Among the conserved ORFs, B385R, H339R, and O61R-p12 showed 100 per cent amino acid identity. The same was true for the hypervariable ORFs, with regard to X69R, DP96R, DP60R, EP153R, B407L, I10L, and L60L genes. The EP402R and B602L genes showed, as expected, an amino acid identity range of 98.5 per cent to 100 per cent and 91 per cent to 100 per cent, respectively. In addition, all of the isolates displayed variable intergenic sequences. As a whole, the results from our studies confirmed a remarkable genetic stability of the ASFV/p72 genotype I viruses circulating in Sardinia.

A51 Genetic variability of small ruminant lentiviruses in sheep and goats from single-species flocks from Poland

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Previous phylogenetic analyses of small ruminant lentivirus (SRLV) sequences found in Poland revealed the circulation of subtype A1 in both sheep and goats, subtypes B1 in goats, and subtypes B2, A12, and A13 in sheep only. This study aimed to analyze the genetic nature of SRLV circulating in sheep and goats from single-species flocks. In order to analyze the degree of genetic variability, the fragments of *gag* and *env* genes of 24 SRLV strains were amplified by PCR, cloned into plasmid vectors, sequenced, and consensus sequences were aligned to each other and to reference sequences available from GenBank. Phylogenetic analysis was performed using the Geneious tree-builder tool, and phylogenetic trees were constructed using Mr Bayes (using the general time reversible substitution model) within Geneious Pro 5.3. Pairwise genetic distances were calculated in MEGA 6. Phylogenetic analysis revealed that the strains were highly heterogeneous and represented ovine strains belonging to subtypes A12 and B2 and caprine strains grouped in subtypes B1, B2, A1, and A12. In addition, two novel subtypes, A16 and A17, were found in goats. The mean pairwise genetic distances of *gag* and *env* sequences of both clusters were above 15 per cent nucleotide divergence when compared to all other subtypes within group A, which is a criterion required to distinguish a new subtype. Additionally, the existence of two separated clusters was confirmed by high bootstrap values. Co-infections with strains belonging to different subtypes within A and B groups were detected in one sheep and four goats originating from four flocks. Since the co-infection with more than one lentivirus genotype offers an opportunity for viral recombination, the possible recombination events were tested based on RDP analysis. For all co-infected animals, no evidence of recombination was found within the *gag* gene; however, *env* sequences showed some recombination patterns in three samples. In conclusion, we have demonstrated extended genetic variability of SRLV in sheep and goats from Poland with the existence of co-infection and recombination events.

A52 MERS coronaviruses from camels in Africa exhibit region-dependent genetic diversity

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Middle East respiratory syndrome coronavirus (MERS-CoV) causes a zoonotic respiratory disease of global public health concern, and dromedary camels are the only proven source of this zoonotic infection. Although MERS-CoV infection is ubiquitous in dromedaries across Africa and the Arabian Peninsula, the continuous appearance of zoonotic MERS cases in humans is confined to the Arabian Peninsula. MERS-CoV from Africa has hitherto been poorly studied. Here, we report the genetic and phenotypic characterization of MERS-CoV from dromedaries in African countries. Phylogenetically, viruses from dromedaries in Africa formed a monophyletic clade, which we have provisionally designated as virus clade C. Molecular dating analyses of MERS-CoV, including clade C viruses, suggests that the ancestral MERS-CoV in dromedaries could have spread to the two continents within a short timeframe. Camel MERS-CoVs from west and north African countries form a subclade (C1) that shares genetic signatures of a major deletion in the accessory gene ORF4b. Compared with human and camel MERS-CoV from Saudi Arabia, virus isolates from Burkina Faso (BF785) and Nigeria (Nig1657) had lower virus replication competence in Calu-3 cells and in *ex vivo* cultures of human bronchus and lung, and BF785 replicated to lower titer in lungs of human DPP4-transduced mice. However, it is still inconclusive whether ORF4b deletions may lead to the reduced replication competence of BF785 and Nig1657. Genetic and phenotypic differences in West African viruses may be relevant to the zoonotic potential of MERS-CoV.

A53 MERS-CoV in East African dromedary camels

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Human Middle East respiratory syndrome is a zoonotic respiratory disease caused by Middle East respiratory syndrome coronavirus (MERS-CoV) originating from camels in the Arabian Peninsula. While there are a large number of camels in East Africa, often traded to the Arabian Peninsula, no autochthonous human MERS-CoV case is reported in East Africa. Furthermore, there is limited information of MERS-CoV in East Africa. In this study, MERS-CoV in dromedary camels from Ethiopia was detected using RT-qPCR. Next-generation sequencing was used to obtain the full genome of MERS-CoV. MERS-CoV antibodies were also detected through MERS-spike pseudoparticle neutralization assay. Phylogenetic analysis of full-genome sequences and spike-genome antibodies indicates that MERS-CoV in East Africa is genetically distinct from those in the Arabian Peninsula. The results from this study show that MERS-CoV circulating in dromedary camels in East Africa are genetically distinct from those in the Arabian Peninsula. Further studies are needed to evaluate the risk of zoonotic transmission in East Africa.

A54 Genomic analysis of camel-HKU23 in Nigeria dromedary camels reveals strain-specific cross-species recombination

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Coronaviruses (CoVs) are enveloped, single stranded, positive-sense RNA viruses with a large genomic size of 26–32 kilobases. The first human CoV identified in the 1960s was isolated from patients presenting with common cold symptoms. Subsequent epidemic outbreaks of novel zoonotic CoV transmission were reported, examples including HCoV-229E (229E), HCoV-OC43 (OC43), severe acute respiratory syndrome, and Middle East respiratory syndrome (MERS). The ongoing outbreak of MERS in the Middle East is originating from a zoonotic source of dromedary camels. Surveillance later revealed that three CoV species—HCoV-229E (229E), camel-HKU23, and MERS-CoV—were co-circulating in Saudi Arabia dromedary camels. Camel-HKU23 belongs to Group 2a CoV, which also includes human coronavirus OC43, bovine coronavirus, and porcine hemagglutinating encephalomyelitis virus. Recombination, resulting in the generation of different novel genotypes, has been reported previously among these CoVs. Our surveillance of dromedary camels slaughtered in a major abattoir in Nigeria identified camel-HKU23 from nasal swab samples with a prevalence of 2.2 per cent. Phylogenetic analysis showed Nigeria camel-HKU23 is distinct from those previously identified in Saudi Arabia, while still genetically similar, as they share a monophyletic origin. Recombination analysis of Nigeria camel-HKU23 revealed two recombination breakpoints at positions of 22774–24100 base pairs (bp) and 28224–29362 bp. Recombination breakpoint at position 22774, encoding the Group 2a CoV-specific hemagglutinin esterase gene, exhibited high bootstrap support for clustering with RbCoV HKU14, which was previously detected in

domestic rabbits in China. The recombination signal is only observed in Nigeria camel-HKU23, suggesting a regional varied evolutionary history of camel-HKU23. Our findings extended the knowledge of the evolutionary relationship among Group 2a CoVs. Further surveillance in other African camels will be important to elucidate the evolution of camel-HKU23.

A55 Molecular systematics of sturgeon nucleocytoplasmic large DNA viruses

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Namao virus (NV) is a sturgeon nucleocytoplasmic large DNA virus (sNCLDV) that can cause a lethal disease of the integumentary system in lake sturgeon *Acipenser fulvescens*. As a group, the sNCLDV have not been assigned to any currently recognized taxonomic family of viruses. In this study, a dataset of NV DNA sequences was generated and assembled as two non-overlapping contigs of 306 and 448 base pairs (bp) and then used to conduct a comprehensive systematics analysis using Bayesian phylogenetic inference for NV, other sNCLDV, and representative members of six families of the NCLDV superfamily. The phylogeny of NV was reconstructed using protein homologues encoded by nine nucleocytoplasmic virus orthologous genes (NCVOGs): NCVOG0022—mcp, NCVOG0038—DNA polymerase B elongation subunit, NCVOG0076—VV A18-type helicase, NCVOG0249—VV A32-type ATPase, NCVOG0262—AL2 VLTF3-like transcription factor, NCVOG0271—RNA polymerase II subunit II, NCVOG0274—RNA polymerase II subunit I, NCVOG0276—ribonucleotide reductase small subunit, and NCVOG1117—mRNA capping enzyme. The accuracy of our phylogenetic method was evaluated using a combination of Bayesian statistical analysis and congruence analysis. Stable tree topologies were obtained with datasets differing in target molecule(s), sequence length, and taxa. Congruent topologies were obtained in phylogenies constructed using individual protein datasets and when four proteins were used in a concatenated approach. The major capsid protein phylogeny indicated that ten representative sNCLDV form a monophyletic group comprised of four lineages within a polyphyletic Mimi-Phycodnaviridae group of taxa. Overall, the analyses revealed that Namao virus is a member of the Mimiviridae family with strong and consistent support for a clade containing NV and CroV as sister taxa.

A56 Visualization of recombination in deformed wing virus infecting bees

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Honey bees suffer increasing colony mortality worldwide, partially caused by the spread of viral pathogens. Among these pathogens, deformed wing virus (DWV) is one of the major, widespread viruses of honey bees resulting in wing deformities and weakening colonies. DWV can be found in honey bees, bumble bees, and other wild bees as three major genotypes named DWV-A, -B (also named Varroa destructor virus 1), and -C. Various recombinants of DWV-A and -B have been previously found in honey bees, some of which have been suggested to have higher virulence over non-recombinant, parental virus. In most of these cases, recombinants were only shown as consensus sequences from previous assemblies and alignments and may not reflect the biological reality of all variants present within a host bee. It is therefore important to build a method of recombinant detection and quantification within mixed infections in single-host individuals, including both parental and various recombinant genomes, so as to evaluate the relevance of recombinants for viral genome evolution and the impact on hosts. Here, we propose to visualize and quantify these recombinants using next-generation sequencing data to better understand how these genomes evolve within bees. Our method will be performed directly from raw sequence reads from various datasets (including field and lab experiments as well as screening of public databases) in order to obtain an overview of DWV recombination in various *in vivo* and *in vitro* conditions. Recombination of viral genomes is a key point for virus evolution. The detection and quantification of recombination will facilitate analysis of the determinants of recombination and help in understanding the routes by which new viral variants emerge. The emergence of new (more virulent) recombinant viruses can result from acquisition of new capabilities, such as escape from host immunity or increased transmission rates. Recombination can also lead to adaptation to new environments and new hosts by a change in cell tropism, allowing cross-species transmission, which may be particularly relevant for bumble bees and wild bees infected by honey bee-derived DWV.

A57 An evolutionary framework to guide the hunt for human dsDNA viruses

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It is becoming increasingly obvious that we only know a fraction of the human virome. The development of next-generation sequencing (NGS) technologies has dramatically increased our ability to hunt viruses. Yet, the small genomes and low copy numbers characteristic of most viruses make undirected (shotgun) hunts a relatively inefficient strategy. Here, we propose to speed-up the rate of double-stranded DNA (dsDNA) virus discovery by combining NGS with evolutionary thinking. dsDNA viruses are thought to have co-diverged with their hosts. As this applies to the hominine lineages (gorillas, humans, chimpanzees, and bonobos), it is theoretically possible to estimate the phylogenetic position of cryptic human viruses by identifying co-divergent viral lineages infecting non-human hominines. Where these lineages do not comprise a human-infecting counterpart, a yet-unknown human virus may be hiding. The first phase of this project will consist in the high-throughput characterization of dsDNA viruses (herpesviruses, papillomaviruses, and polyomaviruses) infecting wild gorillas, chimpanzees, and bonobos. For this, we will use an exhaustive collection of fecal samples (in terms of hominine species/sub-species diversity) and apply a discovery strategy combining in-solution capture and NGS. This strategy has been developed in the ancient DNA field but has a very broad applicability; it will constitute a nice addendum to the institute technical portfolio. Thanks to the massive amount of information collected, we will be able to reconstruct the evolutionary history of many dsDNA virus lineages and to identify those where a human virus would be expected but is still unknown. This will pave the way to the second phase of the project which will consist in a pre-oriented dsDNA human virus hunt based on the use of specific PCR systems implemented in multiplex. We expect that this project will generate an unprecedented amount of data on the processes at play along dsDNA virus evolution (co-divergence versus cross-species transmission), help determine the directionality, frequency, and timing of cross-species transmission events between hominines and unveil the existence of yet-to-be-discovered human viruses.

A58 Epidemic dynamics of ancient disease outbreaks

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Bayesian phylogenetic analysis allows for the estimation of the time to the most recent common ancestor (tMRCA) of sequences sampled at different times, as long as they prove to be 'measurably evolving', which means that the time between sampling dates was long enough to allow the appearance of a measurable amount of genetic changes. This 'temporal signal' can be tested with the software TempEst (Rambaut et al. 2016), which generates a regression of the root-to-tip genetic distance on sampling times and finds the best-fitting root that produces the lowest residual sum of squares. For the case of pathogen single nucleotide