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Rotaviruses of species A (RVA) are a common cause of diarrhea in children and the young of various other mammals worldwide. Interspecies transmission of RVA may lead to the emergency of novel RVA strains which may potentially affect rotavirus vaccine efficacy. The aim of this study was to investigate for possible interspecies transmission of RVAs in Uganda. Whole-genome sequencing of eighteen human (under-fives with diarrhea) and six animal (one bovine, one caprine, and four porcine) RVA strains identified in Uganda in the same geographical region, between 2012 and 2014 was undertaken using the Illumina HiSeq platform. RotaC version 2, a classification tool for RVAs was used to assign genotypes to all eleven genome segments of each isolate. Phylogenetic analysis was carried out using the maximum likelihood method in MEGA 6.06. Human RVA strains had either a Wa- or a DS-1-like genetic constellation. One human strain was a Wa-like mono-reassortant containing a DS-1-like VP2 gene of possible animal origin. In addition, three human RVA strains had one or two genes with possible zoonotic origin. All eleven genes of the bovine RVA strain were closely related to those of human RVAs. The caprine strain had a mixed genotype backbone, suggesting that it emerged from multiple re-assortment events involving different host species. Porcine RVA strains had mixed genotype backbones with possible multiple reassortant events with strains of human and bovine origin. Interspecies transmission of RVA strains occurred in this setting. RVA strains causing diarrhea in children are primarily transmitted from person to person. Rotavirus vaccination in children in Uganda will control rotavirus transmission. It is recommended to continue molecular surveillance of RVAs in humans and animals living in the same geographical region to understand the molecular epidemiology and evolution of RVAs in Uganda and other countries.

A48 Evolutionary history constrains adaptation in vesicular stomatitis virus

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It is unclear how evolutionary history affects the ability of a population to adapt to novel environmental conditions. To explore this question, we use vesicular stomatitis virus (VSV) populations either evolved at a constant temperature of 37 C, or with temperatures randomly changing between 29 C and 37 C. Fitness was subsequently measured at 29 C and 37 C and gains were detected in all constant treatment replicates but the random treatment showed no fitness changes. Consensus genome sequencing revealed that populations in the random treatment had accumulated more mutations than the populations in the constant treatment. In order to determine whether elevated genetic diversity in the randomly evolved populations could facilitate adaptation to a novel environment, we pooled all five replicates of the constant and the random treatments to generate two parental populations with distinct evolutionary histories. Five replicates of each group were then exposed to 40 C for forty generations. Populations derived from the random treatment evolved higher fitness than those derived from the constant treatment when grown at 40 C. The majority of the mutations observed evolved *de novo*, although some alleles that became fixed in the evolved populations were already present at low frequency in the ancestors. Two novel convergent mutations were found in the populations derived from the constant treatment ancestor, while there was no evidence of convergence in the populations derived from the random ancestor. These results suggest that a constant environment could constrain a population to a specific evolutionary pathway when confronted with a novel environment and prevent it from achieving maximum fitness.

A49 Phylogenetic evaluation of the Zika virus emergence in the Americas: 2015–2016

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The introduction of Zika virus (ZIKV) to the Americas caused an unprecedented epidemic with over half a million suspected cases in over forty-eight countries reported to the Pan-American Health Organization to date. Recent phylogenetic studies have proposed that the Asian genotype of ZIKV was introduced into the Americas causing the epidemic, and the most recent ancestor to the American strains originates from a French Polynesian strain circulating in the South Pacific. We evaluated the genetic diversity of ZIKV in the Americas at the population level during the epidemic period using 198 complete genome sequences (including 157 American strains and 41 Asian strains) obtained from GenBank. Our Bayesian maximum clade credibility phylogeny and molecular clock analyses on our dataset confirm that ZIKV was initially introduced into the Americas from the South Pacific but suggest emergence initiated in Haiti prior to Brazil. Analysis of the time of the most recent common ancestor (tMRCA) of the earliest American isolates, including Haiti and Brazil, estimates that this introduction occurred in 2013 (2.011, 4.467 years 95 per cent HPD). The estimated evolutionary rate of the American ZIKV strain compares with other flaviviruses transmitted in the region but on the slower end of the range with a rate of 4.64E-04 nucleotide substitutions per site per year. A preliminary sequence analysis within American isolates did not identify significant mutations or genomic patterns that differentiate viruses isolated from mosquitoes or from humans, or from viruses isolated from different human specimen types including serum, urine, semen, and saliva. Further analyses on sequences and more recent virus isolates will be conducted to provide a better understanding on the evolution and transmission dynamics during early, epidemic, and post-epidemic periods.

A50 Genotypic distribution of HHV-8 in aids individuals without and with Kaposi sarcoma

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AIDS-associated Kaposi's sarcoma (AIDS-KS) caused by human herpes virus 8 (HHV-8) is the most severe and resistant form of KS tumor. Our aim was to verify whether there is an association between HHV-8 variability and development of AIDS-KS in Brazil by comparing the HHV-8 variability between individuals without and with KS. Saliva samples and blood, when available,

were analyzed by polymerase chain reaction techniques for detection of the fragments of ORF K1 of HHV-8, which were then genotyped and analyzed regarding the genetic variability. Our study described 106 positive cases for HHV-8 in the saliva from 751 AIDS patients without previous KS. In addition, we performed a phylogenetic analysis of HHV-8 in 34 of the 106 AIDS patients without KS and in 33 of the 37 patients with active KS. The distribution of HHV-8 genotypes A, B, C, and F in AIDS individuals was indistinguishable by comparing non-KS and KS groups, as well as regarding ethnicity. Considering the KS group, genotype B was associated with better prognosis of KS tumor. Interestingly, we found a particular profile of diversity within clade C and two recombinant patterns of HHV-8 in the saliva of AIDS individuals without KS. We emphasize the need to achieve standard genotyping protocol for ORF K1 amplification, thus allowing for substantial detection of HHV-8 variants. Our findings can shed light on the role of HHV-8 variability in the pathogenesis of AIDS-KS. Our perspective is study polymorphisms and phylogenetic inferences in HHV-8 sequences encoding microRNA.

A51 Rubella genotype 1H is still circulating in Turkey

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Rubella virus, the sole member of the *Rubivirus* genus in the Togaviridae family, is a positive-strand RNA virus. Based on phylogenetic analysis of sequences of the structural coding protein, two virus clades including a total of thirteen genotypes have been identified. Infection with rubella virus generally leads to mild disease with symptoms that include rash and low fever. In pregnancy, however, rubella infection can cause miscarriages and serial birth defects including hearing, vision, mental and heart impairment, which are collectively known as congenital rubella syndrome (CRS). CRS occurs in up to 85 per cent of children born to women with rubella infection during the first trimester of pregnancy. In addition, CRS can lead to neonatal deaths in up to 30 per cent of cases. Laboratory investigation plays an important role in both diagnosis and surveillance of rubella and CRS, since clinical diagnosis is unreliable and up to 50 per cent of infections are estimated to be subclinical. Because phylogenetic analysis of rubella virus genotypes can help determine whether circulating strains result from endemic transmissions or importations, laboratory surveillance for rubella also includes the molecular characterisation of viruses. Rubella genotype 1H was detected in a seven-year-old patient's urine specimen in 2016 (GenBank accession number KY048160). There are only three previous genotype 1H sequences from Turkey which were collected in 2001. No sequences are available from countries bordering Turkey (except for one 2B from Iran). Other 1H sequences are mostly from Russia and Belarus and none have been detected since 2008. The sequences of the recent isolate and three previous isolates cluster as a separate branch of genotype 1H. It seems likely that this lineage of 1H has been circulating in the country (and perhaps bordering countries) during the last fifteen years.

A52 Ebola virus phylogenetic analysis during the 2014–2016 West African outbreak

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Following the conclusion of active Ebolavirus disease (EVD) transmission within West Africa, sporadic EVD-cases continued to re-emerge outside of the expected viral incubation period. Epidemiological evidence suggested that these cases represented sexual transmission from persistently infected, asymptomatic EVD survivors. To address these questions, we directly sequenced EBOV from clinical specimens collected during acute and persistent infection from individuals associated with these re-emerged EVD cases. This sequence analysis was used in conjunction with on-the-ground epidemiological tracing to identify transmission chains and potential routes of infection. Due to a lack of knowledge regarding the effect of persistence on Ebola viral sequences, we were unable to support or refute whether these re-emerged cases represented evidence of transmission from EVD survivors, despite extensive phylogenetic analysis. To address this knowledge gap, we also sequenced Ebola virus directly from the semen of EVD survivors ('SAVS'—semen-acquired viral sequences) and identified molecular characteristics associated with viral persistence. Through extensive use of phylogenetic software and models, we identified that a subset of SAVS exhibited evidence of a slowed or acute-like substitution rates, de novo U-to-T hyper-editing and a moderate change in evolutionary pressure within the viral glycoprotein. Altogether, our data illustrate that phylogenetic analysis and evolutionary hypothesis testing can yield important insights into disease transmission networks and the mechanisms of viral replication.

A53 Systematic application of metagenomics NGS to identify and sequence viral pathogens in infections of the central nervous system

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Unbiased metagenomics sequencing allows the detection of any potential pathogen in a sample using a single methodology. This technique has been used to successfully identify pathogens in case reports of patients with central nervous system (CNS) infection. Metagenomics sequencing also provides genomic information that can be used to classify pathogens and perform studies of molecular epidemiology, especially for viruses, which have small genomes amenable to full-genome sequencing. Here, we apply metagenomics sequencing to detect and sequence viruses in a prospective cohort of patients with CNS infection. We enroll patients with both known (control) and unknown or suspected CNS infection and obtain samples of cerebrospinal fluid. We perform unbiased library construction from both RNA and DNA, followed by deep sequencing and metagenomics analysis. In patients with known infections, we have successfully sequenced Herpes Simplex Virus (HSV)-1 in two cases and HSV-2 in one case, obtaining partial genomes that allowed species classification. We have also successfully sequenced enterovirus in two cases, obtaining full-length viral genomes that allowed strain classification and phylogenetic analysis. In one patient with unknown infection, we identified Powassan virus, an emerging tick-borne flavivirus that causes encephalitis in the Northeastern United States. In that case, our NGS results were obtained three weeks earlier than routine clinical testing by serology, highlighting the potential application of this method for rapid diagnosis of infection. As work in progress, we are currently sequencing the full viral genome, which will be the first Powassan virus genome sequenced directly from a clinical sample. This will allow phylogenetic comparison