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### Phylogenetic analysis of rabies virus (RABV) in Costa Rica

B. León, 1,2, 5 S. Fallas-Rodríguez, 1,2 M. Cordero-Solorzano, 1,2 O. Aguilar-Argueda, 1,2 G.C. Idania, 1 S. Hutter, 5 S.V. Hugo, 1 and R. González-Barrientos 1 6

*Corresponding author
1 Biosecurity Laboratory, Animal Health National Service (SENASA), LANASEVE, Costa Rica, 2 Paternity Laboratory Caja Costarricense del Seguro Social CCSS, Costa Rica, 3 Institute of Veterinary Public Health, Veterinary University, Vienna, Austria and 6 Babies National Program, SENASA, MAG, Costa Rica

Rabies, also known as hydrophobia or lyssa, is a fatal disease that affects both animals and humans and is considered one of the most important zoonotic diseases worldwide. In Costa Rica, there have been no rabies outbreaks associated with canine cases since 1987. However almost every year there are outbreaks in bovids produced by hematophagous bats. In order to establish the relationships of rabies bat viruses in Costa Rica outbreaks, a total of thirty bovine and two human brain tissue samples belonging to twenty-one rabies outbreaks were amplified and sequenced. Samples were obtained and stored by SENASA from 2004 to 2015. The complete nucleoprotein gene sequences were aligned with thirty-five rabies virus sequences downloaded from the GenBank/DDBJ/EMBL database using Biokit software. The phylogenetic analysis was performed using the maximum likelihood method and GTR + I + F model included in Mega 6. The topology of the tree is discussed.

### Enteric virome analysis of non-invasive samples from gorillas by next-generation sequencing and correlation with SIV infection

M. D’arc, 1,2, 3 J.D. Siqueira, 1, 2 A. Ayoub, 3 C. Furtado, 1, 2 M. Peeters, 2 and M.A. Soares 2

*Corresponding author
1 Instituto Nacional de Cancer, Rio de Janeiro, RJ, Brazil and 2 Institut de Recherche pour le Développement - IRD, Montpellier, France

The human immunodeficiency type 1 virus (HIV) is the etiological agent of AIDS and it is estimated that over 39 million people have died due to this disease worldwide. The ancestor of HIV-1 groups M and N have been described from two chimpanzee populations from Cameroon infected with simian immunodeficiency viruses, SIVcpz. Recently, we identified the ancestor of HIV-1 groups O and P in two populations of western lowland gorillas, also from Cameroon. The pathogenicity of SIV in great apes is unclear, but infection in chimpanzees has already been associated with the progression to an AIDS-like disease. The aim of our study is to identify and compare enteric viromes of infected and uninfected gorillas, and to assess their impact on SIV pathogenesis. To pursue this goal, we have used next generation sequencing (NGS) to analyze non-invasive samples of two gorillas, one SIV-infected and the other uninfected. We conducted the NGS in the HiSeq 2,500 illumina platform. Preliminary results suggest that the virome diversity is reduced in the SIV-infected animal, but with some viral taxa expanded. We are currently extending our study and will further correlate their virome profiles to the presence or absence of SIV in the animals.

Identification of induced genes involved in TYLCV transmission putatively responsible for virus gating of begomoviruses through the organs of their whitefly vector Bemisia tabaci

I.F. Fahmy, 1, 2 A. Faisal, 1 R.M. Abou-Ismail, 2 and A.M. Ahmed 3

*Corresponding author
1 Department of Microbiology, Phytopathogen Plant Vector Interaction Laboratory, Agricultural Genetic Engineering Research Institute, Egypt, 2 Genomics Department, Agricultural Genetic Engineering Research Institute, Egypt and 3 Microbiology Department, Gene silencing Lab, Agricultural Genetic Engineering Research Institute, Egypt

Molecular studies of insect disease vectors such as Bemisia tabaci are of increasing importance for understanding pathogen-vector relationships. Discovering the relevant genes that contribute to viral transmission through insect organs will facilitate the development of novel strategies for interfering with vector transmission of plant viruses. The ultimate goal of this study is silencing of putative transmission-responsible genes in the future by the development of transgenics. Information on gene expression and control in the target insect is necessary for this goal. Among the most important plant viruses to be transmitted by B. tabaci are those in the genus Begomovirus (family, Geminiviridae). Unfortunately, little is known about the genome of this vector. This study is investigating molecular aspects of the interaction between Whitefly B. tabaci and begomoviruses. As an initial step in this project differential display reverse transcriptase-PCR and randomly amplified polymorphic DNA-PCR has been applied to characterize differentially expressed mRNA from viruliferous and non-viruliferous insects, and in one case, from a B. tabaci feeding on TYLCV infected plants. Among 120 EST have been sequenced, twenty-seven ESTs show homology to known sequences from GenBank. Of these, fifteen ESTs code for up-regulated genes such as NADP-dependent D-sorbitol-6-phosphate dehydrogenase (Aldoketoreductase family), cell wall associated hydrolase, cytochrome oxidase 3 and 14, mothers against decapentaplegic homolog 4-like gene, alpha satellite repeat, maf Ham1 superfamily, periplasmic-binding proteins, cation-binding domain, odorant-binding protein. There are differentially expressed downregulated genes such as ubiquitin carboxyl-terminal hydrolase/protease ubiquitin specific protease [Culex quinquefasciatus], ATP-binding cassette subfamily B, Apis mellifera, serine/arginine-rich splicing factor 2-like (and in most insects like Apis mellifera aphids, and Anopheles gambiae, saccharopine reductase, sugar (Glycose-Pentose-Hexonide) transporter, aminophospholipid transporter, B. tabaci vitellogenin gene. Expression patterns were verified using qRT-PCR and revealed the accuracy of ESTs in cases of up- and down-regulation.

**Prevalence and genetic diversity of enteric viruses among different green vervet monkey populations on the island of St. Kitts, West Indies**

E. Bajak, 1, 2 C.A. Gallagher, 1 N. Agnes, 1, 2 R. Navarro, 1 M. Lawrence, 1 K.M. Dure, 1, 2 and S. Ghosh 1

*Corresponding author
1 Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, St Kitts & Nevis, West Indies, 2 Ngee Ann Polytechnic, SLSCT, 535 Clementi Road, Singapore, 3 St Kitts Biomedical Research Foundation, St Kitts & Nevis, West Indies and 4 Marist College, School of Science, Poughkeepsie, NY, USA

During 2014–2015, surveillance of enteric viruses in African green vervet monkeys (Chlorocebus aethiops sabaeus) on the island of St. Kitts, West Indies was performed. The population of vervet...
monkeys on St. Kitts is considered to be almost equal to that of humans (around 40,000). The majority of the monkeys are wild. Some juvenile monkeys are caught by locals, and are caged and/or used for entertaining tourists (tourism animals). Several hundreds of monkeys are kept at two of the island’s primate research centers. The present project was designed to study the prevalence of simian rotaviruses and noroviruses in three different green vervet monkey populations on St. Kitts.

A total of 143 fecal samples were collected from three different monkey populations (wild, captive and tourism animals). Viral RNA was extracted from the simian fecal samples by the SDS-pheno- chloroform precipitation method. The presence of rotavirus and picobirnaviruses (PBV) was detected using RNA electrophoresis in polyacrylamide gels (RNA-PAGE). Subsequently, the RNA migration patterns were visualized in polyacrylamide gels by silver staining. RNA-PAGE revealed double- and migration patterns indicative of picobirnaviruses in at least four of screened fecal samples. Picobirnaviruses are small non-enveloped bi-segmented double-stranded RNA viruses that have been reported in humans and in a wide range of animal species, including a single report from monkeys in China. A few studies have provided evidence which suggest that PBVs are zoonotic. In most cases, including this work, these viruses were detected by accident while screening fecal samples for rotoviruses by RNA-PAGE. The presence of rotaviruses and/or picobirnaviruses in the fecal samples will be further confirmed by RT-PCR technique. As the enteric RNA-virome of the gastrointestinal tract of African green vervet monkeys of St. Kitts remains uncharacterized, the genome sequencing and sequence analysis will be performed on select virus strains to study the genetic diversity of rotaviruses and picobirnaviruses in this part of the world. The genetic drift of porcine reproductive and respiratory syndrome virus (PRRSV) in a closed population evaluated by next generation sequencing (NGS) of complete genomes

L.K. Kvasgaard, C.K. Hjulsager, and L.E. Larsen
National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark

Porcine reproductive and respiratory syndrome (PRRS) viruses are divided into two major genotypes (type 1 and type 2). Type 1 PRRSV is further divided into at least three subtypes, but until now only subtype 1 has been detected in Western Europe and North America. Both genotypes are circulating in Denmark and since vaccinations are widely used it is essential to monitor the diversity of circulating PRRSV to secure the vaccines are up-to-date. Prior to the present study, the diversity of circulating viruses in Denmark was virtually unknown. The main objective was to assess the diversity of circulating PRRSV viruses in Danish pigs and to investigate the genetic drift of the virus in a closed population with very limited introductions of new animals. The study included phylogenetic analysis of full genomic sequences of type 1 and type 2 PRRSV viruses, including the very first Danish isolated type 1 virus and the very first Danish type 2 virus, which was isolated from a non-vaccinated pig herd. The results showed a very high genetic diversity among the Danish viruses throughout the genome within the same genotype. A global phylogenetic analysis showed that the Danish type 1 PRRSV formed two major clusters, one vaccine-like clade exclusively containing viruses isolated after the Porcilis vaccine was introduced and another distinct clade consisting mainly of viruses isolated in Denmark. Phylogenetic analysis in a global type 2 PRRSV framework classified all Danish type 2 viruses to a single clade (sub-lineage S.1) which comprised viruses closely related to the type 2 prototype isolate VR2332.

Molecular characterization of T4-like myovirus lytic to avian pathogenic Escherichia coli and extended spectrum β-lactamase producing E. coli isolates from chickens

A.A. Kaikbo, S.M. Abdulkarim, A. Faridah, and C.C. Siew

University Putra Malaysia, UPM 43300 Serdang, Selangor, Malaysia and *National Veterinary Research Institute, Vom, Plateau State, Nigeria

Bacteriophage Φ KAZ14, is a T4-like myovirus which infects Avian pathogenic Escherichia coli O1 (APEC 01) and extended spectrum β-lactamase producing E. coli isolated from chickens. APEC 01 causes colibacillosis in poultry leading to huge economic losses in the poultry industry worldwide and β-lactamase producing E. coli is resistant to commonly used third generation cephalosporins used in human medicine and thus, a threat to public health. The virus ΦKAZ14, which is lytic to APEC 01 and β-lactamase producing E. coli, has been isolated and its partial genome has been sequenced and analyzed. Whole genome sequencing is in progress. Based on the analysis of the partial genome sequences of this virus it belongs to the family Myoviridae. Detailed characteristics of this virus will be presented. It is envisaged that this virus may be useful in biocontrol programs against colibacillosis and susceptible cephalosporin-resistant E. coli in clinical settings.

Unifying viral phylogeography and ecological data to study the origins and spread of H5N8 avian influenza virus

S.C. Hill, Y.-L. Lee, B.-M. Song, H.-M. Kang, E.-K. Lee, M. Gilbert, A. Hanna, I. Brown, and O.G. Pybus

*Corresponding author
Department of Zoology, University of Oxford, UK, 2Animal and Plant Quarantine Agency, Anyang, Republic of Korea, 3Biological Control and Spatial Ecology, Université Libre de Bruxelles, Brussels, Belgium and 4Animal and Plant Health Agency, Weybridge, UK

Highly pathogenic avian influenza (HPAI) viruses pose a global threat to human and animal health, and cause considerable economic damage. The factors behind the emergence of these viruses are poorly understood, in part because of sparse sampling immediately after the identification of H5N1 in 1996. Since 2009 there has been a surge in novel reassortant HPAI H5 viruses, most notably H5N8 in 2013. The H5N8 virus epidemic provides an opportunity to investigate the factors behind HPAI emergence in much more detail than before. Our dataset consists of 110 H5 HA segment sequences sampled during H5N8 outbreaks from late 2013 to December 2014. Eighty-five of these samples were isolated from birds in twelve different provinces in the Republic of Korea, the second country to report outbreaks of H5N8. Forty-six of these are new unpublished sequences. The host species is known for almost all sequences. Ecological data (including domestic duck and chicken density and wintering waterfowl numbers) are available for all provinces. I hope to use phylogeographic and molecular clock methods to reconstruct the spread of H5N8 by integrating the trajectory of the virus with existing ecological data. I will try to characterize the factors behind the spread of H5N8 in the Republic of Korea. The results of this study will help illuminate key drivers of the emergence of novel and historical HPAI in Asia, and their subsequent global spread.
Prevalence and molecular epidemiology of feline calicivirus in European cats (Felis catus)

M. Afonso, 1,2* G. Pincheck, 1 S. Bonner, 1 S. Dawson, 1 J. Daly, 3 R. Gaskell, 3 and A. Radford 1

*Corresponding author

1Institute for Infection and Global Health, University of Liverpool, UK, 2School of Veterinary Science, University of Liverpool, UK and 3School of Veterinary Medicine and Science, University of Nottingham, UK

Feline calicivirus (FCV) is a highly diverse RNA virus causing acute respiratory disease in cats. Vaccination is widely used to control disease but does not prevent infection, with ~10% of household cats shedding virus. The objectives of this study are to estimate the prevalence of FCV and describe its molecular epidemiology at a European level. Sixty-three randomly selected veterinary practices in five European countries were asked to collect oropharyngeal (OP) swabs from their feline patients. Diagnosis of FCV was by isolation in cell culture. RT-PCR was conducted on all isolates followed by consensus sequencing. Neighbor-joining phylogenetic trees were constructed based on partial capsid and polymerase sequences. Fifty (79.4%) of the recruited practices returned a total of 1,521 OP swabs. A total of 140 samples tested positive for FCV (9.2%). Phylogenetic analyses showed high strain diversity evidenced by a radial phylogeny containing 109 strains, with more than one strain isolated in each country. Field strains were restricted to one country with no evidence of widespread international transmission as seen for other caliciviruses. Taken together, FCV remains an ongoing threat to cats, with high prevalence and strain diversity emphasizing the need for ongoing surveillance and vaccination.

Identification of the Culicoides species existing in Trinidad and determination of their impact as viral vectors for bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) in livestock

T. Brown-Joseph, 1,2*, L. Harrup, 1,2 C. Baten, 1,2 L. Frost, 3 H. Hicks 3
J. Flannery, 1,4 V. Ramkisson, 1,4 R. Ramdeen, 1,4 C.V.F. Carrington, 1,4 and C. Oura 1,4

*Corresponding author

1School of Veterinary Medicine, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago, 2Entomology Unit, The Pirbright Institute, UK, 3Non-vascular Diagnostic Laboratory, The Pirbright Institute, Surrey, UK and 4Department of Preclinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St Augustine, Trinidad and Tobago

Culicoides midges are very small, hematophagous insects that live in moist, organic environments. There are ~1,300 known species, some of which are vectors for important livestock viruses (e.g. bluetongue virus [BTV] and epizootic hemorrhagic disease virus [EHDV]) and human viruses (e.g. Oropouche virus). To determine which species currently exist in Trinidad, insect light-trapping was performed in different ecocozones throughout Trinidad. Classification was first performed morphologically using established biological keys, then unidentified specimens were classified using molecular methods involving non-destructive DNA extractions, followed by PCR amplification, sequencing and phylogenetic analysis of the mitochondrial Cytochrome oxidase I gene.

Sero logical survey and molecular characterization of herpesvirus papio 2 in wild-caught olive baboons from selected regions in Kenya

A. Nyachieo, 1,3, N. Ologun 1,3, S. Chepkwony, 1,3 N. Kiulia, 1 and M. Gicheru 1,3

1Department of Reproductive Health and Biology, Institute of Primate Research, Nairobi, Kenya and 2Department of Zoological Sciences, Kenyatta University, Nairobi, Kenya

Herpes simplex virus (HSV) is caused by two Herpes simplex virus subtypes, HSV-1 and HSV-2. HSV has been associated with the risk of HIV acquisition and transmission, miscarriages, premature labor, low fetal growth rate, meningitis, chronic skin infection and sometimes physical disability. The prevalence of HSV has been found to be higher among females than males, thus the detrimental effects seen in pregnant women and their offspring. HSV has no cure. Available drugs only lengthen recurrence period and hence development of an infection animal model phylogenetically close to human such as baboons is crucial for testing of new interventions. The prevalence of HSV in baboons captured from different regions in Kenya is not known. In this study, the prevalence of HSV2 in baboons was determined by detection of anti-HSV 2 antibodies in sera from 189 baboons captured from different regions, using ELISA. Molecular characterization of the ELISA positive samples was done by PCR with specific primers targeting the thymidine kinase region of the virus followed by sequencing. In total, 87% of the baboons had been exposed to HSV2 infection. About 90% of the female and 83% of the male baboons were seropositive for HSV2 antibodies. A PCR and sequencing followed by phylogenetic analysis evaluation confirmed the presence of HSV 2 strain A951 as the circulating strain in seropositive baboons. This information on circulating strain of HSV2 will immensely enhance the use of baboons as models to study the pathogenesis of HSV and test vaccine strategies.

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*Corresponding author

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Phylogenetic analysis of human immunodeficiency virus type 1 (HIV-1) seroconverters in Sydney, Australia: 2004–2013

A. Pinto, 1,3, F. Di Giannonaro, 1 A. Carrera, 1 A. Shaik, 1 P. Cunningham, 3 D. Cooper, 3 and A. Kelleher 1

*Corresponding author

1The Kirby Institute, Sydney, New South Wales, NSW, Australia, 2University of Sydney, Sydney, NSW, Australia, 3SydPath, St. Vincent’s Hospital, Sydney, NSW, Australia and 4St Vincent’s Centre for Applied Medical Research, Sydney, NSW, Australia

In Australia, NSW is the state with the largest burden of HIV, and has seen the highest rates of new infections in 20 years. Previous local studies have differed in rates and identifications of transmitted drug resistance (TDR). We analyzed genotypes of seroconverters to evaluate the impact of transmission networks on TDR. All genotypic antiretroviral resistance testing from 2004 to 2013 was included in a stratified database, and 28% of seroconverters identified from one site using strict laboratory criteria. Duplicates were excluded and sequences analyzed using
HIV-1 in the country is fundamental to provide a better understanding of subtype C epidemic spread and to inform the design of optimal intervention strategies in Mozambique.

A13 The human immunodeficiency virus type 1 (HIV-1) epidemic in the Caribbean: new data sets complicate the story

G. Dos Santos,* M. Ouka, and R. Césaire

*Corresponding author
Service de virologie CHU de Martinique, Fort de France, Martinique

The human immunodeficiency virus type 1 (HIV-1) epidemic in the Caribbean is mostly driven by the dissemination of the pandemic clade B and of older non-pandemic B lineages (BCAR). However, the low number of sequences from most Caribbean Islands limits the information about the timing and the migration patterns of HIV-1B in the region. We used REGA (version 3) to subtype HIV-1 B PR/RT sequences from Martinique (MART, n = 1,189, 1995–2015), Guadeloupe (GUA, n = 1,095, 1999–2014), St Martin/St Maarten (SMART, n = 169, 2002–2007), Haiti (HAI, n = 131, 2010), AKN (AKN, n = 37). HIV-1 subtype B is dominating (MART, 71%; GUA, 68%, SMART, 72%; HAI, 60%, AKN, 62%) but B/D recombinants were identified in 1.2% of infected children. Phylogenetic analyses revealed the epidemic origins from multiple independent introductions and distributed in different lineages that are widely dispersed in other southwestern African countries. This is the first national HIV-1 molecular epidemiology survey performed in Mozambique and it demonstrates that HIV-1 subtype C is the most prevalent in the country and suggests the existence of autochthonous transmission networks of subtype C in Mozambique. Continuous surveillance of the genetic diversity of HIV-1 in the country is fundamental to provide a better understanding of subtype C epidemic spread and to inform the design of optimal intervention strategies in Mozambique.

A14 Phylogenetic analysis of human immunodeficiency virus type 1 (HIV-1) subtype C epidemic in Mozambique

A. Vubli,¹,² N. Mabunda,² D. Bila,¹ J.C.C. Fernandez,² and I. Jan³

¹Corresponding author
2Instituto Nacional de Saúde- Mozambique and 3Instituto Oswaldo Cruz, Fiocruz, Brasil

Human immunodeficiency virus type 1 (HIV-1) subtype C is responsible for the majority of HIV-1 infection in Mozambique, but little information is available about the genetic diversity and evolutionary history of this epidemic in the country. In order to reconstruct the origin of HIV-1 subtype C clades circulating in Mozambique, a total of 496 specimens collected in dried blood spots from children under 18 months old were obtained from eleven Provinces of Mozambique during 2013. HIV-1 genetic subtypes were defined by genotyping from the entire PR gene and 3.0 algorithm. In total, 426 samples (86.5%) generated sequences for genetic characterization. The majority of HIV-1 strains were classified as subtype C (97.0%), subtype A1 (12.7%), subtype G (0.5%) and subtype D (0.2%). The intersubtype recombinant form B/D was identified in 1.2% of infected children. Phylogenetic analyses revealed that the epidemic origins from multiple independent introductions and distributed in different lineages that are widely dispersed in other southwestern African countries. This is the first national HIV-1 molecular epidemiology survey performed in Mozambique and it demonstrates that HIV-1 subtype C is the most prevalent in the country and suggests the existence of autochthonous transmission networks of subtype C in Mozambique. Continuous surveillance of the genetic diversity of HIV-1 in the country is fundamental to provide a better understanding of subtype C epidemic spread and to inform the design of optimal intervention strategies in Mozambique.

A15 Development of a bioinformatics framework for detection of transmission of human immunodeficiency virus type 1 (HIV-1) subtype G infection

E. Vanden Eynde,¹,² K. Theys,² R. Winand,³ A.M. Vandamme,¹,⁴ and A. Abecasis⁵

¹Corresponding author
1KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, Leuven, Belgium, 2KU Leuven, Department of Electrical Engineering (ESAT), STADIUS Center for Dynamical Systems, Signal Processing and Data Analytics, Leuven, Belgium, 3Minds Medical IT, Leuven, Belgium, 4Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal, 5Unidade de Microbiologia, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal and Unidade de Saúde Pública Internacional e Bioestatística, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal

High evolutionary rates of human immunodeficiency virus type 1 (HIV-1) enable rapid adaptation to antiviral treatment by selection of resistance mutations. In addition, transmission of drug resistance (TDR) can challenge the effectiveness of first-line treatment of newly diagnosed, drug-naïve patients. Although most research on TDR has been done in the context of HIV-1 subtype B, we directed ourselves to subtype G infected patients from Portugal. We developed a framework to investigate transmission dynamics, including both drug-treated and drug-naïve patients. After subtyping the database using REGA subtyping tool version 2, and selecting subtype G infected patients, a maximum-likelihood phylogenetic tree was built to identify HIV-1 transmission clusters and patterns of TDR present in those clusters. Given that a consensus definition for a transmission cluster is lacking we investigated the impact of different combinations of bootstrap (70–90%) and distance values (0.015–0.05) on the identification of transmission clusters. A population of 2,529 subtype G infected patients was studied, with 1,189 being drug-naïve and 1,340 being drug-treated. Using the most strict settings (bootstrap of 90% and genetic distance of 0.015) we did not detect any TDR clusters. When the bootstrap support was lowered to 70% and genetic distance to 0.05, seven clusters of TDR were identified, each including two drug naïve patients with resistance mutations on M41L (1%), K103N (1%), K103N/S (1%), F53L/Y (1%). A low number of clusters with subtype G patients displaying TDR, indicates a different pattern in the subtype G epidemic compared with subtype B, highlighting the importance of further investigations.

A16 A population-structured human immunodeficiency virus (HIV) epidemic: roles of risk and ethnicity

Z. Grossman,¹,² B. Avidor,³ Z. Mor,¹ M. Chowers,¹ I. Levy,¹ W. Shao,⁴ S. Girshengorn,³ D. Turner,⁷ and F. Maladecki⁶

¹Corresponding author
1School of Public Health, Tel-Aviv University, Tel-Aviv, Israel, 2National Cancer Institute, Frederick, MD, USA, 3Crusaid Kobler AIDS Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, 4Laboratory of Viruses and Molecular Biology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel, 5Meir Medical Center, Kfar Saba, Israel, 6Infectious Diseases Unit, Sheba Medical Center, Ramat-Gan Israel and 7Advanced Biomedical Computing Center, SAIC-Frederick, Inc, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA

A general aspect of the human immunodeficiency virus (HIV) epidemic in developed areas is that the infection spreads with different dynamics across ethnically and culturally diverse populations, reflecting differences in risk behavior. HIV in Israel started with a subtype-B epidemic among men who have sex with men (MSM), followed in the 1980s and 1990s by introductions of subtype-C (largely acquired by heterosexual transmission) from Ethiopia and subtype-A from the former Soviet Union (FSU, largely acquired by intravenous drug use). The epidemic matured over the last 15 years without additional large influx of exogenous infections. Between 2005 and 2013 the number of infected MSM increased 2.89-fold, compared with 1.18-fold in drug-naive HIV-infected patients with resistance mutations on M41L (1%), K103N (1%), K103N/S (1%) and F53L/Y (1%). A low number of clusters with subtype G patients displaying TDR, indicates a different pattern in the subtype G epidemic compared with subtype B, highlighting the importance of further investigations.
Subtype-B viruses, but not A or C, demonstrated a striking number of large clusters with common ancestors, including some suggesting presence of transmission networks. Transmitted drug resistance was high in subtype B (13%). In cross-ethnic transmission, demonstrated by the presence of Israeli-born with non-B viruses and Fsu immigrants with non-A, MSM represented a frequent risk factor. Reconstructed phylogenetic trees demonstrated substantial grouping in subtype B but not in non-MSM subtype-A and not in subtype C, reflect differences in transmission dynamics linked to risk-behavior. Cross-ethnic spread was due to multiple independent introductions, with a prevalent role of MSM in transmissions. Such data provide a baseline to track epidemic trends and will be useful in informing and quantifying efforts to reduce HIV transmission.

**A38** Human immunodeficiency virus (HIV) transmission dynamics and primary drug resistance in Washington DC

S. Kassaye,1,2 Z. Grossman,1 B. Johnston,1 M. Balaname,1,2 R. Teran,1 M. Young,3 W. Meyer III,4 P. Kumar,1 R. Shafer,2 F. Maldarelli,2 and D. Katzenstein4

1Corresponding author
2Department of Medicine, Georgetown University, Washington DC, USA
3Tel Aviv University, Tel Aviv, Israel
4Department of Medicine, Stanford University, Stanford, CA, USA
5Quest Diagnostics, Baltimore, MD, USA and 6HIV Drug Resistance Program, National Cancer Institute, Frederick, MD, USA

In the era of improved biomedical interventions for prevention, the identification of human immunodeficiency virus (HIV) clusters using molecular epidemiology can inform public health efforts to interrupt ongoing HIV transmission. This retrospective study uses sequence and HIV drug-resistance data from individuals enrolled in clinical research studies at Georgetown University in Washington DC, which has 2.5% HIV prevalence. A total of 314 HIV pol gene sequences were collected from 1994 to 2013. Median age was 38 years, (71% female; 70% African American). HIV exposure was heterosexual for 33%, MSM for 15%, IDU for 17%, blood transfusion for 3% and unidentified or unknown for the remaining 30%. Transmitted drug resistance positions were removed and analyses performed using BEAST (GTR-30%). Transmitted drug resistance positions were removed and analyses performed using BEAST (GTR-30%). Transmitted drug resistance positions were removed and analyses performed using BEAST (GTR-30%).

**A39** Impact of combination of chemotherapy and autologous hematopoietic stem cell transplantation for lymphoma on human immunodeficiency virus (HIV) reservoir

M. Salome,1,2 H. Delagrange,1 L. Gerard,1 M. L. Chalix,1 M. L. Nere,1 L. Galicier,1 F. Simon,1 E. Oksenhendler,2 and C. Delaugerre1

1Corresponding author
2Department of Virology, St. Louis Hospital, Paris - University Paris Diderot, France
3Department of Immuno-Hematology, St Louis Hospital, Paris - University Paris Diderot, France
4School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA and 5Presbyterian Church (USA), Kinshasa, DRC

Myeloablation and autologous stem cell transplantation (ASCT) lead to significant depletion of circulating CD4+ T cells and could impact the human immunodeficiency virus (HIV)-1 reservoir. The analysis of the viral population before and after ASCT could help address the origin of HIV blood reservoir after ASCT. We studied the longitudinal effect of combination chemotherapy and ASCT for HIV-related lymphoma on cellular HIV-1 DNA quantification and diversity in patients on antiretroviral therapy. We analyzed thirteen antiretroviral successfully treated-HIV-infected patients who received myeloablative chemotherapy and ASCT for relapsed or refractory lymphoma. HIV-DNA was quantified longitudinally using real-time PCR assay on whole blood samples at different time points before, during, and after ASCT. No significant difference in median HIV-DNA for each patient before and after ASCT was observed.

Furthermore, HIV-1 envelope C2V3 genomes from longitudinal blood samples from two patients were sequenced with ultra-deep pyrosequencing (UDPS). Four time points were tested for each patient, two before and two after ASCT. Viral variants reconstructed from UDPS sequences using a heuristic algorithm and viral dynamics were evaluated using nucleotide diversity. Sequences were evaluated for viral compartmentalization between viral population before and after ASCT. Analysis showed viral compartmentalization and an emergence of new viral quasispecies after ASCT in the patient who has been virologically controlled by 7 years of antiretroviral treatment. This result suggested that virus found in blood after ASCT came from long-lived ancient reservoirs, or a different compartment such as the gut. Analysis of additional patients with other compartment than blood and sorted cells in the graft is ongoing.

**A40** Transmitted human immunodeficiency virus (HIV) drug resistance in Washington DC, 1994–2013

M. Balaname,1,2 B. Johnston,1 Z. Grossman,1 R. Teran,1 M. Young,3 W. Meyer III,4 P. Kumar,1 R. Shafer,2 F. Maldarelli,2 D. Katzenstein,4 and S. Kassaye1

1Corresponding author
2Department of Medicine, Georgetown University, Washington DC, USA
3Tel Aviv University, Tel Aviv, Israel
4Department of Medicine, Stanford University, Stanford, CA, USA
5Quest Diagnostics, Baltimore, MD, USA and 6HIV Drug Resistance Program, National Cancer Institute, Frederick, MD, USA

Primary human immunodeficiency virus (HIV) drug resistance increases with availability of antiretroviral therapy and demonstrates ~16% transmitted drug resistance (TDR) in the USA with regional heterogeneity. Little is known about TDR in Washington DC despite high HIV prevalence of 2.5%. HIV resistance data for 218 treatment-naive individuals in Washington DC were included in this analysis. TDR was estimated using the WHO-2009 surveillance list. Median age was 37, African-American (57%) and female (65%). HIV risk groups included heterosexual (54%), MSM (20%) IDU (14%), blood transfusion (4%) and unknown (28%). A total of 118 sequences were from 1994 to 1995, twenty-nine from 1996 to 2006 and seventy-one from 2007 to 2013. TDR prevalence was 22% (95% CI: 16.7–27.8%), 19.2% (CI: 14.2–25.1) nucleoside reverse transcriptase inhibitors (NRTI); 3.7% (CI: 1.6–7.1) non-NRTI (NNRTI); 2.8% (CI: 1–6) protease inhibitors (PIs); 1.4% (CI: 0.28–4) dual class NRTI/NNRTI and 0.9% (CI: 0.1–3.3) triple class PI/NRTI/NNRTI. Forty had TAMs: 41L (6.4%), 67N (8.7%), 70R (6.6%), 210W (5.5%), 215Y/F (11.5%), 219Q (5%). Thirty-one had > 1 TAM, and decreasing prevalence between 1994–5 and 2007–13. M184V/I was present in 2.8% of sequences. The most common NNRTI mutations were 103N (2.8%), and 181C (1.4%), and PI mutations were 54V (1.8%) and 90M (2.3%) primarily from 2007 to 2013. Additional sequencing and prospective enrollment of participants is planned to build on the contemporary cohort to permit a time-trend analysis. Phylogenetic methods will be applied to analyze viral diversity and evolution and analyses performed to determine the long-term outcomes among those with TDR.

**A41** Searching for rare human immunodeficiency virus (HIV) strains in rural Democratic Republic of Congo (2001–3)

M.A. Rodgers,1 A. Vallari,1 C. McArthur,2 L. Shiresley,3 and C.A. Brennan1

1Corresponding author
2Infectious Diseases Research, Abbott Diagnostic, Abbott Park, IL, USA
3School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA and 4Presbyterian Church (USA), Kinshasa, DRC

As a region within the epidemiological epicenter of the human immunodeficiency virus (HIV) pandemic, the Democratic Republic of Congo (DRC) is a reservoir of circulating HIV strains exhibiting high levels of strain diversity and intersubtype recombination. In this study, we characterized HIV strains collected in two rural areas of the DRC between 2001 and 2003 to identify and obtain rare subtypes and recombinants. A total of 262 HIV-infected specimens from voluntary testing and pregnant women participating in a PMTCT program were characterized. Classification was determined by RT-PCR amplification and phylogenetic analysis of the vpu gene. Phylogenetic trees showed a high level of strain diversity. Subtype A predominated (43.6%) but eight different subtypes (33.1%), five CRFs (18.6%) and unclassified (4.7%) sequences were also found. Of the rare subtypes, eight specimens were selected for HIV-specific primer based next generation sequencing to obtain full genome sequences. Near complete genome sequences with >86%
Molecular epidemiology of human immunodeficiency virus (HIV) outbreaks among people who inject drugs in the Philippines

N. Siripong,1,* A. Dennis,2 G.M.J. Samonte,3 I.P. Abellanosa-Tac-An,4 A. van Rie,5 K.A. Power,1,6 J. Moody,6,7 and B.W. Pence1

1Corresponding author
2Gilling School of Global Public Health, UNC-Chapel Hill, NC, USA
3Department of Infectious Diseases, UNC-Chapel Hill, NC, USA
4Department of Health, National Epidemiology Center, Manila, Philippines
5Cebu City Health Office, Cebu City, Philippines
6Unit of International Health, Epidemiology and Social Medicine, Faculty of Medicine, University of Antwerp, Belgium
7Department of Sociology, University of Durham, Durham, NC, USA
8King Abdulaziz University, Saudi Arabia

Within a metropolitan area of the Philippines, surveillance surveys documented two separate HIV outbreaks with rapid spread among people who inject drugs (PWID). The first occurred in Cebu City, where HIV prevalence in PWID rose from <1% in 2009 to 56% in 2011. The second took place in the neighboring Mandaue City, where HIV prevalence in PWID increased from 3.5% in 2011 to 38% in 2013. Although the delay between the two epidemics may suggest separate populations, we hypothesized that the transmission of HIV started in Cebu City and spread to Mandaue by people who shared needles in both cities. Phylogenetic analysis offers an important tool to test this hypothesis by assessing genetic similarities and differences within and among the two groups. Reverse transcriptase (RT) sequences collected from people who inject drugs and men who have sex with men during surveillance in 2013. Another 278 sequences sampled in the Philippines or closely related sequences from a BLAST search were also included in the analysis. Sequences were aligned with HXB2 and a maximum-likelihood phylogenetic tree was built using the GTR mode in FastTree. We sequences from a BLAST search were also included in the analysis. Phylogenetic and Simplot analysis identified pure subtypes D (n = 1), F1 (n = 1), H (n = 2) and CRF25 (n = 1). The remaining eight genomes were complex recombinants of three or more subtypes, including A, C, F, G, H1, H2, J, K and unclassified (n = 7). The complexity of these URFs makes recombination analysis challenging. These complete genomes are a valuable addition to surveillance of URFs in this region.

High frequency of TDR in newly diagnosed human immunodeficiency virus type 1 (HIV-1) patients from São Paulo/Brazil

V.F. Pimentel,1,* A.B. Abecasis,2 L. Portes,3 A.C. Pineda-Peña,2,3,4,5 E.M. Matsuda,2 P.M.S. Guimarães,2 C.M. Hars,3 J.L. De Paula,1 A.M. Vandelamme,2,4 and L.F.M. Brígido2

1University of São Paulo, São Paulo, Brazil
2Department of Saúde Pública Internacional e Bioestatística, Departamento de Microbiologia and Center for Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal
3Institute of Biomedical Sciences, São Paulo, Brazil
4IU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, Leuven, Belgium
5Molecular Biology and Immunology Department, Fundación Instituto de Inmunología de Colombia (FIDIC) and Basic Sciences Department, Universidad del Rosario, Bogotá, Colombia
6Clinical of Infectious Diseases Reference, Santo André, Brazil

The human immunodeficiency virus type 1 (HIV-1) epidemic in São Paulo is dominated by subtypes B, F1 and C. The aim of this study was to characterize genotypes and transmitted drug resistance (TDR) among newly diagnosed HIV-1 individuals from January 2014 to February 2015 in São Paulo. Population sequencing (Pr + RT) was performed on viral plasma RNA for 179 patients. For subtyping, we tested different automated tools—Rega HIV Subtyping Tool (RHST), HXB2, Baf Los Alamos, Sceale, UCD v1.0 and jPhMM—and performed phylogenetic analysis using the Neighbor-Joining method under K2-P model with MEGA and bootscanning using SimPlot. Sequences were submitted to the Stanford HIV Database CPR tool (http://cpr.stanford.edu/cpr.cgi) to investigate TDR. Statistical analysis was performed using Graphpad Prism software. Among the 179 sequences, the more prevalent subtypes were B (74%), C (12.3%), F (5.6%) and BF1 recombinants (5.6%). BC recombinants (1%) and subtypes D and G (< 1%) were also found. In total, 36 patients (15.6%) were identified as having TDR to all NNRTIs, 14.2% to PI and 10% to NRTIs.

Identification of multiple unique recombinants forms of the human immunodeficiency virus type 1 (HIV-1) in newly diagnosed Cuban patients

L.Y. Machado,1,* M. Blanco,1 H.M. Díaz,2 O. Martínez,2 D. Romay,3 N. Ruiz,1 and M. Dubed1

1AIDS Research Laboratory, Mayabeque, Cuba
2University of Informatics Sciences, La Lisa, La Habana, Cuba
3Research Laboratory of AIDS, San José de las Lajas, La Habana, Cuba
4National Center of Agricultural Health, San José de las Lajas, La Habana, Cuba

In Cuba, the human immunodeficiency virus type 1 (HIV-1) epidemic is characterized by a high genetic diversity with several circulating subtypes. Previous studies have shown that subtype B is the predominant genetic form in Cuba. However, the evolutionary history of this viral variant in Cuba is still not clear. The aim of this study was to estimate the origin and evolution of HIV-1 subtype B in the Cuban epidemic. Phylogenetic relationships among HIV-1 subtype B pol Cuban sequences isolated between 2009 and 2012 and from different geographic locations were estimated. Bayesian statistical inference was used to estimate the time of HIV-1 subtype B introduction, the nucleotide substitution rate and the demographic history. The phylogenetic relationships revealed multiple introductions of subtype B (n = 40) in Cuba. The most recent common ancestor of Cuban HIV-1 subtype B was dated back to about 1977 (1974-82). Estimated nucleotide substitution rate was 2.7 × 10−3 subs/site/year (2.37 × 10−3 to 3.09 × 10−3). The effective number of HIV-1 subtype B infections grew exponentially between 1980 and 1995 but decreased in infections since the year 2000. The decrease in infections by HIV-1 subtype B appeared to have coincided with the emergence of CRF 20, 23, 24, BG and CRF19_cpx in Cuba. The HIV-1 subtype B was introduced in Cuba in the late 1970s. This supported the idea of subtype B introduction in Cuba from North America and Europe. The results presented herein, provide new insights concerning the epidemic of HIV-1 subtype B in our region.

Identification of multiple unique recombinants forms of the human immunodeficiency virus type 1 (HIV-1) in newly diagnosed Cuban patients

L.Y. Machado,1,* M. Blanco,1 H.M. Díaz,2 O. Martínez,2 D. Romay,3 N. Ruiz,1 and M. Dubed1

1AIDS Research Laboratory, Mayabeque, Cuba
2University of Informatics Sciences, La Lisa, La Habana, Cuba
3National Center of Agricultural Health, San José de las Lajas, La Habana, Cuba

The human immunodeficiency virus type 1 (HIV-1) epidemic in Cuba is characterized by a high genetic diversity with circulation of several subtypes and circulating recombinant forms (CRF). The aim of this study is to identify the presence of unique recombinant forms (URFs) of HIV-1 in newly diagnosed Cuban patients. Three hundred and thirty two (332) HIV-infected patients diagnosed between 2009 and 2014 were included. The viral RNA was isolated from plasma and used to amplify the pol gene by reverse transcriptase-nested PCR. PCR products were sequenced and the data generated was used to determine viral subtype by REGA HIV subtyping tool and phylogenetic analysis. The sequences showed evidence of recombination, according to the bootscanning approach of the REGA HIV subtyping tool, were analyzed by Simplot, RDP v3.0, jPhMM and RIP.

A total of 8.4% of sequences were identified as URFs (CRF19_cpx/CRF18_cpx, CRF19_cpx/B, CRF06_cpx/G, DF1, BC, CRF24_BG). Analysis of the sequences confirmed the presence of several subtypes and CRFs. A total of 132 sequences were included in the current study. The frequency of TDR was significantly different between subtypes B, C and F1: 16% (20.4-36.9), 0% (0-14.9) and 50% (23.7-76.3), respectively (p = 0.0016).

The HIV-1 epidemic in São Paulo is driven by subtype B, however—compared to previous studies—there is an increased prevalence of subtype C. The prevalence of TDR shows important differences between subtypes, with low prevalence of TDR in subtype C compared to significantly higher prevalence in subtypes B and F.
region of CRF06_cpx may explain this result. BLAST analysis showed a high identity percentage with strain 02AG/G.

Our results confirm the presence of multiple URFs of HIV-1 in the recently diagnosed seropositive population. Analysis of the full-length genomes of the respective viruses would be required to confirm whether they are the result of recombination in single dually infected individuals or are new CRFs.

**Human immunodeficiency virus type 1 (HIV-1) subtype G among newly diagnosed HIV-infecting drug users (IDUs) from Romania**

A. Temereanca,1,2,*, C. Oprea,1,3, L. Iancache,1, E. Ceausu,4, S. Mehta,6 and S. Rutu1,3

*Corresponding author

1Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, 2Stefan S. Nicolau Virology Institute, Bucharest, Romania, 3Dr Victor Babes Hospital for Infectious and Tropical Diseases, Bucharest, Romania and 4University of California, San Diego, CA, USA

Although in Europe there is a significant decrease in the number of newly diagnosed human immunodeficiency virus (HIV) infections related to injecting drug use, Romania exhibits important HIV outbreaks in this vulnerable population: until 2010, the percentage of newly diagnosed HIV cases attributable to IDU was insignificant (~1.5%) reaching 30% in 2012 and 2013 and 21% in 2014. Our aim was to analyze the pattern of HIV subtypes and the prevalence of transmitted resistance (TDR) in a group of treatment-naive injecting drug users, newly diagnosed with HIV infection between 2010 and 2014.

We studied a group of fifty injecting drug users, out of which thirty-two (64%) were HIV-1 infected. Pol gene sequencing was performed using the ViroSeq HIV-1 Genotyping System (Abbott Laboratories, USA) and HIV subtype was determined using the REGA HIV-1 subtyping tool. TDR-associated mutations were defined by the WHO 2009 surveillance mutation list. For phylogenetic analysis maximum likelihood trees were generated for each subtype, using PHYML implemented in Geneious (Biomatters, Auckland); for network clustering analysis, a pairwise distance comparison of all pairwise combinations was used.

Our results confirm the presence of multiple URFs of HIV-1 in the recently diagnosed seropositive population. Analysis of the full-length genomes of the respective viruses would be required to confirm whether they are the result of recombination in single dually infected individuals or are new CRFs.

**Phylogenetic reconstruction of the human immunodeficiency virus (HIV) epidemic from a native community in Argentina unravels the impact of viral adaptation on disease progression**

D.C. Monaco,1,2, D.A. Derville,1,2 M. Quipildor,1 A. Di Paolo,3 L. Yue,1 H. Salomon,2 and E. Hunter2

*Corresponding author

1Emory Vaccine Center, Emory University, Atlanta, GA, USA, 2INBIRS, University of Buenos Aires, Argentina and 3San Vicente de Paul Hospital, Oran, Salta, Argentina

Our objective was to study a recently initiated (~10 years) HIV epidemic in a native community that exhibits a restricted human leukocyte antigen (HLA) diversity, with the initial hypothesis that HIV viral adaptation was closely linked to the limited number of HLA alleles and this increased adaptation would impact HIV pathogenesis. We performed high-resolution HLA Class-I typing and near-full length HIV genome sequencing from sixty-five chronically infected HIV-positive individuals. Phylogenetic reconstruction was performed by neighbor-joining and only bootstrap scores >90% were accepted. Implementing statistical and phylogenetic-based methods, we identified HLA-linked viral polymorphisms associated with escape from the most frequent HLA alleles. Considering them as signatures of viral adaptation, we correlated their presence with viral load and CD4 count. We identified twenty-four HLA-linked viral escape mutations (P < 0.05; q < 0.1) distributed across the entire HIV proteome. On the phylogenetic reconstruction, we observed highly supported (bootstrap support ~ 100%) monophyletic clades that suggest independent introductions of HIV. Classifying the viral variants based on their prevalence at the population-level, we found that variants present at higher prevalence exhibited a higher number of escape mutations compared with those found at low prevalence (P = 0.0114). In a subset of forty-one antiretroviral-naïve patients, the percentage of escape mutations was positively correlated with CD4 count (P = 0.044) and negatively correlated with viral load (P = 0.023). The ability to reconstruct the phylogenetic relationships among the variants allowed us to show a rapid adaptation of HIV to the HLA-I mediated immune response that could be leading to less pathogenic infections.

**Viral escape pathways and determinants of neutralization breadth in early human immunodeficiency virus type 1 (HIV-1) infection**

A.S.A. Smith,1,2,*, S.L. Burton,1,2 K.M. Kilgore,1, J. Mulenga,3 E. Karita,3 S. Allen,4 E. Hunter,1,4,5 and C.A. Derdeyn1,4,5

*Corresponding author

1Yerkes National Primate Research Center, Atlanta, GA, USA, 2Emory HIV Research Project, Lusaka, Zambia, 3Rwanda-HIV Research Project, Project San Francisco, Kigali, Rwanda, USA, 4Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, USA and 5Emory Vaccine Center, Atlanta, GA, USA

Human immunodeficiency virus type 1 (HIV-1) has proven difficult to protect against by vaccination, due in part to its ability to induce and tolerate high levels of mutation. This hurdle could be overcome if a vaccine could induce antibodies that broadly neutralize diverse HIV-1 strains. In natural HIV-1 infection, a majority of patients generate moderate breadth after 2–4 years. Understanding how and why antibody breadth develops in some patients, but not others, could inform HIV-1 vaccine design. We hypothesized that the initial targeting of certain epitopes on the envelope glycoprotein gp120, combined with the subsequent influence of viral escape pathways and evolution of the B cell response, programs the development of antibody breadth. We have identified and characterized the transmitted/founder and longitudinal escape Envelope variants for ten subtype A and C HIV-1 infected individuals who developed varying levels of antibody breadth. Preliminary sequence analysis of the transmitted/founder Envelope Envelope did not associate breadth with an amino acid signature, differences in gp120 variable loop length, or differences in the number of N-linked glycosylation sites in gp120. We did find evidence for early nAb pressure in three major regions of gp120 that are targeted by antibodies: the V1V2 hyper-variable domain, the CD4-binding site, and the N332-glycan patch, but with considerable overlap between the higher and lower neutralizers.
Therefore, we postulate that analysis of the ensuing viral escape pathways, and the co-evolving B cell immunoglobulin variable domains, are key to understanding how early, strain-specific autologous antibodies broaden their specificities over time.

**A32** Molecular surveillance of recent human immunodeficiency virus type 1 (HIV-1) infections in Germany allows the detection of transmission networks and putative new subtype clades

K. Hanke,*, A. Hauser,*, S. Somogyi,*, K. Meixenberg,*, A. Hofman,*, B. Bartmeyer,*, N. Bannert,*, and C. Kürcher*

*Corresponding author

**A29** Assessing human immunodeficiency virus (HIV) transmission cluster dynamics in North Carolina using a large statewide dataset

A.M. Dennis,*, S. Hué,*, J. Sebastian,*, W.C. Miller,*, and J.J. Eron*

*Corresponding author

**A31** The contribution of epidemiological predictors in unraveling the phylogeographic history of human immunodeficiency virus type 1 (HIV-1) subtype C in Brazil.

T. Gräf,*, B. Vrancken,*, D.M. Junqueira,*, R.M. de Medeiros,*, F. Leamy,*, S.E.M. Almeida,*, and A.R. Pinto*

*Corresponding author

**A33** Prevalence and distribution of human immunodeficiency virus type 1 (HIV-1) non-B subtypes in the United States.

W.M. Switzer, H.J. Kabore, E. Campbell, A. Shankar, A. Hernandez, H.I. Hall, and A.M. Oster

Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA

Human immunodeficiency virus type 1 (HIV-1) evolves rapidly, increasing its genetic diversity and complexity, and is classified into four distinct lineages (groups M, N, O, P) with group M containing more than eighty subtypes and circulating recombinant forms (CRFs). Subtype determination is important epidemiologically, and subtype can impact pathogenesis, treatment and vaccine development. Little is known about the prevalence of non-B subtypes in the USA, where subtype B predominates. We determined subtypes for 102,153 polymerase sequences reported to the US National HIV Surveillance System between 2001 and 2014. Most patients were men (69.8%) with median age 40 years at sampling; a total of 7,647 (50%) sequences were identified in 2,318 clusters (median size 3). Factors associated with clustering included: younger age (median 38 vs 42 years), more recent sample (median year 2009 vs 2007), and sampling from Raleigh and Charlotte metropolitan areas [p < 0.001]. Among eight largest clusters (n = 22-36 members), clusters originated 1997-2004, spanned mean 12.7 years, and all grew from 2009 to 2014. Large clusters were predominantly young men (mean age 29 years); however one cluster (n = 23) was 57% female.

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A large proportion of sequences clustered, indicating significant local transmission. Large clusters have expanded for >10 years, and continue to grow, particularly among young men. Cluster expansion by cluster size, geographic spread and drug resistance will be further evaluated.

**A34 Toward a resource and time consumption reduction on human immunodeficiency virus (HIV) drug resistance algorithms**

J. Florencio da Silva and V. Garcia

Center of Informatics, Federal University of Pernambuco, Brazil

Analyzing and comparing biomolecular information can present problems that are very time and resource consuming. These kinds of data can be used the field of virology to interpret viral drug resistance. The databases from which these data come present are complex and traditional data management techniques are not useful since they can sometimes present inconsistencies. This research aims to optimize the response time and computational resource consumption of the RegaDB human immunodeficiency virus (HIV) drug resistance interpretation algorithms using big data management and manipulation techniques. To achieve this, it will be necessary to know the performance of these algorithms and evaluate their complexity in order to determine the consumption of time and computational resources under the worst, median and better case scenarios. Finally, big data management and manipulation techniques will be used to modify the algorithms. We expect to offer empirical or mathematical proof of a reduction in time or computational resource consumption in at least one of the algorithms.

**A35 Human immunodeficiency virus type 1 (HIV-1) genetic diversity and drug resistance in the Caribbean.**

G.G. Delva,1,4 C. Gutierrez,1 G. Ravasi,2 M. Charles,3 and G.A. Alemoñji1

*Corresponding author

1Caribbean Public Health Agency, Port of Spain, Trinidad and Tobago, 2Pan American Health Organization, Washington DC, USA and 3Centers for Disease Control and Prevention, Atlanta, Georgia, USA

There have been few phylogenetic analyses on human immunodeficiency virus type 1 (HIV-1) drug resistant strains in the Caribbean region. The primary objective of this study is to analyze the patterns of genetic diversity of HIV-1 in the Caribbean and describe the molecular epidemiology of HIV-1 drug resistance (HIV-DR) in the region. The pol and env gene sequences obtained from HIV-1 genotyping for drug resistance will be collected within the country member states of the Caribbean Public health agency and the Caribbean HIV-DR network and will be used to perform phylogenetic analyses. The Stanford University Database (http://hivdb.stanford.edu/ HIV) will be used to analyze the protease-RT sequences for mutations associated with resistance to antiretroviral drugs. This study will provide a global view of HIV-1 genetic diversity and drug resistant strains in the Caribbean region. Phylogenetic analysis of HIV-DR strains is absolutely necessary to monitor HIV-DR strain evolution, and will also contribute to strengthening the implementation of public health strategies to prevent and address HIV-DR in the region.

**A36 Evolutionary history of dengue virus serotype 2 (DENV-2) in Santander, a dengue endemic region in Colombia.**

C. Jimenez-Silva1* and R.E. Ocazionez1,2

*Corresponding author

1Centro de Investigaciones en Enfermedades Tropicales (CINTROP), Universidad Industrial de Santander, Bucaramanga, Colombia and 2Grupo de Investigación en Enfermedades Infecciosas y Metabólicas (GINEM), Universidad Industrial de Santander, Bucaramanga, Colombia

Santander, Colombia is an endemic region for outbreaks of dengue virus (DENV). DENV-2 predominated in the Bucaramanga metropolitan area of Santander since 1998. However, the evolutionary history of DENV-2 that has been circulating in the Bucaramanga metropolitan area are unknown. In this study, we present a reconstruction of the evolutionary relationships of DENV-2 using the E gene sequences sampled from 1998 to 2015. We include sequences from other regions of Colombia and the world that are available in Genbank. We reconstructed a phylogenetic tree based on Bayesian analysis. The analyses revealed that Colombian strains were grouped in four viral lineages showing different dispersal routes toward Colombia and that two genotypes (American and American/Asian) circulated in Colombia for different periods of time. Lineage 1 was of American genotype and lineages 2, 3 and 4 were of American/Asian genotypes. The Colombian strains in lineage 2 were related to strains formed Venezuela, Puerto Rico and Brazil from the 1990’s. The Colombian lineages 3 and 4 were closely related with Venezuelan isolates from 1996 to 2008. Colombian strains isolated in Norte de Santander in 2005, Guaviare in 2005 and Antioquia in 2004 were included in this two lineages, for this reason, we supposed that this lineages was introduced from Venezuela through Norte de Santander/Santander and has circulated in different areas of Colombia.

**A37 Ecology of the dengue virus (DENV) in domiciliary environments: is the bat a reservoir, a host or accidentally involved in the transmission of dengue?**

A. Vicente-Santos,1,2,3* A. Moreira-Soto,1 C. Soto-Garita,1 L.G. Chaverrí,1 A. Chaves,1 J.A. Morales,1 A. Alfaro,4 and E. Corrales-Aguilar2

*Corresponding author

1Centro de Investigación en Enfermedades Tropicales. Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica, 2Laboratorio de Genética de la Conservación, Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica, 3Escuela de Ciencias Exactas y Naturales, Universidad Estatal a Distancia, San José, Costa Rica and 4Laboratorio de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

Dengue is the most important vector transmitted disease in Costa Rica, but research on the local factors that affect the disease system is scarce. Recent studies show the presence of dengue virus (DENV) in wildlife, including bats, but the role they play in the transmission cycle is unknown. The significance of wildlife in (re)emerging infectious diseases has been increasingly appreciated, and it is possible that some species of bats are susceptible to DENV. This research aims to evaluate the presence of DENV in domiciliary bats that inhabit areas of high and low incidence of dengue, in wet and dry season, in Costa Rica to identify ecological, environmental, anthropogenic and virological factors that could involve the bat in a possible viral transmission cycle. We sampled houses in which humans and bats cohabit, took samples from bats and blood samples from humans, and collected mosquitoes by EYS-CO2 traps. According to the sample type, we determined the presence (RT-PCR and virus isolation) and frequency (serology) of DENV in bats (blood and pool of organs), mosquitoes and humans to determine the possible virus circulation in them. We performed necropsy on a portion of the bats collected and histopathology on shock organs (heart, lung, liver, spleen, kidney, brain), to observe any possible sign of sickness. Furthermore, we will establish the phylogenetic relationships of the strains of DENV obtained from bats, together with co-circulating human and mosquito strains collected by the reference center, by analyzing the E region sequences.

**A38 Genetic diversity and viral fitness studies of dengue virus serotype 1 (DENV-1) isolated during the 2013 outbreak in Costa Rica**

C. Soto-Garita,1 T. Somogyi, A. Vicente-Santos, A. Moreira-Soto, and E. Corrales-Aguilar2

*Corresponding author

1CIEF (Center for Tropical Diseases), Virology, Faculty of Microbiology, University of Costa Rica, San José, Costa Rica

For a hyperendemic country, Costa Rica has few reports about dengue virus genotypes circulating during outbreaks. With the aim of analyzing the genetic diversity within the circulating strains, we used sera from acute patients to isolate dengue virus using C6/36 cells from the 2013 outbreak in Costa Rica. Sixteen isolates were successfully recovered and their serotyping showed dengue virus serotype 1 (DENV-1) as the main circulating serotype. Molecular and phylogenetic characterizations were conducted using the E gene sequence. Phylogenetic analyses suggested that only genotype V was circulating during the outbreak. Furthermore, viral fitness was determined by in vitro replication in C6/36 cells and Hu7-7 cells. In order to get a better understanding and control of dengue outbreaks in hyperendemic settings it is crucial to have a description of the extent and structure of the genetic diversity of the circulating serotypes.
Intra-host genetic diversity of dengue virus type 4 (DENV-4) circulating in Brazil.

A.S. Ortiz-Baeza,1,2 C.J. Villabona-Arenas,2,3 and P.M. Zanotto1

1Corresponding author
2Laboratory of Bioinformatics and Molecular Evolution (LEMB), Department of Microbiology, Institute of Biomedical Sciences (ICB), University of Sao Paulo, Brazil, 2UMI 233 Transitions epidemiologiques, recherches translationnelles appliquees au VIH et aux maladies infectieuses (TransVHIIM), Institut de Recherche pour le Développement (IRD), Université de Montpellier, France, and 3Laboratoire d’Information, de Robotique et de Modélisation de Montpellier (LIRMM), Institut de Biologie Computationnelle (IBQ), Université de Montpellier, France

Characterizing intra-host genetic variability in dengue virus (DENV) virus is paramount for understanding its evolution and population dynamics in its current status as a major human pathogen. The extent to which viral diversity accrues in infected hosts influences aspects such as pathogenesis, transmission and host immunity. Although there are several studies of intra-host genetic diversity of dengue virus infection, limited data have been reported for DENV-4 so far. In the city of Guarujá in the State of Sao Paulo, the reemergence and spread of this serotype was associated with its co-circulation and with the displacement of serotypes 1–3 during recent outbreaks. Based on this epidemiological framework, we seek to identify the intra-host genetic variation of DENV-4 strains from samples collected during the 2013 outbreak using deep sequencing technologies. We will estimate the level of variability and the evolutionary history of these viral lineages in response to selective pressures in a populated urban area previously exposed to the remaining three dengue serotypes. This study would also be the first effort to investigate the intra-host diversity of DENV-4.

Incidence of dengue virus (DENV) infections in febrile episodes in ILE-IFE, Nigeria.

O.A. Adegbesin1 and A. Johnson2

1Department of Microbiology, Obafemi Awolowo University, P.M.B 13, Ile-Ife 220005, Osun State, Nigeria and 2Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Dengue viruses have been identified as the most important arboviral pathogen in the world. They are transmitted by mosquitoes of Aedes species. Although dengue infection is accompanied by little or no symptoms in many, about 1−2% of infections may produce clinically severe disease. The severe Dengue Haemorrhagic Fever or Dengue Shock Syndrome. Early recognition, appropriate treatment and elimination of mosquito vectors can help control the virus. This study aimed at determining the incidence of dengue infections in Ile-Ife. Three millilitres of venous blood were collected from each of 179 patients presenting with fever in the last 2 weeks, and analyzed for the presence of anti-dengue IgM antibodies using Dengue Virus IgM ELISA kit (DIA.PRO, Italy) according to the manufacturer’s instructions, while the results and demographic data were analyzed using SPSS version 16.

It was observed that forty-six (25.7%) of the 179 patients had detectable IgM antibodies to dengue virus with nine of them having no detectable malaria parasite. Dengue virus incidence was 26.5 and 25% in males and females, respectively. Although we did not seek to investigate whether blood transfusion was related to the transmission of dengue, it was the only risk factor that showed a statistically significant result. Further studies will be necessary to confirm this.

The study established the presence of fresh dengue infections for the first time in Ile-Ife among different groups of people. Clinicians are advised to prioritize laboratory diagnosis, especially of fever.

Dynamics of dengue virus serotype 1 (DENV-1) circulation in a medium size city in Brazil.

B.P. Drumond,1,4 J.M. Biselli-Pécori,1,2 D. Vedovello,1,2 T.E. Colombo,1,3 T.M. Pinheiro,1,2 L.S. Ullman,1 J.P. Araujo Jr,1* and M.L. Nogueira1

1Corresponding author
1Virology Area, School of Biochemistry and Pharmaceutical Sciences, National University of Rosario, Rosario, Argentina and 2Human Virology Group, IBR-CONICET/National University of Rosario, Rosario, Argentina

Chikungunya virus (CHIKV), a mosquito-transmitted alphavirus, causes acute fever and joint pain in humans. Recently, endemic CHIKV infection outbreaks have jeopardized public health in wider geographical regions. Here, we analyze the phylogeographic associations of CHIKV and explore the potential recombination events in 152 genomic isolates deposited in Genbank database. The CHIKV genotypes (West African, Asian, East /Central /South African (ECSA)), and a clear division of ECSA clade into three subgroups (I–III), were defined by Bayesian analysis; similar results were obtained using E1 gene sequences. A nucleotide identity-based approach is provided to facilitate CHIKV classification within the ECSA clade. Using seven methods to detect recombination, we found a statistically significant event (P-values range: 1.14 × 10−7 to 4.45 × 10−3) located within the nsP3 coding region. This finding was further confirmed by phylogenetic networks (PHI Test, P = 0.004) and phylogenetic tree incongruence analysis. The recombinant strain, KJ679578/India/2011 (ECSA III), derives from viruses of ECSA III and ECSA I. Our study demonstrates that recombination is an additional mechanism of genetic diversity in CHIKV that might assist in the cross-species transmission process.

Protective B-cell epitopes in chikungunya virus (CHIKV) infection.

A. Ramjag,4 G. Simmons,2 and C.V.F. Carrington1

1Corresponding author
1Department of Preclinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Republic of Trinidad and Tobago and 2Blood Systems Research Institute, 270 Masonic Ave, San Francisco, CA, USA

Chikungunya virus (CHIKV) (Family Togaviridae, genus Alphavirus) is a zoonotic agent of chikungunya fever (CHIKF) and an important newly emergent virus in the Americas. Although not usually life threatening, CHIKF is very debilitating and is often associated with chronic arthritis, resulting in major economic losses. No licensed therapeutic treatments or vaccines exist. However neutralizing antibodies that can protect animals have been described and there is evidence to suggest that therapeutic antibody therapy may also be helpful in relieving symptoms and interrupting transmission. CHIKV exists as a single serotype (which confers lifelong immunity) with three genotypes (West African, East /Central /South African (ECSA), Asian). In this study, we will generate and characterize new antibodies that block CHIKV, and describe the full B-cell repertoire in response to CHIKV infection—both in acutely infected patients and to individuals from the Trinidad population. To maximize their therapeutic and economic efficiency in a field setting, anti-CHIKV
mAbs would have to be broadly cross-reactive against existing CHIKV variants, and should target epitopes that are refractive to immune escape. We will therefore determine the nature and extent of sequence variation within targeted genes, both within and among the individuals (during consecutive outbreak years) and also compare to global CHIKV sequences derived from the wider Americas with emphasis on detecting variation and signatures of selection at B-cell epitopes. Whole genome sequences derived to date confirm the presence of the Asian genotype in Trinidad and low sequence diversity both within and between individuals.

A45 Evolution of chikungunya (CHIKV) in India: whole genome sequencing and clinical data correlation.

J. Jain,1,* J. Shrinet,1 J.S. Shastri,2 R. Gaind,3 R.K. Bhatnagar,1 and S. Sunil1

*Corresponding author
1Insect Resistance Group, International Center for Genetic Engineering and Biotechnology, New Delhi, India, 2Department of Microbiology, T.N Medical College and B.Y. L. Nair Ck. Hospital, Mumbai, India and 3Department of Medical Genetics, Vardhman Mahavir Medical college and Safdarjung Hospital, New Delhi, India

Chikungunya virus (CHIKV) is a vector-borne disease transmitted by Aedes mosquitoes with a very high morbidity rate and its chronic state can persist for >2 years in some cases. Chikungunya is prevalent in most parts of India and has now become a global issue. We aim to study the evolution of CHIKV in India, and for the same purpose more than 100 clinical field isolates were collected from across India over the span of 4 years (2010–13). All the clinical samples, along with CHIKV passed six times in Vero cells in vitro were subjected to clinical, molecular and next generation whole genome analysis. Activity of the virus was studied via viral load experiments, plaque assays, quantitative PCR and Sanger sequencing, before further processing for whole genome sequencing. The main purpose of this study was to perform in depth analysis of CHIKV genomes that could help to understand the underlying single nucleotide changes and mutation events that might have occurred within the CHIKV genome leading to its evolution. The genomic data and molecular analysis, along with SNPs and mutation information, were further correlated with clinical information in order to determine the evolution and molecular epidemiology of the virus.

A46 Spatiotemporal dynamics of Venezuelan equine encephalitis virus (VEEV) antigenic complex

J.-P. Carrera,1,* N. Forrester,2 A.J. Auguste,2 T. Kautz,2 S. López-Vergez,3 and S. Weaver4

*Corresponding author
1Department of Research in Virology and Biotechnology, Gorgas Memorial Institute of Health Studies, Panama City, Panama and 2Department of Pathology and Institute for Human Infection and Biotechnology, New Delhi, India, 3Department of Research in Virology and Biotechnology, Gorgas Memorial Institute of Health Studies, Panama City, Panama and 4Department of Pathology and Institute for Human Infection and Biotechnology, New Delhi, India

Venezuelan equine encephalitis virus (VEEV) (Alphavirus, Togaviridae) antigenic complex is composed for sixteen single strand RNA virus species. Enzootic strains that circulate in sylvatic, rodent-mosquito enzootic cycles regularly spillover to human and equine epidemics since the 1920s have been associated with clinical information in order to determine the underlying single nucleotide changes and mutation events that might have occurred within the CHIKV genome leading to its evolution. The genomic data and molecular analysis, along with SNPs and mutation information, were further correlated with clinical information in order to determine the evolution and molecular epidemiology of the virus.

A47 Antiviral resistance of influenza viruses isolated in Kenya, 2007–11

G. Kikwai,1,* L. Waiboci,2 T. Shigoli,1 and P. Muthoka3

*Corresponding author
1Kenya Medical Research Institute, Nairobi, Kenya, 2University of Nairobi, Nairobi and 3Kenya Ministry of Public Health and Sanitation, Nairobi, Kenya

The continued threat of emergence of novel influenza viruses with pandemic potential in humans underscores the need for information on effective antivirals for management of influenza infections. Specimens collected from patients with respiratory illness were tested by real time reverse transcription polymerase chain reaction for influenza A and B viruses. Influenza A positive samples were subtyped. Influenza viruses, isolated by cell culture were assessed for susceptibility to neuraminidase inhibitors in neuraminidase inhibition assays. Virus isolates showing elevated IC50s in the NI assay were genetically analyzed by conventional sequencing and/or pyrosequencing to detect molecular changes in the neuraminidase protein associated with reduced susceptibility to neuraminidase inhibitors and to detect markers of resistance to adamantanes. All influenza A and B isolates tested were susceptible to oseltamivir, while 668 (91.2%) were susceptible to zanamivir. Influenza B, A (H3N2) and A (H1N1)pdm09 isolates tested were susceptible to oseltamivir. Of the 350 influenza A isolates tested for resistance to adamantanes, 224 had the most commonly detected marker, S31N. A single (H1N1)pdm09 isolate contained a combination of two markers of resistance to adamantanes, A30T and S31N, and one influenza A/H1N1 contained a L26F marker. More than 100% of the viruses characterized were resistant to adamantanes. However, >90% of the viruses circulating during the period were susceptible to oseltamivir and zanamivir. That there would be benefit of antiviral prophylaxis and treatment in Kenya. Further surveillance and characterization is important for detection of drifts and shifts to inform on formulation of policies by the key stakeholders.

A48 High incidence of bacterial co-infections in patients infected with pandemic H1N1/09 influenza virus in Mexico

C. Rodríguez-Padilla,1 S. Saavedra-Alonso,1 L.R. Ramirez-Palacios,2 A.M. Rodríguez-Márquez,1* C. Willis-Rodriguez,1 M.A. Bermúdez-de Leon,4 I.C. Rodriguez-Luna,3 C. Reséndez-Pérez,1 and R.S. Tamez-Guerra1

*Corresponding author
1Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, 2Laboratorio Estatal de Salud Pública de Oaxaca, Carretera a Sola de Vega, Km 18.5, Reyes Mantecon, Oaxaca, 71257, 3Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Blvd. del Maestro esquina Elías Piña S/N, Colonia Narciso Mendoza, 88710, Reynosa, Tamaulipas, México and 4Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, IMSS, 64720 Monterrey, NL, México

There have been at least four waves of pandemic H1N1/09 virus in Mexico since 2009. One factor linked to the severity of the disease is viral/bacterial co-infections. Therefore, monitoring co-infecting pathogens along with influenza virus could help to improve medical care for influenza patients. We collected 104 throat swab samples from Mexican patients showing influenza-like signs or symptoms and analyzed them using real-time PCR and the TessoArray RPM-Flu assay to determine respiratory pathogens. Multiple bacterial co-infections together with pandemic H1N1/09 virus predominated in patients. Main pathogens were Streptococcus spp., Pseudomonas spp. and Haemophilus influenzae from which Streptococcus pneumoniae seems to have preference of co-infection with pandemic H1N1/09 virus. We detected three cases of viral co-infection with Coronavirus, Flu B and Coxsackievirus. The seasonal A/H3N2 virus, parainfluenza virus and rhinovirus were not present in co-infections associated to pandemic H1N1/09 virus. Patients infected with 2009 pandemic influenza reported severe symptoms such as myalgia, fever, rhinorrhea, malaise, headaches and coughing. The number of patients hospitalized and ambulatory patients with multiple infections was more than 10 times greater than the number with single infection by pandemic H1N1/09 virus. This indicates that infection by multiple pathogens relates to the severity of disease. Our findings indicate a high prevalence of bacterial co-infection with pandemic H1N1/09 virus, which leads to a greater number of patients with severe symptoms.

A49 Phylogeography of the Eurasian ‘avian-like’ H1N1 swine influenza virus in Europe

R.S. Tamez-Guerra1, M.A. Bermudez-de Leon,4 I.C. Rodriguez-Luna,3 C. Reséndez-Pérez,1* C. Rodríguez-Padilla,1 S. Saavedra-Alonso,1 L.R. Ramirez-Palacios,2 A.M. Rodríguez-Márquez,1* C. Willis-Rodriguez,1 M.A. Bermúdez-de Leon,4 I.C. Rodriguez-Luna,3 C. Reséndez-Pérez,1 and R.S. Tamez-Guerra1

*Corresponding author
1Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, 2Laboratorio Estatal de Salud Pública de Oaxaca, Carretera a Sola de Vega, Km 18.5, Reyes Mantecon, Oaxaca, 71257, 3Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Blvd. del Maestro esquina Elías Piña S/N, Colonia Narciso Mendoza, 88710, Reynosa, Tamaulipas, México and 4Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, IMSS, 64720 Monterrey, NL, México

Chikungunya virus (CHIKV) is a vector-borne disease transmitted by Aedes mosquitoes with a very high morbidity rate and its chronic state can persist for >2 years in some cases. There have been at least four waves of pandemic H1N1/09 virus in Mexico since 2009. One factor linked to the severity of the disease is viral/bacterial co-infections. Therefore, monitoring co-infecting pathogens along with influenza virus could help to improve medical care for influenza patients. We collected 104 throat swab samples from Mexican patients showing influenza-like signs or symptoms and analyzed them using real-time PCR and the TessoArray RPM-Flu assay to determine respiratory pathogens. Multiple bacterial co-infections together with pandemic H1N1/09 virus predominated in patients. Main pathogens were Streptococcus spp., Pseudomonas spp. and Haemophilus influenzae from which Streptococcus pneumoniae seems to have preference of co-infection with pandemic H1N1/09 virus. We detected three cases of viral co-infection with Coronavirus, Flu B and Coxsackievirus. The seasonal A/H3N2 virus, parainfluenza virus and rhinovirus were not present in co-infections associated to pandemic H1N1/09 virus. Patients infected with 2009 pandemic influenza reported severe symptoms such as myalgia, fever, rhinorrhea, malaise, headaches and coughing. The number of patients hospitalized and ambulatory patients with multiple infections was more than 10 times greater than the number with single infection by pandemic H1N1/09 virus. This indicates that infection by multiple pathogens relates to the severity of disease. Our findings indicate a high prevalence of bacterial co-infection with pandemic H1N1/09 virus, which leads to a greater number of patients with severe symptoms.
EA viruses in Europe; for example, calculating the intra-lineage allowance for analysis of the phylogeography and molecular evolution of lineage, and seventy-nine have complete EA genotypes. These data platforms. Of these, 180 contain internal genes derived from the EA viruses (SIV) circulating in different regions of the world. Two of the eight viral segments were derived from the Eurasianavian-like (EA) H1N1 lineage. Viruses from this lineage have circulated enzootically in European swine since 1979, and despite the emergence of other, more recent SIV lineages, it remains the most prevalent genotype isolated from swine across mainland Europe. Despite this prevalence, there are scarce genomic data from European SIV, and little is known about the molecular epidemiology of this lineage in Europe. Through the European Surveillance Network for Influenza in Pigs project, we have sequenced 243 SIV isolates from fourteen countries across Europe between 2009 and 2013 using high-throughput sequencing platforms. Of these, 180 contain internal genes derived from the EA lineages, and 79 have EA sequences. These data allow for analysis of the phylogeography and molecular evolution of EA viruses in Europe; for example, calculating the intra-lineage reassortment rate, inferring the evolutionary rate of the lineage since the introduction of the A(H1N1)pdm09 virus into European swine, inferring the spatial dynamics of the virus across Europe, and determining predictors associated with these patterns.

Full-length genome characterization and quasispecies distribution of hepatitis A virus (HAV) isolates in China

H. Wang, X. Wang, J. Cao, Y. Gao, W. Zhou, and S. Bi

Corresponding author
National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China

Hepatitis A virus (HAV) infection is the most common cause of acute viral hepatitis and has significant implications for public health worldwide. To characterize HAV strains circulating in China, five samples collected in different provinces from 2006 to 2009 were entirely sequenced. Phylogenetic analysis based on distinct segments showed that all five sequences belonged to subgenotype IA, but with slight differences in some fragments. No amino acid mutations were found at the known neutralizing epitope sites, and one unique substitution was identified near the immunodominant site. While no intratypic recombination was detected, intratypic recombination signals were found in the study. Molecular evolution analyses showed the estimated mean substitution rate of genotype I worldwide is 3.7 × 10⁻⁷ substitutions/site/year, and the time to most recent common ancestor was about 267 years ago. The quasispecies distribution across the complete genome was also evaluated and the nucleotide mutation frequency was found to range from 7.26 × 10⁻⁴ to 1.0 × 10⁻³ substitutions per nucleotide. The amino acid mutation frequency ranged from 1.38 × 10⁻³ to 0.27 × 10⁻³ substitutions per amino acid, and the high mutation frequency regions were mainly in the nonstructural protein coding sequences. This study contributed information on the genotype distribution, selection pressure, neutralizing epitope site mutations, recombination events and quasispecies distribution of HAV strains in China. The evolutionary status of genotype I worldwide was also analyzed, which will provide a reference for future HAV molecular epidemiology studies.

Comparative analysis of canonical phylodynamic data: hepatitis C virus (HCV) in Egypt

K. Parag and O.G. Pybus

Corresponding author
Department of Zoology, University of Oxford, Oxford, UK

Hepatitis C virus (HCV) is a global health threat with unusually high prevalence in Egypt. Previous epidemiological studies have implicated inadequate needle sterilization, during a large-scale 2000–2002 national antischistosomal treatment (PAT) campaign, as the primary cause of the Egyptian HCV epidemic. The well-characterized epidemic history of Egyptian HCV and its interesting iatrogenic cause makes it appropriate for appraising various phylodynamic models. Comparative analysis of HCV sequences from Egypt (Ray et al. 2000) from the dominant HCV-4a subtype has been used to evaluate many phylodynamic techniques for estimating population history, including skyline, skygrid and skygrid methods, as well as death–birth model based approaches. However, recent work (Cuadros et al. 2014) has suggested that PAT infection may be overstated significantly. For example, many HCV-4a gene sequences have been obtained since 1993, including whole genomes, and these have not been integrated with the 1993 data. Moreover, the different phylodynamic approaches sometimes disagree on virus dynamics prior to PAT initiation. I propose to combine and align all available HCV-4a sequences to generate an updated benchmark dataset for testing phylodynamical techniques. I aim to apply methods, taught in this workshop, on evolutionary hypothesis testing, to resolve uncertainty surrounding the epidemic history of Egyptian HCV and hopefully clarify the importance of the PAT campaign. Furthermore, by contrasting and comparing results of existing models on a canonical data set, I hope to gain insight into their relative merits as statistical estimators. This would support my long-term aim of developing richer and more informative Bayesian phylodynamic models.

An evolutionary approach of interspousal transmission of hepatitis C virus (HCV) infection

S.A. Pereira, M.P. Espirito Santo, G.M. Lauer, E. Lampe, and L.L. Lewis-Ximenez

Corresponding author
Viral Hepatitis Laboratory, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil and Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Even though there is evidence of sexual transmission of hepatitis C virus (HCV) the actual risks in different types of sexual behavior have been difficult to assess and it is still unclear as to which factors actually contribute to this route of transmission. In this study, we report transmission associated with lack of lubrication during sex from fifteen HCV-infected individuals to their heterosexual partners. Forty female patients with symptomatic acute HCV infection were identified at the Viral Hepatitis Laboratory, Fiocruz, Rio de Janeiro, Brazil. To confirm HCV transmission between spouses, nested reverse transcription polymerase chain reaction products were submitted to direct nucleotide sequencing of the NS5B region. The obtained sequences were aligned with corresponding nucleotide sequences of twenty-three HCV reference sequences retrieved from GenBank and thirteen local unrelated HCV sequences and phylogenetic tree was constructed with Mega 4 software using Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and their reliability was assessed by bootstrap resampling 1,000 replicates. Among the forty subjects that reported sexual risk behavior, twenty-nine had sexual partners that volunteered samples to investigate possible HCV genomic similarities. The phylogenetic analysis of the nucleotide sequence of HCV genome was limited to the remaining fifteen subjects and their sexual partners, which revealed nucleotide identity >95% among fourteen of the fifteen couples, which strongly suggests these partners to be the source of infection, after ruling out possible contamination through personal item sharing. These findings provide strong molecular evidence that the women had acquired HCV infection most likely by interspousal sexual transmission.

Rare genetic variation in hepatitis delta virus (HDV) that influences genotype determination

S. Perneen, A. Azhar, and O.Y. Khan

Corresponding author
The Karachi Institute of Biotechnology & Genetic Engineering (KIBGE), University of Karachi, Karachi-Pakistan and Department of Genetics, University of Karachi, Karachi-Pakistan

Hepatitis delta virus (HDV) only infects hepatitis cells already infected with Hepatitis B virus (HBV). The delta virus infection leads to a clinical outcome that is more severe than HBV infection alone, with severity of infection varying with the HDV genotype. During sampling of HDV in Karachi, Pakistan, a variant of genotype 1 HDV was encountered that was initially misdiagnosed as genotype II, based on Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis of Small Open Reading Frame (S0) domain sequence. Correct genotype determination of the newly found strains was carried out using phylogenetic analysis and an attempt was made to analyze its origin and propagation in our population. Blood from HDV positive patients were collected from 8 patients with Hepatitis B Surface Antigen (HBSAg) →ve and Hepatitis Delta Antigen (HDAg) →ve patients and viral RNA was extracted, reverse transcribed and used to amplify

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the HDV R0 region by Reverse Transcriptase (RT)-nested PCR. The PCR products were screened with RFLP using Smal restriction enzymes. Nucleotide sequences were used for phylogenetic and analysis for determination. In silico analysis for evidence of recombination was also carried out in order to determine the origin of the HDV isolates of interest. PCR-RFLP analysis using Smal enzyme showed that three of our HDV isolates belonged to genotype II. A deeper nucleotide analysis exposed a single base pair selectively neutral mutation at the single Smal restriction site within the B cell epitope, which caused these three strains to be falsely categorized as genotype II. Sequences were examined in silico for recombination showed exchange of genetic material within and across genotype I and II. Phylogenetic analysis of our sequences have shown a clear misclassification of genotype in our HDV isolates and proved the shortcomings of HDV genotyping based on PCR-RFLP. Recombination analyses have suggested a possible reason for the high frequency of occurrence of this mutation in our sample.

**A53** Lassa virus: codon usage and bias along with its host

L.E. Okoro1,2,* and A.A. Elduie1,2

*Corresponding author
1Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria and 2Department of Computer Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria

Lassa virus continues to be endemic with frequent outbreak in areas of endemicity which is of a public health concern due to its fast evolutionary rate. There have been reports of new strains in different epidemic outbreaks. We used seven different codon usage bias tools and indexes targeting synonymous codon analysis, which included GC content, ENC, SCUO, Codon Volatility, RSCU, Odds ratios and Graphical Codon Usage Analysis tool. This study observed evolutionary patterns in Lassa virus from humans, rodents and bats. It also observed the evolutionary pattern and influence of different geographical locations and periodic outbreaks. There was variation in GC content in the glycoprotein gene, nucleocapsid gene, E2 protein gene and polyprotein gene. The RSCU value was positively correlated with the Odds Ratio of dinucleotides in the codons. RSCU values of humans, rodents and bats were slightly different, though this result was not completely true for odds ratios. The GC content, ENC, SCUO and Codon Volatility indices were similar across all the hosts. However, there was slight variation of genes from different geographical locations, thereby supporting reports that Lassa virus strains vary among different locations. Though there was slight variation among years of isolation the difference in hosts confirms why the virus causes fever in humans as against being normal in other hosts.

**A54** Molecular detection and genotyping of sapovirus (SaV) and norovirus (NoV) in children with acute gastroenteritis in a hospital in Lima

G. Sánchez1,4, V. Saravia,1 H. Jahuira,1 G. Oyola,1 K. Neira,1 H. Mayta,1 R. Gilman,1,2 and S. Baillard2

*Corresponding author
1Laboratorio de Investigación en Enfermedades Infecciosas, LID-UPCH, Peru and 2Bloomberg School of Public Health, Johns Hopkins University, MD, USA

Gastroenteritis remains one of the main problems for children under five. Most studies of gastroenteritis caused by viruses are limited to Rotavirus and norovirus (NoV). In this study, we look for sapovirus (SaV) and NoV in 695 children (416 cases and 279 controls) sampled from November 2013 to October 2014. Preliminary results of the ongoing project are presented. Stool samples were collected from children and stored frozen at –70°C until processed. Aliquots of 0.1 g (formed) or 0.1 ml (watery) stools will be diluted 1:10 and RNA extracted using QIAamp viral RNA kt. RNA was used for detection of NoV GI and GII in a duplex one step PCR by using Cog 1F, Cog 1R, Cog 2F, Cog 2R, Cog 3F and Cog 3R as primers and Ring 1A, Ring 1B and Ring 2TP as probes. genomic typing was performed using the sequence from the product of GI SKF/SKR and G2 SKF/SPK primers for capsid N/S domain. cDNA synthesis was performed with SuperScript III reverse transcriptase, SaV detection was performed using SaV124F, SaV1F, SaV5F and SaV1245R as primers and SaV124TP and SaV5TP as probes, genomic typing was performed using the sequence from the product of SaVF22 SaV R2 primers for a partial capsid region. Preliminary data indicates that prevalence of NoV was 38%, with NoV GI as the main genotype found in the specimens, and the prevalence of SaV was 9.6%. The genotyping for twenty-two isolates according the partial capsid region was GI = 7, GII = 9, GIV = 4 and GV = 2, and from these data six samples show co-infection for SaV and NoV. Control non-diarrhetic samples were negative for NoV and SaV. According to these preliminary results, NoV remains an important pathogen and SaV seems to be responsible for a substantial number of cases in Lima. SaV detection should be included in future research of viral gastroenteritis epidemiology.

**A55** Diversity and dynamics of rotavirus in humans, pigs and rats in Vietnam using antigenic whole-genome deep sequencing

M.V.T. Phan,1,* B.B.B.O. Munnink,2 P.H. Anh, N.V. Cuong,3 T.T.N. Dung,1 V.V. Phat,1 M. Rabaa,4 L. van der Hoek,5 S. Baker,5 P. Kellam,1,4 and M. Cotten1

*Corresponding author
1Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, 2Laboratory of Experimental Virology, Center for Infection and Immunity Amsterdam, Academic Medical Center, Amsterdam, The Netherlands, 3Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam and 4Department of Infection, University College London, London, UK

Rotavirus is the major diarrhoeal pathogen in humans and other mammals. Given the propensity of rotavirus for genetic reassortment and interspecies movement, it is important to sequence the entire eleven-segment genome to understand diversity, evolution and antigenic chatter. Current rotavirus sequencing approaches are challenging by the segmented genome, particularly the primer design step. Using an antigenic deep sequencing method for enteric viruses that requires no prior knowledge of the virus contents for primer design, rotavirus genome sequences were obtained from human, porcine and rat stools, in addition to full genomes from peripheral enteric viruses in the samples. Particularly, thirty-six genomes of rotavirus group A were documented in fifty human stool samples (forty-five rotavirus A-positive by real-time polymerase chain reaction) with more than one genotype detected in six samples. Rotavirus was found in 36/150 random porcine stool samples (thirteen group A, ten group B, eight group C and five newly identified group D). Rotavirus B was found in four/ten rats with one sample of mixed infections. Maximum-likelihood phylogenetic analyses of the new Vietnamese rotavirus A sequences showed that the viruses were closely related to existing Vietnamese and global strains, yet were different from the vaccine strains. Importantly, several amino acid differences from vaccine strains were observed in neutralizing epitopes of VP4 and VP7. The method also provided genomes for other enteric viruses in the samples, such as norovirus, sapovirus, astrovirus and various picornaviruses. This detailed viral sequence data will provide an important basis for examining the rate and properties of virus host switching.

**A56** First enterovirus D68 (EV-D68) cases detected in the Caribbean region

S. Nathaniel,1,* P. Salazar,1 C. Gutierrez,1 D.D. Chadee,2 and W.A. Nix3

*Corresponding author
1Caribbean Public Health Agency, 16-18 Jamaica Boulevard, Port of Spain, Trinidad and Tobago, 2The University of the West Indies, St. Augustine, Trinidad and Tobago and 3The Center for Disease Control and Prevention, Atlanta, GA, USA

Enteroviruses are a common cause of illness in infants and children, with over 100 types in existence that cause a range of respiratory, gastrointestinal and neurological diseases. In 2014, the global reemergence of human enterovirus 68 (EV-D68) commonly associated with severe respiratory illness was identified with outbreaks occurring in the USA and Canada. With the recent awareness regarding EV-D68, and as part of the continuing surveillance program on respiratory illness, respiratory samples sent to the Caribbean Public Health Agency that initially tested negative for influenza and non-influenza viruses were tested for EV-D68 using molecular diagnostics. In October and December 2014 enteroviruses were detected in respiratory samples from patients coming from Bermuda and Dominica, respectively. The patients were all children under 5 years old and symptoms were mainly respiratory. No patient exhibited neurological symptoms. Genetic analysis of partial VP1 sequences confirmed that the detected enteroviruses belonged to the D68 subtype, making these the first EV-D68 cases detected in the Caribbean region. By phylogenetic analysis the strains from Bermuda were shown to be closely related to the recent outbreak in the USA, confirming the circulation of that strain in regions outside of USA. The strains
from Dominica showed, however, some level of diversity when compared with the strains from USA. The isolation of EF-D68 in the Caribbean region may represent a major public health challenge for the region but also for the rest of the Americas due to the potential spread of the virus through the continent, the wide range of symptoms at presentation, the need of molecular diagnosis to confirm the isolation and the expected morbidity mostly in young children.

**A57** Full genome sequences and molecular characterization of ten arboviruses from Brazil

W.M. Souza,1 M.F. Romeiro,1 A.L. Tolardo,1 O. Reis,2 L.C. Vieira,1 and L.T.M. Figueiredo1

1Virology Research Center, School of Medicine of Ribeirão Preto of University of São Paulo, Ribeirão Preto, São Paulo, Brazil and 2Laboratory Central de Tecnologias de Alto Desempenho em Ciências da Vida (LaCTAD), parte da Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil.

Arboviruses (arthropod-borne viruses) are maintained in a cycle of alternating transmission between vertebrate hosts and arthropod vectors and possess RNA genomes capable of rapid diversification and adaptation. These viruses emerged from their sylvatic reservoirs and dispersed globally due to changing factors that include anthropological behavior, climatic, social, commercial transportation and land remediation. Thus, many arboviruses are an important emerging problem in public health worldwide. However, there is currently a gap in genomic information for arboviruses other than dengue virus in South America. In this study, we performed sequencing and molecular characterization of ten viruses discovered in the 1950s–90s in Brazil. The viruses includes: Catu, Itaqui, Enseada, Tacaima, Capim and Apeu (Orthobunyavirus), Bujaru and Anhanga virus (Phlebovirus) Pyr virus (Vesiculovirus) and Cacipore virus (Favilivirus). The full-length genomes of arboviruses were sequencing using Illumina HiSeq 2,500 system with paired-end 2×150 paired-end bases. The genomes were obtained by employing a de novo assembly strategy. The reads in fastq format were quality filtered using the program FastQC v0.11.3 and any adapter sequences were removed using Trimmomatic-0.33 software. The de novo assembly program IDBA-UD-1.1.1 was used to assemble the reads into contigs. The longest contigs were submitted to BLAST-based searches to identify viruses. Afterwards, annotations of putative ORF genes were identified by prediction in Geneious 8.0.3. All viruses that were characterized were molecularly and structurally similar and showed similarity to viruses from their respective genera. These data will represent the first complete coding region sequences for each species of virus. Our results will provide the molecular basis for the development of diagnostics, further genetic analyses, and future epidemiologic studies of these arboviruses in South America, especially Brazil.

**A55** Sequence analysis of the hepatitis B virus (HBV) PreC/C region by ultra-deep pyrosequencing as a predictor of nucleus colonies (Nuc) treatment outcome in patients with HBeAg-positive chronic hepatitis B

C. Rodríguez,1**,2 E. Audureau,2 S. Chevaliez,1 F. Darthuy,1 and J.M. Pawlotsky1

**Corresponding author**

1Microbiology Department, University Hospital Henri Mondor, INSERM U955 Team 18, Paris–Est Creteil University, France, and 2Clinical Investigation Laboratory, LIC- EA 4393, Paris–Est Creteil University, France.

One of the main goals of hepatitis B virus (HBV) antiviral therapy in patients with HBeAg-positive chronic hepatitis B is HBe seroconversion with sustained inhibition of viral replication. Whether and when such patients receiving nucleos(t)ide analogues can stop treatment without exposing the patient to a relapse is unknown. Thus, predictive markers are needed, both at baseline to evaluate the risk of treatment failure and during therapy to predict treatment success and evaluate the likelihood of a viral rebound at its withdrawal. We studied a cohort of 156 treatment-naïve patients with chronic HBeAg-positive chronic hepatitis B treated with adefovir dipivoxil and followed for a maximum of 180 weeks. HBV PreC/C domain which overlaps with X gene was sequenced in sequential samples by means of ultra-deep pyrosequencing using Genome Sequencer FLX (Roche Molecular systems/454) and analyzed by pyroseq (in-house software). Then, each mutation found was logit modeled over time, clustered (methods k-means, STEM-FLAME) and visualized by heat-map. From 337 serial samples from the 156 patients, we found four groups of patients harboring patterns of mutations located in the critical domain of HBV (promoters, replication regulation domain) associated with phenotypic characteristics of viral load decrease or coreversion. In conclusion, we used ultra-deep pyrosequencing to assess whether signature sequences in the PreC/C region could help tailor antiviral treatment of HBeAg-positive chronic hepatitis B. Results are promising to better understand the evolution of HBV under nucleotide inhibitor treatment and to better manage treatment of patients.

**A59** Cervical microbiome diversity is associated with cervical precancer using Next-Gen sequencing

Z. Chen,1,2 P.K.S. Chan,1 and R.D. Burk1,3

**Corresponding author**

1Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, 2Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY, USA and 3Departments of Microbiology & Immunology, Epidemiology & Population Health, and Obstetrics, Gynecology & Woman’s Health, Albert Einstein College of Medicine, Bronx, NY, USA.

A variety of microbial communities exist throughout human ecosystems, such as the gut, skin, oral cavity and vagina, with fundamental roles in human development, physiology, community and nutrition. The cervicovaginal microbiome plays an important role in female reproductive health, affecting rates of preterm-birth and neonate mortality; prevalence, susceptibility to, and transmissibility of sexually transmitted infections (including HIV); and other important clinical conditions. Despite its importance, however, little is known about how cervicovaginal microbiome communities differ in function and, more importantly, how their constituent members interact with each other and the host to form a dynamic ecosystem that responds to various host and environmental factors.

We used a nested case-control design to investigate the cervical microbiome in ninety-four HPV16-positive cervical samples from the Persistence and Progression (P&P) Cohort, a collaborative study with the NCI and KPNC. There were forty-four controls and fifty cases with Cervical intraepithelial Neoplasia Grade 3 (CIN3). To characterize the microbiome, we PCR amplified an approximately 145 bp fragment spanning the V6 region of the bacterial 16s RNA gene. Each sample was amplified using a unique barcoded primer set. Barcoded PCR products were pooled at approximately equal molar DNA concentrations and sequenced on an Illumina HiSeq 3,000 platform at Einstein. The NGS reads (~100 million) were processed using two-third part taxonomy algorithms, usearch and pplacer, and additional bioinformatics tools developed in-house. Classification tables were generated from input data at the species-level, based on the vaginal 16s RNA sequence database. The characterized reads were organized using a heat map and sample clustering. Pairwise comparisons were calculated for principal component analysis and Simpson diversity indices to evaluate the difference of cervical microbiome communities between case and control groups.

The cervical microbiome of the forty-four controls revealed two predominant genera, Lactobacillus and Gardnerella, consistent with previous reports of female reproductive tract microbiota. Thirty-five (80%) control samples were dominated by one or more species of Lactobacillus that constituted 86% of all Illumina reads. All five types of vaginal communities previously defined that are predominated by Lactobacillus crispatus (type I, 27%), Lactobacillus gasseri (type II, 5%), Lactobacillus iners (type III, 43%) and Lactobacillus jensenii (type V, 5%). The type IV community group (20%) lacked detectable Lactobacillus spp., but contained other bacterial groups with high proportions of anaerobic bacteria, including Gardnerella, Prevotella, Solobacter, Atopobium, Sneathia, Eggerthella, Pseudomonas, Acinetobacter and Streptococcus. The CIN3 group had similar cervical microbiome communities, but showed significantly higher measures of diversity compared with the control group (Mann-Whitney-U test P < 0.05).

**A60** Next-generation sequencing highlights unique aspects of Ebola virus (EBOV) biology and provides novel diagnostic platforms.

R. Shabman,1* K. Dilleby, R.A. Halpin, X. Lin, S.R. Das, T.B. Stockwell, and K.E. Nelson

**Corresponding author**

1Infectious Disease group, J. Craig Venter Institute, Rockville, MD, USA.
Filoviruses (Ebola and Marburg viruses) are a significant threat to human health, as indicated by the recent Ebola outbreak in West Africa. The mortality rate from filovirus infection can approach 90% and there are currently no approved filovirus vaccines or therapeutics. We seek to better understand filovirus biology to aid in our ability to design effective antiviral treatments. Through next generation sequencing (NGS), our collaborative work has uncovered unique features of filovirus transcription, replication, protein translation and innate immune evasion. We have deep sequenced RNAs produced by Zaire Ebola virus (EBOV) and the Angola strain of Marburg virus (MARV) to identify novel viral and cellular mechanisms that diversify the coding and non-coding sequences of filovirus RNAs (PMID: 25370975). Moreover, we have applied sequence-independent NGS methods to further define how the EBOV protein VP35 antagonizes the human innate immune response, specifically by detecting intrahost single nucleotide polymorphisms (iSNPs) at viral RNA from multiple serum samples from West African patients infected with Ebola virus strain Zaire, and patients infected with Ebola virus strain Sudan, as well as from within-host variants that are shared among two or more patients, often spanning several months of the Ebola epidemic. We conclude that these shared variants are due—at least in part—to the transmission of multiple virus genomes, which implies that the transmission bottleneck is wide enough to facilitate the transmission of intermediate-frequency variants. This finding can be used to better resolve transmission chains during an Ebola outbreak.

**A83** Time-dependent dynamics of intrahost variation during Ebola virus infection

A.E. Lin,1,2,3 S. Whitmer,4 A.N. Honko,4 S.K. Gire,2,4 C.B. Matranga,1,2 S. Wohl,1,2 K.G. Andersen,1,2 D. Park,1,2 L.E. Hensley,1 U. Stroeher,3 and P.C. Sabeti1,2

*Corresponding author
1Department of Organismic and Evolutionary Biology, Harvard University, 2FAS Center for Systems Biology, Cambridge, MA, USA, 3Broad Institute, Cambridge MA, USA, 4Viral Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, USA and 5Integrated Research Facility, National Institutes of Health, 6Frederick MD, USA

Lack of proofreading during RNA virus replication generates diverse viral quasispecies within a single host. Strong bottlenecks (e.g. viral entry, virion production, immune evasion) shape the emergence and frequency of viral variants and new phenotypes. Therefore, deep sequencing of the viral quasispecies over a time course of infection provides invaluable information regarding biological processes and outcome in a single host. We sequenced viral RNA from multiple serum samples from West African patients infected with Ebola virus strain Zaire, and patients infected with Ebola virus strain Sudan, as well as from non-human primates infected with Ebola virus strain Kikwit, and identified intrahost single nucleotide polymorphisms (iSNPs) at >0.5% frequency. Using phylogenetic tools, we aim to gauge the amount of viral diversity in the quasispecies and determine if diversity increases (diversifying selection) or decreases (purifying selection) over time. Moreover, emergence or disappearance of iSNPs can help us identify biological bottlenecks shaping the viral quasispecies. Taken together, these studies will shed light on the forces molding Ebola virus evolution in individual hosts during the course of infection, and will help identify biological processes to disrupt during design of vaccines and therapeutics.

**A85** Within-host diversity informs Ebola virus transmission analysis

S. Wohl,1,2,3 D. Park,1 G. Dudas,2 K.G. Andersen,1 C.B. Matranga,1 and P.C. Sabeti1,2

*Corresponding author
1Broad Institute of Harvard and MIT, Cambridge, MA, USA, 2Harvard University, Cambridge, MA, USA, 3University of Edinburgh, Edinburgh, UK and 4The Scripps Research Institute, La Jolla, CA, USA

During a disease outbreak, reconstructing transmission chains is an important tool for curtailting disease and evaluating control measures. Recent studies have shown that inferring outbreak transmission trees from both epidemiologic and genomic data produces better results than epidemiological tracing alone, especially during large outbreaks with sparse epidemiological data. Transmission tree investigation could be further improved by incorporating within-host variation, which has yet to be done, largely because extensive intrahost data have not typically been available during an outbreak. We have deep sequencing data available for over 200 Ebola virus genomes from 2013 to 2015 epidemic in Western Africa and have identified sites of intrahost variation in these samples. In this dataset, we observe several within-host variants that are shared among two or more patients, often spanning several months of the ebola epidemic. We conclude that these shared variants are due—at least in part—to the transmission of multiple viral genomes, which implies that the transmission bottleneck is wide enough to facilitate the transmission of intermediate-frequency variants. This finding can be used to better resolve transmission chains during an Ebola outbreak.

**A86** Diversity and evolution of human metapneumovirus (HMPV)

S. Shirivastava,1 H.A. Lorenz,1 R. Halpin,2 I.M. Mackay,1 M.D. Nissen,3 P. Sloots,3 S.C. Tollefson,3 G. Melendi,4 F. Polack,4 M.A. Staat,4 M. Bose,6 K. Henrickson,5 K.M. Edwards,3 and J.V. Williams5,7

1J. Craig Venter Institute, Rockville, MD, USA, 2Queensland Children’s Medical Research Institute (QCMRI), University of Queensland, Australia, 3Departments of Pediatrics and Pathology, Microbiology and Immunology, Milwaukee, WI, USA and 4Scripps Translational Science Institute, The Scripps Research Institute, La Jolla, CA, USA

Human metapneumovirus (HMPV), a paramyxovirus associated with acute respiratory infection is the leading cause of serious lower respiratory tract infection in young children worldwide and is associated with severe disease in immunocompromised hosts or persons with underlying conditions. HMPV is divided into four different subgroups: A1, A2, B1 and B2 and based on phylogenetics, the closest virus to HMPV is the Avian metapneumovirus type C. Partial sequencing of the glycoprotein and the fusion protein genes from several groups suggest that the lineages of HMPV are preserved over time. We have sequenced and analyzed fifty-nine complete genomes and their individual coding regions from several global locations to determine the diversity of HMPV at the genomic level, and viral evolution over time. Lineages B and B2 were found to circulate globally, and host gender or age had no bearing on the type of subgroup infecting the host. Analysis of selection pressure for individual coding regions suggested very few positively selected sites, although a higher dN/dS ratio for the B lineage in six of the nine coding regions were observed. Further analysis to identify clade specific amino acid changes did not establish any definitive differences. The presence of both variants A and B in our study raises questions about whether the variable residue changes in HMPV are due to purifying selection or because of the genetic diversity of HMPV.

**A87** Viruses associated with acute febrile illnesses in Trinidad and Tobago (T&T)

J.Y. Ngogang1

1Environmental Health, Kwara State University, Malete, Federal Republic of Nigeria

In the absence of integrated disease surveillance data and contact tracing in most African countries, understanding of EVD incidence and frequency of viral variants and new phenotypes. Lack of proofreading during RNA virus replication generates translational and innate immune evasion. We have deep sequenced RNAs produced by Zaire Ebola virus (EBOV) and the Angola strain of Marburg virus (ANG) to identify novel viral and cellular mechanisms that diversify the coding and non-coding sequences of filovirus RNAs (PMID: 25370975). Moreover, we have applied sequence-independent NGS methods to further define how the EBOV protein VP35 antagonizes the human innate immune response, specifically by detecting intrahost single nucleotide polymorphisms (iSNPs) at viral RNA from multiple serum samples from West African patients infected with Ebola virus strain Zaire, and patients infected with Ebola virus strain Sudan, as well as from non-human primates infected with Ebola virus strain Kikwit, and identified intrahost single nucleotide polymorphisms (iSNPs) at >0.5% frequency. Using phylogenetic tools, we aim to gauge the amount of viral diversity in the quasispecies and determine if diversity increases (diversifying selection) or decreases (purifying selection) over time. Moreover, emergence or disappearance of iSNPs can help us identify biological bottlenecks shaping the viral quasispecies. Taken together, these studies will shed light on the forces molding Ebola virus evolution in individual hosts during the course of infection, and will help identify biological processes to disrupt during design of vaccines and therapeutics.
Dengue viruses (DENVs) and now chikungunya virus (CHIKV) are important public health problems in Trinidad and Tobago (T&T). Both present as acute fevers, clinically indistinguishable from one another and from a range of other febrile illnesses. Accurate, up-to-date information on the nature, prevalence and distribution of viruses circulating in a given population is critical for efficient and effective targeting of public health interventions. In this regard, we have been screening individuals presenting with acute undifferentiated febrile illnesses (AUFIs), presenting at a major hospital in T&T, in order to determine the rate of DENV and CHIKV as well as identify other viruses associated with AUFIs. Of 158 individuals screened using DENV and CHIKV specific reverse transcription quantitative polymerase chain reactions (RT-qPCRs), CHIKV was detected in 19% (n = 30) and DENV in 5.1% (n = 8; six DENV-1, one DENV-3, one DENV-4) of cases. Using an illumina platform, eight CHIKV sequences were derived from the aforementioned samples. Phylogenetic analysis of their complete coding regions confirmed that they belonged to the Asian genotype and clustered together with the British Virgin Islands sequence (accession no. KJ451624) isolated in 2014 at the beginning of the Caribbean outbreak. Serum samples from thirty individuals who were RT-qPCR negative for DENV and CHIKV were also subjected to illumina sequencing resulting in the detection of CHIKV in three additional individuals. Viral sequence reads also included several herpesvirus related sequences, and Human Immunodeficiency Virus 1 in one individual in whom Teno Torque virus 3 was also detected.

Metagenomic profiles of the Amazon river

C.L. Dupont,1,2 G. Oliveira,3 J. Hoffman,1 S. Cuadros,2 R.A. Richter,1 S. Yooshef,1 R. Friedman,1 C. Lee,1 and T. Harkins2

*Corresponding author
1J. Craig Venter Institute, La Jolla, CA, USA, 2FIOCRUZ Minas CEBio, Belo Horizonte, Brazil and 3Life Technologies, Inc. Oyster Point, CA, USA

In February 2014, size fractionated metagenomic samples were collected at twenty locations of the Amazon River watershed. These include samples just upstream of Manaus all the way to the mouth at Belem. Samples were collected just upstream of confluences as well as after significant mixing between the main branch and the tributaries. Detailed analyses of the water chemistry have revealed dramatic differences in the samples, particularly in terms of carbon, iron and calcium content, which corresponds with the drainage basin and land usage. Sequencing is underway. The analyses of the sixty metagenomes (twenty samples, three technical fractions with regard to changes in water chemistry and land usage will be presented.

Deep sequencing of the small RNAs of sweet potato leaf phytobiome reveals potentially new virus disease causing complexes in Barbados

A.T. Alleyne* and C. Cummins

*Corresponding author
The University of the West Indies Cave Hill Campus, Bridgetown, Barbados

In 2000-3, a decline in yields by >50% was observed in some sweet potato fields in Barbados and symptoms of yellowing, swollen leaf veins and deep grooves on the tubers suggested the possibility of Sweet potato virus disease (SPVD). SPVD is a complex syndrome of several viral pathologies belonging to the two main genera: Potyvirus and Custerivirus and other minor viruses. This study sought to characterize the viruses in the SPVD complex affecting sweet potato varieties in Barbados by using PCR amplification of viral RNA and genomic siRNA sequencing of affected sweet potato leaves. Initial PCR of viral RNA using specific primers only detected the Potyvirus SPMV. Deep sequencing however revealed the presence of several DNA viruses. Geminiviridae (genus Begomovirus) and Caulimoviridae (genus Betaflexivirus) were accounted for >75% in the sweet potato leaf phytobiome in Barbados. The results possibly suggest new virus associations in the leaf phytobiome, largely composed of symptomless DNA viruses previously unreported in the Caribbean, in sweet potato.
which agrees with the fact that arthropod vectors targeting 
_Ferroplasma_ are currently unknown. Due to the lack of sequence 
homology, further in situ analysis may be required to target and 
identify the MGEs within the host's environment. This research 
represents the first documented analysis on the CRISPR-Cas 
operon structure and spacer elements in _F. acidarmanus_. Future 
research in this area has the potential to reveal the MGE-host 
dynamics in extreme environments.

### 6.7 Genetic variation and population dynamics of members of the _Fusarium incarnatum-equiseti_ species complex (FIESC)

R.K. Latchoo, H. Ramdial, F.N. Hosein, and S.N. Rampersad

*Corresponding author
Department of Life Sciences, Faculty of Science and Technology, 
The University of the West Indies, St. Augustine, Trinidad and 
Tobago, West Indies

Correct identification of Fusarium species in food products allow 
for more accurate prediction of mycotoxicogen risk. Members of the _Fusarium incarnatum-equiseti_ species complex (FIESC) are known to be trichothecene producers. Trichothecenes are potent inhibitors of 
protein synthesis in eukaryotic cells as they interrupt peptidyl 
transferase activity during the elongation phase of translation. These 
fungi cause different acute and severe diseases in humans and 
animals depending on the type of trichothecene ingested. The 
*Trinidad* strains belonged to at least eight different phylogenetic 
species of the FIESC. _F. equiseti_ strains belonged to three phylogenetic 
species. The partial sequences of the translation elongation factor 
gene (EF-1a) and of the internally transcribed spacers of the rDNA 
region (ITS-1 and ITS-2) were used in a multi-locus sequence 
comparison. Additionally, the genetic diversity of ninety-five strains 
of the FIESC belonging to different global populations was 
investigated. Sequence exploration indicated that the aligned DNA 
sequences of the EF-1a gene were more informative than the ITS 
sequences based on DNA polymorphism indicators. Phylogenetic 
relationships, gene flow and the potential for migration of the 
pathogen across continents are to be determined.

### 6.7.1 The role of mainland-island vampire bat movement and 
population dynamics in rabies virus (RABV) activity in Trinidad

J.F.R. Seethal, 1,2 O.M. Allicock, 1,2,3 V. Ramkissoon, 1,2 C. Oura, 2 and C.V.F. 
Carrington 1

*Corresponding author
1Department of Preclinical Sciences, Faculty of Medical Sciences, 
The University of the West Indies, St. Augustine, Trinidad and 
Tobago and 2School of Veterinary Medicine, Faculty of Medical 
Sciences, The University of the West Indies, St. Augustine, 
Tobago and Tobago

Our previous phylogenetic studies implicate the vampire bat species _Desmodus rotundus_ as the source of rabies virus (RABV) outbreaks in the Caribbean island of Trinidad and provide evidence for RABV importation from the nearby South American mainland on at least three occasions between 1972 and 2010, each with subsequent in 
situ lineage expansion. RABV activity in Trinidad is greatest in the 
southern peninsula closest to the mainland. The main method of 
transmission is chemical culling of vampire populations, which can 
indiscriminately affect co-roosting non-vampire species. The aim of 
this study is to investigate the role of _D. rotundus_ population 
dynamics and mainland-island movement in determining patterns of 
RABV activity. Between February 2012 and August 2013, 103 _D. rotundus_ were 
collected at various locations in Trinidad (primarily during routine 
eradication exercises by the Anti-Rabies Unit of the Ministry of 
Food Production). They were humanely euthanized and swabs 
(oral and rectal), blood and tissue samples were harvested using 
appropriate laboratory safety precautions. Samples were then 
frozen (–80 °C) until further use. _D. rotundus_ from selected areas in 
South America (Venezuela, Suriname, Guyana) will be captured and 
similary processed.

Genomic DNA will be extracted from tissue samples and 
comparative bat population genetic analysis will be performed using 
the vampire bat mitochondrial cytochrome b gene and selected 
microsatellite markers. Bat tissues will be screened for RABV using 
rabies-specific reverse transcriptase-polymerase chain reaction. 
RABV seroprevalence will also be determined. The BEAST software 
package will be used on data sets of derived and previously 
published viral sequences to infer evolutionary relationships, 
population dynamics and patterns of gene flow. _D. rotundus_ population 
sizes will also be estimated using partial roost counts and 
capture/recapture data. The relationship between bat 
population dynamics in Trinidad and movements estimated from 
the bat population genetic analyses, and patterns of RABV gene 
flow and outbreaks will then be investigated.

### 6.7.2 The frequency of the N348I mutation in patients 
falling combination antiretroviral treatment in Botswana

B. Seraise, 1,2,3 K. Andreu-Marabela, 1 S. Mayo, 2 R. Musonda, 7 
J. Makhma, 2 M. Essex, 3,4 and S. Gaseiwi4

*Corresponding author
1Department of Biological Sciences, University of Botswana, 
Gaborone, Botswana, 2Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana and 3Department of 
Immunology and Infectious Diseases, Harvard School of Public 
Health, Boston, USA

The N348I mutation in the connection subdomain of HIV-1 
Reverse Transcriptase (RT) has been reported to reduce 
susceptibility to Nevirapine (NVP), Efavirenz (EFV) and Zidovudine 
(AZT), and is frequently selected in HIV-1 patients experiencing 
virologic failure. We aimed to investigate the frequency of the 
N348I mutation in HIV-1 subtype C infected patients in Botswana 
falling different antiretroviral therapy regimens, as there is no 
data on the prevalence of this mutation in patients failing cART in 
Botswana. HIV-1 drug resistance genotyping was performed on samples from 
two previous clinical trials investigating the efficacy of different ART 
regimens, conducted at the Botswana Harvard Partnership. 
Plasma samples from forty-three patients from the two studies 
who experienced virologic failure were available for genotyping. 
Thirty-four of the forty-three (79.1%) virologic failure samples were 
successfully genotyped. Amongst the thirty-four, nine (26.5%) were 
found to harbor the N348I mutation. The N348I mutation emerged in 
10% of patients failing AZT containing cART and 33.2% of 
participants failing regimens containing NVP or EFV.

We found a frequency of 26.5% for N348I mutation among HIV-1C 
patients experiencing virologic failure in Botswana. The association of the N348I mutation with virologic failure in this 
population warrants further investigation but in this cohort it 
seemed to be more closely linked with non-nucleoside RT inhibitor 
failure than with AZT failure. The kinetics of the mutation in 
relation to other RT mutations also needs to be investigated to 
better understand its impact.

### 6.7.3 Integrated, enriched data and diverse bioinformatics 
-analysis tools for comparative genomics in the Influenza 
Research Database (IRD) and Virus Pathogen Resource (ViPR)

R.H. Scheuermann, 1,2 Y. Zhang, 1 D.S. Greer, 1 B. Evermann, 1 A. Lee, 1 
L. Stewart, 1 L. Zhou, 2,3 G. Sun, 3,4 C. N. Larson, 2 W. Jen, 2 and E.B. Klemp 1

*Corresponding author
1J. Craig Venter Institute, 4120 Capricorn Ln, La Jolla, CA 92037, 
USA, 2Northrop Grumman Health Solutions, Rockville, MD, USA 
and 3Vecna Technologies, Greenbelt MD, USA

The Influenza Research Database (IRD, www.fludb.org) and Virus 
Pathogen Resource (ViPR, www.viprbrc.org) are freely available, 
online resources supported by the US National Institute of Allergy 
and Infectious Diseases to search, analyze, visualize, save and 
share infectious disease research data for a broad range of human 
virus pathogens, including influenza virus and other viruses in the 
Arenaviridae, Bunyaviridae, Caliciviridae, Coronaviridae, Flaviviridae, 
Foliviridae, Hepeviridae, Herpesviridae, Paramyxoviridae, Picornaviridae, 
Poxviridae, Reoviridae, Rhabdoviridae and Togaviridae families. IRD 
and ViPR provide access to sequence resources, BLAST, multiple 
sequence alignment, phylogenetic tree construction leveraging 
supercomputing resources, metadata-driven comparative 
analysis, sequence variation determination, PCR primer design, 
Sequence Feature Variant Type analysis, 3D protein structure 
visualization, gene enrichment analysis, genome annotation, 
genotype reconstruction detection, sequence format conversion

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and genome viewing in GBrowse. A personal Workbench space is also provided to the user for saving and sharing sequences, searches and analysis results for future use. The data management and analysis tools have been designed to facilitate the research and development of diagnostics, prophylactics and therapeutics against these human virus pathogens.

**A75 Molecular epidemiology and evolutionary dynamics of echovirus 3 (E3) serotype**

Z. Kyriakopoulou,1 M. Bletsa,2,* D. Tsakogiannis,2 T.G. Dimitriou,2 G.D. Amoutzias,3 C. Gartzonika,3 S. Levidiotou-Stefanou,3 and P. Markoulatos2

*Corresponding author

1Department of Biochemistry and Biotechnology, University of Thessaly, 26, Ploutonos & Aeolou Str., Larissa 41 221, Greece, 2Department of Biochemistry and Biotechnology, University of Thessaly, 26, Ploutonos & Aeolou str., Larissa 41 221, Greece and 3Department of Microbiology, University of Ioannina, Medical School, Ioannina, Greece

The echovirus 3 (E3) serotype has been associated with several neurologic diseases, although it constitutes one of the most rarely isolated serotypes, with no report of epidemics in Europe. The aim of this study was to provide insights into the molecular epidemiology and evolution of this enterovirus serotype; an E3 strain was isolated from sewage in Greece, 4 years after the initial isolation of the only reported E3 strain in the same geographical region. Phylogenetic analysis of the complete VP1 genomic region of that E3 strain and of those available in GenBank suggested three main genogroups that were further subdivided into seven subgenogroups. Further evolutionary analysis suggested that the VP1 genomic region of E3 was dominated by purifying selection, as the vast majority of genetic diversity presumably occurred through synonymous nucleotide substitutions and the substitution rate for complete and partial VP1 sequences was calculated to be $8.13 \times 10^{-7}$ and $7.72 \times 10^{-8}$ substitutions/site/year, respectively. The partial VP1 sequence analysis revealed the composite epidemiology of this serotype, as the strains of the three genogroups presented different epidemiological characteristics.

**A76 Impact of TasP in Brazil: Setting a reference dataset for future comparison analysis**

M.P. Silva,1,2 L.B. Arruda,3,4 L. Paixão,5 M. Oliveira,1 T. Haguíhara,1 A. Oliveira,1 A.T.L. Queroiz,7 J. Casseb7 A.J. Duarte,1 I.C. Siqueira,6 F. Grassi,2 R. Camacho,1 A.M. Vandamme,3,6 R. Khouri,2,5

*Corresponding author

1CEDAP – Specialized Center of Diagnosis, Assistance and Research - Salvador-Bahia, Brazil, 2LIMI-LIP, CPqGM, Oswaldo Cruz Foundation (FIOCRUZ), Salvador-Bahia, Brazil, 3Institute of Tropical Medicine of São Paulo, University of São Paulo, São Paulo, SP, Brazil., 4LASP, CPqGM, Oswaldo Cruz Foundation (FIOCRUZ), Salvador-Bahia, Brazil, 5KU Leuven - University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, Leuven, Belgium and 6Global Health and Tropical Medicine, Unidade de Microbiologia, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal

Recently, the World Health Organization (WHO) HIV/AIDS guidelines proposed CD4 $>500$ cells/ul as the threshold to start HAART. Last year, the Brazilian government introduced Therapy as Prevention (TasP) in the HIV/AIDS guidelines and will treat all HIV infected patients at diagnosis, independent of the CD4 values. Notwithstanding the benefits the early treatment might bring to the control of HIV-1 transmission; TasP might also contribute to the increase of transmission of resistance mutations in Brazil and reduce the effects of the program in medium-term. We reviewed $>5000$ patients with a stored serum/plasma sample at HIV-1 diagnosis between 2003-2014, and included 2% of them in a study aiming at evaluating HIV-1 genetic diversity, transmission of resistance mutation, coreceptor use, and clinical outcome. For this purpose, we performed pol and V3 loop genotyping, collected epidemiological information and reviewed the clinical history. We plan to investigate subtype distribution, TDR prevalence and potential associated transmission chains, in silico fitness using protease fitness landscape predictions, predicted coreceptor use using Geno2Pheno, and associate these variables with epidemiological and clinical information. Thus, the data generated from this analysis will be used to establish a reference dataset to study the impact of TasP in Brazil prospectively.