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EVALUATION OF IMMUNOLOGICAL MECHANISMS INVOLVED IN RESISTANCE TO HIV-1 EXHIBITED BY CHRONICALLY INFECTED INDIVIDUALS WHO CONTROL VIRAL REPLICATION WITHOUT ANTIRETROVIRALS

N.A. Taborda¹, S.M. González¹, C.J. Montoya¹, K.R. Fowke², M.T. Rugeles¹

¹Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. ²Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada.

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Objective: To establish the association between spontaneous control of HIV-1 replication and the following factors in peripheral blood (PB) and gut-associated lymphoid tissue (GALT): i) immune cell frequency; ii) immune hyperactivation status; iii) soluble proteins with anti-HIV-1 activity; iv) cytotoxic activity of NK and CD8+ T cells (CTLs); and v) cytokine production.

Material and methods: PB and GALT samples were obtained from 10 HIV controllers (viral load (VL) < 2,000 copies/mL); 10 HIV progressors (VL > 10,000 copies/mL) and 10 healthy controls. Mononuclear cells were cultured in presence of HIV gag pool peptides for CTLs or IL-12/ IL-15 for NK cells stimulation. Flow cytometry was performed to determine frequency, phenotype and cytotoxic activity of immune cells. Plasma cytokines were determined by bead array assay. Comparison between groups was performed using the Mann Whitney test.

Results: HIV controllers have higher percentage of CD16⁻ CD56^{bright} NK cells, and lower percentage of CD16⁺ CD56⁻ NK cells (dysfunctional cells) and activated CD4⁺ T in PB, compared to HIV progressors. Compared to HIV progressors, HIV controllers have a higher CD107a expression in CTLs, granzyme production by NK cells and a lower percentage of regulatory T-cells in GALT. Lastly, HIV controllers have lower expression of immune activation molecules, such as IP-10 and TNF- α . No significant differences were observed between HIV controllers and healthy controls.

Conclusions: These results suggest that HIV controllers conserved normal frequency and phenotype of immune cells in both peripheral blood and GALT. In addition, these patients have a lower immune activation status, which has been previously associated with AIDS progression.

THE PERCEPTION OF MALE PARTNERS RELATED TO PREGNANCY DECISION AND OUTCOMES IN RURAL INDIA

D. Gupta

Public Health Foundation of India, New Delhi, India.

Financial support: This research study is a part of the partial fulfillment of the PhD. program supported by a Fellowship from University of Manitoba, Canada under the International Infectious Disease & Global Health Training Program.

Objective: The objective of the study is to explore the links between male roles in pregnancy decision-making and women's access to abortion information and services in the rural areas villages of Uttar Pradesh, India.

Material and methods: The study combined both quantitative (survey) and qualitative research methods and its Sampling universe comprised all married couples (age 15-45 years) who reported that they experienced an unintended pregnancy in the last three years preceding the date of the survey in four selected districts of Uttar Pradesh, India. The proposed study will cover all the married women (ever pregnant) to identify the women experienced unintended pregnancies in last three years. The husbands of selected women who have experienced unintended pregnancies will be covered and interviewed to find out the final outcome of the pregnancies. In-depth interviews and focus group discussions will be recorded for analysis with the help of Atlas-TI software.

Results: The study will explore the associated factors affecting decision making on pregnancy outcomes among young married men. The research will also focus on the perception, knowledge and attitude on abortion legislation among men in the selected districts of Uttar Pradesh.

Conclusions: The proposed study is a major policy-focused research on men's role in abortion decision-making in India. The research findings will help to design community based male involvement intervention to reduce the incidence of unplanned pregnancies and unsafe abortions.

PROTEOMIC ANALYSIS OF HIGHLY EXPOSED HIV-SERONEGATIVE WOMEN FROM THE PUMWANI SEX WORKER COHORT IN NAIROBI, KENYA

D.R. Stein¹, S.J. McCorrister³, G.R. Westmacott³, F.A. Plummer^{1,4}, T.B. Ball^{1,2,4}

¹University of Manitoba, Department of Medical Microbiology and ²Immunology, Winnipeg, Manitoba, Canada. ³Mass Spectrometry and Proteomics Core Facility, Public Health Agency of Canada, Winnipeg, Manitoba, Canada. ⁴National HIV and Retrovirology Laboratories, Public Health Agency of Canada, Winnipeg, Manitoba, Canada.

Financial support: This research is supported by the Canadian Institute of Health Research and the Public Health Agency of Canada, and the Banting and Best Canada Graduate Grant awarded to Derek Stein as well as support from the International Infectious Disease and Global Health Training Program.

Objective: Over the past 25 years the Manitoba HIV research group has been studying a cohort of commercial sex workers from the Pumwani district in Nairobi, Kenya. We have shown epidemiologically that despite intense exposure, some women appear to be resistant to HIV-1 infection. In order to understand how Highly Exposed HIV Seronegative (HESN) women resist infection we have taken a proteomic approach to identify biomarkers that characterize this phenotype, which might one day lead to the development of vaccines or microbicides for HIV treatment or prevention.

Material and methods: PBMC were isolated from HESN women along with HIV negative and positive controls. PBMC lysates were subjected to trypsin digestion followed

by iTRAQ isobaric labeling. iTRAQ labeled peptides were fractionated by high pressure liquid chromatography followed by mass spectrometry analysis.

Results: Initial results identified and quantified 2,552 proteins of which 12 were significantly regulated between HESN and negative controls ($p = 0.05$, $FC > 1.5$). Several proteins including MX1, HLA-C, and RGS6, were chosen based on biological significance for validation by Western blot. MX1, HLA-C, and RGS6 were no longer significantly expressed after Western blot analysis between HESN and negative individuals. However, MX1 and HLA-C protein levels significantly correlated with the initial mass spectrometry results indicating that the protein levels were correctly measured by both techniques.

Conclusions: This study has identified several key immune response proteins as potential markers describing the HESN phenotype. Further study is needed to place these markers into the overall context of HIV infection and ultimately use them to inform future vaccine and microbicide design.

HIV EPIDEMIC DIVERSITY AMONG HIGH RISK POPULATIONS IN PAKISTAN

L.H. Thompson¹, P. Sandstrom², J.F. Blanchard¹

¹Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada. ²National HIV & Retrovirology Laboratories, National Microbiology Laboratory, Public Health Agency of Canada, Ottawa, Ontario, Canada.

Financial support: This project has received funding from Canadian Institutes for Health Research [grant reference number MOP-123575]. LHT is partially supported by a joint University of Manitoba Graduate Fellowship/ Manitoba Health Research Council Studentship and the Canadian Institutes of Health Research International Infectious Disease & Global Health Training Program.

Objective: Pakistan has substantial heterogeneity in HIV prevalence and risk behaviors in different geographic areas and high risk populations. Little is understood about the diversity and trajectory of HIV epidemics in Pakistan. This project will investigate and characterize HIV transmission chains in Pakistan using molecular, behavioural and social network data.

Material and methods: Sequencing and molecular phylogenetic analysis of the gag and pol genes will be performed on HIV specimens isolated from dry blood spots from injection drug users in 2011 and 2013 and female, male, and transgender sex workers in 2011 in six cities across Pakistan. These results will be linked to accompanying behavioural, geographic, and social network data collected through questionnaires administered in parallel with dry blood spot collection.

Results: By sequencing particular genes isolated from HIV specimens of a sample of infected individuals and grouping them according to sequence similarities, clusters of closely related HIV strains can be identified. Clusters represent specimens that share a common ancestor and therefore are part of the same transmission chain. Transmission routes may be further described using network data, which indicates sexual and injection bridging between individuals.

Conclusions: These results will provide a uniquely comprehensive understanding of the heterogeneity and population dynamics of HIV subepidemics in Pakistan, which will be critical to strategically design targeted HIV prevention programs.

BARRIERS AND FACILITATORS FOR ADHERENCE IN HIV PREVENTION TRIALS

J. Ambia¹, J. Olenja², K. Agot³, O. Anzala¹

¹KAVI-Institute of Clinical Research, University of Nairobi, Nairobi, Kenya. ²School of Public Health, University of Nairobi, Nairobi, Kenya. ³Impact Research and Development Organization, Kisumu, Kenya.

Financial support: This investigation was supported by International Development Research Centre/ KAVI-Institute of Clinical Research grant.

Objective: To determine how barriers and facilitators for adherence influenced product use among trial participants and draw implications that can be used to optimize adherence in future trials.

Material and methods: Study design: cross-sectional study design. Both quantitative and qualitative data collection methods will be employed. Location: Kisumu and Bondo in Western Kenya. Population: comprised of former trial participants in FEM-PrEP (n = 273) where poor adherence (< 40%) led to ineffectual outcomes and Partners PrEP (n = 196) where high adherence (82%) demonstrated PrEP's protective effect against HIV. Both trials were evaluating effectiveness of pre-exposure prophylaxis (PrEP) in preventing HIV acquisition among women and sero-discordant couples. Analysis of data: for the descriptive analysis, frequencies, percentages, means, and standard deviations will be computed. Covariates of participants in FEM-PrEP will be compared with those in Partners PrEP trial. Covariates whose univariate association with sub-optimal adherence will reach statistical significance at $p < 0.1$ will be included in an initial multivariate model and retained if significant at $p < 0.05$. For qualitative data, a code tree on emerging themes will be developed and analysed using NVivo.

Results: Understanding factors that promote or hinder adherence will provide meaningful insight in assessing likelihood of adherence among potential trial participants. In addition, customize adherence approaches and messages to suit different needs of enrolled participants.

SURVEILLANCE FOR ARTEMETHER-LUMEFANTRINE CLINICAL RESPONSE, LUMEFANTRINE IN VITRO SENSITIVITY AND MOLECULAR MARKERS OF TOLERANCE IN CURRENTLY CIRCULATING PLASMODIUM FALCIPARUM ISOLATES IN KENYA

J. Gitaka

Introduction: Malaria continues to be a major public health burden with about 3 billion people at risk worldwide, 28 million of them in Kenya. Following the introduction of artemisinin combination therapy (ACT) in the malaria control

armamentarium, declining rates have been reported in many areas. The recent emergence of artemisinin resistant *P. falciparum* malaria in South East Asia, therefore, is a big threat towards realising elimination and eventual eradication. Strategies to protect artemisinins from parasite resistance include use of effective combination partner drugs that have longer half lives to ensure complete parasite clearance and safeguard against selection for tolerant clones. In Kenya, artemether-lumefantrine (AL) combination has been in use in the public sector since 2006 and has largely been effective. Nonetheless, reports of 4%AL clinical failure and *in vitro* tolerance to lumefantrine point to the need for keener front-line surveillance in order to inform control strategies, antimalarial drug policies and patient management practices.

Objective: This study aims to evaluate the current circulating *P. falciparum* isolates in Kenya for AL therapeutic efficacy, *in vitro* sensitivity to lumefantrine, correlate these with established molecular markers of lumefantrine tolerance and provide baseline data for future reference.

Material and methods: Eighty eight malaria infected patients from 6 sentinel surveillance sites across Kenya will be enrolled in this study. Therapeutic efficacy of AL will be evaluated on days 3, 14 and 28. Blood samples will be obtained pre-treatment and on these days and parasites subjected to *in vitro* lumefantrine sensitivity. Parasite DNA will also be extracted and genetic analysis of *Plasmodium falciparum* multi drug resistance 1 (*Pfmdr1*) gene for single nucleotide polymorphisms at codon 86 and amplification. Statistical data analysis for correlation will then be done using Stata version 9 (Stata Inc.). Ethical approvals will be sought from KEMRI ethical review committee.

Results: Data on therapeutic efficacy of AL will inform on suitability of this combination in treating uncomplicated malaria. *In vitro* sensitivity data of parasites to lumefantrine will evaluate its choice as a partner drug in artemisinin based combinations and the correlation of these phenotypes to molecular markers will validate their use in surveillance. These findings will also provide comparative data for future studies.

LONGITUDINAL ANALYSIS OF IMMUNE QUIESCENCE AND IMPACT OF COMMERCIAL SEX WORK IN HIV EXPOSED SERONEGATIVE (HESN) SEX WORKERS FROM NAIROBI, KENYA

G. Boily-Larouche¹, J. Lajoie¹, S. Kirwan¹, J. Kimani³, K.R. Fowke^{1,2,4}

¹Departments of Medical Microbiology, ²Community Health Sciences, University of Manitoba, Winnipeg, Canada; ³Institute for Tropical and Infectious Diseases, ⁴Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya. Canadian Institute of Health Research (CIHR), Fond de Recherche du Québec-Santé (FRQS), International Infectious Diseases and Global Health Training Program (IID&GHTP).

Objective: To determine what drives the protective low levels of immune activation in HIV exposed seronegative (HESN) commercial sex workers (CSW), we will assess HESN capacity to control sex work driven immune activation.

Material and methods: 40 HESN, 40 newly enrolled HIV negative and 40 HIV positive CSWs will be enrolled. 17 participants have already been recruited. Blood is drawn and cervicovaginal lavages and cervical cells are collected at 6 different time points. Two biopsy samples are collected and women are asked to abstain from sex for 4 weeks to allow the healing. Sampling occurs before, during and after interruption in sexual activities. Location: Nairobi, Kenya. Population: Pumwani Sex Worker Cohort. Analysis of data: the impact of sex break on cervical and peripheral immunity and variation in HIV susceptibility will be studied. At each time point, activation of cervical and peripheral T cells, phenotype and frequency of innate and regulatory populations and levels of vaginal and peripheral cytokines, chemokines or hormones will be analyzed and compared between groups.

Results: Preliminary data showed that a sex break results in a reduction of immune activation in HIV-positive women. In HESNs, cervical levels of immune activation remain constant while in controls, they increase after resumption of sex work to reach higher levels compared to HESNs.

Conclusions: A better capacity to maintain low levels of immune activation in the vaginal tract following sex break may contribute to protect HESNs from acquiring HIV infection by reducing the number of available targets at the site of entry.

GENETIC ANALYSIS OF ADVERSE DRUG REACTIONS ASSOCIATED WITH ANTIRETROVIRAL THERAPY IN KENYA

F. Ndakala

University of Nairobi Institute of Tropical Infectious Diseases

Financial support: Funding was provided by the State Department of Science and Technology, Kenya, International Infectious Diseases and Global Health Training Program (IID&GHTP) and University Hospital Hannover, Germany.

Objective: To assess the influence of Single Nucleotide Polymorphisms (SNPs) on observed differences in Antiretroviral Therapy (ART) response in terms of Adverse Drug Reactions (ADRs) in HIV patients. SNPs in Cytochrome P450 (*CYP450*) and Multidrug Resistance 1 (*MDR1*) genes were investigated.

Material and methods: A case control study was designed to recruit 362 adult HIV individuals undertaking ART from SWOP clinics in Nairobi. Saliva and blood samples collected from patients were used for restriction enzyme genotyping. The influence of SNPs was established by measuring messenger ribonucleic acid (mRNA) levels and drug concentrations using both samples. Data analysis: Hardy-Weinberg equilibrium (HWE) for SNPs genotypes and allele frequencies was undertaken. The influence of SNPs on ADRs among individuals was analyzed using uni-variate two-way analysis of variance (ANOVA) testing.

Results: Will include prevalence of ADRs in SWOP clinics at baseline, the distribution of SNPs commonly associated with Africans and the expression levels of SNPs in blood and saliva.

Conclusions: Using a less invasive biological material like saliva in generating pharmacogenomic data could enhance the ART outcomes in Kenya.

PUBLIC VS PRIVATE HEALTH CARE SYSTEMS: MISTRUST AS BARRIER TO HIV VACCINE ACCEPTABILITY

E. du Plessis¹, R. Kaddu¹, M. Doshi¹, M. Gichuhi², J. Kimani², L. Avery¹, R. Lorway¹

¹University of Manitoba. ²University of Nairobi.

Financial support: This research was funded by a grant from the Canadian Institutes of Health Research/Canadian HIV Vaccine Initiative –CIHR Team in the socio-cultural aspects of implementing HIV vaccine programs among MSM and FSWs in Asia and Africa (VSR-107742).

Introduction: An efficacious HIV prevention vaccine could significantly affect the HIV pandemic by providing vulnerable groups with access to a prevention method within their control. However, vaccine acceptance could be influenced by many factors including attitudes towards the state and the health care system. The research presented here forms part of a larger study examining the social and cultural issues related to introducing future HIV vaccines among men who have sex with men (MSM) and female sex workers (FSW) in China, India and Kenya.

Objective: To document perceptions and attitudes towards the state, western biomedicine and the private and public health care system.

Material and methods: Participatory research including peer ethnography and fieldnote writing pertaining to “informal chatting”. Location: three districts (Kisumu, Nairobi, Mombasa) in Kenya. Population: female sex workers and men who have sex with men. The sample included 36 MSM and 35 FSWs. Analysis of data: thematic analysis of 71 transcripts.

Results: Preliminary analysis show that MSM and FSW access services at both public and private health care facilities, although FSW visit public health care facilities more frequently. Mistrust of both public and private health care facilities is evident in both groups fuelled in part by the government’s perceived lack of regulation of both systems.

Conclusions: Members of vulnerable populations may not consider private and public health care facilities as clearly distinct health care systems but rather separate elements of the health care system. This has implications for how and where a potential HIV vaccine will be rolled out in Kenya.

IDENTIFICATION AND CHARACTERIZATION OF IMMUNOLOGICALLY RELEVANT T-CELL EPITOPES IN HIV-1 NEF

E. Shadabi¹, R. Sampathkumar¹, J. Ho², D. La², B. Liang^{1,2}, F.A. Plummer^{1,2}, M. Luo^{1,2}

¹Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada. ²National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada.

Financial support: Canadian Institute of Health Research (CIHR).

Objective: Human Immunodeficiency Virus type 1 (HIV-1) evades the host cytotoxic T-lymphocytes (CTL) by escape mutations. Identifying these mutations and whether they have beneficial or detrimental effects on the host can provide us with clues as to what immunogen can be included in an effective vaccine. Nef, one of the regulatory proteins of HIV-1, enhances the pathogenicity of the virus by downregulating the expression of CD4 and HLA molecule on surface of infected cell and by interfering with cell signaling pathways. Escape mutations in nef have been reported but their functional roles on disease outcome have not been investigated.

Material and methods: Using 454 pyrosequencing technology we have sequenced 326 subtype A nef from HIV-1 positive, treatment naïve women enrolled in the Pumwani sex-workers cohort established in Nairobi, Kenya in 1985. The positive selected mutations were analyzed using quasi analysis, a bioinformatics approach. The positive selected mutations were correlated with patient CD4+ T cell counts using Kaplan-Meier survival analysis.

Results: We have identified five positive selected mutations in nef that were correlated with different disease outcomes. E70D, I109V and I176M were associated with rapid CD4 decline ($p = 0.010$, $p = 0.015$, and $p = 0.025$ respectively); H124N and K190M were associated with slow CD4 decline ($p = 0.001$ and $p = 0.029$).

Conclusions: HIV-1 nef contains positively selected mutations that are associated with different disease outcomes, some are beneficial for the host and others are detrimental. Analysis of the T cell responses to such mutations could determine the best immunogen to be included in a T-cell based vaccine.

MIGRATION PATTERNS AND HIV/AIDS VULNERABILITIES AMONG URBAN AND RURAL FEMALE SEX WORKERS IN KARNATAKA, INDIA

S. Ramanai^{1,2}, V. Annigeri², P. Banandur¹, K. Gurav¹, M.L. Becker³, R. Lorway³, S.S. Halli³, J.F. Blanchard³

¹Karnataka Health Promotion Trust, Bangalore, India.

²Center for Multidisciplinary Development Research, Dharwad, India. ³Center of Global Public Health, University of Manitoba, Winnipeg, Canada.

Financial support: This study has been supported by Bill and Melinda Gates Foundation.

Objective: The overall objective of this study is to compare the migration and mobility patterns of urban and rural female sex workers (FSWs) and understanding individual, structural, societal and contextual factors that contribute to their risk and vulnerability to HIV/AIDS.

Material and methods: A cross-sectional face-to-face interview of 1,567 FSWS from 142 rural villages and 1,306 FSWS from 25 urban towns in 3 districts of northern Karnataka, India was conducted between 2007-09. For rural FSWS, villages having 10+ FSWS, a large number of who were migrant, were selected following mapping of FSWS and for the urban FSWS were randomly selected from all urban towns in 3 districts. Government of India definitions were used to classify the urban town and villages and data analyzed in SPSS

17.0. Ethics approval has taken from institutional review board at St. Johns medical college, Bangalore, India and University of Manitoba, Canada.

Results: Compared with urban FSWS, rural FSWS are young (mean age: rural, urban), start sex work at their early age and entered sex work through mainly traditional devada system. Urban FSWS are having better education and moving within the district or state as compared to rural FSWS and stay for lesser duration. Rural FSWS are migrating outside the state and stay for longer duration.

Conclusions: The study findings are indicating the differences in the migration and mobility patterns of urban and rural female sex workers and recommend have a most effective and setting specific interventions strategies for both urban and rural FSWS.

ANALYSES OF NATURAL KILLER CELL MIGRATIONS IN A REAL-TIME AND DYNAMIC MICROFLUIDIC SYSTEM

S. Mahmood¹, S. Nandagopal², F. Lin², S.K.P. Kung¹

¹Department of Immunology and ²Department of Physics, University of Manitoba, Winnipeg, MB, Canada.

Financial support: NSERC discovery grant (to S.K.P.Kung), CIHR iid&ghtp (to S.M).

Introduction: Bidirectional interactions between mature natural killer (NK) cells and dendritic cells (DC) are important in coordinating innate and adaptive immune responses that shape anti-tumor and anti-microbial responses in vivo. Role of this association in regulating NK trafficking, however, is still not well characterized in vitro.

Material and methods: The conventional migration assays lack the ability to provide stable chemokine gradient environment for single cell level analysis. Emerging microfluidic based approach has been developed to provide better control over stable gradient generation and to mimic complex microenvironment for the quantitative analyses of chemotaxis, chemokinesis and, potentially, chemorepulsion at a single cell level. In the present study, we established a novel real-time, live cell imaging microfluidic platform to study IL-2 activated NK-cells migration in vitro. We generated immature and lipopolysaccharide (LPS)-matured bone marrow derived DC (BMDC). Supernatant of these DC cultures was collected and assayed for its ability to regulate NK migration in the microfluidic system.

Results: We found that soluble factors released by immature and matured BMDC contained chemotactic signals which induced respectively, a modest and high level of chemotactic movements of NK cells in vitro. We confirmed these findings in a standard transwell migration assay and identified that CXCR3 is a key receptor on NK-cells that regulated the migration. More interestingly, using this microfluidic system, we observed also chemorepulsion of NK-cell migration for the first time.

Conclusions: This microfluidic system should prove useful in elucidating factors that regulate NK trafficking behaviour and in studying NK migration in microenvironments that mimics inflamed peripheral sites.

MOLECULAR EPIDEMIOLOGY OF MYCOBACTERIUM TUBERCULOSIS IN PRISONS, COLOMBIA 2010-2012

Z.V. Rueda^{1,2}, L. López³, D. Marín¹, M.P. Arbeláez^{1,4}

¹Facultad Nacional de Salud Pública, Universidad de Antioquia.

²Grupo Investigador de Problemas en Enfermedades Infecciosas, Universidad de Antioquia.

³Escuela de Microbiología, Universidad de Antioquia.

⁴Grupo de Epidemiología, Universidad de Antioquia.

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Objective: To describe the genotypes of *Mycobacterium tuberculosis* isolated from patients with tuberculosis (TB) in four prisons in Medellín and Bucaramanga, Colombia.

Material and methods: Prospective cohort study. 65 patients diagnosed with TB and incarcerated in four prisons in two states in Colombia, Medellín and Bucaramanga, were followed-up monthly for six months, bimonthly for the next six months and quarterly for the second year. All isolates identified at diagnosis or during follow-up were genotyped using MIRUs-VNTR typing, and analyzed using BioNumerics®. A cluster was considered if two or more isolates had identical pattern, and were isolated from patients recruited in the same prison. Endogenous reactivation was defined if the isolates from two TB episodes, after effective short-course therapy for the first active TB episode, have the same genotype; otherwise, it was defined as exogenous reinfection.

Results: Among 65 patients diagnosed with TB, there were 133 strains isolated at diagnosis and during follow-up. 45.3% (29/ 64) of the patients were clustered. There were 10 clusters, and one of them had 10 patients. 10.9% (7/ 64) of the patients changed the *M. tuberculosis* strain during follow-up compared with the strain isolated at diagnosis. During the two-year follow-up there was one patient with exogenous reinfection, and another with endogenous reactivation.

Conclusions: Recently there has been transmission of *M. tuberculosis* in our prisons, and it is not only due to an acquired infection in the community or a reactivation. It is important to establish effective control measures to stop TB transmission inside prisons.

ASSOCIATION OF VARIANTS IN VITAMIN D PATHWAY AND INNATE IMMUNE RESPONSE GENES WITH NATURAL RESISTANCE TO HIV-1 INFECTION

W. Aguilar-Jiménez¹, W. Zapata^{1,2}, A. Caruz³, J. Fibla⁴, M. Clerici^{5,6}, M.T. Rugeles¹

¹Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. ²Grupo Infettare, Facultad de Medicina, Universidad Cooperativa de Colombia, Medellín, Colombia. ³Unidad de Inmunogenética,

Departamento de Biología Experimental, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, España. ⁴Unitat de Genètica Humana, Departament de Ciències Mèdiques Bàsiques, IRBLleida, Universitat de Lleida, Lleida, España. ⁵Department of Physiopatology Medical-Surgery and Transplantation, University of Milan, Milan, Italy. ⁶Don C. Gnocchi Foundation ONLUS Milan, Italy.

Objective: Vitamin D (VitD) could protect from HIV-1 infection reducing immune activation and inducing expression of anti-HIV-1 peptides. The aim of this study is to associate variants in VitD pathway genes and the antiviral response with resistance/ susceptibility (R/S) to HIV-1 infection in cohorts of HIV-1-exposed but seronegative (HESN) individuals from Colombia, Spain and Italia, and to explore the mechanisms of those associations.

Material and methods: One-hundred-forty variants were genotyped by GoldenGate-assay (Illumina). mRNA levels of genes with associated variants are being quantified by qRT-PCR in blood and mucosa of the Colombian cohort. Finally, the effects of these variants on HIV-1 infection will be evaluated using primary *in vitro* cultures.

Results: Thirty-seven variants in 7 genes of the VitD pathway and in 10 genes of the antiviral response were associated with R/S to HIV-1 infection ($p < 0.05$). In the Colombian cohort, higher levels of plasma VitD and mRNA of VDR and IL-10 in peripheral-blood-mononuclear-cells (PBMCs) and genital mucosa from HESN, compared to healthy controls were found. Positive correlations between VDR and IL-10 mRNA in PBMCs and genital mucosa of HESNs were detected. Moreover, mRNA levels of β -defensin -2 and -3, were higher in oral mucosa of HESNs, and positively correlated with VDR mRNA expression.

Conclusions: These results suggest that variants in genes of VitD pathway, high levels of plasma VitD and its receptor could regulate inflammation and induce defensins, influencing the resistance phenotype of Colombian HESNs. Further genetic analysis and exploration of the mechanisms behind these associations are required to confirm these results.

ECONOMIC IMPACT OF HIV/ AIDS ON WOMEN LIVING WITH HIV/ AIDS IN KARNATAKA INDIA

S.S. Chitrapu¹, E.C. Joby², E. Chandrashekar³

¹Vinayaka University, Salem, India. ²Karnataka Health Promotion Trust, Bangalore, India.

Objective: To find out the economic consequences of HIV infection on women living with HIV/ AIDS

Material and methods: This is a descriptive study. The population included HIV infected women residing in the State of Karnataka India. The sample size includes 300 infected women who were selected through purposive sampling. The structured interview schedule was used as a tool for data collection. Informed consent was obtained. The collected data was verified, edited and tabulated for analysis and interpretation.

Results: Study findings denotes the mean age of respondents as 30.1 years with more than 79.6% of them from rural area. 44% depended on daily wages. Husband is income

generator in 59.33% of the families. Nearly 79.33% women depended on their family members' income. Vast majority of 77.17 opined their performance of work ability has come down after HIV infection and 69 percent lost interest in work. About 73.67% do not have any savings of their own whereas 26.33 percent have savings with Mean savings of Rs.20000. About 40% respondents having debt with mean of Rs. 43,000 per head. The maximum investment from debt happened for medical treatment.

Conclusions: These results suggest that HIV infection exacerbate the economic condition of the women living with HIV/ AIDS

FOLLOWING THE DIVINE IN A TIME OF AIDS: JOGAPPAS CONFRONT THE HIV EPIDEMIC IN KARNATAKA

D. Sumit¹, L. Robert²

¹Department of Sociology, Dr. K. N. Modi University, Rajasthan and Karnataka Health Promotion Trust, Karnataka, India. ²Department of Community Health Sciences, University of Manitoba, Winnipeg, Canada.

Financial support: The research project is partially supported by the CIHR International Infectious Diseases & Global Health (IID&GH) Training Program.

Introduction: The HIV epidemic acted as a catalyst in raising the visibility of transgender communities in India. Although much has been written about indigenous cultural categories for male to female transgender identities such as *Hijra* and *Aravani*, which have existed since ancient times, 'Jogappa', a prominent cultural identity in the south Indian state of Karnataka, has received little mention in the literature.

Objective: The study will explore the social and cultural dimensions of the Jogappa community through a systematic documentation of their everyday cultural lives. More specifically, the study will examine how Jogappa identities take shape within the context of the HIV & AIDS movement and will assess their sexual and psychosocial health needs.

Material and methods: The study will follow a mixed methods approach and will be conducted in three districts of Karnataka where Jogappa communities predominate. The study will employ the anthropological technique of 'participant observation', daily ethnographic field note writing, in-depth qualitative interviews and quantitative surveys. Qualitative research will capture perceptions and lived experiences while the quantitative component will enable broader generalizations. Analysis will be conducted using SPSS 20 and NVivo9 software.

Conclusions: The expected outcome and aim of the study is to generate new knowledge about Jogappa communities that are highly marginalized and socially disenfranchised. We expect this study to make a unique contribution to gender and sexuality studies in Southeast Asia and generate invaluable demographic and health information that will enable policy makers to address the specific social and health inequities encountered by Jogappas.

SIMULATING THE IMPACT OF NETWORK STRUCTURE AND NETWORK DYNAMICS ON THE TRANSMISSION OF SEXUALLY TRANSMITTED AND OTHER BLOODBORNE INFECTIONS

S.Y. Shaw¹, J.W. Wylie^{1,2}, A. Gumel³, J.F. Blanchard¹

¹Department of Community Health Sciences; ²Department of Medical Microbiology; ³Department of Mathematics, University of Manitoba.

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Objective: To understand the interplay between network structure and network dynamics on epidemic phase and the epidemic trajectory of sexually transmitted and other bloodborne infections (STBBI). To develop rapid network assessment tools to ascertain network structure and dynamics.

Material and methods: Agent-based modeling will be used to simulate social and sexual networks. A variety of sampling schemes will be applied to data derived from simulation models. Sampling estimates will then be evaluated against their population values. Data will be parameterized to contact tracing data available from Manitoba. Analysis of data: adjacency matrices from simulated networks will be collected and characterized using basic social network analysis metrics, such as degree, density, and centrality measures. Mixing patterns will be characterized by a variety of assortativity measures (e.g., Q). To develop rapid network assessment tools, several sampling schemes (simple random sampling, snowball sampling and time-location-cluster sampling) will be utilized. Network metrics and assortativity measures derived from sampling schemes will be compared to their population values in order to evaluate bias from different sampling schemes.

Results: The interaction between network structure and network dynamics, and how this ultimately impacts the severity and amplitude of different STBBI epidemics will be characterized. Biases in sampling schemes will be understood.

Conclusions: Simulation of social and sexual networks provide insight into the complex dynamics associated with the spread of STBBIs. Knowledge of biases inherent in sampling schemes will help guide public health approaches to characterize STBBI epidemics, and the design of targeted interventions.

INTRACELLULAR ZINC LEVELS AND NUTRITIONAL ASSESSMENT IN HIV-EXPOSED AND HIV-INFECTED CHILDREN FROM MEDELLIN, COLOMBIA

E. Gómez, G. Posada

Escuela Nutrición y Dietética, Universidad de Antioquia, Colombia.

Financial support: Escuela Nutrición y Dietética, Universidad de Antioquia.

Objective: To explore the association between nutritional status and intracellular zinc levels in peripheral blood mononuclear cells (PBMCs) of children infected with human immunodeficiency virus (HIV) and exposed, uninfected children.

Materials and methods: A cross-sectional study was performed in 34 children (17 infected, 17 exposed) aged 2-10 years, recruited from AIDS care institutions in Medellín, Colombia. Population: 34 children (17 infected, 17 exposed), 2-10 years old. The following assessments were made: anthropometric measurements, nutrient intake based on a nutritional survey (24 hour dietary recall) and physical activity assessed with the three Day physical activity record (3DPAR) questionnaire, and associated to intracellular zinc levels measured by flow cytometry in PBMCs.

Results: Direct association was found between weight for age, height for age and body-mass index (BMI) for age and the zinc level in the cells studied ($p < 0.000$). Height for the age, energy intake, food intake and adequacy (R) of energy, protein and zinc were significantly higher ($p < 0.05$) in exposed uninfected children than in infected children. A clear inverse association of zinc levels with weight for age and BMI for age was visible. No significant differences between groups were observed in BMI or zinc levels in monocytes, CD4+ and CD4- lymphocytes.

Conclusions: There was no association between zinc intake and intracellular zinc levels in either group. Exposed uninfected children had better food intake and adequacy for energy, protein and zinc than children with HIV. Decline in nutritional status and growth retardation were mostly associated with HIV infection.

HIV PATIENTS WITH MUCOSAL LEISHMANIASIS TREATED WITH MILTEFOSINE IN COLOMBIA: A CASE REPORT

L. Mestra^{1,2}, A.M. Díaz², D.A. Vásquez², I.D. Vélez²

¹Septentrional Research and Development. CEO. Medellín, Colombia. ²Program for the Study and Control in Tropical Diseases -PECET, Medellín, Colombia.

Objective: To report the case of a 27 year-old male patient, resident in northeast Colombia, who years before had been diagnosed with HIV alongside a history of cutaneous leishmaniasis (CL), then presented leishmaniasis in nasal mucosa.

Material and methods: Experimental design: descriptive (case report). Location: Medellín, Antioquia, Colombia. Population: one Colombian patient. Analysis of data; a few cases of cutaneous manifestations of leishmaniasis in patients infected with HIV have been reported. This group of patients expresses atypical manifestations of the disease and are at higher risk of leishmaniasis dissemination due to depletion of both humoral and cellular response to the organism. The patient discussed here was treated with miltefosine and after finishing treatment relapsed, needing to be treated with antimonials. This patient had a low CD4 count during treatment and bad compliance with the therapy.

Results: The atypical presentations of the disease caused by *Leishmania* and HIV co-infection force one to adopt a comprehensive approach of diagnostic possibilities so as to

understand the pathology involved and provide adequate and timely management.

Conclusions: The information reported is important not only to create more awareness within the scientific community, but also to highlight the fact that we need to acquire a better therapeutic arsenal to treat neglected tropical diseases, since co-infection is increasing all around the world.

PREVALENCE OF SEXUALLY TRANSMITTED DISEASES IN 3 TO 14 YEAR-OLD CHILDREN, VICTIMS OF SEXUAL ABUSE IN ANTIOQUIA (COLOMBIA) BETWEEN 2009 AND 2011

N. Betancur¹, P. Fonseca¹, D. Echeverri¹, A. Calderón²

¹Facultad de Medicina. Universidad Cooperativa de Colombia. Medellín, Colombia. ²Facultad de Psicología. Universidad de San Buenaventura, Medellín, Colombia.

Introduction: Sexual exploitation of children is a crime that violates the fundamental rights of children, besides being one of the most deplorable forms of child abuse. This type of abuse of infants has great negative social consequences including psychological, social, family, moral and health issues. The latter because this population is at high risk of suffering from sexual transmitted diseases (STD).

Objective: This research focuses on measuring the prevalence of STD in children between 3 and 14 years of age, victims of sexual abuse in the department of Antioquia between 2009 and 2011. The prevalence of exposure and disease was measured on a sample of 100 children in the department of Antioquia.

Material and methods: The data was supplied by authorized personnel at the foundation focused in child care, and taken from databases from the corresponding areas at the Institute of Legal Medicine, that were compared to avoid biases and collect reliable information.

Results: Out of the total number of samples from sexually accosted and assaulted children, the accosted children made up 88% and sexually assaulted children corresponded to 17%. Among the sexually assaulted children 50% had an STD. **Conclusions:** We conclude that the prevalence of STD in sexually abused children in the age range mentioned above is high. Public health care programs should focus on providing early child care to combat this type of problem.

CLINICAL AND EPIDEMIOLOGICAL DESCRIPTION OF AN OUTBREAK OF KPC PRODUCING *KLEBSIELLA PNEUMONIAE* INFECTION IN A IV LEVEL INSTITUTION IN BOGOTÁ, COLOMBIA

I.G. Bustos Moya, D.F. Josa Montero, J.J. Perea Ronco

Department of Pathology and Clinical Laboratory, Fundación Clínica Shaio. Bogotá, Colombia.

Objective: The aim of this study was to describe the clinical, epidemiological and microbiological characteristics of a nosocomial outbreak of carbapenemase (KPC)- producing

Klebsiella pneumoniae in the Intensive Care Unit in the "Fundación Abood Shaió", Bogotá, Colombia.

Material and methods: Retrospective, descriptive and observational study. We recorded clinical, epidemiological and microbiological data of patients with KPC-producing *Klebsiella pneumoniae* infection. Bacterial identification and antimicrobial susceptibility was performed by an automated method (MicroScan Walk Away Plus®). Confirmation for *K. pneumoniae* carbapenemase was performed by the modified Hodge test and confirmed by PCR (bla_{KPC-1} and bla_{KPC-2}). Sixteen samples were analyzed for the detection of the New Delhi metalobetalactamase gene (NDM-1). The clonal study was performed by pulsed field gel electrophoresis (PFGE).

Results: Three patients were confirmed as having a nosocomial infection (8 isolates) and fifteen as patients colonized by KPC-producing *K. pneumoniae*. The PCR revealed the presence of the gene bla_{KPC-1} and bla_{KPC-2} in all isolates. Through PFGE the presence of a clone was identified with a greatest similarity over than 75%. We found no band pattern of the gene NDM-1.

Conclusions: The clone presented revealed that the main mechanism of transmission in this outbreak was patient-to-patient. Strategies implemented in cleaning and disinfection processes, intensification of hand washing, the intensification of hand washing, performing environmental cultures and patient cultures (looking for KPC-producing *K. pneumoniae*), relocation of the patients and access control of hospital personal to the ICU, were essential steps taken to prevent propagation of the microorganism.

DEVELOPMENT OF AN ELISA TEST USING RECOMBINANT PROTEINS FOR THE DETECTION OF BRUCELLACANIS

M.M. Sánchez-Jiménez^{1,2}, N. Dabral², M. Olivera-Angel¹, R. Vemulapalli²

¹Grupo de investigación Vericel-Biogénesis. Facultad de Ciencias Agrarias, Escuela de Medicina Veterinaria. Universidad de Antioquia. ²Department of Comparative Pathobiology. College of Veterinary Medicine. Purdue University. West Lafayette, Indiana, United States.

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Introduction: The diagnosis of *Brucella canis* infection in humans and dogs is infrequent in Colombia due to the unavailability of highly specific and sensitive diagnostic assays for the detection of infection.

Objective: To develop ELISA tests for the diagnosis of *Brucellacanis* in humans and dogs.

Material and methods: Four proteins of *B. canis*: Inosine 5' phosphate dehydrogenase (52 kDa), Pyruvate dehydrogenase E1 subunit beta (49 kDa), Elongation factor Tu (42.6 kDa) and TetR-family transcriptional regulator (27.4kDa), were previously identified as immunogenic in humans and dogs using mass spectrometry analysis and bioinformatics tools. The genes encoding these proteins

were PCR-amplified from DNA of a *B. canis* strain isolated from a dog from Medellín, Colombia. These genes were cloned in the pGEM-vector, subcloned in the pRSET A and/or pGEX-4T plasmids, and *Escherichia coli* BL21 cells were transformed with the resulting constructs. The expression of these proteins was induced and the proteins were purified. The purified proteins were evaluated as diagnostic antigens in indirect ELISA to detect IgG antibodies in sera obtained from dogs and IgG, IgM and IgA antibodies in human serum samples. The conditions for the test were standardized.

Results: All four proteins showed the potential to be used as target antigens for identification of *B. canis* infection in humans and dogs.

Conclusions: The use of recombinant antigens for the development of ELISA tests to detect *B. canis*-specific antibodies in serum of humans and dogs, can be a potential tool to improve the specific serological diagnosis of this infection.

MIRNA EXPRESSION PROFILING OF HUMAN MACROPHAGES DURING DENV INFECTION

M. Diosa-Toro^{1,2}, J. Flipse¹, S. Urcuqui², J. Smit¹

¹Molecular Virology Section, University Medical Center Groningen, The Netherlands. ²Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia, Colombia.

Objective: Dengue virus (DENV) is the most prevalent mosquito-borne virus worldwide and there is no vaccine nor antiviral drug therapy available against it. MicroRNAs (miRNAs) are regulators of host-pathogen interaction by controlling gene expression. It is the aim of this project to determine the expression profile of cellular miRNAs in macrophages infected with DENV-2.

Material and methods: *Bioinformatic screening.* The software MicroInspector was used to identify miRNAs with candidate target sites in the 3' UTR of DENV. *Macrophage studies.* Monocytes were isolated from PBMCs and cultured for 6 days in presence of M-CSF to induce differentiation from macrophages (MDM). Cells were phenotyped by FACS using common cell markers. *Screening.* MDM of three healthy donors were mock infected or infected with a GFP reporter DENV. RNA extraction was performed 24 hours post infection and deep sequencing for small RNAs was performed on an Illumina platform.

Results: Several human miRNAs were predicted by MicroInspector to target the 3' UTR of four DENV serotypes. The target sequences are located in the 3' stem loop, a highly conserved region within the four DENV serotypes. It was observed in the screening that 40 miRNAs were down-regulated and 2 miRNAs were up-regulated more than two fold in DENV-infected macrophages. One of the down-regulated miRNAs was predicted by MicroInspector to target the 3' UTR of DENV.

Conclusions: DENV infection induces a profound change in the expression profile of cellular miRNAs of primary macrophages. Further analysis will be conducted to determine the possible antiviral activity of those miRNA regulated.

DEVELOPMENT OF NOVEL MOLECULAR ASSAYS FOR THE IDENTIFICATION OF *HISTOPLASMA CAPSULATUM*: TARGETING PROTEIN-CODING GENES AND RRNA

C. Muñoz¹, M. Brandt², V. Loparev³, O. Clay⁴, B.L. Gómez^{1,4}

¹Medical and Experimental Mycology Group, Corporación para Investigaciones Biológicas, CIB, Medellín, Colombia. ²Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta GA, USA. ³Biotechnology Core Facility Branch, Centers for Disease Control and Prevention, Atlanta GA, USA. ⁴School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia.

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Objective: The primary objective of this international collaboration is to develop and validate new molecular methods for the rapid diagnosis of histoplasmosis and for the follow up of patients during therapy.

Material and methods: In order to obtain a representative *H. capsulatum* sequence database, we extracted DNA from 30 reference cultures of this fungal pathogen that corresponded to eight of the clades previously reported, and amplified known sequences of four loci: the genes encoding the 100 kDa protein and the H and M antigens, all specific for *H. capsulatum*, and the internal transcribed spacers (ITS) between the rRNA genes. All PCR products were sequenced and edited using Sequencher 5.0. To design specific primers and probes for identifying *H. capsulatum* by quantitative PCR (qPCR), we aligned all sequences obtained for each target locus using MEGA 5.

Results: Ten qPCR protocols for amplification were standardized and evaluated using DNA from the *H. capsulatum* cultures, as well as DNA extracted from 37 other medically relevant pathogens. All PCR assays under evaluation were positive when performed with purified DNA from the various *H. capsulatum* yeast cultures, the amplified sequences exhibited 100% identity to the reference sequences for this fungus, and none of the DNAs obtained from cultures of the other microorganisms gave positive results.

Conclusions: These assays could be useful in our quest to develop a qPCR assay for the detection of *H. capsulatum* in human samples.