Oral Abstract Presentations

17th International Congress on Infectious Diseases
Hyderabad • India • 2016
Expression of glycoprotein gene of Rabies virus and evaluation of recombinant protein for
seromonitoring of vaccinal antibodies in dogs

R. Sharada¹, S. I. Isloor², V. Balamurugan³, B. Veeresh⁴, V. Suryanarayana⁵, R. Manisha³, D.
Rathnamma³, M. Satyanarayana³
¹Veterinary college, Hassan, India, ²Veterinary college, Bangalore, Karnataka, India, ³NIVEDI,
Bangalore, India, ⁴Veterinary College, Bangalore, India, ⁵IVRI, Bangalore, India, ⁶Veterinary
college, Bangalore, India

Background: In India, control of rabies in dogs by mass vaccination is the practical
approach. Currently, viable infectious Rabies virus (RABV) dependent and laborious Rapid
Fluorescent Focus Inhibition Test (RFFIT) is employed in selected laboratories in India to
monitor the vaccinal antibodies. This situation demands an alternative rapid, sensitive,
specific and user friendly test. Hence, this study was undertaken to express the recombinant
glycoprotein (rRVL-G) of Dr. Larghi’s strain of RABV and evaluate its diagnostic potential.

Methods & Materials: The RABV propagated in BHK 21 cells was used as the source of G
gene. The Polymerase Chain Reaction (PCR) product of G gene was cloned into pGEM®-T
Easy Vector and transferred into competent Top 10 E. coli. The insert from the recombinants
(pGRVL-G) was subcloned into pET32a vector, transferred into TOP 10 cells and
recombinants (pETRVL-G) obtained. These recombinants were transferred into E.coli
BL21 and screened for expression and immunogenicity by SDS-PAGE and Western Blotting
respectively. Indirect ELISA was standardized by Checkerboard titration of complete RABV-
G protein (RV-G) and diagnostic potentials of rRVL-G confirmed.

Results: The PCR product of RABV complete G gene revealed a band of 1596 bp.
The PCR product cloned in pGEM®-T Easy Vector and subcloned in pET32a vector was
confirmed by Restriction Enzyme (RE) digestion and colony PCR (Fig.1a, 1b) and
sequencing. Further, SDS-PAGE of the IPTG induced clones revealed a fusion protein of 75
kDa which was confirmed to be immunogenic by Western blotting (Fig.2a, 2b) when probed
with anti rabies vaccinal dog sera. Standardization of indirect ELISA and application using
RV-G and rRVL-G by testing of 40 anti rabies vaccinal dog sera of varying RFFIT
titres revealed a significant correlation in the performance of both the antigens.

Conclusion: The outcome of the work suggested the diagnostic potentials of the
recombinant protein in ELISA for seromonitoring of antirabies vaccinal antibodies. In view of
the availability of ELISA facilities, expertise and limitations of RFFIT in India, the
results encourage the development of recombinant G-protein based ELISA as a newer
diagnostics for sero monitoring of antirabies vaccinal antibodies in both domestic and street
dogs at a regular interval of time.
The EpiCore Project: Using innovative surveillance methods to verify outbreaks of emerging infectious diseases

Z. Haddad¹, L. Madoff², E. Cohn³, J. Olsen⁴, A. Crawley⁴, J. Brownstein⁵, M. Smolinski⁶, J. Shao⁷, M. Pollack⁸, D. Herrera-Guibert⁹

¹International Society for Infectious Diseases, Brookline, MA, USA, ²ProMED-mail, Boston, MA, USA, ³Boston Children’s Hospital, Boston, USA, ⁴Skoll Global Threats Fund, San Francisco, USA, ⁵Children’s Hospital, Boston, USA, ⁶Skoll Global Threats Fund, San Francisco, CA, USA, ⁷International Society for Infectious Disease, New York, USA, ⁸Task Force for Global Health, Atlanta, USA

Background: Over the past two decades, initial disease outbreak reports were increasingly based on nontraditional sources such as news media, online search queries, social media and participatory systems. Strategically tapping into these resources can help speed up detection, reporting and responses to new outbreaks of emerging infectious diseases allowing earlier interventions.

The EpiCore Project – a joint venture between the Skoll Global Threats Fund, HealthMap, ProMED-mail, and TEPHINET – seeks to maximize the advantage of these nontraditional sources by creating a system for field-based verification of reports from these sources. This system creates a cadre of trained health professionals from around the world and leverages their expertise to verify reports they receive in their geographic proximity through innovative surveillance approaches.

Methods & Materials: The EpiCore project developed a specialized expert-led training that aims to highlight current disease surveillance challenges, demonstrate innovative surveillance methods, explain their role in complementing traditional surveillance, introduce different online sources for outbreak surveillance, and allow the participants to assess challenges in current innovative surveillance approaches before introducing the unique online platform specifically developed by the EpiCore project to connect health professionals to verify outbreak reports.

The EpiCore Project provided the training in two formats, online – for accepted applicants to the project, and in-person, which was conducted at 6 TEPHINET Conferences, including the Global Conference and the regional conferences in Asia, Africa, the Middle East, and Latin America over the past two years.

Results: 174 people attended a series of TEPHINET regional workshops and provided feedback through surveys, interviews, and focus groups. This feedback helped mold a specialized online training that was necessary to reach global audiences, and further understand the mechanism by which to recruit and engage participants worldwide. Since the launch in October of 2015, 80% accepted applicants successfully finished the online training and were granted privileges to access the EpiCore platform where they could start receiving and responding to requests for verification of disease outbreaks.

Conclusion: The EpiCore project’s training and online platform are developing a cadre of trained health professionals to verify reports of potential outbreaks from nontraditional sources, complementing traditional surveillance, and contributing to finding outbreaks faster.
Effect of non-steroidal anti-inflammatory drugs (NSAIDS) on bleeding and liver in dengue infection

A. Wijewickrama1, G. Abeyrathna2, S. Gunasena3, D. Idampitiya2
1Infectious Diseases Hospital, Sri Lanka, Colombo, Sri Lanka, 2Infectious Diseases Hospital, Colombo, Sri Lanka, 3Medical Research Institute, Colombo, Sri Lanka

Background: Many treatment guidelines for Dengue prohibit using Non-Steroidal Anti-inflammatory Drugs (NSAIDS). However, these are still used either prescribed by general practitioners or taken 'over the counter' by general public, for symptomatic relief. Side effects of NSAIDs include hepatitis and increased bleeding. But, there is no study evidence on how NSAIDS affects bleeding and liver in Dengue infection.

Methods & Materials: A prospective Case Control Study was conducted to determine the actual effect of NSAIDs on bleeding and liver in Dengue. All patients admitted to Dengue Management Unit at the Infectious Diseases Hospital, Colombo for four months from 1st of July 2014 were included in the study. Dengue infection was confirmed by NS1 antigen or Dengue specific IgM antibodies. A history of NSAID treatment prior to hospital admission was looked for. These patients were followed up to see the development of bleeding and/or hepatitis.

Results: There were 919 patients with confirmed Dengue infection with 499 males and 420 females. Age ranged from 12 to 86 years. (mean 31 years.) 57.2% (n=526) had DF; 42.8% (n=393) had DHF. 6.6% (n=65) were definitely treated with NSAIDs prior to admission, while 57% (n=577) have not used NSAIDs definitely; the rest (35.6%) were not certain. Out of DF patients 28% developed bleeding in the non-NSAIDS group while 36.7% had bleeding in the NSAIDS group (<0.05). Among the DHF patients 34% had bleeding in non-NSAIDS group while 51% had bleeding in the NSAIDS group (<0.05).

Conclusion: There is significant increase in bleeding and in liver enzyme derangement in Dengue patients treated with NSAIDS compared to those didn’t have NSAIDS. Hence, NSAIDS should not be used in fever patients when Dengue is a possibility.
Clinical features and virology of hand foot mouth disease in Southern Vietnam, July 2013 - March 2015

V. M. T. Hoang1, T. A. Nguyen2, T. T. Tran3, M. T. Ha3, V. Do4, V. Ho5, T. H. Nguyen6, K. Truong Huu7, N. Le7, C. Nguyen Van Vinh6, Q. Phan6, L. Thwaites2, S. Sabanthan9, T. Le10, H. R. van Doorn11

1 Oxford Clinical Research Unit, Ho Chi Minh City, Ho Chi Minh City, Viet Nam, 2 Oxford Clinical Research Unit, Ho Chi Minh City, Viet Nam, 3 Children's Hospital 2, Ho Chi Minh, Viet Nam, 4 Children's Hospital 1, Ho Chi Minh, Viet Nam, 5 Children's Hospital 1, Ho Chi Minh City, Viet Nam, 6 Children's Hospital 1, Ho Chi Minh City, Viet Nam, 7 Hospital for Tropical Disease, Ho Chi Minh, Viet Nam, 8 Oxford Clinical Research Unit, Ho Chi Minh City, Viet Nam, 9 Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam, 10 Oxford University, Ho Chi Minh City, Viet Nam

Background: In Asia, hand, foot and mouth disease (HFMD) is associated with large and sometimes severe outbreaks since 1997, and is caused by enterovirus A (EV-A), in particular EV-A71. Monitoring the pattern of replacement between EV-A serotypes, the associated clinical profiles and pathogen evolution are essential for understanding the progress of outbreak/epidemics and development of intervention strategies.

Methods & Materials: A large prospective study was conducted at three referral hospitals in southern Vietnam since July 2013: Children’s Hospital 1, Children’s Hospital 2 and Hospital for Tropical Diseases in Ho Chi Minh City. Clinical data, throat and rectal swabs were collected and analysed using multiplex real-time and nested RT-PCRs to detect and identify specific EV serotypes. Selected positive swabs were then subjected to VP1 or whole-genome deep sequencing.

Results: Over 18 months, 1350 HFMD patients were enrolled. The most common detected pathogens included CV-A6 (24%), EV-A71 (23%), CV-A16 (11%) and CV-A10 (9%), followed by CV-A2/A4/A12 and EV-Bs. A total of 295 genome sequences were obtained. B5 (n=156) was the predominant EV-A71 subgenogroup. Phylogenetic analysis further showed that all CV-A16 (n=25), CV-A2 (n=7), CV-A5 (n=3), CV-A8 (n=4), CV-A12 (n=10) and CV-A14 (n=1) were closely related to those from China and the region, while CV-A4 (n=10), CV-A6 (n=26) and CV-A10 (n=43) clustered with viruses belonging to genogroups collected in other parts of the world. Two separate introductions were observed for CV-A4.

clinically, there was no significant difference between CV-A6, CV-A16 and CV-A10 groups. Patients with EV-A71 infection were older than those with non-EV-A71 infection (21.7 vs. 17.3 months old, p<0.001). Other differences included myoclonus (21% vs. 13%, p=0.001), irritability (17% vs. 70%, p<0.001) and location of erythema. There was a trend toward EV-A71 detection and clinical severity: 23% grade 1, 17% (2A), 39% (2B group 1), 71% (2B group 2), 64% (3) and 67% (4).

Conclusion: Our study represents the most comprehensive descriptive HFMD study from Vietnam to date. The analysis of 1350 patients revealed important insights into the epidemic patterns of this multi-pathogen disease, including pathogen associated phenotypes and viral evolution, which are essential for public health strategies and of clinical significance.
Functional Dipeptidyl Peptidase 4 (DPP4) in mink supports entry and replication of Middle Eastern respiratory syndrome coronavirus: American Mink (Neovision vision), a novel in vivo model of MERS-CoV infection

S. K. Naveen¹, C. Kannadka², M.-C. Chen², S.-C. Lin², G. J. N. Nichols², M. Patterson², M. Kappes², T. G. Voss²  
¹SRI International, Harrisonburg, Virginia, USA, ²SRI International, Harrisonburg, USA

Background: A novel coronavirus, named the Middle East Respiratory Syndrome coronavirus (MERS-CoV), was first identified in humans in 2012. MERS infections are characterized by acute respiratory distress with fatal cases often diagnosed with comorbidity factors including diabetes, cardiovascular disease, or obesity. Continued emergence of MERS-CoV, coupled with a lack of understanding of the natural history of MERS highlights the importance of identifying therapeutics for treatment of MERS-CoV infections in humans. Like other coronaviruses, the MERS-CoV virion utilizes a large surface spike (S) glycoprotein for interaction with and entry into the target cell. The host cell protein dipeptidyl peptidase 4 (DPP4, aka CD26) was identified as a cellular receptor for MERS-CoV and the specific interaction of the receptor-binding domain (RBD) of MERS-CoV spike protein and DPP4 was determined recently by crystallography.

Methods & Materials: Our laboratory has been working to develop rational in vitro and in vivo models of MERS-CoV infection to allow better understanding of the pathogenesis and transmission potential of virus, and also to evaluate potential therapeutic and vaccine approaches to treat or prevent MERS in humans. Here we present studies focused on characterization of the MERS-CoV receptor, DPP4 in cells from the American mink (Neovision vision) as well evaluation of mink as an animal model of MERS-CoV infection. Using multiple approaches we have shown a cell line derived from mink lung epithelium to be susceptible to infection by MERS-CoV.

Results: Western blot and PCR analysis of mink lung epithelium cells demonstrate the presence of DPP4, the receptor for MERS-CoV expressed in mink, suggesting a role for this receptor in viral entry in this species. Characterization of the expression of DPP4 in mink cells reveal multiple isoforms, which show varying patterns of expression in cells transfected with each DPP4 isoform using confocal microscopy.

Conclusion: In conclusion, evaluation of known DDP4 inhibitors for antiviral activity against MERS-CoV reveal potential therapeutic approaches to treatment of MERS with existing, licensed compounds. Studies underway using mink as an in vivo model of MERS-CoV infection and pathogenesis support it’s use in discovery and development of therapeutic and vaccines for MERS-CoV in humans.
Multiple introductions of MERS-CoV in a 2014 hospital outbreak in Riyadh, Saudi Arabia

S. Fagbo1, L. Skakni1, D. K. Chu2, M. Garbati3, M. Peiris2, A. M. Hakawi4
1King Fahad Medical City, Riyadh, Saudi Arabia, 2The University of Hong Kong, Hong Kong, China, 3King Fahad Medical City, Riyadh, Saudi Arabia, 4KFMC, Riyadh, Saudi Arabia

Background: In 2012, the Middle East respiratory syndrome (MERS), a zoonotic disease caused by the MERS Coronavirus (MERS-CoV) emerged in Saudi Arabia as a disease with global health implications. Though much of its transmission dynamics remain unclarified, most cases (over 1250) have occurred in Saudi Arabia with substantial contribution from hospital outbreaks. This molecular epidemiological study investigated a MERS outbreak at the King Fahad Medical City (KFMC), a 1,200-bed tertiary care hospital in Riyadh that occurred between March 29 and May 21, 2014.

Methods & Materials: Following MOH guidelines, respiratory specimens (nasopharyngeal swab, tracheal aspirate or bronchoalveolar lavage) from persons including health care workers (HCWs) suspected to have MERS-CoV infection were tested using a reverse transcription PCR targeting the Orf1a and upstream E-gene (upE). In parallel, these samples were tested for 15 other respiratory viruses using a Multiplex PCR platform. Epidemiologic data were extracted from electronic health records, sick leave records of HCWs and direct interviews. Stored remnants of PCR positive samples were retrieved and sent to The University of Hong Kong where overlapping PCR products generated from multiple sets of PCR on synthesized cDNA were used to sequenced the entire MERS-CoV genome with >3–5 times genome coverage. BEAST (version 1.8) was used for phylogenetic analysis.

Results: 45 MERS-CoV infected persons were documented during this period: 23 HCWs; 13 long stay in-patients; and 8 patients infected outside KFMC. The source of infection for 1 person was inconclusive. Comparison of retrieved full-length MERS-CoV sequences from 10 patients and a partial sequence from another with other publicly available MERS-CoV sequences revealed that this outbreak was part of a larger outbreak that involved several hospitals in Riyadh. The larger outbreak seemed to have originated from a zoonotic transmission event around December 2013 (95% highest posterior density interval November 8, 2013–February 10, 2014) thus indicative of probable sustained human-to-human spread over a 5-month period.

Conclusion: Using whole genome sequencing, we demonstrated multiple introductions of MERS-CoV in a hospital outbreak that was hitherto thought to be otherwise.
Moving Leptospira to the focus of One Health epidemiology: Lessons from large scale genome analysis of pathogenic species

N. Ahmed, K. Nalam
University of Hyderabad, Hyderabad, India

Background: With their dynamic and agile genomes, pathogenic *Leptospira* have shown tremendous acumen to adapt to changing host niches and the environment. Next generation sequencing (NGS) of their genomes has provided a lot of insight in to the ability of *Leptospira* to evolve fitness advantage and survival mechanisms which have direct bearing on the development of diagnostic and control strategies such as discovery of global vaccine candidates. NGS data analysis of highly diverse Leptospirae has been a formidable challenge although the automation of data handling and analysis could impart sophistication in commandeering the translational exploitation of genomic co-ordinates.

Methods & Materials: Using Leptospiral NGS data with in-house pipelines, we assembled 172 full genomes of pathogenic species and strains obtained from a wide range of human and animal hosts representing all continents. Pan and core genomes were computed and analyzed for all pathogenic species and functional classification and conserved domain search of proteins were performed for all core genomes to understand predicted fitness advantages of different species with respect to different hosts or geographical regions. Pathogen restricted proteins were analyzed and compared to individual species/genomes to get insights into the pathogenicity of the organism.

Results: A total of 427 pathogen specific proteins were identified and their characteristics were deduced. Out of these, 83 unique proteins with signal peptides were identified with predicted roles in host pathogen interactions. A total of 16 genomic islands with proteins arranged in operons were identified indicating possible evolution and acquisition of virulence and survival functions through lateral gene transfer mechanisms. Some of the virulence factors and downstream mechanisms gleaned by us appear to be entailing crucial roles for *Leptospira* relevant to long-term colonization in the host and transmission propensity with wide host tropism.

Conclusion: The potential virulence factors, diagnostic markers and vaccine candidates as identified in our study could lead to devising much sought after strategies relevant to control of Leptospirosis. Due to the identification of a genomic basis for extended survival acumen (and vast host tropism), *Leptospira* would certainly occupy significant focus of attention under the One Health regimen.
Integrated human and animal vaccination delivery to Nomadic Fulani communities in Northern Nigeria 2015

I. M. Bomoi¹, N. E. Waziri¹, P. Nguku¹, A. Tsofo²
¹Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Abuja, Nigeria, ²Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria

Background: Nigeria remains the only African country yet to interrupt transmission of wild polio virus and has a high incidence of childhood vaccine preventable diseases. Routine immunization (RI) services have not achieved sufficient coverages in remote rural settings especially among nomadic Fulani pastoralist. The high mobility and dispersion of these pastoralists makes easily missed by healthcare services yet these pastoralist often seek medical care for their animals regardless of the cost. We adopted an integrated human and animal vaccination strategy with the aim of increasing access and demand for RI services among nomadic pastoralist.

Methods & Materials: Nomadic communities and stock routes in Bauchi were identified and geocoded using GPS enabled phones. We assessed RI and animal vaccination coverage in these communities. Three rounds of targeted joint human and animal vaccination campaigns were carried out. Vaccinations and other healthcare services were administered to children, women of child bearing age, cattle and dogs. Vaccination teams were composed of local veterinary officers, healthcare workers and health promotion officials.

Results: A total of 4285 children less than one year of age and 1624 women were vaccinated in 49 settlements. Vaccination coverage increased from 22.7% to 80.1%. Of the 1271 children given oral polio vaccine (OPV) and 836 given pentavalent vaccine, 311 (24.5%) and 348 (41.6%) respectively were zero dose. Dropout rate was 42%. A total of 496 (30.5%) women that received Tetanus Toxoid (TT) received it for the first time. The animal component of the study gave intervention to a total of 28581 cattle increasing the coverage from 41% to 61%, 26 dogs were vaccinated against rabies.

Conclusion: Routine immunization coverage increased in these communities largely due to the administration of human vaccinations alongside animal vaccination and other healthcare services. High dropout rate recorded can be attributed to the high mobility of these pastoralists. We recommend that the government adopt this strategy in nomadic pastoralist communities.
A synthetic consensus anti-Spike protein DNA vaccine induces protective immunity against Middle East Respiratory Syndrome Coronavirus in non-human primates


1Perelman School of Medicine at the University of Pennsylvania, Philadelphia, USA, 2University of Saskatchewan, Saskatoon, Canada, 3Inovio Pharmaceuticals Inc, Plymouth Meeting, USA, 4University of South Florida Morsani College of Medicine, Tampa, USA, 5GeneOne Life Science, Seoul, Korea, Republic of, 6Special Pathogens, National Microbiology Laboratory, Winnipeg, MB, Canada, 7National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, USA, 8University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Background: First identified in 2012, Middle East respiratory syndrome (MERS) is caused by an emerging human coronavirus, which is distinct from SARS-CoV, and represents a novel member of lineage C betacoronoviruses. Since its identification, MERS-CoV has been linked to over 964 infections manifesting with severe morbidity and often mortality (i.e. approximately 400+ deaths) in the Arabian Peninsula, Europe, in the US and in Korea. Human-to-human transmission has been documented with nosocomial transmission appearing to be an important route of infection. The significant recent increase in cases of MERS in the Middle East, coupled with the lack of effective antiviral therapies or vaccines to treat or prevent this infection are significant causes for concern.

Methods & Materials: A synthetic DNA plasmid based vaccine containing a full-length consensus MERS-S protein sequence was constructed and the cellular and humoral immunogenicity of MERS-vaccine was evaluated in mice, macaques, and camels. Following immunization, NHPs were challenged with infectious MERS-CoV (EMC/2012) and monitored for signs of infection by clinical scoring and examinations. Viral load was measured by qRT-PCR and tissue sections were stained with H&E.

Results: An optimized DNA vaccine encoding the MERS spike protein induced potent cellular immunity and antigen specific neutralizing antibodies in mice, macaques, and camels. Vaccinated rhesus macaque monkeys seroconverted rapidly and exhibited high levels of virus-neutralizing activity. Upon MERS viral challenge all of the monkeys in the control-vaccinated group developed characteristic disease, including pneumonia. Vaccinated macaques were protected and failed to demonstrate any clinical or radiographic signs of pneumonia.

Conclusion: A consensus DNA MERS-vaccine was able to generate both a strong T cell and neutralizing antibody response in multiple animal models, including camels, a natural host for MERS-CoV and a probable source of human infection. MERS-vaccine was also able to protect NHPs from an infectious MERS-CoV challenge. These results demonstrate the promise of this consensus DNA MERS-vaccine as a candidate for vaccine modality against this emerging pathogen.
Climate change and disease dynamics - A big data perspective
D. Lopez¹, G. Sekaran²
¹VIT University, vellore, Tamil Nadu, India, ²VIT University, Vellore, India

Background: The objective of this research is to predict disease scenarios based on environmental conditions change and climatic variability by combining regional climate models with mathematical models for disease transmission. Malaria and dengue fever are the most important vector borne diseases in the tropical and sub-tropical countries. Integration of large repositories of geospatial and health data derived from traditional stream as vital statistics, surveillance and hospitalization, and non-traditional sources including social media networks provide valuable insights into the spatio-temporal determinants of health and wellbeing.

Methods & Materials: Data on infectious affected by vector borne diseases (Malaria) are collected from various private and public health centres, for the period starting January 1998 to December 2013 in Tamil Nadu, India. Daily weather data is collected from Regional Meteorological Centre, Chennai (Figure 1). The suggested approach is implemented as a Big Data system using lambda architecture and MapReduce data processing model (Figure 2). Pearson correlation coefficient is computed in the proposed framework to find the climatic factors that greatly influence the transmission of the vector borne diseases.

Results: This paper proposes a new architecture for modeling the climate change and vector borne diseases in real-time. A variety of big data analytical algorithms and data visualization approaches were used in the proposed big data based disease surveillance system to present the geographic regions at risk during this century. We found that maximum temperature is positively correlated while incidences of malaria and minimum temperature, wind, rainfall, humidity are negatively correlated with malaria incidence (Figure 3).

Conclusion: The proposed early warning system is developed for continuous monitoring of information related to climatic change and public health as they unfold. These systems are in most instances, timely surveillance systems that collect information on epidemic prone diseases in order to trigger prompt public health interventions. Developing countries like India needs effective surveillance system and equity in health delivery programs for taking corrective actions to improve health conditions of vulnerable populations.
Pattern of HIV-1 drug resistance mutations among patients failing thymidine analogue and non-thymidine analogue based first-line failure in South India
S. Sivamalar¹, T. R. Dinesha², S. Gomathi³, J. Boobalan⁴, A. Pradeep⁵, S. Poongulali⁶, S. S. Solomon⁵, S. Solomon⁵, P. Balakrishnan⁵, S. Saravanan⁵
¹YRG CARE infectious Diseases Laboratory, chennai, Tamil Nadu, India, ²YRG CARE, chennai, Tamil Nadu, India, ³YRG CARE, chennai, India, ⁴Y.R. Gahtine Centre for AIDS research and Education, Chennai, Tamilnadu, India, ⁵YRG CARE, Chennai, India

Background: HIV-1 Drug Resistance Mutations (DRMs) among individuals with Immunological failure (IF) on different NRTI based first-line regimen, Thymidine analogue (TA) - AZT & D4T and Non-Thymidine Analogue (NTA) - TDF; and predict viral drug susceptibility to gain vision about optimal treatment strategies for second-line.

Methods & Materials: Cross-sectionaly, 400 HIV-1 infected patients, failing first-line HAART were included in the analysis. HIV-1 pol gene spanning 20 – 240 codons of Reverse Transcriptase was genotyped by validated homebrew method and mutation pattern was examined, (IAS-USA 2014 and Stanford HIV drug resistance database v7.0)

Results: Out of 400, majority had subtype C infection 97.2% (n=389) and any DRM was seen in n=379. On analyzing two groups, duration of failure was longer for TA (n=279), with the median of 57.6 (IQR 35.2-76.2) months compared to NTA (n=121), 24 (IQR 11.2-51.8) months, (p<0.05). Among individual mutation analyzed K65R (23.2% vs. 4.6%), L74V (10.7% vs. 0.3%) and Y115F (11.6% vs. 0.7%) was significantly higher among NTA compared to TA, (p<0.001); whereas M184V and TAM accumulation was significantly high in TA (p <0.05), Among NNRTI mutation K101E/P/H (18.1% vs. 8.5%), G190A (32.7% vs. 27.6%) was higher among NVP (p<0.05) and K103N (57% vs. 35.1%), V106M (30.2% vs. 12.1%) was higher among EFV (p <0.05).

Based on the mutation pattern observed in both groups, we analyzed the impact of DRMs on future therapy and found as; in TA high level of resistance was observed to all drugs, except TDF (71.5%) >AZT (56.3%) >ABC (50%). In the same way among NTA AZT (78.6%) >TDF (63.7%) and >ABC (38.1%) was observed. On combining both, among TA failures still 56.7% and 71.4% can be considered for AZT and TDF based second-line. Similarly, among NTA failures still 63.7% and 78.6% can be considered for TDF and AZT based second-line regimen.

Conclusion: As expected TA based first-line failure had developed more cross resistance compared to NTA group, but still majority can be recycled in the same regimen as second-line option. Further, in resource limited settings, treatment outcome is monitored immunologically; when virological based treatment monitoring comes in practice further emergence of DRMs can be avoided by early switching.
The impact of HIV infection on the burden and severity of influenza illness in Malawian adults
A. Ho¹, S. Aston², H. Jary¹, M. Alaerts³, M. Menyere¹, J. Mallewa¹, M. Nyirenda³, D. Everett¹,
N. French⁴, R. Heyderman⁵
¹Malawi Liverpool Wellcome Trust Clinical Research Programme, Blantyre 3, Malawi,
²Liverpool School of Tropical Medicine, Liverpool, United Kingdom, ³Queen Elizabeth Central
Hospital, Blantyre, Malawi, ⁴Institute of Infection and Global Health, Liverpool, United
Kingdom, ⁵Malawi Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi

Background: The impact of HIV infection on the incidence and severity of influenza illness in adults in sub-Saharan Africa (SSA) is unclear. Although annual seasonal influenza vaccination is recommended for HIV-infected persons in developed settings, it is not part of routine HIV care in SSA.

Methods & Materials: At the Queen Elizabeth Central Hospital in Blantyre, Malawi, we conducted: i) a prospective cohort study, to compare the incidence of influenza illness between HIV-infected and HIV-uninfected adults; ii) a case control study, to determine risk factors for severe influenza presentation. Cases were adults with severe influenza illness (lower respiratory tract infection (LRTI) requiring hospitalization). Controls were adults with mild influenza presentation (influenza-like illness (ILI) managed as outpatients). Influenza was identified from nasopharyngeal specimens by real-time reverse transcription polymerase chain reactions (RT-PCR).

Results: The cohort study enrolled 360 HIV-infected (median CD4 count 390 cells/cm³) and 248 HIV-uninfected adults, providing 520 and 348 person-years of observation, respectively. Between April 2013 and March 2015, 24/229 (10.5%) ILI episodes in HIV-infected and 5/119 (4.2%) in HIV-uninfected adults were influenza PCR-positive. HIV-infected adults had almost three times increased risk of laboratory-confirmed influenza illness compared to HIV-uninfected adults (incidence rates 46.0 vs. 14.5 per 1000 person-years; incidence rate ratio 2.75, 95% confidence interval (CI) 1.02-7.44). In the case control study, 56/518 (10.8%) patients with hospitalised LRTI, and 88/642 (13.7%) with ILI were influenza PCR-positive. HIV prevalence among the influenza-positive cases and controls were 70% and 30% respectively. On multivariable analysis, HIV infection was the most important risk factor for severe influenza presentations (odds ratio (OR) 4.98, 95%CI 2.09-11.88), with a population attributable fraction (PAF) of 57%. Unimproved sanitation (OR 3.14, 95%CI 1.25-7.84) and food insecurity (OR 20.85, 95% CI 1.97-221.16) were also associated with hospitalised influenza.

Conclusion: A substantial burden of influenza was identified in both HIV-infected and uninfected Malawian adults. Moreover, HIV-infected adults appear to have increased susceptibility and severity of influenza presentations. Influenza vaccination in this at-risk group is likely to be beneficial. However, the optimal mechanism for vaccine introduction and evaluation in already overstretched health systems in SSA will need to be determined.
Background: Studies assessing the impact of Highly Active Antiretroviral Therapy (HAART) on B cell subpopulations in HIV infected children are scarce. Hence, we undertook this study to describe the B cell compartment and the effect of HAART in a cohort of HIV-infected children (<5 years of age).

Methods & Materials: Treatment-naïve HIV infected children were enrolled and followed regularly till 12 months; HIV uninfected children with no major illness were recruited as healthy controls (n=51). CD10-CD20+ B cells were characterized as naïve (CD21+CD27-), resting memory (21+CD27+), activated memory (CD21-CD27+) and tissue like memory (CD21-CD27-) B cells. The frequency of these B cell subpopulations was evaluated in HIV-1 infected children at baseline, 6 months and 12 months of HAART. The percentage of B cell subpopulations at baseline were compared between HIV infected and uninfected children.

Results: Twenty-seven HIV-1 infected HAART naïve children of median age 22 (12-44) months [boys: 63%] were enrolled. At baseline, Marked differences were observed in B cell subpopulation distribution among HIV infected children and healthy controls. The median frequencies of naïve B cells and resting memory B cells were significantly lower (p=0.001; p=0.0005 respectively), while activated memory and tissue like memory B cell pool were significantly expanded in HIV infected children compared to healthy controls (p<0.0001 for both). At 12 months of HAART, no significant differences were observed in the frequencies of naïve (p=0.88) and activated memory (p=0.13) B cells between HIV infected HAART treated children and healthy controls. However, the frequency of tissue like memory B cells was still significantly higher in HIV infected HAART treated children (p=0.001) and significantly inferior resting memory B cell pool was observed compared to their healthy counterparts (p=0.002).

Conclusion: This study re-iterates the effectiveness of HAART in pediatric population; however, it raises concern about the inadequate reconstitution of B cell compartment at 12 months of treatment.
Evaluation of novel rapid bead based method for capturing of Mycobacterium tuberculosis in sputum

S. Verma^1, T. Dhole^2, S. Kashyap^3, M. Kumar^4

^1Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, ^2SANJAY GANDHI POSTGRADUATE INSITUTE OF MEDICAL SCIENCES, LUCKNOW, India,

^3Integral Institute of Medical Sciences & Research, Lucknow, India, ^4SGPGIMS, Lucknow, India

**Background:** Tuberculosis is one of the major global health problems. The diagnosis of TB relies primarily on the identification of acid fast bacilli (AFB) by microscopy. Microscopic examination of direct specimen smear stained with Ziehl-Neelsen (ZN) staining and studies have found this assay to be less sensitive. TB-Beads can be used as an alternative to centrifugation for concentration of TB bacilli. The current study intends to compare the conventional specimen pre-treatment with the novel technique of sample pre-treatment based on magnetic beads in single tube.

**Methods & Materials:** We evaluated the feasibility of a ligand-coated magnetic bead technology to concentrate *M. tuberculosis* in single tube (decontamination, concentration and staining) prior to detection by LED-based fluorescence microscopy. We compared the quality of this method with the Ziehl-Neelsen (ZN) microscopy performed after modified Petroff (MP) method and direct fluorescence microscopy (FM) in sputum samples at a reference laboratory in India. The kappa coefficient ($K_c$) analysis performed for positive correlation between the tests and LJ culture taken as a gold standard for evaluation to sensitivity and specificity of performed tests.

**Results:** The head to head comparison on all performed tests were performed, concentrated magnetic bead-FM possess higher positivity rate (58.7%) than and direct microscopy (45.3%) and MP-ZN microscopy (41%) among all 150 sputum samples. By comparing with culture the concentrated magnetic bead-FM (88.5%) had significantly higher sensitivity than MP-ZN (73%) and direct FM (74.4%). The specificities of magnetic bead FM direct FM and MP-ZN microscopy were 74%, 86% and 94% respectively. The fair correlation found between culture and ZN microscopy ($K_c = 0.67$), magnetic bead-FM ($K_c = 0.62$) and $K_c = 0.60$ with direct FM.

**Conclusion:** Newer Magnetic bead concentration of Mycobacteria in clinical samples showed a significant improvement in the sensitivity of microscopy compared to direct FM and concentrated ZN microscopy. These methods will be improving the diagnostic performance of smear microscopy and reducing the tuberculosis burden by single tube processing.
Comparative evaluation of in-house Real time IS 6110, nested MPT 64 PCR and Roche AMPLICOR 16s rRNA PCR for diagnosing tuberculous meningitis
R. Gupta, R. Thakur, N. Jalan, R. Pumanshi, M. Paul, S. Kushwaha
Institute of Human Behavior and Allied Sciences, Delhi, India

Background: Early diagnosis of tuberculous meningitis (TBM) is still a diagnostic challenge due to paucibacillary nature of the disease and conventional methods being quite insensitive and time consuming. We evaluated performance of three real time PCR assays targeting MPT64, IS6110 and 16s rRNA gene sequences for early diagnosis of TBM

Methods & Materials: A total of 100 consecutive probable TBM patients and 50 non TBM patients were enrolled from an ongoing prospective study on tuberculous meningitis (July 2012 to Dec 2014). CSF specimens were subjected to microscopy and automated BACTEC MGIT culture. In-house Real time nested MPT64 and IS6110 PCR were standardized using H37Rv spiked CSF and evaluated for diagnostic utility along with commercially available Roche Amplicor Real time PCR assay.

Results: Out of 100 clinical specimens processed, the sensitivity of smear microscopy and culture was 4% and 42% respectively. The sensitivity, specificity of Real time IS6110 PCR, Nested MPT 64 assay and Roche Amplicor Real time PCR assay was 71 %, 98% ; 69%, 98% and 25%, 100% respectively against probable TBM as reference standard. The Real time IS6110 PCR, Nested MPT 64 assay and Roche Amplicor Real time PCR assay could detect M. tuberculosis in only 84%, 77% and 30% of culture positive patient’s respectively

Conclusion: Real time IS6110 PCR proved to be a simple and rapid method to diagnose TBM with sensitivity, specificity of 71%, 98% only. Nested MPT 64 assay though had comparable sensitivity and specificity was much more difficult due to nested design and higher risks of contamination. None of the PCR was 100% sensitive for detecting all culture positive samples suggesting that for TBM diagnosis there is no single rule out test and all the tests are contingent upon their ability to pick the target in tested volume of CSF.
Evolutionary patterns of T cell epitopes in Mycobacterium tuberculosis strains isolated in India

A. Ramaiah1, S. Nayak1, S. Rakshit1, A. McGuire2, S. Shanmugam3, J. Chandrabose3, S. Narayanan1, A. Earl2, S. Swaminathan3, A. Vyakarnam1

1Indian Institute of Science, Bangalore, India, 2Broad Institute of MIT and Harvard, Cambridge, USA, 3National Institute for Research in Tuberculosis (ICMR), Chennai, India

**Background:** Mycobacterium tuberculosis (Mtb) is an obligate, persistent, intracellular human pathogen. Host immune pressure and associated Mtb immune evasion that drive the evolution of Mtb, is crucial for diagnosing and designing effective vaccines. Human T cell responses are essential for containment of Mtb through secretion of IFNγ and TNFα. Previous studies have shown that Mtb T cell epitopes (TCEs) are hyperconserved, suggesting there is little evidence for immune selection pressure and indicating that antigenic variation may contribute to bacterial persistence. Thus, the aim of this study was to provide an in-depth analysis of the pattern of changes in TCEs in circulating Indian Mtb strains.

**Methods & Materials:** We analyzed 79 Mtb whole genome sequences generated from strains isolated from South Indian pulmonary TB patients. Extensive bioinformatic analyses were employed, but not limited to determine i) phylogenetic relationships ii) extent and nature of mutations harbored within TCEs and iii) binding affinity of novel mutated TCEs (mTCEs).

**Results:** Phylogenetic trees revealed clustering of 79 strains to three lineages: EAI, CAS and Beijing. We identified that 13% of 1101 examined TCEs within the Mtb genome were mutated in at least one strain, with 67% of mutations resulting in single amino acid changes. We report for the first time, focused mutations in 16 of 66 highly immunodominant CD4 T cell antigens comprising experimentally verified epitopes. More than 80% of the sequenced strains had common mutations across this antigenic group, with 4-6 mutated immunodominant antigens occurring per strain. The most impacted functional category of mutated immunodominant antigens belonged to the cell wall and cell processes. Furthermore, we identified three mTCEs (one each in mce2A, hemK and espA antigens) that have not previously been identified to be immunodominant, but present in all the strains studied. Binding affinity of the majority of mTCEs to common Indian HLA alleles was marginally higher than the corresponding parent TCEs.

**Conclusion:** Presently, investigating the impact of these novel mutants and their corresponding parent epitopes in CD4 T cell functional assays that enable the evaluation of the immunogenicity of candidates peptides. Together, our data provide novel insights into the importance of immune selection pressure in Mtb evolution in India.
Molecular evidence of melioidosis among patients suspected for tuberculosis

E. Jayakumar\textsuperscript{1}, R. Barani\textsuperscript{1}, M. Mani\textsuperscript{1}, V. Seshan\textsuperscript{1}, S. Muthiah Kothandaramanujam\textsuperscript{2}, R. Balakrishnan\textsuperscript{1}, P. Srikanth\textsuperscript{1}

\textsuperscript{1}Sri Ramachandra University, Porur, Chennai – 600116, Tamil Nadu, India, Chennai, India, \textsuperscript{2}(fmr) Sri Ramachandra University, Porur, Chennai – 600116, Tamil Nadu, India, Chennai, India

\textbf{Background:} Melioidosis is an emerging infectious disease caused by \textit{Burkholderia pseudomallei}. It is endemic in South East Asia and Northern Australia. Clinical manifestation of the infection mimics tuberculosis (TB) by causing acute to sub-acute to chronic disease. We aimed to detect the presence of \textit{B. pseudomallei} among patients suspected with tuberculosis using a nested Polymerase chain reaction (PCR) and to analyze its risk factors.

\textbf{Methods & Materials:} The retrospective study was conducted in a tertiary care centre. Samples that tested negative by Microscopy and PCR (targeting IS6110 & TRC4) specific to \textit{Mycobacterium tuberculosis} were included in the study. The nested PCR method was performed by targeting the 16S-23S spacer region of \textit{B. pseudomallei}. The PCR products were analyzed by agarose gel electrophoresis for a band size of 251 bp. Plasmid DNA from cloned PCR product was used as positive control for the nested PCR. Clinical details of those samples that tested positive were reviewed.

\textbf{Results:} A total of 55 patient samples were included in this study, of which 36(65.45 \%) were sputum and 19(34.5 \%) were pus samples. Nine samples (16.3 \%) were detected positive for \textit{B. pseudomallei} DNA by nested PCR. They were from 4 (7.27 \%) sputum and 5 (9 \%) pus samples. Among these, 2(3.6 \%) sputum samples were from patients attending DOTS centre. The demographic profile of the patients showing positivity to \textit{B. pseudomallei} (n=9) showed Male: Female ratio of 1.25:1 and they were from coastal regions of India such as Tamil Nadu 8(88.8 \%) and West Bengal 1(11\%). Major clinical manifestations in the positive patients were weight loss (44\%), fever (33\%), cough (33\%), breathlessness (33\%), loss of appetite (22\%) and sepsis (22\%). The co-morbid conditions encountered included chronic kidney disease (33\%), cancer (11\%), history of trauma (11\%) & HIV (11\%).

\textbf{Conclusion:} Evidence of \textit{B. pseudomallei} in patients suspected for TB shows that Melioidosis masquerades as TB. Unless looked for actively, patients possibly may receive unnecessary empirical Anti-tubercular therapy. Rapid tests such as PCR for \textit{B. pseudomallei} may be used to rule in a diagnosis of Melioidosis especially in patients with no lab evidence of TB.
Missed pulmonary TB screening opportunities at Primary Healthcare Facilities: An Exit Study, Eastern Cape Province, South Africa

P. F. Kweza¹, N. Abraham¹, M. M. Claassens², C. Van Schalkwyk³, A. Medino-Marino¹
¹Foundation for Professional Development, Pretoria, South Africa, ²Desmond Tutu TB Centre, Cape Town, South Africa, ³South African DST/NRF Centre of Excellence for Epidemiological Modelling and Analysis (SACEMA), Cape Town, South Africa

Background: A key driver of TB transmission is the significant number of “missed” TB cases who are undiagnosed. In 2013, WHO estimated 3.3 million missing cases of TB. Though South African national guidelines recommend universal TB screening of all individuals presenting to health facilities, TB suspects continue to be missed. We assessed the magnitude of missed pulmonary TB cases presenting at primary health care (PHC) facilities in Buffalo City Metropolitan District (BCM).

Methods & Materials: A cross sectional study was conducted at 10 randomly selected PHCs. Inclusion criteria: individuals exiting selected PHCs; age ≥18 years; not on TB treatment; positive TB symptom screen. Participants were stratified into two groups, those attending clinics for 1) respiratory symptoms, and 2) other health reasons. Participants were interviewed by study staff. TB symptomatic individuals were asked to provide a sputum for GeneXpert MTB/Rif testing.

Results: A total of 647 individuals were enrolled; 319 with respiratory symptoms and 328 for other health reasons. Of those who attended facilities with respiratory symptoms, 251/319 (78.7%) were screened for TB by clinic staff and 12/319 (3.8%) were asked to provide sputum for testing. Of those who attended for other health reasons and were TB symptomatic, 62/328 (18.9%) were screened for TB and 5/328 (1.5%) were asked to provide sputum for testing. A total of 541 (83.6%) participants provided a sputum sample; 31 (5.7%) tested positive for MTB (n=27 rifampicin sensitive; n=3 MDR; n=1 rifampicin inconclusive). Of those infected with TB, 21 (67.7%) were men, 6 (19.4%) had contact with a known TB patient within the last year, 4 (12.9%) had diabetes mellitus, 7 (22.6%) self-reported HIV infection, and 5 (16.1%) previously had TB. Nineteen (61.3%) of those who tested positive for MTB were screened but not asked to provide sputum for testing by clinic staff. Eighteen (58.1%) of those who tested positive for TB had sought care for respiratory symptoms but were not asked to provide sputum for testing by clinic staff.

Conclusion: Missed screening opportunities within health facilities are contributing to the 3.3 million missing cases of TB. Universal screening within South African PHCs must be conducted as mandated by national guidelines.
Performance evaluation of Anyplex™ MTB/MDR/XDR for detection of first and second-line drug resistance in Mycobacterium tuberculosis

L. A. Malinga¹, B. Sibandze², R. Tsireledzo³, N. Makhado⁴, C. Maluleka⁴, B. Magazi⁵
¹South African Medical Research Council, Pretoria, Gauteng, South Africa, ²University of Pretoria, Pretoria, South Africa, ³Sefako Makgatho Health Science University, Pretoria, South Africa, ⁴National Health Laboratory Service/Dr George Mukhari Hospital, Pretoria, South Africa, ⁵National Health Laboratory Service/Tshwane Academic Hospital/University of Pretoria, Pretoria, South Africa

Background: The Anyplex™ MTB/MDR/XDR (Seegene) detection assay is a real-time multiplex PCR that can detect Mycobacterium tuberculosis (M.tb) and resistance to first line drugs (Rifampicin (RIF) and Isoniazid (INH)) and second-line drugs (Fluoroquinolone and injectables). Currently rapid diagnosis of drug resistance is performed through molecular assays that have limited detection probes. Anyplex assay has a high number of probes that are used to detect first and second-line drugs. We compared the performance of Anyplex™ MTB/MDR/XDR with culture and Genotype MTBDR plus 2.0 (Hain Lifescience) for detection of M.tb drug-resistance to first and second-line drugs.

Methods & Materials: We retrospectively sampled 34 culture isolates and prospectively collected 51 clinical sediments. The 34 culture strains were tested for RIF, INH, OFL (Ofloxacin) and kanamycin (KAN). Of the 51 clinical sediments evaluated, microscopy results were positive (45), negative (2) and scanty (1) while 3 had no results. The clinical sediments were further tested on Genotype MTBDRplus. DNA sequencing was performed on gyrA and eis genes detected by Anyplex™ MTB/MDR/XDR assay.

Results: The concordance between culture and Anyplex™ MTB/MDR/XDR respectively for INH, RIF, OFL and KAN was 26/26 (100%), 26/27 (96.0%), 15/16 (81.25%) and KAN (63.6%). Clinical specimens had a concordance between Genotype MTBDRplus and Anyplex™ MTB/MDR/XDR for INH and RIF was 4/4 (100%) and 16/17 (85.7%). The specificity for RIF and INH in clinical sediments was 95.0% and 95.3% respectively. Analysis of discordant results of KAN resistant samples revealed eis –C12T mutation not detected by Anyplex™ MTB/MDR/XDR assay.

Conclusion: Anyplex™ MTB/MDR/XDR has good overall performance for detection of drug resistance in culture and clinical sediments. High sensitivity and specificity for detection of INH makes it an alternative method to Genotype MTBDRplus which is based on conventional PCR.
Impact of Type I IFN dysregulation in M. tuberculosis infection on T cell responses

A. Ahmed¹, S. Nayak², S. Babu³, A. Vyakarnam²
¹Indian Institute of Science, Bangalore, Karnataka, India, ²Indian Institute of Science, Bangalore, India, ³National Institutes of Health-NIRT-International Center for Excellence in Research, Chennai, India

Background: It is only recently that the role of Type I Interferon (IFN) α/β signalling in tuberculosis (TB) is being appreciated. An exaggerated Type I IFN induced response is associated with TB disease severity. However, the mechanisms by which an elevated IFN response impacts TB disease severity are not clear. It is also unclear whether type I IFN induced responses modulate regulatory T (Treg) cell function in tuberculosis. The specific objectives of our study therefore were to firstly, validate the Type I Interferon driven gene signature in blood of Indian patients with active TB and identification of unique gene signatures that help characterise latent and active tuberculosis using RNA (transcriptome) sequencing which has not been carried out so far. Secondly, to characterise Treg frequency and function in individuals with TB and further identify molecular signatures in whole blood that might specifically impact Treg frequency and function.

Methods & Materials: Towards the above listed objectives, RNA isolated from whole blood samples from healthy, latently infected, and active TB individuals before and after treatment was subjected to sequencing and a comprehensive list of differentially expressed genes was obtained which was mined to obtain information about modulation of Type I IFN genes. For the next part of the study frequency of Treg cells from healthy, latent and active TB individuals were studied by flow cytometry and an assessment of Treg function was carried out with the help of in vitro suppression assays.

Results: Data obtained from RNA transcriptome sequencing revealed up-regulation of several Type I IFN genes like STAT2, IFIH1, IFITM3, GBP4, TrafD1, UBE2L6, IFI44, BATF2, OASL, ISG15 etc in active tuberculosis. Interestingly, the expression of some of these genes was found to diminish with treatment. A comparison of Treg frequencies revealed that latently infected and individuals suffering from active TB infection had elevated Treg cell numbers. Also, the suppressive function of Treg cells from active TB afflicted individuals was diminished compared to latently infected and healthy controls.

Conclusion: We are currently investigating if this disparity in Treg suppressive function is a consequence of a differential Type I IFN response. Together, these data provide novel insights to TB pathogenesis.
Ps20: A novel correlate of inflammation and infection in TB?
1Indian Institute of Science, Bangalore, India, 2St. Johns Research Institute, Bangalore, India, 3Arogyavaram Medical Centre, Madanapalle, India, 4National Institute for Research in TB, Chennai, India, 5National Institute for Research in Tuberculosis (ICMR), Chennai, India, 6National AIDS Research Institute, Pune, India

Background: The soluble factor ps20 encoded by the human WFDC1 gene on Chromosome 16, is an ancient whey acidic protein (WAP) family member, characterized by highly evolutionarily conserved domain comprising eight cysteines that make 4 disulphide bonds. WAPs are soluble innate immune mediators implicated in homeostatic control of inflammation and broad anti-infective activities. Previous studies in our laboratory highlighted a novel function of ps20. We demonstrated ps20 expression in CD4+ T cells, which rendered these cells highly susceptible to HIV infection through up-regulation of ICAM-1. Consistent with this observation, we showed that plasma ps20 levels positively correlated to CD4+ T cell count. We also demonstrated that ps20 levels at the CD4+ T cell level showed a strikingly inverse relationship with IFNg and silencing of ps20 in CD4+T cell clones led to upregulation of IFNg. This study was designed to further examine the role of ps20 in IFNg regulation in a chronic infection, such as TB, where IFNg levels are known to be impaired.

Methods & Materials: (i) An in-house ps20-specific sandwich ELISA was calibrated and used to confirm ps20 levels in plasma of 30 treatment naïve active TB, 15 TB treated (12 months post treatment), 12 IGRA+ and 10 IGRA- subjects.
(ii) To further test if raised plasma ps20 in active TB correlated with reduced IFNg expression, we measured the ps20 and IFNg mRNA and protein levels in PBMC cultured activated with PHA/IL-2 in time-course assays.
(iii) Rapamycin, a known regulator of the mTOR pathway is well established inhibitor of IFNg expression. We therefore used this regulator to further determine if suppression of IFNg leads to induction of ps20.

Results: (i) The ELISA data showed active TB subjects had a significantly higher (p=0.0356) plasma ps20 compared to IGRA+ and IGRA- subjects.
(ii) PHA/IL-2 immunomodulation data confirm active TB subjects to have lower IFNg than IGRA+ and IGRA- subjects with concomitantly higher ps20 expression.
(iii) Rapamycin Inhibition assay confirms IFNg expression to be significantly reduced in the presence of Rapamycin with a concomitant marginal but consistent induction of ps20.

Conclusion: These studies highlight ps20 may be a novel regulator of IFNg and provide novel insights on the possible role of ps20 in TB pathogenesis.
Safety, immune lot-to-lot consistency and non-inferiority of a fully liquid pentavalent DTwP-HepB-Hib vaccine: Results from Phase III licensure study of Shan5™

A. Sil, B. N. Patnaik, V. J. Midde
Shantha Biotechnics Private Ltd (A Sanofi Company), Hyderabad, India

**Background:** Pentavalent combination vaccines perform a key role in increasing vaccine coverage rate; and provide an efficient and reliable method of implementing WHO recommendations for controlling diphtheria, tetanus, pertussis, hepatitis B and Hib infections on a worldwide basis.

**Methods & Materials: Study design:** A phase III, multi-center, randomized, single blinded study of a fully liquid pentavalent DTwP-HepB-Hib investigational vaccine (Shan5™) was conducted across India in healthy toddlers and infants. Cohort 1 consisted of 15 toddlers aged 15-18 months, administered with a single booster dose; Cohort 2 consisted of 1085 infants aged 6-8 weeks, administered 3 doses at 6-8, 10-12 and 14-16 weeks of age.

**Objectives:** Subjects in Cohort 1 were evaluated for safety and immunogenicity. Immunogenicity and safety were evaluated in Cohort 2 subjects vaccinated with a three-dose primary immunization of either the investigational vaccine or a locally licensed comparator vaccine (Pentavac™ SD). Immune consistency analysis among three lots of the investigational vaccine, and immune non-inferiority analysis of pooled (three lots) data of investigational vaccine versus comparator vaccine were also evaluated in cohort 2.

**Results:** The vaccines demonstrated comparable safety and immune responses in cohort 1. In cohort 2, immune non-inferiority against the comparator vaccine (primary endpoint) was demonstrated for all five antigens and safety results (secondary endpoint) were comparable between vaccine groups. Equivalent immune consistency among three lots was observed for all antigens except whole cell pertussis antigens, where a marginal variation was observed. The variation was linked to the low power of the test and concluded to be clinically insignificant.

**Conclusion:** The investigational, fully-liquid, whole-cell pertussis (wP) containing pentavalent vaccine has a good safety profile and immunologically non-inferior to the licensed comparator vaccine. This study was the basis for licensure in India and also WHO- prequalification for international use.
Correlates of county-level non-viral sexually transmitted infection hot spots in the US

B. Chang¹, W. Pearson², K. Owusu-Edusei Jr.²
¹Icahn School of Medicine at Mount Sinai, New York City, USA, ²Centers for Disease Control and Prevention, Atlanta, USA

**Background:** Studies on county-level hot spots of sexually transmitted infections (STIs) in the entire U.S. and their association with socio-economic factors are lacking. In this study, we used a combination of hot spot analysis (HSA) and spatial regression to examine the county-level correlates of the most commonly reported curable sexually transmitted infections (STIs) in the U.S.

**Methods & Materials:** We obtained reported county-level total case rates of chlamydia, gonorrhea, and primary and secondary (P&S) syphilis in all counties in the 48 contiguous states using the National Notifiable Disease Surveillance System (NNDDS). We computed temporally-smoothed rates using 2008–2012 data. Covariates were obtained from county-level multiyear (2008-2012) American Community Surveys (ACS) from the US census. We conducted HSA (applying the false discovery rate (FDR) correction) to identify hot spot counties for all three STIs. Hot spots were defined as counties or clusters of counties with rates above the global mean (p< 0.05). We used logistic spatial regression with the spatial error model (SEM) to determine the association between hot spots and the covariates and variance inflation factor (VIF<10) analysis to reduce the effect of multicollinearity on the coefficients.

**Results:** HSA indicated that ≥ 80% of hot spots for each STI were in the South. Spatial regression results indicated that, compared to White non-Hispanics, a 1% increase in the percentage Black non-Hispanic was associated with a 3.3% (p<0.01; chlamydia), 3.8% (p<0.01; gonorrhea) and 2.5% (p<0.01; P&S syphilis) increase in the odds of being a hot spot county. Compared to the other regions (West, Midwest and Northeast), counties in the South were 6.6 (p<0.01; chlamydia), 6.7 (p<0.01; gonorrhea) and 4.0 (p<0.01; P&S syphilis) times more likely to be hot spots (Table 1).

**Conclusion:** Our study provides important information on hot spot clusters of non-viral STIs in the entire US, including associations between hot spot counties and socio-economic/demographic factors.
Recombinant accessory cholera enterotoxin of *Vibrio cholerae* activate ANO6 via RhoA-ROCK-PIP2 signaling to induce secretory diarrhea

I. A. Sheikh¹, J. Aoun², P. Sarkar³, T. Saha², M. H. Kazi²

¹National Institute of Cholera & Enteric Diseases, Kolkata, West Bengal, India, ²National Institute of Cholera & Enteric Diseases, Kolkata, India, ³National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India

Background: *Vibrio cholerae* accessory cholera enterotoxin (ACE) is the third toxin alone with cholera toxin and zonula occludens toxin that cause secretory diarrhea by activating Ca²⁺-dependent Cl⁻/HCO₃⁻ symporters. However, the identity of the underlying signalosomes and Cl⁻ channel specifically activated by ACE is unknown because of the low amount of toxin produced by *V. cholerae*.

Methods & Materials: Using previously established biologically active recombinant ACE, Ussing chamber and patch clamp techniques, we examine the identity of unknown apical Cl⁻ channel activated by ACE to cause diarrhea.

Results: We observed ACE induce apical Cl conductance (ICl⁻) sensitive to classical calcium activated Cl channel (CaCC) blockers such as NPPB, niflumic acid, and AO1 but was neither affected by ANO1 (TMEM16A) specific inhibitor T16A-AO1 nor by CFTR blocker, CFTRinh-172. In vivo mice ileal loop experiment reveal similar sensitivity of ACE induced fluid accumulation in presence of various CaCC inhibitors. Based on these pharmacological studies, we hypothesized ACE activated CaCC ANO6 (TMEM16F) to induce apical Cl⁻ secretion. This hypothesis validated by robust ANO6 expression in intestinal epithelial cell model Caco2 cell line and reduced ICl in ANO6 knockout Caco2 cell relative to wild type Caco2 cells. The role of ANO6 in ACE induced Cl⁻ secretion was further confirmed by measuring whole cell patch clamp recordings of Cl⁻ currents upon ACE exposure in HEK293 cells transiently expressing ANO6-GFP but not ANO1-mCherry. However, treatment with calcium ionophore A23187 induced strong outwardly rectifying ANO1 and ANO6 currents in these HEK 293 cells. Surprisingly, ACE was not able to induce [Ca²⁺]i rise in these cells. Together, these data indicate ACE elicits whole cell Cl⁻ currents by calcium-independent mechanism of ANO6 activation by ACE. Further investigation reveal Caco2 cells expose to ACE cause significant RhoA translocation to plasma membrane while ACE evoke ICl⁻ decreases in presence of RhoA kinase (ROCK) inhibitor, H1152 and PI4K inhibitor, wortmannin and PAO which reduces plasma membrane PIP2 level. We have also identify PIP2 binding motif at the N-terminal sequence among human and mouse ANO6 variants.

Conclusion: We conclude increased plasma membrane PIP2 level via RhoA-ROCK-PIP2 signaling cascade induced specific activation of ANO6 by during ACE mediated secretory diarrhea.
A decade of antimicrobial stewardship at the University of Florida - Challenges, strategies and outcomes

K. cherabuddi¹, K. Klinker²
¹University of Florida, Gainesville, FL, USA, ²University of Florida, Gainesville, USA

Background: Antimicrobial Management Teams (AMT) play an integral role in modifying antimicrobial utilization (AU). In 2004, AMT at Shands UF developed restricted antimicrobial policy (RAP) with institutional criteria for carbapenems (CAR), piperacillin/tazobactam (P/T), daptomycin (DAP), echinocandins (EC), cefepime (CEF) and fluoroquinolones (FQ). In 2008, AMT was disassembled due to pharmacist attrition but RAP remained under Infectious Diseases (ID). AMT was re-initiated in 2010 and later further expanded. We report the experience of the AMT over a 10-year period and the impact on consumption, resistance, drug costs and various opportunities, challenges and strategies.

Methods & Materials: A retrospective evaluation was performed to compare the consumption of targeted antimicrobials, measured as defined daily dose per 1000 patient days, before and after AU management strategies were initiated and also when AMT was disassembled and again when it was re-initiated. AU, Acquisition costs (AC) and Resistance rates (RR) from 2003 were compared to 2006/7 and also from 2008 was compared to 2010. Following re-implementation, AMT's central role focused on RAP adherence with direct prescriber feedback on Days 3 and 7 of therapy.

Results: With Initiation of AMT in 2004, consumption of most anti-pseudomonal antibiotics fell: IMI (-80%), P/T (-22%), T/C (-60%), CEF (-3.8%) and FQs (-37%). Resistance among Pseudomonas to targeted antibiotics has stabilized and displays a downward trend. Antimicrobial drug acquisition costs decreased by 18%.

Following loss of AMT, antimicrobial expenditure (AE) and antibiotic cost per patient day (ACPD) increased by 33 and 22% respectively. Increased consumption of aztreonam (78%), CEF (6%), EC (25%), FQ (20%), CAR (4%), and DAP (133%) occurred. In 2010, AMT was re-implemented and resulted in 12 and 15% reduction in total AE. Cost savings exceeded $300,000.

From 2010 to 2014, we have implemented multiple strategies to further control AU.

Conclusion: Since 2004, AMT has implemented strategies that reduced the consumption of RAP agents and Pseudomonas resistance has not increased. Re-implementation of AMT responsible for administering institutional guidelines and providing direct feedback resulted in rapid decrease in AE, ACPD. The most gains in the AMT implementation occur early and sustaining an effective AMT requires administrative support and adapting strategies to challenges faced and anticipated.
Guillain–Barré syndrome in Bangladesh: The role TLR4 Asp299Gly and Thr399Ile polymorphisms

I. Jahan¹, R. U. Ahammad¹, M. M. Khalid¹, S. K. Sarker¹, M. B. Islam¹, H. P. Endtz², Z. Islam¹

¹International Centre for Diarrhoeal Disease Research, (icddr,b), Dhaka, Bangladesh, Dhaka, Bangladesh, ²Erasmus University Medical Centre, Rotterdam, The Netherlands, Rotterdam, Netherlands

Background: Bangladesh has achieved a remarkable success to eradicate poliomyelitis, however, Guillain–Barré syndrome (GBS) is frequently diagnosed. GBS is an autoimmune mediated disease of the peripheral nervous system preceded by infections. *Campylobacter jejuni* has been identified as the predominant cause of antecedent infection in GBS. *C. jejuni* lipopolysaccharide (LPS) induces antibodies cross-reactive with gangliosides and has shown to be involved in peripheral nerve damage. Toll-like receptor 4 (TLR4) is an important pathogen recognition receptor that recognizes mainly lipopolysaccharide (LPS) of gram-negative bacteria. In this study, we investigated functional single nucleotide polymorphisms (SNPs) in the extracellular domain of TLR4 (Asp299Gly and Thr399Ile), and assessed their association for GBS susceptibility, disease pathogenesis and disease outcome.

Methods & Materials: A hospital based case controlled study was conducted in Dhaka Medical College Hospital (DMCH) in Dhaka, Bangladesh in between 2010 to 2013. A total of 210 genomic DNA (105 consecutive patients with GBS and 105 healthy controls of Bangladeshi population) were isolated using QIAGEN (DNA) blood midi kit and genotyped by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: TLR4 (Asp299Gly) polymorphism were significantly associated with GBS patients compared with healthy controls (p <0.05). Gly299Gly homozygote increased the susceptibility of GBS patients compared with healthy controls (p=0.0365, OR=8.9, 95% CI=1.1-73.2). Acute motor axonal neuropathy (AMAN) was significantly associated with Gly299Gly homozygote (p=0.0093, OR=14.6, 95% CI=1.6-130.7). TLR4 variant genotype Asp/Gly (p= 0.0441, OR=2.6, 95% CI= 1.1-6.5) was associated with poor outcome (unable to walk after 6 months); suggesting that these genotype might be one of the factors contributing to a severe form of GBS. No significant association of TLR4 polymorphism (Asp299Gly and Thr399Ile) with anti-ganglioside antibodies was found. In addition, TLR4 Thr399Ile polymorphism had no role in GBS susceptibility as compared with controls.

Conclusion: TLR4 Gly299Gly homozygote is associated with the increased disease susceptibility to GBS. Gly299Gly homozygote is also associated with the axonal variant of GBS. However, TLR4 Asp299Gly polymorphism is prevalently significant with the disease outcome of GBS. Therefore, further study is required to confirm this association using a large cohort.
Identification of biofilm-stage specific proteins associated with multidrug resistance and quorum sensing pathway in a pandemic strain of Vibrio parahaemolyticus isolated from India

A. Dharmaprakash, S. Thomas
Rajiv Gandhi Center for Biotechnology, Thiruvananthapuram, Tamil nadu, India

**Background:** Vibrio parahaemolyticus, a Gram negative halophilic bacterium, is rated as one of the leading etiological agent of food borne diseases in humans. Gastroenteritis is the most common clinical manifestation and specific serotypes of this pathogen were associated with pandemic outbreaks in several parts of the world since 1996. Recent studies conducted in the related Vibrio pathogens has revealed the role of biofilm mode of life in the emergence of multidrug resistance and pathogenicity. Present study was conducted to identify the genes and pathways specific to the biofilm stage of *V. parahaemolyticus* employing high throughput global proteomic approaches.

**Methods & Materials:** Total protein extracted at various time points of planktonic and biofilm stages from a pandemic strain of *V. parahaemolyticus* were analyzed by high resolution mass spectrometry employing qualitative and label-free quantitative proteomic approaches. Acquired mass spectra data was analysed using ProteinLynx Global Server™ v2.5.3 (PLGS) software against the NCBI reference sequence *V. parahaemolyticus* RIMD2210633 database. Generated proteomic data was analyzed using DAVID Bioinformatics Resource site to identify biofilm-stage specific functions and pathways.

**Results:** In the present study, 45.5% of the total proteome of *V. parahaemolyticus* was identified which is the largest proteome coverage obtained till date. Comparative proteome analysis revealed 52 down-regulated and 47 up-regulated proteins in biofilm stage compared to log-phase and stationary-phase planktonic stage [Figure.1]. Integration of quantitative and qualitative proteomic results identified 246 proteins specific to the biofilm stage which on functional analysis provided evidence for the expression of proteins associated with multidrug resistance and quorum sensing pathway during biofilm stage [Figure.2]. Proteome analysis also provided evidence for expression of 17 proteins in tdh pathogenicity island and 11 proteins in the super integron region.

**Conclusion:** Proteomic data generated in the present investigation provided fundamental information and set of interesting targets for a multifaceted analysis of biofilm formation mechanism. First study to provide evidence for quorum sensing (QS) regulated biofilm formation and the involvement of genes related to multidrug resistance during biofilm stage. Study provided potential drug targets which could be used in virtual high-throughput screening method for accelerating the discovery of anti-virulence drugs that do not provoke resistance.
Acquired 16s methyl transferase associated high level aminoglycoside resistance in Acinetobacter baumannii recovered from ICU patients from a tertiary referral hospital of northeast India

S. Upadhyay¹, S. R. Joshi¹, A. B. Khryiem², P. Bhattacharyya²
¹North Eastern Hill University, Shillong, India, ²NEIGRIHMS, Shillong, India

Background: Acinetobacter baumannii is an emerging pathogen associated with hospital acquired infections across the globe. In last one decade the therapeutic options against this pathogen became complicated due to acquisition of multidrug resistant trait. Aminoglycoside, which have been used successfully for treatment of hospital infection, is severely compromised as the acquired 16S rRNA methylases have emerged as an important mechanism of high-level resistance to aminoglycosides in clinical isolates of A. baumannii. Current investigation deals with the occurrence of acquired 16s methyl transferase genes associated with high-level aminoglycoside resistance (HLAR) in A. baumannii obtained from intensive care unit of a tertiary referral hospital in north-east India.

Methods & Materials: We analysed a total of 164 multidrug-resistant A. baumannii obtained from ICU patients admitted in a referral hospital of Shillong, north-east India, from April-September 2015. 16S rRNA methyl transferase genes; npmA, armA, rmtA, rmtB, rmtC and rmtD, were amplified by PCR among the isolates resistant to aminoglycosides by disk-diffusion method. To determine the HLAR [gentamicin and amikacin ≥ 512µg/ml], MIC against gentamicin and amikacin was recorded. Horizontal transferability and plasmid stability were performed by conjugation and serial passage. Plasmid elimination was performed by treating the isolate with 10% SDS. Clonal dissemination/differentiation of the isolates was analysed by REP-PCR.

Results: A total of 157 (95.7%) isolates were found to exhibit HLAR, among them carriage of acquired 16s methyl transferase was observed in 109 (69.4%) isolates. ArmA was found to be the predominant gene followed by rmtD and rmtA. All the gene types were horizontally transferable. The isolates retained the resistance genes from 89th to 95th consecutive serial passages. Plasmids were eliminated with a single treatment of SDS (4%). REP-PCR analysis indicated that 17 different haplotypes were responsible for infection.

Conclusion: The current study underscores polyclonal spread of HLAR A. baumannii within ICU patients. The study has revealed the presence of different acquired 16s methyl transferase genes which is not being frequently reported from this geographical region. Further, the study could predict stability of these resistance determinants which is helpful in predicting a future treatment option and formulating infection control strategy in this region.
Helicobacter pylori infection: Correlation to disease severity and Clarithromycin resistance in a Sri Lankan setting

N. L. Ubhayawardana, M. Weerasekera, C. Gunasekera, D. Weerasekera, K. Samarasinghe, N. Fernando
University of Sri Jayawardenepura, Colombo, Sri Lanka

Background: Helicobacter pylori is a causative agent of gastritis, gastric ulcer, duodenal ulcer and gastric cancer. Clarithromycin is often used in the treatment of H. pylori infections in Sri Lanka. Although resistance of H. pylori to clarithromycin has been reported in other countries the situation in Sri Lanka is an enigma. We determined clarithromycin resistance of H. pylori by detecting two major point mutations (A2142G and A2143G) in the 23S rRNA gene. Further we assessed the histology of gastric mucosa of dyspeptic patients as a reasonably good predictor of cancer risk specially, in H. pylori positive patients.

Methods & Materials: The study was a cross-sectional, descriptive study where 138 dyspeptic patients undergoing endoscopy examination were included. Ethical approval was granted from the ethical review committee, University of Sri Jayewardenepura (No-723). H. pylori infection was diagnosed by Polymerase chain reaction (PCR) amplification of the glmM gene of H. pylori. A2142G and A2143G point mutations associated with clarithromycin resistance were determined by PCR restriction fragment length polymorphism (RFLP). Histological features of the gastric mucosa were examined using H & E stain and gastritis was classified microscopically according to the updated Sydney system.

Results: Seventeen percent (24/138) of the dyspeptic patients were positive for H. pylori by PCR. Of them 13 were males (54%) while 11 were females (46%). All H. pylori strains had a point mutation at A2142G, while A2143G mutation was not detected. Based on histological findings, 15 patients were diagnosed as H. pylori associated chronic active gastritis. Though mild to moderate infiltration of polymorphonuclear and mononuclear cells were observed in all H. pylori positive patients, gastric atrophy and metaplasia were not observed.

Conclusion: This is the first report describing the presence of A2142G point mutation which is associated with clarithromycin resistance in a Sri Lankan population. It is therefore important to determine the eradication efficacy of H. pylori following clarithromycin treatment in Sri Lanka which can give an insight regarding the pheno-typical expression of the A2142G mutation. Further the proportion of H. pylori infections was found to be 17% in Sri Lanka.
Recurrent spontaneous abortion: Significance of early non-invasive detection of Chlamydia trachomatis infection

N. Singh¹, P. PRASAD², B. Das³, S. Rastogi⁴
¹National Institute of Pathology, New Delhi, India, ²NIP, New Delhi, India, ³Safdarjung hospital, New Delhi, India, ⁴National Institute of Pathology (ICMR), New Delhi, India

Background: Sexually transmitted Chlamydia trachomatis infection is common widespread public health concern worldwide, chiefly in women because of chronic oligosymptomatic/asymptomatic course of infection. These “silent infections” lead to devastating reproductive consequences such as spontaneous abortion. During pregnancy, collection of endocervical sample causes discomfort; also chorionic villous sampling is not done in India. It is thus important to investigate whether molecular diagnosis of C. trachomatis in non-invasive sample such as urine can assist in early detection of infection in recurrent aborters as this will result in development of better diagnostic modalities and control of this STD. Hence, the study aimed to investigate the frequency of C. trachomatis in urine/Endometrial Curettage Tissue (ECT) by PCR during early gestation in Recurrent Spontaneous Aborters (RSA).

Methods & Materials: With hospital ethics permission, ECT was collected from 90 women undergoing recurrent spontaneous abortion and 45 age-matched healthy pregnant women (control) undergoing induced abortion at Department of Obstetrics and Gynaecology, Safdarjung hospital (SJH), New Delhi (India). Urine was collected from 50 asymptomatic pregnant women (with history of two or more recurrent abortions) attending OPD in Department of Obstetrics and Gynaecology at SJH for routine check-up. Nucleic acid amplification test by C. trachomatis gene-specific PCRs was performed for C. trachomatis diagnosis in the ECT/urine.

Results: Overall, 15.5% (14/90) RSA were found to be C. trachomatis-positive for either MOMP/plasmid gene in the ECT. Among these, 10% (9/90) were positive for plasmid gene while the MOMP gene of C. trachomatis was present in 13.3% (12/90) RSA. Further, in the urine specimens of RSA, the prevalence of C. trachomatis infection was 20% (10/50) for either MOMP/plasmid gene. MOMP and plasmid were found in the urine of 20% (10/50) and 4% (2/50) patients, respectively. None of controls was found positive for either chlamydial MOMP/plasmid gene in ECT/urine.

Conclusion: Results indicate that urine PCR for chlamydial MOMP gene detected greater number of infected RSA. Apparently, PCR technique is a useful method for detecting C. trachomatis in urine because it represents a non-invasive and more convenient method for better clinical management/maintenance of pregnancy in first trimester RSA.
Zinc restores altered intestinal ion-transport, barrier functions and counteract inflammatory mediators induced by Shigella infection in T84 cells

P. Sarkar¹, I. A. Sheikh², T. Saha³, J. Aoun³, M. H. Kazi³

¹National Institute of Cholera and Enteric Diseases, Kolkata, West Bengal, India, ²National Institute of Cholera & Enteric Diseases, Kolkata, West Bengal, India, ³National Institute of Cholera & Enteric Diseases, Kolkata, India

**Background:** In recent years Zinc (Zn) has emerged as a major anti-diarrheal therapeutic and preventive strategy in the current management of diarrhea; however, the mechanisms behind this protective effect are yet to be elucidated. We investigated the potential benefits of Zn in restoring paracellular and transcellular ion transport by modulating tight junction protein expression and chloride (Cl⁻) secretion altered upon Shigella infection.

**Methods & Materials:** T84 monolayers were used for measurement of transepithelial electrical resistance (TER), permeability to non-charged particles, dilution potential (DP) as well as electrogenic ion transport in using chamber. Bacterial adherence and invasion was quantified and inflammatory responses were studied by cytokine assay. Adult C57BL/6 (B6) mice were intraperitoneally challenged with virulent S. flexneri 2a, oral Zn supplementation was given after the onset of diarrhea and faecal pathology was determined. Electrophysiological properties of the colonized colon were characterized in using experiments.

**Results:** Cells infected with Shigella flexneri 2a caused reduction of TER by ~71% [3500±1.2 vs. 1000±1.5Ω.cm²] and DP by ~65% [4±1.2 vs. 1.5±0.3 mV]. This was prevented in the presence of apical Zn. An increase of paracellular flux of 4kDa dextran [2.1±0.6 vs. 0.8±0.2 µg/3.5hr/cm²] compared to 70kDa version in infected cells while in the presence of Zn, flux of 4kDa dextran was reversed by 52%. Immunofluorescence study revealed removal of Claudin 4 and Claudin 2 from the level of TJ, which correlated with the reduction of TER and decreased permeation of Na⁺ upon Shigella infection. Electrogenic studies in Ussing chambers demonstrate reduced cAMP and Ca²⁺ induced Chloride secretion (Cl⁻) in infected cells. Zn ameliorate this perturbed transport function. Zn treatment reduced Shigella invasion and counteract the up-regulated secretion of intestinal specific inflammatory cytokines IL-6 and IL-8 by inhibiting MAPK signal transduction pathways. C57BL/6 (B6) mice challenged with virulent S. flexneri showed significant bacterial colonization and barrier defects, while oral Zn supplementation diminished bacterial translocation and restore barrier function in mouse intestinal tissue.

**Conclusion:** We conclude that Shigella infection caused altered barrier function, reduced Cl⁻ secretion and stimulate inflammatory cytokines, thus inducing acute dysentery. Zn resituates these transport function along with pro-inflammatory cytokines and bacterial translocation, thus having a potential therapeutic value in acute inflammatory diarrhea.
Performance of the matrix assisted laser desorption / ionization time of flight mass spectrometry (MALDI-TOF MS) for accurate identification of routine gram negative bacteria - A reference laboratory experience from Mumbai

S. Athalye Shetye
Metropolis Healthcare Ltd, Mumbai, India

Background: The Vitek MS (Biomeriux France) is a recently launched FDA approved MALDI TOF system for rapid identification of bacteria and yeasts. Rapid and reliable organism identification is crucial in the current multidrug resistant infection era. The objective of this study was to evaluate the performance of the VITEK MS in a large clinical microbiology laboratory across a variety of routinely encountered gram negative bacterial isolates.

Methods & Materials: A total of 10267 gram negative clinical isolates representative of 26 genera and 41 species were tested. Vitek MS based identification was performed by means of a single deposit on a MALDI disposable target slide without any prior extraction step. Identifications proposed by Vitek MS were referenced to standard laboratory methods such as phenotypic and Vitek 2 identification. Discordant results were resolved by molecular methods (16S rDNA sequencing).

Results: Of the 10267 gram negative isolates studied, 10085 were identified to the species level as a single choice organism and 56 isolates were found to be correctly identified after reference comparison with Vitek 2 identification. Therefore, 10141 isolates (98.8%) were correctly identified up to the species level. 109 (1%) isolates were identified up to the Genus level and 17 (0.12%) were misidentified/not identified.

Of the Enterobacteriaceae (8814), 98.95% isolates were identified as single choice up to species level; 0.93% isolates were correctly identified at the Genus level while 0.12% isolates were mis/not identified. All Shigella spp. isolates could not be identified by the Vitek MS system.

For the other gram negatives including nonfermenters; correct identifications were 97.8% and genus level / mis-identifications were 1.8 and 0.4% respectively.

Conclusion: The Vitek MS was found to be highly accurate and reliable for routine identification of gram negatives. Vitek MS technology is very simple, convenient and technologist friendly for routine incorporation in the microbiology laboratory. Rapid identification has great potential for reduced turnaround time, improved patient care; timely calling of critical identification; targeted antibiotic therapy and enhanced identification of some challenging organisms. Upgradation of database would further improve the efficiency of the system.
Challenges and opportunities in antibacterial drug discovery

K. P. Purnapatre, S. Dube
Daiichi Sankyo India Pharma Private Limited, Gurgaon, Haryana, India

Background: The past decade has been a lean period for the discovery of antibiotics with very few approvals of new antibacterial agents by FDA. However, emergence of Multi Drug Resistant organisms (MDRO) and Extensively Drug Resistant organisms (XDRO) pathogens have led to increased attention to this field. Center for Disease Control and Prevention (CDC) has declared infections caused by MDR ESKAPE pathogens as a critical area of unmet medical need. Further, FDA has also announced various incentives to support the Infectious Diseases Society of America (IDSA) aspiration to develop 10 new antibiotics by 2020. In this presentation, I will discuss challenges in the discovery of antibacterial compounds with a case study of Leucine tRNA synthetase inhibitor (LRSI).

Methods & Materials: Various properties of antibacterial standard of care agents analyzed with respect to physicochemical, safety and activity against MDROs/ XDROs. Also, merits and demerits of novel targets vs. novel approaches were considered. In order to explore novel targets we have designed, synthesized & profiled novel LRSI. The profile of our compound was compared with reported LRSI to identify the differentials.

Results: Unlike general drugs, antibacterial compounds need distinct physicochemical properties in order to work efficiently in different cellular milieu of host & pathogen. Safety (selectivity & toxicity) is critical as doses required to control the infection are usually high. New compounds should not only be effective against MDROs but also possess lower risk of resistance development and relapse. We have identified novel selective Leucine tRNA synthetase inhibitor (LRSI) for Gram negative MDROs. On analysis this compound shows distinct advantages over previously reported inhibitor.

Conclusion: To circumvent the challenges in the discovery of antibacterial compounds, the Pharma industry recognizes the need of novel targets and approaches to tackle the ever evolving pathogens. Towards this pursuit we identified a novel Leucine tRNA synthetase inhibitor (LRSI) with potent activity against MDROs. Promising profile of this novel inhibitor merits further development for clinical use.
From evidence to impact: Improving treatment for Kala Azar patients in India

R. Mahajan¹, T. Sunyoto², K. Malakyan³, G. Mitra¹, D. Kumar⁴, M. A. Lima⁵, P. Mathew⁶, S. Burza¹

¹Medecins Sans Frontieres, Delhi, New Delhi, India, ²Medecins Sans frontieres, New delhi, India, ³medecins sand Frontieres, New Delhi, India, ⁴Medecins Sans Frontieres, Hajipur, India, ⁵Medecins Sans Frontieres, Barcelona, Spain, ⁶Medecins Sans Frontieres, New Delhi, India

Background: Visceral leishmaniasis (VL), or Kala-Azar is a life-threatening systemic infection caused by protozoan Leishmania, with sand flies as its vectors affecting the poorest of the poor people. India bears 50% of global VL burden and was committed to eliminate VL elimination by 2015. New treatment modalities were proposed by WHO since 2010, yet first line treatment in India has remained unchanged since 2006. The 2014 release of India National Road Map of Kala azar Elimination marked a milestone in the progress towards the elimination goal: Single Dose liposomal amphotericin B (Ambisome®) or SDA was adopted in the national programme. Role of evidence in the policy change is explored.

Methods & Materials: Médecins Sans Frontières (MSF) started a VL treatment programme in Bihar, India since 2007, using 20 mg/kg intravenous Ambisome and treated over 11,000 patients with excellent results. With lack of high quality evidence, a phase 4 trial of new treatment modalities including SDA was commenced in August 2012 by MSF, Drug for Neglected disease initiative (DNDi) and Rajendra Memorial research Institute (RMRI). Effectiveness and safety data presented in December 2013. Additionally, a qualitative study to determine patient perspectives of these new treatments was conducted. At the same time, advocacy wise, a successful negotiation with Gilead as Ambisome producer has led to free donation of the drug to the Indian Government, further facilitating the process towards policy change.

Results: By supporting MoH facilities in implementing alternative regimen such as Ambisome, MSF demonstrated its feasibility at different level of health care, including primary health centres. Evidence from formal trial is indispensable; however, debunking the perception that such a ´complex´ treatment could not be implemented as first line treatment was as important. Various factors play a role in the policy change, including availability of resources and formation of kala-core consortium. The technical expertise of MSF has proven invaluable in supporting government during initial phase of treatment policy change implementation.

Conclusion: High quality and contextualized evidence is crucial in policy change process. Policy change more likely to happen when funding, tools and required inputs for implementing evidence are available and MSF project contributes to all that.
In silico and experimental studies of Plasmodium serpine receptor predicts its role as putative purineric receptor

S. Gupta¹, N. Joshi¹, S. Singh²
¹Shiv Nadar University, Dadri, Uttar Pradesh, India, ²Shiv Nadar University, Noida, India

**Background:** Invasion of red blood cells by *Plasmodium* merozoites involves specific receptor-ligand interactions. Previous reports suggest the role of secondary messengers like calcium and cAMP in invasion and egress of *Plasmodium*. However, the receptors associated with calcium signaling and their relation with parasite growth remains undefined. Recently, serpine receptors with G-protein coupled receptor (GPCR) like seven transmembrane (7 TM) topology are identified in *Plasmodium*. A class of GPCR known as purineric receptors binds to purines such as ADP, ATP and UTP and mediates important physiological functions including regulation of calcium signaling.

**Methods & Materials:** Here we performed in silico analysis of *P. falciparum* serpine receptors to investigate the presence of conserved seven transmembrane domains and a consensus nucleotide binding sequence (P-loop). The interaction of serpine receptor PfSR12 with ATP was analysed using docking programmes. The expression of PfSR12 in blood stages of life cycle was analysed by confocal microscopy. We also used agonists and antagonists of purineric signaling in the growth inhibition assays to understand the role of this receptor in *Plasmodium*.

**Results:** Computational analysis of *P. falciparum* serpine receptors showed that one of the *P. falciparum* serpine receptors, PfSR12 possess nucleotide binding consensus P-loop sequence in addition to seven transmembrane domains. The presence of conserved seven transmembrane domains and a consensus nucleotide binding sequence (P-loop) suggest that PfSR12 is a putative purineric receptor. On further analysis using docking programmes we found active binding residues in P-loop of PfSR12, interact with ATP. This work gives insights into the interactions between putative purineric receptor PfSR12 and its ligand ATP which can be explored in structure based drug designing against malaria. Localization studies using antibodies against PfSR12, we have found that this receptor is expressed in malaria parasite. Our results highlighted that various antagonists used in study have a good inhibitory effect on growth cycle of malaria parasites suggesting the importance of purineric receptors in growth of parasite.

**Conclusion:** Together our findings demonstrate that the approach that we have applied here is a powerful strategy to identify new inhibitory scaffolds suitable for further development of anti parasitic drug against these targets.
Spatiotemporal epidemiology of malaria in Madagascar between 2006 and 2015
F. A. Ihantamalala¹, V. Herbreteau², J. M. Rakotondramanga¹, G. Pennober², B. Raholijiana³, C. J. E. Metcalf³, C. O. Buckee⁴, F. Rakotomanana¹, C. Rogier¹, A. Wesolowski⁴
¹Institut Pasteur de Madagascar, Antananarivo, Madagascar, ²IRD, Saint-Pierre, La Réunion, France, ³Woodrow Wilson School, Princeton University, Princeton, USA, ⁴Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, USA

Background: Malaria is endemic in Madagascar, with local specificities. Its transmission occurs throughout the year along the eastern coast, while it is unstable and seasonal on Central Highlands. In this study, we investigate the spatiotemporal patterns of the occurrence of malaria in relation to bioclimatic conditions.

Methods & Materials: The Service for Health and Demographic Statistics of the Ministry of Public Health provided epidemiological data related to complicated and uncomplicated malaria cases from 2006 to April 2015. We integrated these data into a Geographic Information System to map monthly incidence for each health district and identify spatiotemporal clusters. We also acquired environmental information (meteorological and vegetation indices) in order to assess relations with malaria incidences.

Results: Since 2010, the report of malaria cases has improved and malaria incidence shows more regular trends. Malaria transmission generally starts with the rainy season and has a distinct peak on February and March. Children under 15 years old are the most vulnerable over the country. Coastal districts can be considered as a source of malaria because of their high incidence all over the year.

Conclusion: The quality of epidemiological data is discussed regarding the provision and access to health services. Case reports show weaknesses for some remote areas and at the end of each year. The persistence of malaria on the coast could induce the emergence of malaria in Central Highlands following reintroduction by travelers.
Assessment of effect of intermittent preventive treatment of malaria in pregnancy on birth weight of babies in Nigeria: Life-saving dynamics

Y. F. Oke¹, M. Salihu²
¹Malaria Consortium, Abuja, FCT, Nigeria, ²Malaria Consortium, Abuja, Nigeria

Background: Malaria infection during pregnancy, although preventable and treatable, still has adverse effects on both the mother and fetus in Nigeria. These adverse effects; intrauterine growth retardation, low birth weight and maternal anemia are significant risk factors for neonatal and infant mortality. The 2014 national guidelines and strategies for control of malaria during pregnancy recommend administration of at least 3 doses of Sulphadoxine-Pyrimethamine (SP) as Intermittent Preventive Treatment in pregnancy (IPTp) to pregnant women attending Antenatal Care Clinic (ANC). However, implementation of the guidelines is still sub-optimal. The objective of the study was to assess the effect of scaled implementation of prevention of malaria in pregnancy (MiP) with IPTp on birth weight of babies born in states supported by the US President’s Malaria Initiative.

Methods & Materials: The study used secondary data collected from July 2013 to June 2015 in 7 states where routine ANC data from all the health facilities are reported through the National District Health Information System to analyze trend and differences in reported birth weight following implementation of IPTp with SP. The interventions provided by the project include capacity building on control of malaria in pregnancy; strengthening of logistics management systems for SP, monitoring and supportive supervision.

Results: Between July 2014 and June 2015, 636,600 health facility ANC visits and 191,104 births were reported. The observed trend in the available data showed that the birth weight of babies improved as the IPTp uptake increased. Mean percentage of ANC revisits who received IPTp2 increased from 29% to 38%; the mean percentage of babies with low birth weight decreased from 14% to 10%; while the mean percentage of babies with birth weight higher than 2,500g increased from 86% to 90% between the previous year and the intervention period.

Conclusion: Though many confounders might contribute to the improved birth weight of babies reported within the period, however the contribution of the scaled implementation of IPTp is significant as previously documented in other malaria endemic countries. Concerted efforts are needed to scale up this intervention nationwide and strengthen health system in order to improve the birth weight of babies and consequently reducing neonatal and infant mortality.
Rickettsial disease IFA-IgG titres in auto-immune diseases: What do they imply?

P. Balasooriya¹, N. B. Bandara², T. Chandrasena³, R. Premaratna⁴

¹Professorial medical unit North Colombo Teaching Hospital, Ragama, Ragama, Sri Lanka, ²University of Kelaniya, Ragama, Sri Lanka, ³Faculty of Medicine Ragama, Ragama, Sri Lanka, ⁴Faculty of Medicine University of Kelaniya, Ragama, Sri Lanka

Background: Rickettsial infections are known to present mimicking autoimmune disorders. The gold standard diagnostic test for rickettsial diseases is based on the detection of IgM and/or IgG antibodies against these infections by immuno-fluorescent technique (IFA). While confirmation of rickettsial diseases warrant demonstration of rising or declining antibody titres between acute and convalescent samples, high titres of either IFA-IgM or IFA-IgG in acute phase serum in patients with a compatible clinical illness may help in the presumptive diagnosis and introduction of anti-rickettsial antibiotics. During the IFA test, patient sera containing anti rickettsial antibodies are made to react with rickettsial antigens that are grown in cell culture media. However, presence of nuclear material in these cell cultures may react with anti-nuclear antibodies that are produced in autoimmune disorders and cause a false positive immunofluorescent signal.

Methods & Materials: In order to evaluate the reactivity of rickettsial disease IFA-IgG test [IFA-IgG-OT (Orientia tsutsugamushi) and IFA-IgG-SFG (spotted fever group)] among patients with autoimmune diseases, an analytical cross-sectional study was carried out using sera of 38 patients with confirmed auto-immune diseases.

Results: The 38 patients included 15 systemic lupus erythematosus (SLE), 5 autoimmune-thyroiditis, 13 idiopathic-thrombocytopenia (ITP), 4 autoimmune-haemolytic-anaemia (AIHA), 1 polymyositis, 1 polyglandular syndrome and 1 Anti-phospholipid syndrome. The IFA-IgG reactivity of ≥ 1:128 was noted in 14/38 (37%); IFA-IgG-SFG in 7, IFA-IgG-OT in 3 and for both in 4. Of the 14; titre of 1:128 in 2, 1:256 in 4, 1:512 in 5, >1: 1024 in 3 and 8/14 (57%) were SLE, 3/14 (21.4%%) were ITP, 2/14 (14.3%) were AIHA, 1/14 (7.1%) were polymyositis and none were thyroiditis. 8/14 had received anti-rickettsial antibiotics during the early stages of illness based on the clinical presentation and high IFA-IgG titres.

Conclusion: There was a significant reactivity of Rickettsial disease IFA-IgG assay in auto-immune diseases. Further studies are needed in order to ascertain whether this is due to recent rickettsial infections, false positive cross reactivity of autoimmune antibodies with rickettsial antigens or with cell culture nuclear antigens. We did not carry out IFA-IgM due to non-availability and non-affordability.
Novel tick-borne Rickettsia sp. from wild ticks of Kenya: Implications for emerging vector-borne disease outbreaks

M. M. Mwamuye¹, E. Kariuki², D. Omondi³, J. Kabii³, D. Odongo¹, D. Masiga³, J. Villinger³
¹University of Nairobi, Nairobi, Kenya, ²Kenya Wildlife Service, Nairobi, Kenya, ³International Center of insect Physiology & Ecology, Nairobi, Kenya

**Background:** *Rickettsia* sp. causes rickettsioses, which despite being among the oldest known arthropod-borne diseases, are now recognized as important emerging vector-borne infections of humans worldwide. In Kenya, the prevalence of these diseases is poorly understood leading to far-reaching public health implications such as the unexplained fevers problem, some of which are caused by different spotted fever group (SFG) rickettsiae.

**Methods & Materials:** We used a multi-genic approach to identify rickettsia from 4,324 questing ticks (209 adult ticks, 586 nymphs and 3,502 larvae) processed into 270 pools of varying sizes, depending on species and life-cycle stages. We first performed PCR-high resolution melting point (HRM) based on *Rickettsia* 16S rRNA gene followed by sequencing of unique melt profiles. Sequences were identified by comparisons using BLASTn method. Thereafter, we re-amplified and sequenced citrate synthase (*gltA*) and outer membrane protein B (*ompB*) genes, as well as the highly variable *rpmE*-tRNA\(^{Met}\) intergenic spacer for samples with unique rickettsial 16S amplicon HRM profiles.

**Results:** We report the molecular detection of *Rickettsia africae* in *Amblyomma eburneum* ticks and novel *Rickettsia*-like species in *Rhipicephalus maculatus* ticks for the first time. We detected *R. africae* DNA with 99-100% identity for all the amplified gene loci while identities of two *Rickettsia*-like species could not be ascertained due to identical similarities associated with more than one *Rickettsia* species across the amplified gene loci. Sequence analysis of *gltA* gene for these two *Rickettsia*-like species showed 98% identity across several *Rickettsia* sp. The *ompB* gene could not amplify for one of the species which also had 97% identity with *R. bellii* based on the *rpmE*-tRNA\(^{Met}\) intergenic spacer sequence.

**Conclusion:** The detection of *R. africae*, an important emerging pathogen in Sub-Saharan Africa as well as novel *Rickettsia* sp. with unknown pathogenicity in this study represent significant findings that may explain the occurrence of some unidentified febrile illnesses contributing to human morbidity. Additionally, our study outlines the indispensable role of molecular methods in routine surveillance to monitor both known and novel pathogens likely to be emerging threats.
Twelve months outcome in kala-azar patients treated with 3 novel regimens, at public health care facilities in Bihar


1Drugs for Neglected Diseases initiative, New Delhi, Delhi, India, 2Medecins Sans Frontieres, Delhi, New Delhi, India, 3DNDi, Geneva, Switzerland, 4MSF, Barcelona, Spain, 5Rajindra Memorial Research Institute, Patna, India

Background: Kala-azar elimination initiative launched in 2005 in South Asia aims to reach the target by 2017. Early diagnosis and effective treatment is one of the key strategies for control along with integrated vector management. Single dose Ambisome (SDA) and combination regimens are the recommended treatments in South Asia. Our objective was to assess feasibility of using these treatments within the public health facilities and document 12 month outcome.

Methods & Materials: This was an open label, prospective, non-randomised, non-comparative, multicentric trial conducted at public health facilities. The study was conducted from Aug 2012 to Sep 2015 at 02 districts (Vaishali and Saran) in Bihar and at kala-azar referral hospital (Rajendra Memorial Research Institute of Medical Sciences) in Patna. In Vaishali district, patients were treated with SDA (10mg/kg) at the District hospital and A+M (single dose Ambisome 5mg/kg + miltefosine 7 days) at 5 primary healthcare centres (PHC). In Saran District, M+P (Miltefosine and Paromomycin) for 10 days at district hospital and 3 PHC.

All patients were followed up to document outcome at 6 months and cohort of them were followed for 12 months.

Results: 1761 patients were treated in the study, achieved cure rate of > 99% at initial outcome (Day 10) in each treatment arm. The cure rates at 6 months were 90.9% (95% CI 89.0-92.8) for SDA (n=892), 88.8% (CI 85.5-92.1) for A+M (n=357) and 97.0% (CI 95.6-98.5) for M+P arm (n=512). During 12 month FU (n=1386) there were 11 additional relapses, 2 in SDA (n=706) and 6 in A+M (n=294) and 3 in M+P (n=386). 12 patients developed PKDL in M+P arm, 1 in A+M arm and 2 in SDA. Five SAE occurred in SDA arm, 2 considered related and 3 non-related to Ambisome, all of them resolved.

Conclusion: The new treatment regimens showed excellent outcome and safety profile to be used within the programme settings. On the basis of these results, Indian national program have revised the treatment policy in Sep 2014 where SDA has been recommended as the 1st option and M+P as the 2nd option. Extension of FU beyond the standard 6 months yielded additional relapses and PKDL cases.
Post kala-azar dermal leishmaniasis treated with liposomal amphotericin B (AmBisome)
S. Burza¹, M. D. Boer², R. Mahajan¹, A. K. Das³, G. Mitra¹, P. Almeida³, M. A. Lima⁴, B.-N. Ahmed⁵, T. Sunyoto¹, K. Ritmeijer²
¹Medecins Sans Frontieres, Delhi, New Delhi, India, ²Médecins Sans Frontières, Amsterdam, Netherlands, ³Médecins Sans Frontières, Dhaka, Bangladesh, ⁴Medecins Sans Frontieres, Barcelona, Spain, ⁵Directorate General of Health Services, Dhaka, Bangladesh

Background: Post-kala-azar dermal leishmaniasis (PKDL), a cutaneous sequela of visceral leishmaniasis (VL), develops in approximately 10% of treated VL patients in the Indian subcontinent and may act as a reservoir of disease. Current evidence for treatment is limited; only long and toxic treatments currently exist for a condition that is more a public health rather than an individual health problem. We describe the characteristics and outcome of PKDL treated with liposomal amphotericin B (AmBisome®) in MSF-supported facilities in India and Bangladesh.

Methods & Materials: We administered intravenous liposomal amphotericin B 30 mg/kg body weight in 6 divided doses over 3 weeks on an ambulatory basis to patients with PKDL. To assess the treatment response, trained physicians regularly scored the severity of lesions and took medical photographs at baseline and during follow-up visits. The main end-points were safety of treatment, with an emphasis on development of hypokalaemia, and efficacy at 12 months' follow-up. Following safety concerns in Bangladesh, we introduced a lower dose of 15 mg/kg, given in 5 doses of 3 mg/kg over 3 weeks.

Results: 223 patients initiated treatment with the 30mg/kg regimen, 110 in Bangladesh and 113 in India. In India, of 72 patients who completed 12 months' follow-up, 59 (82.2%) cases showed substantial or complete cure, with excellent tolerance and safety. In Bangladesh, of 88 patients who completed 12 months' follow-up, 70 (79.5%) showed substantial or complete cure; however, 6.5% developed severe hypokalaemia. No patients in either group developed rhabdomyolysis or further sequelae. In Bangladesh, 278 patients were subsequently treated with the 15mg/kg regimen; 12-month follow-up results are available for 167. Of these, 147 (83%) showed substantial or complete cure. The safety profile was excellent with no severe hypokalaemia. We expect all results to be available by March.

Conclusion: Short-course liposomal amphotericin B treatment regimens for PKDL appeared to be effective and may be considered as an option for the treatment of PKDL patients in India when biochemical monitoring is available. However, levels of hypokalaemia seen in Bangladeshi patients meant that a lower dosage regimen is necessary, which appears to be safe and effective.
Multilocus sequence typing of seven genetic loci to discriminate strains of L. donovani isolated from Bangladesh

S. S. Banu
University of Sydney, Westmead, NSW, Australia

Background: Leishmania (L.) donovani, a species of the L. donovani complex, causes fatal visceral leishmaniasis (VL) disease in the Indian Subcontinent, parts of Asia and a large part of Africa. Genetic variations in Leishmania cause heterogeneity, spread of virulent strains, resistance to chemotherapeutics and exploitation of different hosts and vectors. Previously, L. donovani strains in the Indian subcontinent were detected genetically homogeneous by MLMT. Later study using genome-wide single nucleotide polymorphism (SNP) markers showed that genetic variation existed in L. donovani strains isolated from Nepal and India. We aimed to reveal intra-species genetic variation between strains of L. donovani derived from Bangladesh.

Methods & Materials: Twenty two newly isolated L. donovani strains from VL cases were investigated in a cross sectional study by MLST of seven functionally independent housekeeping genes located in different chromosomes. Target loci were rhomboid like serine protease, fatty acid/sphingolipid δ-4 desaturase, serine/threonine-protein kinase, 5′ A2rel-related gene, ubiquitin-activating enzyme e1, mannosyltransferase and splicing factor 3B subunit1 gene. Sequences of respective genes of L. donovani retrieved from GenBank and that obtained after sequencing of reference strains of L. donovani and L. infantum were used for comparison.

Results: Unambiguous sites of SNPs were detected in analysed sequences across all loci. The 5′ a2rel-related locus was the most diverge region with 26 SNPs, followed by mannosyltransferase with 10, splicing factor 3B subunit1 with 6, ubiquitin-activating enzyme e1 with 5, rhomboid like serine protease with 4, fatty acid/sphingolipid δ-4 desaturase and serine/threonine-protein kinase possessed 1 SNP each. Two large closely related clusters (one contained 10 and the other 7 isolates) and five separate individual groups among 22 Bangladeshi isolates were revealed by phylogenetic analysis showing the presence of 7 genotypes among L. donovani strains in the country.

Conclusion: The study illustrates that intra-species genetic diversity between strains of L. donovani does exist in Bangladesh. A combination of sequencing of 5′ a2rel-related gene and mannosyltransferase gene are sufficient to differentiate strains of the L. donovani. The MLST can be a future tool to determine intra- and inter-species genetic variation of Leishmania.
The effect of TNF-α neutralization on parasite load and cytokine production in human visceral leishmaniasis

**N. Singh**¹, R. Kumar², S. Nylén ³, D. Sacks⁴, S. Sundar⁵

¹Institute of Medical Sciences, Banaras Hindu University, Varanasi, Varanasi, India, ²Netaji Subhas Institute of Technology, New Delhi, India, ³Karolinska Institutet, Stockholm, Sweden, ⁴National Institute of Allergy and Infectious Diseases, Bethesda, USA, ⁵Institute of Medical Sciences, BHU, Varanasi, Varanasi, India

**Background:** The pro-inflammatory cytokine tumor necrosis factor (TNF)-α has an important role in control of experimental *Leishmania donovani* infection. Less is known about the role of TNF-α in human visceral leishmaniasis (VL). Direct evidence is primarily based on case reports of development of VL in individuals treated with TNF-α neutralizing antibody. Blockade of TNF-α can result in increased susceptibility to infections such as TB and leishmaniasis or reactivation of disease following cure. This indicates that TNF-α plays an important role in the control of intracellular pathogens, especially those that infect macrophages. In mice, TNF-α was found to be required for the control of *L. donovani* infection and for the formation of granulomas. To better understand the role of TNF-α in host defence against human *L. donovani* infection, we tested the effect of TNF-α blockade on splenic aspirate (SA) cell cultures, a cell culture system previously established to examine the role of cells and cytokines in tissue from an important site of disease in VL patients.

**Methods & Materials:** The effect of TNF-α on parasite burden was measured by neutralization of TNF-α and/or Enbrel in SA cultures from active VL patients. The SA suspension was divided into three equal parts and treated with purified mouse monoclonal antibodies against TNF-α or Enbrel. Control SA were incubated with IgG or IgG1 isotype antibody. SA were incubated for 3 days at 37°C in 5% CO₂. SA culture supernatants were used to measure IFN-γ and IL-10 levels. Left over SA was then cultured in 1:3 serial dilution to determine the number of viable parasites in the culture. Cell mediated antigen specific responses was also evaluated using TNF-α blocking in SLA stimulated whole blood cultures.

**Results:** Neutralization of TNF-α did not affect parasite numbers in ex vivo SA cultures. Interferon(IFN)-γ levels were significantly reduced in these cultures, but there was no effect on interleukin (IL)-10 levels.

**Conclusion:** In contrast to expectations, we found no direct effect of TNF-α neutralization on parasite burden. However, there was significant decrease in IFN-γ levels, which suggests that during active human VL, TNF-α stimulates IFN-γ production, but with no direct effect on parasite replication.
The baboon (Papio anubis)-Plasmodium knowlesi model of placental malaria
F. I. Onditi
Institute of Primate Research, Nairobi, Kenya

Background: About 24 million pregnant women in sub-Saharan Africa are exposed to malaria in pregnancy, a condition referred to as placental malaria. This condition affects both the mother and infant causing adverse pregnancy outcomes like low birth weight, intrauterine growth retardation, abortion, still birth, anaemia, mortality, just to mention a few. Comprehensive studies of placental malaria cannot be done in humans due to confounding variables that include mother’s health status, inaccurate estimation of infection, inadequate tissue for analysis, patient compliance, socio-economic conditions and moral, ethical and financial limitations. Reproducible animal models are required to overcome these challenges. The human-like structure of the baboon placenta and the cyto-adherent property of Plasmodium knowlesi have informed the choice for baboon-P. knowlesi model. Work in our malaria laboratory at the Institute of Primate Research in Nairobi, Kenya, has demonstrated that baboons are susceptible to placental malaria due to experimental infection by P. knowlesi. The baboon model of placental malaria is therefore useful in understanding pathophysiology of the disease in humans.

Methods & Materials: This study sought to validate and apply baboon-P. knowlesi model of placental malaria. Pregnant baboons were experimentally infected by P. knowlesi blood stage parasites in their first, second and third trimesters. The animals were sampled and analyzed for clinical and haematological changes, pathological changes, parasitaemia profile, immunological profile, and infant characteristics.

Results: Data generated from this study demonstrates for the first time the infiltration of infected P. knowlesi parasitized red blood cells and inflammatory cells in the placenta of non-immune baboons and their association with the underlying pathology of placental malaria. In addition, this study reveals that protective immunity against placental malaria in baboons involves both antibody dependent and cytokine dependent immune mechanisms. Maternal immunoglobulin G is important in infant survival against P. knowlesi malaria while a balanced type-1 and type-2 cytokine responses are displayed at the peripheral and placental maternal levels respectively.

Conclusion: Therefore, these findings validate the baboon-P. knowlesi model of placental malaria for the characterization of malaria during pregnancy and for further exploration in the field of reproductive immunology.