we did not find the medium segment or a homologue gene. Lastly, we describe the nearly complete sequence of the Wuhan Tick Virus-like (WTV-like), which is comprised of one negative-sense single stranded RNA molecule, with 11,208 nucleotides. This virus was classified into Chuviridae family and encodes the polymerase, glycoprotein, nucleoprotein, and VP4. Interestingly, the tick pools from all the four sites of collection were positive for MGTV, LT2V-like, and WTV-like viruses, indicating that these viruses can be found in the tick population of a large area of the Brazilian territory, which is an important cattle producing region in the country and one of the leading regions in animal produce exportation. On the other hand, only the MGTV was simultaneously detected in 19.4 per cent (7/36) serum cattle, indicating viremia in these animals. In summary, we have identified three potentially novel tick-borne viruses with broad distributions in South of Brazil, which include potential novel pathogens for cattle.

A55 Expansion of genetic diversity and interspecies transmission dynamics of swine influenza viruses in China

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Swine influenza viruses (SIVs) pose a continuous threat to agriculture and public health, as indicated by the emergence of 2009 pandemic H1N1 virus (pdm/09). Our previous evolutionary studies of SIVs isolated from long-term surveillance in China during 1998–2010 revealed co-circulation of four major swine lineages, all of which were introduced from North America or Europe. The introduction and reassortment of pdm/09 with these major swine virus lineages have led to the expansion of genetic diversity in the study area. Frequent influenza virus transmission from human to swine also drives evolution of SIVs, facilitating the genesis of novel variants with increased human infectious potentials, as evidenced by the human cases caused by infection of zoonotic H3N2 variants. Repeated transmission of H3 human influenza viruses (hIVs) to pigs has been observed in China since 1998, but their long-term impact on the ecology and development of SIVs were not systematically explored. Using whole-genome sequences of 1,631 SIVs isolated from our influenza surveillance program during 1998–2015, we aim to investigate the following scientific questions: 1) the continuous development of pdm/09-like and hIV-like H3 viruses in pigs; 2) population dynamics of co-circulating swine lineages in China; 3) global migration of SIVs of various origins; 4) viral determinants of increasing diversity and human-to-swine transmissibility. Phylogenetic analyses in this project may provide insights into the risk posed by circulating SIVs and understanding of the mechanism of interspecies transmission.

A56 Molecular epidemiology of canine parvovirus type 2 (CPV-2) in Italy

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In this study, we performed phylodynamic analysis of 107 VP2 sequences of CPV strains detected in dogs showing clinical signs of gastroenteritis, collected during the period 1994–2013. The age of these animals ranged between two months and ten years, with or without vaccination history from different breeds and both genders. The majority of faecal samples were collected in the continental area of North and Central Italy and twenty-nine faecal samples came from Sicily. VP2 genes were sequenced and a variety of statistical analyses of nucleotide polymorphism and sequence variability were performed on the sequence data. Maximum likelihood phylogenetic trees were estimated using PAUP* version 4.0, with the best-fit model of nucleotide substitution determined using JModeltest 2. The HKY + I + G substitution model was optimal for all the sequence data (including reference strains), whereas the GTR + I + G substitution model was used for the sequences analysed in this study; the key parameter values (the HKY and GTR substitution matrix, the proportion of invariant sites I and the gamma distribution of rate variation with eight categories) were estimated from the data. To assess support for individual nodes, bootstrap resampling values were estimated with 1,000 neighbor-joining trees, again employing PAUP*. Molecular phylogenetic analysis of the amino acid sequences with a maximum likelihood method was carried out using the software PHYLP version 3.695. The best substitution model for amino acid sequences was estimated using MEGA version 5.2.2. The JTT + F substitution model was optimal for the protein dataset and used to build phylogenetic trees. Typing of CPV strains detected fifty-six CPV-2 strains, twelve CPV-2b strains, and thirty-nine strains characterized as CPV-2c. Sixty-one genetically distinct sequences or nucleotide sequence types (ntSTs) and nineteen amino acid sequence types (aaSTs) were identified among the 107 viruses sequenced. In the ten-year observation period, the frequency of the CPV variants showed rapid oscillations.

A57 High prevalences and a wide genetic diversity of simian retroviruses in non-human primate bushmeat in rural areas of the democratic Republic of Congo

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Like the majority of emerging infectious diseases, HIV and HTLV are of zoonotic origin. Here, we assess the risk of cross-species transmissions of their simian counterparts, SIV and STLV, from non-human primates (NHP) to humans in the Democratic Republic of Congo (DRC). A total of 331 samples, derived from NHP bushmeat, were collected as dried blood spots (n = 283) or as tissue samples (n = 36) at remote forest sites mainly in northern and eastern DRC. SIV antibody prevalences in dried blood spots were estimated with a novel high-throughput immunoassay with antigens representing the actual known diversity of HIV/SIV lineages. Antibody-positive samples were confirmed by PCR and sequence analysis. Screening for STLV infection was done with universal primers in tax, and new strains were further characterized in LTR. SIV and STLV infection in tissue samples was done by PCR only. Overall, 5 and 15.4 per cent of NHP bushmeat was infected with SIV and STLV, respectively.
A new SIV lineage was identified in Allen’s swamp monkeys (Allenopithecus nigroviridis). Three new STLV-1 subtypes were identified in Allen’s swamp monkeys (Allenopithecus nigroviridis), blue monkeys (Cercopithecus mitis), red-tailed guenons (Cercopithecus ascanius schmidti), and agile mangabeys (Cercocebus agilis). SIV and STLV prevalences varied according to species and geographic region. Our study illustrates clearly, even on a small sample size from a limited number of geographic areas, that our knowledge on the genetic diversity and geographic distribution of simian retroviruses is still limited, and that humans continue to be exposed to relative high proportions of infected NHP bushmeat.

Molecular characterization of emerging variants of bovine leukemia virus

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Bovine leukemia virus (BLV) is the etiological agent of the enzootic bovine leukosis. The BLV transcriptional promoter is located in 5’ long terminal repeat (LTR), which is composed of the U3, R, and U5 regions. One of the limiting factors in the study of BLV is the lack of sequence data in relation to geographical origin of strains and the potential effects of genetic variability on virus infectivity and disease progression. We would like to address this question by analysing LTR variability of BLV variants isolated from emerging cases of infection, recorded in already-cured herds. Our hypothesis is that the genetic variability in the LTR sequences of these isolates can be associated with transcriptionally down regulated provirus variants. DNA samples were isolated from PBLs of sixty cattle serologically positive for BLV, which were classified as a newly infected animals in herds having BLV free status. Additionally, twenty-five archival DNA samples were collected from East European countries: Poland, Russia, Ukraine, Moldova, and Croatia, representing endemic cases of BLV infection. Fusion PCR was developed and used for amplification of 531-bp fragment corresponding to the full length BLV LTR. Amplified fragments were sequenced and sequences were aligned using Muscle. The following steps of phylogenetic analysis were performed: (1) creation of trait information for the sequences, different molecular clock models, tree priors and parameters for the MCMC chain, (2) computation of Bayesian Markov chain Monte–Carlo simulations of 100 million steps in BEAST, (3) creation of a maximum clade credibility (MCC) tree in TreeAnnotator, (4) creation a Google Earth file of the MCC tree using SPREAD. The Shimodaira–Hasegawa (SH) test was used to simultaneously compare sets tree topologies based on the partial env gene and LTR sequences. Comparative analysis of eighty-five sequences and sequences available from GenBank allowed the analysis of a new mutation in Geneious Pro 5.3. Sequence analysis of the BLV variants from new infections revealed 96.4 to 99.5 per cent homology, when compared to the archival DNA samples. Detailed analysis of LTR sequences showed ninety-one different mutations dispersed mainly along U3 and U5 regions of the LTR. About thirteen of these mutations were located within promotor contained in the U3 region of BLV and these could be associated with diminished transcriptional activity.

Equine infectious anaemia virus in Great Britain: Molecular characterisation of cases from 1975 to 2012

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Equine infectious anaemia virus (EIAV) is the aetiological agent of equine infectious anaemia (EIA), a notifiable disease within the EU and to the OIE. In Great Britain, EIA cases were reported in 1975–1978; then followed a disease-free period until 2010–2012, when six positive animals were identified. As a member of the family Retroviridae and genus Lentivirus, EIAV shares the lentiviral trait of considerable genetic variation which has hampered development of molecular diagnostics. In addition, there is a lack of publically available sequence data, hence our intention was to conduct molecular characterisation of the British cases. A combination of Sanger and next-generation sequencing (NGS) was employed in order to determine viral sequences. Using Sanger sequencing only small genomic fragments could be recovered, hence efforts were refocused on NGS. Phylogenetic analysis and evaluation of sequence diversity of the British sequences were assessed using the MEGA5 software. Full-genome sequences were obtained from symptomatic cases in 1975, 2010, and 2012. Phylogenetic analysis of these sequences revealed that each British case formed its own branch on the tree as did sequences from America, China, Ireland, and Japan. Almost an equal distance was observed between each of the isolates, with nucleotide homology of 75–79 per cent. Nucleic acid identity of the full-genome sequences varied between individual genes and ranged from 46 per cent (P9) to 98 per cent (protease). EIAV’s variable nature made the use of primer walking sequencing strategies laborious, whereas attaining full genome via NGS was relatively straightforward. However, it is not without its problems as significant viral load is required to overcome the high host background typical of clinical extractions rendering sequencing of asymptomatic cases problematic. As the British asymptomatic cases provided limited or no sequence, investigation is ongoing into strategies to elucidate these sequences. Currently EIAV diagnosis relies solely on...