A47 Reconstructing the evolutionary history of pandemic foot-and-mouth disease viruses: The impact of recombination within the emerging O/ME-SA/Ind-2001 lineage

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Foot-and-mouth disease (FMD) is a highly contagious disease of livestock affecting animal production and trade throughout Asia and Africa. Understanding FMD virus (FMDV) global movements and evolution can help to reconstruct the disease spread between endemic regions and predict the risks of incursion into FMD-free countries. Global expansion of a single FMDV lineage is rare but can result in severe economic consequences. Using extensive sequence data, we have reconstructed the global space-time transmission history of the O/ME-SA/Ind-2001 lineage (which normally circulates in the Indian sub-continent) providing evidence of at least fifteen independent escapes during 2013–7 that have led to outbreaks in North Africa, the Middle East, Southeast Asia, and the Far East and the FMD-free islands of Mauritius. We demonstrated that sequence heterogeneity of this emerging FMDV lineage is accommodated within two co-evolving divergent sublineages, and that recombination by exchange of capsid-coding sequences can impact upon the reconstructed evolutionary histories. Thus, we recommend that only sequences encoding the outer capsid proteins should be used for broad-scale phylogeographical reconstruction. These data emphasize the importance of the Indian subcontinent as a source of FMDV that can spread across large distances and illustrates the impact of FMDV genome recombination on FMDV molecular epidemiology.

A48 Identification and full-genome characterization of Alpha- and Beta-Coronaviruses viruses from bats in Italy

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Bats are the natural reservoir of Coronaviruses (CoVs). Human CoVs cause mild respiratory diseases worldwide, but, in the last decade, two Beta-CoVs [Middle East respiratory syndrome (MERS)-CoV and severe acute respiratory syndrome] caused thousands of deaths and cases worldwide. Phylogenetic analysis suggested the evolutionary origin of mammalian CoVs is derived from bats. In this study, we characterized three Alpha-CoVs and two Beta-CoVs demonstrating the circulation of bat strains in Italy. Isolates were sequenced using a next-generation sequencing approach and genomes reconstructed using the online tool Galaxy Aries. Phylogenetic analyses were conducted using MEGA7 and MrBayes. Similarity plots were generated using SSE v1.2. The structure of the receptor binding domain (RBD) in the S protein was predicted by sequence-homology method using the protein data bank. Bioinformatics analysis permitted the identification of 2 Beta-CoV complete genomes of 30 kb and three Alpha-CoV of 28 kb (named BatCoV-ITA1-5). BatCoV-ITA1 and 2 formed a monophyletic group with MERS-CoV sequences. The comparison of the concatenated domains within ORF1ab confirmed their classification into the MERS-CoV species. The 3D structure of RBD of Italian strains showed two amino acid deletions located in a region corresponding to the external subdomain of MERS-RBD. BatCoV-Ita3 and BatCoV-Ita4/5 were classified into two novel Alpha-CoV species by comparison of concatenated domains within ORF1ab. Due to the high divergence with the Alpha human spike protein strains, it was impossible to establish the protein structure and the potential affinity to human receptor. The Italian strains showed the typical organization of Alpha and Beta-CoVs. We reported two Beta-CoVs closely related to MERS-CoVs from bats belonging to common Italian species (*Pipistrellus kuhlii* and *Hypsugo savii*). The analysis of the RBD in the spike protein indicates significant differences from human RBD known to date. The three Alpha-CoV strains were classified into two novel species, confirming the high heterogeneity of CoV strains in bats. Although the studies conducted cannot confirm a risk for humans, surveillance studies are needed to investigate the genetic diversity of CoVs in bats. Because this exceeds what is known for other hosts, it is compatible with bats being the major reservoir of mammalian CoVs.

A49 Emerging rodent-borne viral pathogens in Italy: Overview of seroprevalence and genomic investigations

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Rodents play a key role as reservoirs of many zoonotic pathogens which represent an emerging public health threat worldwide. Among these, Dobrava-Belgrade virus (DOBV) is the most pathogenic hantavirus in Europe with a case-fatality rate of up to 12 per cent, while Lymphocytic choriomeningitis virus (LCMV) has a mortality rate below 1 per cent. Both viruses are predominantly transmitted to humans through the inhalation of infected particles in aerosolized urine, feces, or saliva that are shed in the environment by chronically infected hosts, such as the yellow-necked mouse *Apodemus flavicollis*. Although no human cases of DOBV or LCMV have been reported in the Province of Trento (northeastern Italy) thus far, in order to evaluate the human hazard for these viruses, the prevalence of antibodies to DOBV and LCMV has been monitored using a specific immunofluorescence assay test in a wild population of *A. flavicollis* since 2000. These investigations have shown that the two RNA viruses circulate silently in this species in the study area. In particular, a sudden increase (up to 12.5%) in DOBV seroprevalence was observed in this rodent species between 2010 and 2012. Several efforts have been undertaken to isolate these viruses and characterize their genomes, but it has not yet been possible to detect viral RNA from seropositive mice using traditional methods such as RT-PCR. Since RNA viruses are very diverse and often difficult to isolate, innovative molecular methods based on viral targeted enrichment and high-throughput sequencing have been applied. We intend to report on this long-term seroprevalence study and provide an overview of the molecular approaches adopted in the attempt to confirm the presence of these viruses, and identify which variants are circulating in the region, as well as their pathogenicity.

A50 Whole-genome sequencing of African swine fever isolates from Sardinia

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In order to assess the molecular epidemiology of African swine fever (ASF) in Sardinia, we analyzed a wide range of isolates from wild and domestic pigs over a 31-year period (1978–2009) by genotyping sequence data from the genes encoding the p54 and the p72 proteins and the CVR. On this basis, the analysis of the B602L gene revealed a minor difference, placing the Sardinian isolates into two clusters according to their temporal distribution. As an extension of this study, in order to achieve a higher level of discrimination, three further variable genome regions, namely p30, CD2v, and I73R/I329L, of a large number of isolates collected from outbreaks in the years 2002–14 have been investigated. Sequence analysis of the CD2v region revealed a temporal subdivision of the viruses into two subgroups. These data, together with those from the B602L gene analysis, demonstrated that the viruses circulating in Sardinia belong to p72/genotype I, but since 1990 have undergone minor genetic variations in respect to its ancestor, thus making it impossible to trace isolates, enabling a more accurate assessment of the origin of outbreaks, and extending knowledge of virus evolution. To solve this problem, we have sequenced and annotated the complete genome of nine ASF isolates collected in Sardinia between 1978 and 2012. This was achieved using sequence data determined by next-generation sequencing. The results showed a very high identity with range of nucleotide similarity among isolates of 99.5 per cent to 99.9 per cent. The ASF virus (ASFV) genomes were composed of terminal inverted repeats and conserved and non-conserved ORFs.

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