A33 Using serological and surveillance data to infer the introduction date and unobserved transmission dynamics of Zika virus in Fiji 2013–7

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Zika virus (ZIKV) has been circulating in the South Pacific since 2007, and transmission in Fiji was first confirmed in 2015. To better understand the history and transmission dynamics of ZIKV in Fiji, we combined a transmission dynamic model with serological and surveillance data from Central Division, Fiji. A longitudinal population representative of seroepidemiological data were available from participants sampled in 2013, 2015, and 2017. In addition, ZIKV case reports were available from 2015 and 2016. Using a Bayesian approach, we fitted a transmission dynamic model with a seasonally varying transmission to these data. We also estimated the virus introduction date, given the effect this has on transmission dynamics as it interacts with the observed seasonal pattern of transmission. We found evidence that the virus was introduced in October 2013 (95% credible interval: April 2013–April 2014) and that the strong seasonal transmission pattern meant the virus persisted for several years with multiple waves of infection in consecutive years. It is important to corroborate this evidence against other work done in the same area. A phylogenetic analysis was performed on 5 ZIKV strains obtained from Fiji in 2015 and 2016, which were aligned with 33 E gene sequences from the Pacific, Americas, and Africa. This analysis showed evidence of virus persistence over multiple years in Central Division, Fiji. The estimated most recent common ancestor of the group isolated from Central Division was November 2013 (95% credible interval: March 2013–July 2015). Our modeling estimate is consistent with these results despite the very different methods being used. The availability of detailed case and serology data in an island outbreak setting, combined with mathematical models, presented a unique opportunity to gain crucial insights into these infections. Our analysis provides evidence that seasonal variation in transmission, combined with other co-circulating flaviviruses, means the timing of ZIKV introduction can have a major impact on outbreak transmission dynamics.

A34 Molecular characterization of Zika virus in Cuba

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Until now, three genotypes of Zika virus (ZIKV) have been detected (two African lineages and one Asian lineage). After the declaration of Public Health Emergency of International Concern issued by The Pan American Health Organization and the World Health Organization authors from some Latin American countries have identified the Asian genotype as the lineage responsible for the Zika epidemic in the western hemisphere. However, data from the Caribbean are sparse, and there is no published data regarding the genotypes that produced isolated outbreaks in Cuba. Aiming to realize the molecular characterization of ZIKV in Cuba, we will sequence by next-generation sequencing the full genome of the ZIKV identified in samples from Cuban patients of different provinces in which ZIKV produced outbreaks. All samples required for this study have been collected during the molecular surveillance of Arboviral diseases conducted at the National Reference Laboratory at Pedro Kourí Tropical Medicine Institute. Viral RNA will be purified from urine and serum samples collected from patients with confirmed ZIKV infection by real time PCR. Using evolutionary dynamics studies, we will map the spread of a virus or of particular variants in time and space in order to understand how frequently ZIKV has been introduced into Cuba. Moreover, we will evaluate the amino acid diversity of each ZIKV proteins. Further, we will evaluate the population dynamics of ZIKV in samples from patients with varying clinical outcomes. The results will allow us to characterize the ZIKV genome and its evolution into the Cuban population that would also have impact for vaccine development, diagnosis, and pathogenesis studies.

A35 The first laboratory confirmation of chikungunya outbreak in Ethiopia

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Chikungunya is a viral disease (genus Alphavirus) which is transmitted to humans by infected mosquitoes—including *Aedes aegypti* and *A. albopictus*. An outbreak of febrile illness, suspected to have been caused by chikungunya, was reported in June 2016 from Dolloado district, Suuf Kebele, in the Somalia regional state of Ethiopia that borders the Mandera county of Kenya where a confirmed chikungunya outbreak was ongoing. Laboratory investigation was carried out to confirm if the outbreak in Ethiopia was caused by Chikungunya virus. Ten serum samples were collected from suspected patients visiting a health center in Suuf Kebel, who were then sent to the Nation laboratory in Ethiopian Public Health Institute. RNA was extracted from the serum samples using QIAgene RNA Mini kit, and PCR detection of dengue, chikungunya, and Zika virus nucleic acid was done using Trioplex Real-time RT-PCR Assay following the protocol from the Center for Disease Control (CDC). The Trioplex Real-time RT-PCR assay, for detection and differentiation of RNA from dengue, Chikungunya and Zika, was provided by CDC as part of the zika emergency preparedness effort. Of the nine samples tested, eight (88.88%) were found to be positive for chikungunya virus nucleic acid but negative for dengue and Zika virus nucleic acids. The median age of the affected sampled patients was 40 years, and males appear to be more affected (66.6% of sampled patients). The laboratory investigation confirmed that the outbreak was caused by chikungunya virus. Even though further molecular characterization of the positive isolates will provide more information as to the circulating genotypes and elucidate the origin of the outbreak virus, it is also possible to assume that the outbreak was an extension of the outbreak in neighboring countries in Kenya and, therefore, warrants that cross-border integration efforts to control chikungunya should be implemented by the concerned countries.

A36 Analysis of CHIKV evolution during the Caribbean outbreak, 2013–5, using complete genome sequences

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Chikungunya virus (CHIKV) is a re-emerging, mosquito-borne alphavirus that causes chikungunya fever, a febrile illness characterized by severe acute and persistent arthralgia. At the end of 2013, autochthonous CHIKV transmission was detected for the first time in the Americas, on the Caribbean island of Saint Martin. Subsequently, CHIKV rapidly spread through the Caribbean Islands and onto the American mainland, causing millions of cases of chikungunya fever. During the outbreak, the Dutch National Institute of Health performed diagnostics on patient samples originating from the six Caribbean islands that belong to the Kingdom of the Netherlands. Using a subset of PCR-positive patient samples, we aimed to retrospectively analyze the 2013–5 CHIKV outbreak on the Dutch Caribbean islands using whole-genome sequences. Twenty-five CHIKV-positive sera were selected for next-generation sequencing based on viral load, location, and date of sampling. Sera were subjected to high speed centrifugation, filtration, and nuclease treatment to reduce the amount of background sequences from human and bacterial origin. Total RNA was extracted, primed with random nanomers for reverse transcription, after which dsDNA was produced and purified. Libraries were created using Nextera XT library preparation kit, and samples were run on a MiSeq desktop sequencer. Reads were trimmed and mapped to a reference sequence using the CLC Genomics workbench. To date, eight

full-genome sequences were obtained, originating from four different islands and dating from the start of the outbreak (December 2013) to April 2015, when the outbreak was waning. High similarity (>99%) between sequences was found; nevertheless, all genome sequences were unique with a minimum of three SNPs differentiating one sequence from another. Thirty-three unique single nucleotide polymorphisms (SNPs) were identified, of which 29 were located in the coding regions of the genome. Eight SNPs were informative, and ten SNPs led to amino acid changes. Of the amino acid changes, nine were located in the non-structural proteins (1× nsP1, 5× nsP2, and 3× nsP3), and one was located in E2. In conclusion, we report the first whole-genome sequences of CHIKV isolates from the 2013 to 2015 outbreak that originated from the Dutch Caribbean islands. Sequencing of the remaining samples is still in progress.

A37 Transmission success of dengue virus type 1 lineages in a dynamic virus population: An evolutionary view

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Arbovirus transmission involves an interplay between host, virus, and environmental factors. Because of the complexity of interactions, the transmission success of arboviruses could either be a function of viral fitness or be stochastic. In the present study, using 1,963 envelope (E) gene sequences and 239 whole genomes, we conducted a large-scale molecular epidemiological analysis of a dengue virus type 1 (DENV-1) population to understand the transmission success, evolution, and dispersal patterns of different lineages of DENV-1 circulating in Singapore from 2011 to 2016. The study population was highly dynamic and heterogeneous. However, only a handful of genetically distinct strains ($n = 6$) established sustained transmission, but at variable levels of dominance. Phylogeographic analysis revealed a weak spatial clustering and 35 well-supported diffusion pathways, implying widespread and complex dispersal of these strains in local settings. Yet, the dominant strains were neither evolving faster than less dominant ones nor under positive selection. These observations suggested that lineage dominance was likely to be stochastic and opportunistically driven by non-viral factors such as host immune pressure and vector abundance. Our findings, therefore, emphasize the implications of understanding the vector and human factors in parallel to virus dynamics on continuing efforts to control the arbovirus disease transmission in endemic regions.

A38 Genomic epidemiology quantifies gaps in Aedes-borne virus transmission in the Americas

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The rapid spread and severity of pathogens, such as Zika (ZIKV) and Chikungunya (CHIKV) viruses in the Americas, demonstrate the need for a better understanding of when and where outbreaks emerge. Sequence evolution of these viral pathogens occurs simultaneously with geographic spread, which allows phylodynamic processes to be recovered from genomic data. Here, we used time-calibrated phylogeographic analyses implemented in a Bayesian phylogenetic framework to characterize the date of introduction of ZIKV, CHIKV, dengue, and yellow fever viruses in different geographic regions of the Americas. To estimate 'surveillance gaps', we compared the estimated dates of introduction of these pathogens to the first confirmations of virus circulation in the region. Datasets included all publicly available geo-referenced and time-stamped genetic data from the Americas. A series of environmental and ecological covariates will be tested to infer what factors are associated with the delayed detection of arbovirus transmission in each geographic region. These results will provide important information on where to concentrate surveillance strengthening measures in order to prevent future mosquito-borne virus epidemics.

A39 Reconstruction of Ebola chains of transmission using sequence and epidemiological data

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Transmission trees can be established through detailed contact histories, statistical inference, phylogenetic inference, or a combination of methods. Each method has its limitations, and the extent to which they succeed in revealing a 'true' transmission history remains unclear. Moreover, the net value of pathogen sequencing in transmission tree reconstruction is yet to be assessed. We explored the accuracy and sensitivity to biases of a range of methods for transmission chain inference. We studied eight transmission chains determined by contact tracing, each one having more than a third of its cases sequenced (87 samples over 199 cases in total). We compared three inference methods on the selected transmission chains: (i) phylogenetic inference: the Ebola virus (EBOV) sequences derived from patients were mapped onto a dated EBOV phylogeny tree including 398 EBOV sequences sampled in Guinea between March 2014 and October 2015; (ii) statistical inference: we used the maximum likelihood framework developed by Wallinga and Teunis to infer the most likely transmitter-recipient relationships from the onset dates; (iii) combined method: we inferred probabilistic transmission events using both pathogen sequences and collection dates with the R package Outbreaker2. The cases coming from each transmission chain were mostly clustered together in the phylogenetic tree. The few misclassified cases were most likely allocated to the wrong chains of transmission because of the timing of their symptom onsets. Probabilistic transmission tree using only onset dates broadly matched the contact tracing data, but multiple potential infectors were identified for each case. The combined method showed that an *a priori* knowledge of the number of independent imports had an important impact on the outcome. Although cases were allocated to the correct transmission chains, discrepancies were found in identifying direct case linkage and transmission generations within a chain. Phylogenetic, epidemiological, and combined approaches for transmission chain reconstructions globally concurred in their output. Sequence data proved useful (if not necessary) to place the sampled cases in a wider context, identify transmission clusters, and misclassified cases when epidemiological chains are inferred from date of symptom onset only, and to identify links between supposedly independent chains of transmission.

A40 Estimation of Lassa virus emergence in Upper Guinea through a time-calibrated phylogeny

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Lassa fever is a hemorrhagic fever caused by an arenavirus, the Lassa virus (LASV), and can affect 150–200,000 persons per year in West Africa. The virus is hosted by several rodents, *Mastomys natalensis* and *M. erythroleucus*, *Hylomyscus pamfi*, and *Mus baoulei*. People can be contaminated at home or in the farms, by touching contaminated surfaces, eating contaminated food, or breathing aerosolized viral particles. Human-to-human transmission is occurring as well through infected bodily fluids. In Upper Guinea in particular, *M. natalensis* is the main host, with LASV prevalence of 14 per cent and IgG prevalence of 27 per cent. In humans, IgG prevalence is 40 per cent. This is, therefore, a hot spot for LASV transmission. In a previous phylogenetic study including 132 partial nucleoprotein (NP) sequences isolated from rodents, we showed that LASV could have emerged 90 years ago in the area. Here, we aim to revise the time of emergence upon analyzing the complete NP and polymerase genes of two strains coming from Upper Guinea: 'Bantou 366', a strain isolated from *M. natalensis* in