to identify the sources of infection, and the virus evolution as well as host/virus interaction. In our study, using 454/Illumina sequencing, we have obtained large amount of whole genome sequences. We designed a preliminary bioinformatics analysis pipeline to classify these NGS reads. First we mapped our nucleotide reads to GenBank reference sequences using BLAST, and classified them by their taxonomic family, such as host, virus and unclassified. Then, for a specific type of virus (e.g. influenza virus, MERS coronavirus), we conducted de novo and reference based assembly of the reads to obtain the full genome sequences for further phylogenetic study. In the future, through advanced bioinformatics tools, we hope to get more detailed information from our large amount of NGS sequences of field/clinical samples, experimental data, especially in the following areas: (i) finding novel pathogens in unclassified sequences; (ii) virus/virus interactions; (iii) pathogen/host interaction.

A25 Phylogenetic analysis of the nucleocapsid and RNA-dependent RNA polymerase fragments of the first imported case of middle east respiratory syndrome coronavirus (MERS-CoV) infection in the Philippines from Saudi Arabia, February 2015

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We report the first laboratory-confirmed case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection from a patient returning to the Philippines from the Kingdom of Saudi Arabia (KSA). MERS-CoV was first identified in 2012 circulating in Middle Eastern countries with outbreaks occurring in KSA, the United Arab Emirates (UAE), and South Korea, plus sporadic imported cases in at least 20 other countries. The Philippines is at risk for MERS-CoV transmission from frequent travelers, such as overseas Filipino workers and Hajj pilgrims, coming from Middle Eastern countries. Throat swabs, sputum samples, and a rectal swab were collected from the index case within 13 to 22 days after the onset of symptoms. MERS-CoV testing was performed using a real-time reverse transcription polymerase chain reaction (RT-qPCR) screening assay targeting regions upstream of the envelope gene (upE) and the nucleocapsid gene (N2), a confirmatory RT-qPCR assay targeting regions within the open reading frame 1a gene (ORF1a) and another region of the N gene (N3), and Sanger sequencing of regions of the N and RNA-dependent polymerase (RdRp) genes. The index case tested weakly positive for MERS-CoV in a sputum sample until day 19 of illness. Sequences of the N and RdRp gene regions reveal 100 and 99% similarity with MERS-CoV sequences obtained in KSA and UAE, respectively, confirming that the infection originated from Middle Eastern strains. Two unique synonymous/silent mutations (T15259A and T15265C) were identified in the RdRp sequence fragments. Whole genome sequencing of the strain may identify other mutations across the genome and determine the most probable origin of the strain.

A26 Transmission patterns and evolution of RSV in a community outbreak identified by genomic analysis

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Detailed information on the source, spread and evolution of respiratory syncytial virus (RSV) during seasonal community outbreaks remains sparse. Molecular analyses of attachment (G) gene sequences from hospitalised cases suggest that multiple genotypes and variants co-circulate during epidemics and that RSV persistence over successive seasons is characterized by replacement and multiple new introductions of variants. No studies have defined the patterns of introduction, spread and evolution of RSV at the local community and household level. We present a whole genome sequence analysis of 131 RSV group A viruses collected during six-month household-based RSV infection surveillance in Coastal Kenya, 2010 within an area of 12 km^2. RSV infections were identified by regularly screening of all household members twice weekly. Phylogenetic analysis revealed that the RSV A viruses in 9 households were closely related to genotype GA2 and fell within a single branch on the global phylogeny. Genomic analysis allowed the detection of household-specific variation in seven households. For comparison, using only G gene analysis, household-specific variation was found only in 1 of the 9 households. Nucleotide changes were observed intra-host (viruses identified from same individual in follow-up sampling) and inter-host (viruses identified from different household members) and these coupled with sampling dates enabled partial reconstruction of the within household transmission chains. The genomic evolutionary rate for the household dataset was estimated as 2.307×10^{-3} (95% highest posterior density: $0.93513–4.1636 \times 10^{-3}$) substitutions/site/year. We conclude that (i) at the household level, most RSV infections arise from the introduction of a single virus variant followed by accumulation of household specific variants and (ii) analysis of complete virus genomes is crucial to better understand viral transmission in the community. A key question arising is whether prevention of RSV introduction or spread within the household by vaccinating key household members in these functions would lead to a reduced onward community wide transmission.

A27 Using whole genome sequence data and minority variant profiles to elucidate transmission patterns during RSV household outbreaks

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Reconstructing transmission chains for outbreaks is important in understanding how viruses spread. Furthermore, defining the main underlying determinants of transmission chains is important for developing effective interventions. Whole

genome consensus sequence data provides information that represents the dominant virus subtype. It does not provide sufficient information to resolve transmission events particularly for rapidly spreading viruses. However, changes in the composition of minor variants between hosts and the pattern of minor variants fixation during outbreaks, could provide additional high-resolution data on who is infecting whom. The same data could also potentially inform the extent of within-host virus diversity as well as the proportion of diversity that is transmitted between individuals. We have developed a reproducible semi-automated whole genome variant calling pipeline to explore the role of minority variants in resolving transmission patterns and within host viral evolution. The pipeline is available as modular Bash scripts that run on a Linux cluster environment.

A28 Frequent co-infection among human group a rotaviruses in Thailand

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Rotavirus (RoV) is a non-enveloped dsRNA virus in the Reoviridae family, with a 18.5-kb genome of 11 segments encoding six structural (VP1–4, VP6 and VP7) and five or six non-structural proteins (NSP1-NSP5/6). Reassortment between human and/or animal RoVs plays an important role in the generation of genetic diversity in these viruses, and is presumed to result from co-infection in human or animal reservoirs. However, co-infection with heterologous RoV has rarely been documented, in part due to inadequate detection methods and a lack of large-scale genomic investigations. Despite the availability of an efficacious vaccine, the burden of rotaviral diarrhea remains high in many developing countries, with rotavirus infection detected in 40–50% of all pediatric patients hospitalized with diarrhea. In addition to its cost, reduced vaccine effectiveness in developing country settings has contributed to its low uptake and the lack of government support for vaccination programs across Southeast Asia. The genetics and dynamics of rotavirus (RoV) have rarely been systematically investigated in these settings. The government of Thailand is preparing to add the rotavirus vaccine to its immunization program but has expressed concern regarding its effectiveness in the population and its long-term impact on rotavirus diversity and disease burden. To investigate the diversity of Group A RoV prior to the initiation of immunization programs, we performed full genome sequencing of 200 RoV from infected children across the country from 2004 to 2010 using novel in-house rotavirus capture and sequencing methods. Whilst the majority of samples (76%) showed infection with RoV of the common G1-P[8]-I1-C1-M1-A1-N1-T1-E1-H1 (Wa-like) genotype constellation (representing VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, respectively), 41% of all samples additionally showed heterologous rotavirus co-infection, containing one or more segments representing an additional, less common genotype. Co-infection of G1-P[8] Wa-like viruses together with segments representing G9-P[19]-I5 lineages was particularly common and phylogenetic analysis suggests that these lineages may have spread through one region of Thailand for three years via serial co-transmission. G1-P[8] Wa-like infection with additional G3 or G9 VP7 segments was also common across the country. Despite the high frequency of RoV co-infection in this setting, reassortant viruses were rarely observed (in ~8% of cases), and none of these appeared to represent reassortment among the common co-infecting viral segments detected within this study. The impacts of co-infection and reassortment on transmission fitness, the epidemiology of rotaviral diarrhea, and vaccine efficacy and long-term viral diversity warrant further investigation via full genome sequencing following the large-scale introduction of immunization programs in Southeast Asia.

A29 Wolbachia for dengue control; will dengue viruses evolve resistance?

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Dengue viruses (DENV) are major human pathogens, transmitted between people mainly by *Aedes aegypti* mosquitoes. Recently, the endosymbiont bacteria Wolbachia was horizontally introduced into Ae. Aegypti; Wolbachia infection non-specifically impairs DENV replication in these mosquitoes opening up a potential new strategy for dengue control. This project will test the hypothesis that DENV cannot easily develop resistance to the mechanisms by which Wolbachia blocks virus replication. Using contemporary viruses currently circulating in Vietnam, we will perform 30 cycles of repeated passage of DENV in wild-type and Wolbachia-infected Ae. Aegypti (wMel strain). This experimental system maximizes the possibility of observing selective pressure by wMel on DENV populations in mosquito tissues. We will use intermittent quantitative virology and virus genome sequencing to detect changes over time in the genotype and phenotype of DENV in Ae. aegypti. The results will provide valuable insights into how easily DENV adapt to Wolbachia in a controlled system. If Wolbachia specific evolution is observed, then we will test the infectiousness and replication profile of such viruses in cultured human cells. The results will deliver the first evidence of the likelihood of DENV "escape" from wMel, with implications for future and ongoing field releases of wMel in dengue-endemic countries.

A30 Recombination & evolution in two viral families: effective steps or a random walk?

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Recombination or reassortment occurs in many different viral families and is understood as one of the predominant mechanisms of evolution. We perform a broad analysis of the mechanisms and recombinants of two viral families, Picornaviridae and Hepadnaviridae, which exhibit very different viral behaviour. Combining information about recombinants with knowledge of the evolutionary selection pressures on the genome and insights from the conservation of functional aspects of the genome, we find that viruses can dynamically exploit recombination in different ways, either to evade antigens and prolong infection, or to enhance viral diversity and allow the virus to infect new hosts. Better understanding of the mechanisms of