2] Stephen Baker[1,2,7]

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[5] Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam;
[6] University of Queensland, School of Population Health, Queensland, Australia;
[7] The London School of Hygiene and Tropical Medicine, London, United Kingdom.

Background

Diarrhea is a major cause of illness in low-income countries and is responsible for a significant burden of disease. Despite being projected to remain within the top ten leading causes of DALYs, diarrheal disease is often overlooked as a major global health issue and does not command the same media attention as many of the other "big infections". The etiological agents of diarrhea include multiple viral, bacterial and parasitic pathogens. Enteric viruses, including rotavirus, norovirus, enteric adenovirus and astrovirus, play the leading role in terms of both morbidity and mortality of severe diarrhea cases in developing countries [1]. Despite widespread evidence that diarrhea has a broad range of etiologies, a relatively finite etiology is often assumed and syndromic diagnoses predominate in Vietnam.

Noroviruses (NoV) are positive-sense single-stranded RNA viruses belonging to the taxonomic family Caliciviridae and are considered to be the second most common cause of severe gastroenteritis in children under the age of five years [2]. The virus is a well-recognized cause of gastrointestinal infections in all ages, responsible for 90 % of diarrhea outbreaks worldwide [3]. However, whilst the role of NoV as an important cause of sporadic and endemic gastrointestinal infections in developed countries, the burden and the understanding of the pathogen in developing countries is less well understood [2, 3].

Vietnam, in particular, Ho Chi Minh City (HCMC) is transitioning through a period of rapid economic development. Such an economic change is bringing about a shift in the spectrum of pathogens causing infectious diseases, such as diarrhea [4]. We know very little about the burden, epidemiology, genetics or population structure of norovirus in HCMC and in other locations in Vietnam, as routine etiological diagnosis of diarrheal pathogens is seldom performed. Studies on prevalence of NoV infections in various settings suggest the presence of asymptomatic NoV carriers in the community, we are currently unsure of the prevalence of NoV carriage in healthy children in Vietnam. Such individuals represent a latent but mobile reservoir of the virus circulating in the community. This study aimed to describe the NoV prevalence, genotype and spatiotemporal dynamics of this pathogen in children in HCMC.

Results and Discussion

All NoV identified (N = 315) from symptomatic patients and healthy controls (N = 2,054) were subjected to direct sequencing and genotyping. NoV GII and GI were detected in 304 (96.5 %) and in 11 (3.5 %) of the NoV-positive stools, respectively. Among the GII strains, GII.4 was the most prevalent genotype (81.3 %; 247/304), belonging to 2 major genotypes, the GII.4-2006b (Minerva) and the novel GII.4-2010 (New Orleans) variant. The remaining genotypes included GII.2, GII.3, GII.6, GII.7, GII.9, GII.12, GII.13 and unassigned types within GII.4 lineage.

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There was a positive linear correlation between NoV infections and monthly rainfall ($R = 0.550$, $p = 0.029$), but no similar correlation with temperature ($R = 0.308$, $p = 0.330$). This association of NoV infections with the tropical rainy season may reflect differential transmission between climates as NoV infections are classically associated with the winter in temperate countries [5].

We investigated sequences of the two GII.4 variants from HCMC, comparing them with global sequences and previously published GII.4 sequences from Vietnam [6]. Using Bayesian phylogenetics, the evolutionary rate of NoV was calculated as 8.072×10^{-3} substitutions/site/year. The GII.4-2006b sequences from NoV originating in Vietnam [6] fell in the same genocluster as global GII.4-2006b viruses, clustering was independent of their time or place of isolation. This GII.4-2006b lineage could be further divided into two sub-lineages, strains from HCMC could be found in both, confirming co-circulation of divergent GII.4-2006b viruses. The GII.4-2010 strains clustered in a single lineage, separate from the GII.4-2006b genocluster lineage. The GII.4-2010 lineage could be differentiated partially by location, with Vietnamese and Belgian sub-lineages stemming from the New Orleans GII.4-2010 variant.

The Mantel test confirmed evidence of an association between isolation time and genotype within GII sequences ($p < 0.0001$) and GII.4 sequences ($p < 0.0001$). Yet, there was no similar association between geographical distance and genetic distance, or between isolation date and geographical distance. SaTScan spatiotemporal cluster detection analysis supported our original hypothesis, detected a cluster of six GII.4-1010 NoV (over other NoV GIIs (0.59 expected)) in a 3.8km radius in the northeast of the City (relative risk = 12.65, $p = 0.0003$), indicating that the initial dynamics of GII.4-2010 upon introduction were highly localized.

Conclusion

This study is the first investigating the prevalence of NoV in children with and without diarrhea in HCMC (Vietnam). NoV GII.4 variants predominate among a diversity of NoV strains co-circulating in this location and are highly endemic in HCMC. We also report the novel emergence of NoV GII.4-2010 variant in Asia, which exhibited a spatiotemporal phylogenetic signal upon strain introduction to the local population.

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ISID Report of My Phan

The prevalence and phylodynamics of norovirus, an agent of diarrhea, in symptomatic and asymptomatic children in Ho Chi Minh City, Vietnam

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

Dodi Safari

Dr. Safari joined the Eijkman Institute for Molecular Biology, Jakarta, Indonesia in 2001 as a research assistant. In 2006, he left for Utrecht in the Netherlands as a PhD student in the Department of Medical Microbiology, University Medical Center Utrecht. He returned to Indonesia in 2010 and was eager to continue his research on molecular epidemiology of Streptococcus pneumoniae *in the Indonesian population at the Eijkman Institute as a research fellow.*

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Small Grant Report

Serotype distribution and antimicrobial drugs susceptibility of *Streptococcus pnuemoniae* in human immunodeficiency virus infected children

Dodi Safari • Eijkman Institute for Molecular Biology • Jakarta, Indonesia

Background

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis worldwide. Incidence of invasive pneumococcal disease (IPD) varies substantially by age, socioeconomics, immune status, and geographical location [1]. Capsular polysaccharide is considered the ultimate virulence factor of *S. pneumoniae*. With over 90 serotypes identified based on the polysaccharide structure and immunogenicity, there is no universal pneumococcal vaccine and those available cover only selected serotypes. Knowledge on the exact serotypes distribution in disease is critical for making an informed decisions about vaccination policies, especially in high risk groups (like HIV-infected individuals) or in national immunization programs.

Nasopharyngeal colonisation is the obligatory first step in the pathogenesis of pneumococcal disease and therefore considered the most important risk factor for IPD. It also provides the basis for horizontal spread of pneumococci in the community, making it an important target for preventive measures [2]. In present study, a cross sectional study was performed to investigate *S. streptococcus* carriage in human immunodeficiency virus (HIV)-infected children in Jakarta, Indonesia. Currently, the publish data on *S. pneumoniae* carriage or invasive from Indonesian population was still limited. In 2001, Soewignjo et at was reported that prevalence of *S. pneumoniae* carriage in healthy children in Lombok Island, Indonesia was 48% [3].

Materials and Methods

A cross-sectional study was performed among human immunodeficiency virus (HIV)-infected children group from January to July 2012 in Jakarta, Indonesia in collaboration with Nia Kurniati, MD from the CiptoMangun Kusumo Hospital/Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. During this study, we collected 116 of sample swabs and we confirmed that 90 of 116 swabs obtained from positive HIV-infected children based on medical information. Nasopharyngeal (NP) swabs were collected from children using a flexible nasopharyngeal floxed swab with deep nasopharyngeal swab as described previously [4]. These NP swabs were placed into 1.0 ml of skim milk tryptone glucose glycerol (STGG) transport medium and shipped on wet ice within 4 h to Microbiology Laboratory, Eijkman Institute. Samples of NP-STGG (20 µl) were plated onto a 5% sheep blood agar plate supplemented with 5 mg/L gentamicin and incubated at 35°C for 24–48 h with 5% CO2. *S. pneumoniae* was identified based on strain susceptibility to optochin. All pneumococcal isolates were tested in PCR for presence of genes coding pneumococcal surface antigen A (psaA) and major pneumoccal autolysin (lytA) and capsule transcriptional regulator (wzg) [5-7]. Strains PCR-negative for wzg were tested for polymorphism within recA in order to determine the phylogenetic relation with *S. pneumoniae*. Serotyping was performed by a multiplex sequential PCR (SM-PCR) [5]. All *S. pneumoniae* isolates were tested for susceptibility to a set of antimicrobial drug discs: Ampicillin, amoxycillin, choramphenicol, cefixime, erythromycin, penicillin, sulfamethoxazole/ trimethoprim, cefotaxime, cefprozil, spiramyin, and clindamycin by use the disc diffusion method. *S. pneumonia*e strain (ATCC 49619) was also grown and used as a control test. Definition of non-susceptibility was interpreted based on the size of the zone inhibition.

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ISID Report of Dodi Safari

Serotype distribution and antimicrobial drugs susceptibility of *Streptococcus pnuemoniae* in human immunodeficiency virus infected children

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

Results and Discussion

Nasopharyngeal samples from 42 children were culture-positive for *S. pneumoniae*. All pneumococcal strains were pspA-, lytA- and wzg-positive except for 5 strains negative for wzg alone. Eleven strains were untypeable in SM-PCR including all negative for wzg. The recA analysis revealed all but one of those to be *S. pseudopneumonia*. It lowers the number of carriers to 41 (46%). Serotype 19F was identified as most common (8 isolates, 20%carriers) followed by 9A and 6A/B (4 carriers each), 23F (3 carriers), 9V, 35B, 11A (two carriers each) and serotpes 18C, 12F, 15B/C and 35F (single carrier each). Presence of *S. pneumoniae* in carriage correlated negatively with CD4 count. There was no correlation between carriage and patient age, previous antibiotic use, exposure to cigarette smoke or crowding at home. The coverage for conjugated pneumococcal vaccines immunogenic in children but targeting selected few serotype was between 62% to 74% for only serotyped strains isolated by us from carriage for 7- and 13-valent vaccines, respectively. We found that more than a half of isolates were non susceptible to cefixime and sulfamethoxazole/ trimethoprim and 12.5 % of isolates were non-susceptible to pencilillin. Serotype 19F has more non susceptible to different antimicrobial drugs excepted to amoxyllin and ceferoxime. No significantly correlation between carriage of *S. pneumoniae* and risk factors of age, antibiotic use, exposure to cigarette, or number of family member, only risk factor of CD4 percentage negatively correlated with *S. pneumoniae* carriage.

Our study gives insight into population of *S. pneumoniae* strains circulating in carriage in patients at high risk for IPD due to age and comorbidity. We expect our results to be helpful in shaping preventive strategies targeting IPD in Indonesia both on national and on local level.

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Kamlesh Gidwani

Dr. Gidwani has been developing his scientific career at the Infectious Disease Research Laboratory of Institute of Medical Sciences, BHU-India, working on marker of Leishmania donovani parasite infection in asymptomatic infected individuals and also marker of exposer of its vector sand fly in endemic region of Visceral Leishmaniasis. In his initial three year of doctorate work he was involved in a European Commission KALANET project in which he used sand fly saliva ELISA to measure the efficacy of insecticidal bed nets in intervention clusters incomparision to controls. Later, he took on the challenge to adapt the Quantiferon commercial assay into an application for leishmaniasis. He will be working with an immune response in the saliva of sand fly at the London School of Tropical Medicine and Hygiene.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Fellowship Report

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify novel salivary peptides with serum of asymptomatic infection in VL.

Kamlesh Gidwani
PI Dr. Shyam Sundar, Institute of Medical Sciences, Banaras Hindu University, Varanasi India
PI Dr. Matthew Edwards Rogers, London School Hygiene Tropical Medicine, London, UK

Training Program at the *London School Hygiene Tropical Medicine, London, UK*

Background

In Indian subcontinents visceral leishmaniasis (VL or kala-azar) caused by obligate intracellular parasite *Leishmania donovani* is transmitted exclusively by the bite of female *Phlebotomus argentipes* sand flies (Swaminath CS, et al 1942). The disease is fatal if not treated, and it is responsible for an estimated 60,000 deaths per year worldwide (WHO 1998). The annual incidence of kala-azar cases is estimated to be 0.5 million and the prevalence to be 2.5 million (WHO, 1998). More than 90% of the world's reported VL cases are in Indian subcontinents, Sudan and Brazil. Every year, more than 100,000 cases of VL occur in India alone, with the state of Bihar accounting for more than 90% of these cases, followed by West Bengal and Eastern Uttar Pradesh (WHO 2009). Importantly 80-90% of *L. donovani* infections living in endemic areas are asymptomatic usually associated with strong cell-mediated immunity (Blackwell JM et al 2009). Annual incidence rates and prevalence of asymptomatic infection of *L. donovani* are known to be much higher than the frequency of clinical cases (Bern C et al and Bart O et al 2011).

Sand fly always injects saliva to the host during a bite irrespective of uninfected or infected with leishmania parasites. It contains a variety of anti-haemostatic, vasodilatory and immunomodulatory compounds. Sand fly saliva antibodies correlate with sand fly exposure in: Turkey (*P. sergenti*), Tunisia (*P. papatasi*), Brazil (*Lu. longipalpis*) (Rohousova I *et al* 2005, Barral A *et al* 2000 and Volf P 2001). Sand fly saliva antibodies in children correlate with 'protection' to VL in Brazil (seroconversion from a-saliva –ve to +ve correlates with a-*L. infantum* DTH) (Gomes RB et al 2002). Vaccination with either whole saliva, defined salivary proteins (PpSP15) or pre-exposure to the bites of uninfected sand fly have all shown to protect against *L. major* infection (Kamhawi, S et al 2000).

Recently, we developed pre-adsorbed *P. argentipes* saliva ELISA method as a marker of exposure which distinguishes people from a sand fly free country (UK) or people from urban areas of India where VL does not exist, from those sera collected from the VL-endemic State of Bihar. Densities of *P. argentipes* and *P. papatasi* correlated well with the antibody response to saliva of sand flies (Clements et al 2009). And in preliminary study we found highest anti salivary protein antibodies among asymptomatic individuals (Rogers ME *et al* Unpublished). So it was proposed by us to compare the western blot profile against saliva of sand fly by using different groups of serum including asymptomatic and to relate it to a protective profile. For better specificity we also want to replace the crude saliva with recombinant salivary protein for better marker of exposer.

To increase the participation rate of blood sampling for any community trial it is better to use finger prick blood on filter paper in comparison to venous blood, for that it is necessary to evaluate the recovery of antibodies against the saliva of the sand fly from blood spots archived on filter paper. To improve the specificity of this assay it is necessary to replace the crude saliva by its recombinant antigen by using western blot profile with different group's sera of endemic and non- endemic region.

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The specific aim of small grant ISID fellowship:

1) To evaluate the recovery of antibodies against the saliva of the sand fly *Phlebotomus argentipes* from blood spots archived on filter paper.

2) To analyse the reactivity of Indian sera from a variety of groups exposed to sand flies and VL patients against sand fly saliva by Western blot to accurately determine the molecular weight of interesting protein bands that were (a) markers of exposure to *P. argentipes* bites and (b) unique to asymptomatic VL patients.

Training Method

To investigate whether recoveries of anti salivary protein antibodies from archived blood spots on FP, we used washed blood cells from heparinized venous blood of healthy volunteer mixed with equal volume of hyper immune sera (known high titer of anti salivary protein Abs) and spotted on what man filter paper number-3 (FP) allowed drying at room temperature then the FP blood spots eluted in diluent at 4C for overnight. FP elute were serially diluted (1:20 to 1:1280) parallel with same hyper immune sera alone and anti *P. argentipes* saliva antibodies detected through ELISA (Clements M. et al 2010).

For specific aim 2, saliva from colonized *P. argentipes* (LSHTM, London UK) was collected from female flies five days old post-emergence and maintained on 70% sucrose solution given *ad libitum* . Pools of 20 sand fly salivary gland pairs were collected in 100 µL PBS on ice and individually pierced to release their saliva. After centrifugation (1,800*g* for 5 minutes), the saliva was collected from the supernatant fraction and frozen at −70°C until used. Saliva of 3-4 flies was run in each lane of SDS PAGE, precast gradient gel (4-12%) used and the protein bands were transfer on nitrocellulose membrane for blotting with different groups of serum and was finally develop it to substrate observe protein band profile and identify the peptide band which only present Indian people and which only in asymptomatic individuals among Indian groups for marker of exposure and protection respectively.

Group of serum samples used for western blot were (a) VL-contact (serologically positive) asymptomatic, sub clinically infected (b) VL-contact (serologically negative); (c) Active VL; (d) 6 month Post-treated VL; (e) Non Endemic (Healthy) Control as negative control (f) UK resident.

Results

Recovery of anti salivary Abs from serially diluted elute of blood spots on FP was excellent and the trend of OD was almost similar to the serially diluted same serum samples with our saliva ELISA. In our another aimSaliva of *P. argentipes* were run on SDS PAGE (precast gradient gel) and after transferring on nitrocellulose membrane blots with different groups of serum mentioned above. A number of antigenic bands were recognized by the serum sample of different groups with different frequency and intensity. In addition, major bands of 15 kDa, 20 kDa, 35 kDa and 62 kDa were frequently recognized. While most of these frequently recognized bands were also reactive with sera from patients with kala-azar and patients who had been cured of kala-azar . Differences in reactivity were observed for the 15 kDa and 62 kda bands, against sera of Indian people while it was absent in UK sera, further 35 and 20 kda band was only present in asymptomatic individuals and absent in all other groups. Among the controls very faint cross-reactions were observed.

Conclusion

In recent years major advances have been made in the development of diagnostic methodologies that focus on evaluation of the patient's antibody response to determine whether infection or exposure has taken place. In continuation of this diagnostic development, we are also developing the salivary protein based diagnostic tools for identification of asymptomatic individuals. Because of the conditions prevailing in areas of endemicity, any sophisticated method cannot be employed on a wider scale. There is a need for a simple rapid and accurate test with good sensitivity and specificity, which can be used without any specific expertise.

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ISID Report of Kamlesh Gidwani

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify a novel salivary peptides with serum of asymptomatic infection in VL

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

The experiments of recovery of anti salivary Abs from FP blood spots confirmed that we can use this methodology for study of exposer of sand fly on archived finger prick blood samples on FP for vector control measure programs on larger series of samples which was not possible on venous blood samples.

Recombinant antigen(s) specially based on 15 and 62 kda bands shown in our western blot experiments with Indian serum samples while it was absent with UK sera can be replaced the whole *P.argentipes* saliva for saliva ELISA for better marker of exposer. This will improve the specificity and sensitivity of the assay and this approach will not require labor-intensive sand fly rearing and salivary gland dissection, this modification will make the assay more amenable for large-scale application.

In endemic region of VL most of the leishmania infected population remains healthy and showed serology and PCR positive. We also used such asymptomatic sera to see the western blot profile against saliva and we identify 20 and 35 kda bands was only present in asymptomatic groups which can be further used as a marker of protection for vaccine candidates.

The results of this fellowship will allow us to (i) publish a new methodology of utilising dried blood spots for studying exposure to sand fly bites and (ii) identify specific sand fly salivary proteins that reflect exposure to *P. argentipes* to improve the specificity and sensitivity of the current saliva ELISA by developing recombinant salivary antigens.

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ISID Report of Kamlesh Gidwani

Immune response of humans to sand fly *P. argentipes* saliva: Western blot approach to identify a novel salivary peptides with serum of asymptomatic infection in VL

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Calendar of Events

2013

12–23 August

13th Edition of the Dengue International Course

Havana, CUBA

http://instituciones.sld.cu/ipk/anuncioc/

Under the auspices of: Tropical Medicine Institute of Havana, 'Pedro Kouri' Tropical Medicine Institute (IPK), Cuban Society of Microbiology & Parasitology, Ministry of Public Health of the Republic of Cuba (MINSAP), Pan American Health Organization (PAHO), World Health Organization (WHO). Objective of the course: Participants—physicians, virologists, immunologists, sociologists, epidemiologists, entomologists, and health managers, among others interested in this field, along with many professors from several prestigious national and international institutions—may have the opportunity to debate on the most relevant and updated aspects of this disease and its control.

5–10 September

Options for the Control of Influenza VIII,

Cape Town, SOUTH AFRICA

http://www.controlinfluenza.com/

In 25+ years, Options for the Control of Influenza has become the largest scientific forum solely focused on the prevention, control, and treatment of influenza. Join your colleagues from academia, industry, and government agencies for this important triennial event. The Options VIII conference will draw an international audience of virologists, infectious disease specialists, physicians, clinicians, scientists, public health specialists, researchers, epidemiologists, healthcare policy experts, and government agency staff to review the most recent advances in influenza science. The Options VIII Conference Chairs welcomes the latest scientific research on influenza for Oral or Poster presentation during the Options for the Control of Influenza VIII conference. Travel awards available.

17–20 September

31st World Veterinary Congress

Prague, CZECH REPUBLIC

http://www.wvc2013.com/en/home

Program: http://www.wvc2013.com/en/scientific-programme

Contact: wvc2013@guarant.cz

Scientific Program: Highly attractive lectures with world class speakers. Sessions include: Canine & Feline Medicine, Canine & Feline Surgery, Equine Medicine & Surgery, Bovine Medicine, Exotic Animals Medicine, Porcine Medicine, Global Veterinary Seminar on Animal Welfare, Aquatic Medicine, Poultry Medicine, Food Hygiene, Epidemiology, Veterinary Professional Wellness.

18–20 September

2nd International Conference on Digital Disease Detection University of San Francisco

San Francisco, CA, USA

http://healthmap.org/ddd/

Email: anna.tomasulo@childrens.harvard.edu

Brought to you by HealthMap at Boston Children's Hospital, Harvard Medical School and Skoll Global Threats Fund. To register and review the schedule, speakers, panels, and workshops, please see website.

28–30 October

Chikungunya 2013

Langkawi Island, MALAYSIA

http://umconference.um.edu.my/CHIKV2013

Email: chikv2013@um.edu.my

Chikungunya 2013 is a major conference focusing on chikungunya and related alphaviruses. A broad range of topics will be addressed by leading experts; the conference will appeal to virologists, clinicians, epidemiologists and entomologists. There will also be opportunities for selected participants to present papers at oral and poster sessions, including a number of Young Investigator Awards.

3–5 November

What Will it Take to Achieve an AIDS-free World?

San Francisco, CA, USA

http://www.translationalmedicine-lancet-cell.com/HIV/

This translational medicine conference on HIV research is organized jointly by the editors of *The Lancet* and *Cell*.

2014

2–5 April

16th International Congress on Infectious Diseases

Capetown, SOUTH AFRICA

http://www.isid.org/icid

Encompassing all of the fields in infectious diseases with particular attention being paid to the major infectious causes of death in Africa and elsewhere, which include AIDS, malaria, TB, pneumonia and enteric infections including typhoid fever and diarrhea. In all of these fields there are exciting interventions underway in Africa, the results of which will be presented in Cape Town. In addition there are major areas of neglected tropical diseases that will be discussed and a particular focus will be on the largely uncounted burden of nosocomial infections in developing countries. The ISID has always had its focus on the global burden of infectious diseases and it is important for us to announce our return to Africa some 20 years after the last meeting of the Society there in Kenya in 1992.

27 July–1 August

International Union of Microbiological Societies 2014 Congresses

Montréal, CANADA

http://www.montrealiums2014.org/

- XIVth International Congress of Mycology
- XIVth International Congress of Bacteriology and Applied Microbiology
- XVIth International Congress of Virology

2015

16–18 January

International Conference on Infectious & Tropical Diseases (ICTID)

Phnom Penh, CAMBODIA

http://ictid.webs.com

Email: geo_stv_goss@hotmail.com

Focusing on infectious and tropical problems in Asia and Africa, and on other global problems such as malaria, tuberculosis, HIV/AIDS, dengue/other viral fevers, melioidosis, schistosomiasis, hemorrhagic fevers, ricketsioses, diarrheal diseases, sepsis, meningitis, respiratory tract infections, avian influenza, neglected infectious diseases, hepatitis and more.