(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

genetic sequences has a fundamental role in the study of a virus with high sequence variability, such HRSV. This methodology could also be used for genome sequencing of other RNA viruses.

A33 Respiratory syncytial virus group B evolutionary trends in the attachment (G) glycoprotein in Kilifi, Kenya, 2003–2015

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Respiratory syncytial virus (RSV) attachment (G) protein mediates virus binding to cells and is a target for human neutralising antibodies. Understanding its evolutionary patterns is relevant to design of vaccines or antiviral therapy. RSV group B genotype BA, characterised by a sixty-nucleotide duplication, was first detected in 1999 in Buenos Aires, Argentina, and has since been spread and become the globally dominant RSV B genotype. BA viruses were first detected in Kilifi, coastal Kenya in 2003 and soon achieved high prevalence, replacing all other RSV-B genotypes. We present in detail, evolutionary patterns of the genotype BA G protein from >600 BA viruses obtained during twelve successive RSV epidemics in Kilifi. Phylogenetic analyses revealed extensive diversification of the BA genotype viruses into multiple genetically distinct variants (~58), some of which persisted locally across sequential epidemics while others were re-introductions into the community. The most recent common ancestor dated back to 1990, and the mean evolutionary rate over their G ectodomain region was 6.51×10^{-4} substitutions/site/year (95 per cent CI $5.65–7.42\times10^{-4}$). Demographic analysis demonstrated two main phases: an early rapid expansion and subsequent seasonal fluctuations of BA populations. Putative positive selection was detected in six codon sites, all located in the second hyper-variable G protein region. Nucleotide substitutions introducing alternative termination codons were observed resulting in up to five different protein lengths. Two potential N-glycosylation sites were conserved in 89.3 per cent sampled viruses while eight other sites were detected in a small proportion of the viruses. Further, four codons tended to revert to previous states over successive epidemics, an indication of adaptive mechanism for immune evasion. These results provide insights into the local genotype BA viral evolutionary dynamics and highlight the importance of continuous molecular surveillance to inform on changes in proteins that may be important to vaccine design.

A34 Spread and evolution of respiratory syncytial virus A genotype ON1, coastal Kenya, 2010–2015

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In February 2012, the novel respiratory syncytial virus (RSV) group A, genotype ON1, was detected in Kilifi County, coastal Kenya. ON1 is characterized by a seventy-two-nt duplication within the highly variable G gene (encoding the immunogenic attachment surface protein). Cases were diagnosed through surveillance of pneumonia in children at the county hospital. Analysis of epidemiologic, clinical, and sequence data of RSV-A viruses detected over five RSV seasons (2010/2011 to 2014/2015) indicated the following: 1) replacement of previously circulating genotype GA2 by ON1, 2) an abrupt expansion in the number of ON1 variants detected in the 2014/2015 epidemic, 3) recent accumulation of amino acid substitutions within the ON1 duplicated sequence, and 4) no clear evidence of altered pathogenicity relative to GA2. The study demonstrates the public health importance of molecular surveillance in defining the spread, clinical effects, and evolution of novel respiratory virus variants.

A35 Molecular epidemiology of respiratory viruses

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Respiratory viruses cause a high burden of disease worldwide. The attributed morbidity and mortality is especially high in infants and young children with lower respiratory tract illness. In contrast to influenza virus, little is known about the circulation patterns of other pathogens that commonly cause respiratory disease in humans, such as respiratory syncytial virus and the human parainfluenza viruses. The recently proposed source-sink and globally migrating metapopulation models have improved our understanding of circulation patterns of influenza virus. We thus aim to investigate the molecular evolution of non-influenza respiratory viruses and understand their geographical transmission dynamics by sequencing viruses and combining them with sequences from public databases. Temporal phylogenetic trees are then inferred within a Bayesian framework to characterise the evolutionary and population dynamics of these viruses. Finally, we use phylogeographic methods to infer global migration patterns. For human parainfluenza virus 3 (HPIV-3), our preliminary analysis indicates that multiple virus lineages co-circulate globally and regionally, with introductions into specific locations that are followed by expansion and endemic circulation within each given location. We find that the HPIV-3 phylogeny displays geographical structuring that may be related to regional outbreaks. However, HPIV-3 can also be transmitted globally as reflected by the inter-mixing of different geographical locations.

A36 Circulating strains of human respiratory syncytial virus in Belgium during six consecutive respiratory seasons (2011–2017)

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Human respiratory syncytial virus (HRSV) is the most common cause of acute respiratory infection in young children. HRSV belongs to the Pneumoviridae family within the order of the Mononegavirales and can be divided into two subtypes: HRSV-A and HRSV-B. The two subtypes co-circulate during the annual HRSV season, which occurs between November and March in Belgium. The aim of this study was to determine the circulating HRSV subtypes and genotypes between the seasons of 2011–2012 to 2016–2017. With this information, we intend to understand the temporal phylogenetic relationships better between the circulating strains over the six seasons. Between October 2011 and February 2017, 1,272 HRSV positive patient samples from the University Hospitals of Leuven were collected. In order to