high-throughput sequencing to observe how SAMHD1 expression alters the mutational profile (frequency and spectra) of integrated proviruses. We will explore how mutation rates of HIV-2 can be manipulated through the use of nucleoside analogs and RNRI drugs to explore what effects these compounds have on the HIV-2 mutation profile. Using single-cycle infectivity assays as well as long-term spreading experiments, we will be able to correlate mutagenesis with viral evolution and infectivity data to explore how sensitive these two viruses are to changes in viral mutation. This work will serve to understand how HIV-2 operates at a lower mutation frequency than HIV-1, elucidate the relationship between mutagenesis and infectivity for the two viruses, and provide insights into the contrasting phenotypes observed between the viruses.

A28 Phylogeographic analysis of HIV-2 ANRS CO5 cohort reveals new trends in HIV-2 epidemic patterns in West Africa

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The early spread of HIV-2 in Western Africa is imperfectly described for group B and the recently identified subtype A2. Recent HIV-2 epidemiological data are also scarce outside of Guinea-Bissau. The sequence database of the HIV-2 ANRS CO5-cohort, one of the largest to date, was used to explore the early migration patterns of these strains by phylodynamic's means. All publicly available (forty-nine and eight for A and B, respectively) and ANRS CO5-cohort (125 and 68 for A and B, respectively) pol sequences with available time of sampling and patient's country of birth were included. Bayesian phylogeographic reconstructions and effective population size estimations were performed under the best fitting combination of evolutionary, demographic, and molecular clock models using BEAST 1.8. The tree topology was assessed with maximum likelihood trees using RAxML 8.0.0. The estimated introduction of group A in humans was 1945 [95 per cent HPD: 1935–1953], as previously reported. Subtype A1, present in Senegal, Gambia, Guinea-Bissau, and Guinea, experienced an early diversification around 1946 [1936–1954] with two distinct early epidemics in Guinea-Bissau and Senegal. Subtype A2, present in Ivory Coast and Mali, experienced a latter diversification (1956 [1947–1963]) in Ivory Coast with two introduction events in Mali (1963 [1957–1969] and 1967 [1960–1974]). Group B was originally introduced in Ivory Coast in 1962 [1953–1913]. Changes in effective population size over time revealed initial exponential growth phases occurring sequentially for the three HIV-2 strains and followed by a population decline starting in the 2000s for all HIV-2 strains. The rate of this decline was slower for A2 and B subtypes (Ivory Coast, Mali) than for A1 (Guinea-Bissau, Senegal). This phylogeographic study is the first to reconstruct the early dispersal of A2 and B HIV-2 clades in Western Africa. Our results suggest that subtype A1 was circulating in Guinea-Bissau and Senegal before the independence war of the former, believed to have contributed to the dispersal of HIV-2. Both A2 and B clades emerged in Ivory Coast and experienced latter diversification and population expansion (starting in 1980 and 1990, respectively) than A1. There is indication of slow decreasing incidence rates of HIV-2 in Ivory Coast or Mali where recent data are scarce.

A29 Development of a full-genome sequencing platform to study norovirus diversity

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Norovirus is a major cause of acute gastroenteritis worldwide. Noroviruses are very infectious and highly diverse, with two different genogroups (GI and GII) and almost thirty different genotypes infecting humans. Over the last two decades a single genotype (GII.4) has been shown to be the predominant cause of viral gastroenteritis outbreaks worldwide, therefore, most of the research was focused on this genotype. However, the epidemiological picture has changed during the last three seasons, where two different genotypes GII.17 (2013–2015) and GII.2 (2015–2016) have emerged as the major causes of gastroenteritis in different countries. Thus, a better understanding of the evolution of all different norovirus genotypes is needed for vaccine development. Much of current research on norovirus evolution has been focused on the major capsid protein (VP1), the major target for vaccine development. The VP1 is encoded by the open reading frame (ORF) 2, which constitutes only about one-fourth of the whole genome. However, much is unknown about the evolution, functional, and immunological roles of ORF1, a 5,000-nucleotide segment of the genome that encodes six nonstructural proteins. Because only 0.3 per cent of the norovirus sequences deposited in public databases correspond to full-length genomes, we developed an RT-PCR assay that amplifies the full-length genome of different norovirus genotypes; the resulting amplicons are sequenced using next-generation sequencing platforms. Using this platform, we successfully sequenced and assembled over fifty norovirus genomes from eleven different genotypes. The full-length sequences of two genotypes, GII.9 and GI.7, were obtained for the first time. Using neighbor-joining phylogenetic trees, we determined that the GII.9 presented an ORF1 region very similar to the one associated to GII.6, GII.7, and GII.14 noroviruses. On the contrary, sequences of GII.17 strains circulating pre-2013 clustered in different branches, for both ORF1 and ORF2 regions, than the ones circulating during 2013–2016. In addition, ORF1 sequences from different GII genotypes showed the clustering into at least two different groups. Together, this suggests independent evolution of the two different regions of the genome. We expect this method will encourage full-genome sequencing in the norovirus field, and create an improved database to expand our genomic analyses.

A30 Norovirus epidemiology and diversity in South Africa, 2009–2016

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Norovirus is a major cause of viral gastroenteritis in all age groups. The virus is classified in the Caliciviridae family of small, icosahedral viruses with a ~7.6-kb linear positive-sense RNA genome. The genome encodes three open reading frames

(ORFs) which code for six non-structural proteins (ORF1) and the major (ORF2) and minor (ORF3) capsid proteins. Noroviruses are very diverse and among the three genogroups (GI, GII, and GIV) that infect humans, >30 genotypes have been described. One genotype (GII.4) causes >80 per cent of norovirus outbreaks worldwide, a new variant of this strain emerges every two to three years, rapidly replaces the circulating variant and then becomes dominant globally. A high mutation rate as well as recombination contributes to high diversity of noroviruses. From April 2009–May 2016, large-scale surveillance, based within the Rotavirus Sentinel Surveillance Programme in South Africa (SA), detected noroviruses in 12.9 per cent of children with severe diarrhoea. Norovirus infections were most frequently detected in children <2 years of age with spring/early summer seasonality. Norovirus genogroup II strains predominated (>80 per cent) and strains were genotyped based on partial RNA-dependent RNA polymerase (RdRp) and capsid nucleotide sequences. To date sixteen RdRp and twenty-two capsid-based genotypes have been identified with GII.4 the overall predominant strain (57 per cent) followed by GII.3. The combined RdRp/capsid genotype was determined for 333 GII strains. Fifteen confirmed recombinant norovirus strains circulated during the study period, including several novel recombinants. The GII.4 New Orleans 2009 variant predominated from 2009 to 2013 after which it was replaced with the GII.4 Sydney 2012 variant. In 2016, the capsid of the Sydney 2012 variant was detected with the GII.P16 RdRp in SA. Phylogenetic analysis based on the capsid gene (1,623 bp) of fifty-two GII.4 variants, circulating between 2009 and 2016, indicated that both pandemic strains (New Orleans 2009 and Sydney 2012) diversified in SA and several sub-clusters within these major variants were identified during the study. In addition, three minor GII.4 variants, restricted to SA were characterised. Continued norovirus surveillance in SA is essential to understand the epidemiology of this diverse group of viruses and to enable further studies on norovirus evolution.

A31 Molecular characterization of circulating human noroviruses in Canada to assess RT-qPCR assays used for the detection of foodborne noroviruses

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Noroviruses (NoV) have been identified as the main cause of acute gastroenteritis in humans of all ages and as the main etiologic agent of foodborne illnesses worldwide. Since human NoV are difficult to grow in vitro, and that alternative diagnostic methods such as electron microscopy and serology lack sensitivity, modern detection methods rely on molecular approaches such as Reverse Transcription qPCR (RT-qPCR). However, the continuous emergence of new NoV strains, coupled with the genetic diversity between and within genotypes and the potential for recombination, represent continuous and significant challenges to clinical and food diagnostic laboratories. In this study, we investigated 215 outbreak-related NoV isolates collected between 2010 and 2016 by the Quebec provincial public health laboratory (Laboratoire de santé publique du Québec). An ~2.4-kb-long amplicon, encompassing the 3′-end of ORF1, polymerase gene, and the complete VP1 gene, was characterized using Next-Generation Sequencing. Paired-end dual-indexed sequencing runs of 2×75 cycles were conducted on pooled libraries on Illumina's MiSeq Instrument and paired reads were assembled de novo into a single contig. Firstly, both the 3′-end of ORF1 and VP1 sequences derived from the contig were genotyped and subtyped by phylogenetic analysis. Typing revealed that multiple NoV genotypes were in circulation between 2010 and 2016, but NoV genotype GII.4 was responsible for most of the gastroenteritis outbreaks. Moreover, genotyping of ORF1 and VP1 did not always match suggesting recombinant noroviruses. Secondly, binding affinity between all the characterized NoV isolates and the primers or probes was assessed in silico. The analysis demonstrated that mismatches in the Taqman probe-binding sites were associated with certain genotypes: GI.P3_GI.3a, GI.P9_GI.9, GI.Pa_GI.3 b, GII.P7_GII.6, and GII.P17_GII.17. The NoV GII Taqman probe was modified accordingly and tested against the original probe using a panel of twelve representative samples. A single mismatch in the NoV GII Taqman probe impeded detection: concurrent testing demonstrated a discrepancy between the CT values. Improved understanding of the genetic diversity of circulating noroviruses is important to develop molecular assays with appropriate inclusivity and exclusivity panels during analytical sensitivity evaluations in food virology laboratories.

A32 Whole-genome sequencing of human respiratory syncytial virus directly from clinical samples by next-generation sequencing

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Human respiratory syncytial virus (RSV) is the main viral cause of acute lower respiratory tract infections in children worldwide. Despite this, no licensed vaccine or effective treatment against RSV are currently available. However, clinical trials with live-attenuated virus vaccines against RSV have been reported and most are coming to the end. This make promising in the near future the possibility of preventing the infection with this virus, and as a consequence molecular surveillance studies should be considered. Taking into account the ability of RSV to generate drastic genetic events it would be necessary to monitor the impact of the evolutionary process on the complete genome. For this purpose, a fast and flexible methodology for whole-viral genome sequencing is required. We describe a methodology developed to obtain the complete genome sequences of RSV directly from nasopharyngeal aspirates by using random amplification followed by next-generation sequencing with Illumina platforms. We analysed the NGS data with bioinformatic tools available on Galaxy (https://usegalaxy.org/) and UGENE software. We obtained RSV genomes with a horizontal coverage of 90–100 per cent in all cases, and an average depth coverage between 19 and 44 on a MiSeq platform and between 259 and 1,420 on a NextSeq500. The coverage profile was not regular and showed different maximums and minimums depending on the evaluated pretreatment. For each library, between 1 and 18 per cent of the reads aligned against HRSV, most of the reads aligned against human genome and rRNA. Metagenomic analyzes showed that 20 per cent of the reads corresponded to sequences of other microorganisms. The best methodology was random amplification from nasopharyngeal aspirate samples with RSV-viral load $\geq 10^6$ copies/ml. Analysed pretreatments showed that without sample filtering, without rRNA depletion, but with a DNase treatment to the extracted RNA, performance was improved. The analysis of whole viral genomes obtained independently of the knowledge of viral