to genomes isolated from tick vectors in order to assess whether there are viral characteristics associated with human infection. Overall, our results highlight the utility of metagenomics NGS to identify and study the molecular epidemiology of viruses that cause CNS infection.

A54 Viral metagenomics: Relative viral enrichment and detection limits in clinical serum and faeces

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Any etiological agent containing nucleic acids should be identifiable using random next-generation sequencing (NGS) of human clinical materials. Unlike PCR based and baiting strategies, random NGS metagenomics is able to identify unknown and genetically drifted agents without relying on prior knowledge. This makes it suitable for the identification of RNA-viruses, which naturally drift due to their error-prone RNA-dependent RNA polymerase. However, NGS applied to virome investigation (viral metagenomics) presents biological and technical barriers. Viruses often cannot be cultured or isolated, therefore, low viral load samples are common. Furthermore, during NGS, all nucleic acid molecules compete for limited sequencing capacity (whether viral or non-viral) and the costs of NGS increases proportional with required sequencing depth. Therefore, to efficiently sequence low viral load samples, a protocol has to be developed that enriches viruses/viral nucleic acid. We first focused on single stranded RNA-viruses in serum and faeces. Different stages of the protocol were tested in the process from RNA-virus positive sample to dsDNA input for NGS: centrifugation, filtration, endonuclease treatment, RNA extraction, reverse transcription, second strand synthesis and library preparation. Different combinations of these methods were applied to human faeces and serum and assessed using qPCR. Subsequently, the optimal method was applied to Chikungunya virus positive serum and norovirus positive faeces ranging from Ct eight and eleven up to Ct 35 and 30, respectively. Lastly, these samples were sequenced using an Illumina MiSeq (PE300, $\sim 10^6$ reads/sample) and analyzed to determine detection limits. Our method reliably generates full (>95 per cent) viral genomes up to Ct 26 in both serum and faeces, while allowing identification of viral agent up to Ct 30. Viral metagenomics proved its merit by also identifying sapovirus, coxsackievirus, parechovirus, and picobirnavirus in faeces. The coxsackievirus and parechovirus were confirmed using qPCR with a Ct of 28 and 29.18, respectively. The identified sapovirus could not be confirmed using our diagnostic qPCR, although NGS data coverage indicated a high viral load. Further analysis of this sapovirus showed many mutations in the qPCR primer binding site, explaining the negative result in our diagnostic assay. These results emphasize the power and promise of viral metagenomics.

A55 Foot-and-mouth disease virus undergoes abundant viral genomic changes at distinct stages of infection of cattle

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The rapid evolution of pathogenic RNA viruses presents a major challenge for scientists and others fighting to control transmission, predict and prevent future epidemics. Foot-and-mouth disease virus (FMDV), the picornavirus responsible for the eponymous disease, is one of the costliest livestock pathogens across much of the globe. Understanding how the virus changes over time both within hosts and through chains of transmission is of central importance for vaccine development, vaccination and quarantine strategies and international trade regulations. Cloven-hoofed animals including swine, cattle, and other domesticated and wild bovids are susceptible to the disease. Importantly, cattle and buffalo can be long-term carriers of the virus with the role of these animals in transmission being an active subject of research. Recent publications examining the full-length FMDV genome have begun to explain the complexities of the quasispecies and its behavior through transmission events and within hosts. Several of our lab's recent publications have addressed the question of which factors are responsible for inducing the carrier state. Our current endeavors build upon these concepts with detailed genomic study of FMDV in experimentally infected cattle through the acute and persistent phases of infection. Beginning with a heterogeneous inoculum mirroring the diversity that might be seen in an intensive farm outbreak, we have followed the progression of consensus genomes in twelve steers through different stages of disease including incubation, clinical disease, and the post-acute carrier state. In this study, we have documented convergent novel mutations at the canonical host cell entry RGD motif. We also characterized divergent minority genomes that, through powerful selective sweeps, became dominant at distinct points of infection. This study included both vaccinated and unvaccinated animals, with protection correlating with different patterns of viral evolution, notably at major antigenic sites. This is the first study to evaluate the full consensus genome of FMDV at distinct stages of infection, thus revealing significant micro-evolutionary events that can be of substantial benefit to disease control strategies and epidemiological modeling. The next stage of this work, supported by preliminary NGS data, will incorporate quasispecies-level analysis, elucidating the dynamic selective and population pressures during viral infection.

A56 Evolutionary analyses of foot-and-mouth disease virus in Southeast Asia using whole-genome sequences

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Foot-and-mouth disease (FMD) is one of the most important diseases of livestock worldwide. The causative agent, FMD virus (FMDV) is an aphthovirus from the Picornaviridae. The FMDV ORF is translated as a single polyprotein that codes for four structural proteins and eight non-structural proteins. Molecular epidemiology and evolution of FMDV have been traditionally studied using the sequence coding for VP1 (639 nt), the capsid protein containing most relevant antigenic domains. Although full-genome sequencing of this virus is not used as a routine diagnostic or surveillance tool, the availability of full-genome sequences in public repositories has increased over recent years.

Previous studies have suggested that recombination breakpoints of FMDV are mostly located in the boundaries between capsid and non-capsid proteins. Here, we investigated the recombination patterns of viral lineages (determined by VP1 phylogeny) known to be endemic to Southeast Asia (SEA): FMDV serotype O lineages PanAsia and Mya-98, and serotype A lineage Sea-97). We analyzed ninety-three full ORF sequences from SEA countries and reference sequences from other Asian regions. Of these, thirty sequences were generated by our laboratory and the remaining were obtained from GenBank. We used maximum likelihood phylogenetic reconstruction for each of the protein coding regions and RDP4 to detect recombination. Specific recombinant viruses were further analyzed using RIP to visualize their mosaic patterns. Three specific mosaic viruses of lineage A/Sea97 and O/Mya98 sequences were detected. Reconstruction of the phylogenies revealed a closer relationship between O/Mya98 and A/Sea97 lineages in the non-structural proteins. We further analyzed intra-lineage recombination, using homoplasy test (after removal of mosaic sequences), revealing hot spots of recombination regions that differ depending on the lineages A/Sea97 (hotspots in VP2, 2 C, and 3 D), O/Mya98 (in Lpro, VP1, 3 C, and 3 D), and PanAsia (in Lpro and 2 C). This study integrates knowledge of molecular FMD epidemiology and the specific implications of viral recombination. Furthermore, these results suggest novel understanding of the evolutionary interdependence of FMDV serotypes and lineages. Unveiling the evolutionary mechanisms of FMDV may help predict emergence of new lineages, and inform the risk posed by co-circulating lineages in FMD-endemic regions.

A57 Clinical features and virology of hand, foot, and mouth disease in southern Vietnam from July 2013 to July 2015

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In Asia, hand, foot, and mouth disease (HFMD) is associated with large and sometimes severe outbreaks since 1997, and is caused by enterovirus A (EV-A), in particular EV-A71. Monitoring the pattern of replacement between EV serotypes, its associated clinical profile and pathogen evolutionary process are essential for understanding the progress of outbreak/epidemics and development of intervention strategies. A large prospective study has been conducted at three referral hospitals in southern Vietnam since July 2013: Children's Hospital 1, Children's Hospital 2 and Hospital for Tropical Diseases in Ho Chi Minh City. For each participant, clinical data, throat and rectal swabs were collected. Multiplex real-time and nested RT-PCRs were employed to detect and identify specific EV serotypes in clinical specimens. Selected EV-A(71) positive swabs were then subject to whole-genome deep sequencing. During two years, there were 1,547 cases enrolled into the study. The most commonly detected pathogens included CV-A6 (21.8 per cent), EV-A71 (24.4 per cent), CV-A16 (10.8 per cent), and CV-A10 (7.9 per cent), followed by CV-A2/A4/A12 and Echovirus. Temporally, the four common enterovirus genotypes (including EV-A71, CV-A6, CV-A10, and CV-A16) replaced each other during the entire study period. A total of 295 genome sequences were obtained, including 156 EV-A71 sequences. EV-A71 B5 ($n = 156$) was the predominant subgenogroup. Phylogenetic analysis showed that all Vietnamese CV-A16 ($n = 25$), CV-A2 ($n = 7$), CV-A5 ($n = 3$), CV-A8 ($n = 4$), CV-A12 ($n = 10$), and CV-A14 ($n = 1$) were closely related to those from China and the region, while CV-A4 ($n = 10$), CV-A6 ($n = 26$), and CV-A10 ($n = 43$) clustered with viruses belonging to genogroups collected from worldwide, and CV-A4 were imported into Vietnam from two independent events. Clinically, there was no significant difference between CV-A6, CV-A16, and CV-A10 groups. Patients with EV-A71 infection were older than those with non-EV-A71 infection (21.7 vs. 17.3 months old, $P < 0.001$). Other differences included myoclonus (21 vs. 13 per cent, $P = 0.001$), irritability (17 vs. 70 per cent, $P < 0.001$), and location of erythema. There was a trend toward EV-A71 detection rate and clinical severity: 23 per cent grade 1, 17 per cent (2 A), 39 per cent (2B group 1), 71 per cent (2B group 2), 64 per cent (3), and 67 per cent (4). Our study represents the most comprehensive descriptive HFMD study in Vietnam. The analysis of 1,547 patients has revealed interesting and important insights into epidemic patterns, pathogen-associated clinical phenotypes, and viral evolution, which are essential for public health and of clinical significance.

A58 Identification of novel viruses in the families Flaviviridae (Jigmenvirus), Chuviridae, and Bunyaviridae (phlebovirus-like) in ticks from the south of Brazil

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Tick-borne viruses are transmitted to humans and animals by tick bites and include many important emerging and re-emerging viruses. In addition, recent studies based on high-throughput sequencing have revealed an unprecedented diversity of tick-borne viruses. The objective of this project was to investigate the viral diversity present in ticks in South of Brazil. To this end, we sampled ~600 ticks (*Rhipicephalus microplus*) and 36 serum from cattle collected in six farms in the South of Brazil between October of 2015 to June of 2016. Samples were distributed in twelve pools based on sample (ticks or serum cattle) and site of collection. Viral RNA was extracted, followed by synthesis of double-stranded cDNA and was sequenced using the Illumina platform. Sequence reads were quality-filtered, the adapter sequences removed and the remaining reads were assembled with *de novo* methods using the MetaVIC pipeline. We identified and characterized the complete genome sequence of three RNA viruses, which were classified into the families Flaviviridae, Bunyaviridae, and Chuviridae. The genome of Mogiana Tick virus (MGTV) comprised four positive sense single stranded RNA molecules, named as segments one to four with 2,672 to 2,994 nucleotides, which encodes five proteins (NSP1, VP1, NSP2, VP2, and VP3). This virus was classified as a member of Jigmenvirus, a possible new genus in the Flaviviridae family. The Lihan Tick 2 virus-like (LT2V-like) possesses two segments, the small segment encodes the nucleoprotein and the large segment encodes the RNA-dependent RNA polymerase. Despite this virus being classified as a phlebovirus-like (Bunyaviridae),