individuals (21.5%). Additional clustering was done by parsing the two datasets by subtype 1a ($n = 714$) and 1b ($n = 151$). The genotype 1a network demonstrates a majority, 65.8 per cent, of participants in clusters. Moreover, two large clusters can be observed with baseline participants towards the center and recent participants on the outskirts indicative of high linkage at baseline. The genotype 1b network produced a single large cluster but subclusters were observed. The sequences between the two time points co-mingled but subclusters were also observed. Interestingly, the two large clusters from 1988 to 1989 were still evident in the 2005–16 viral sequences. We observed greater cluster diversity in more recently diagnosed individuals, indicative of a less connected network of individuals sharing transmission risk, though major viral strains did persist over time in this cohort.

A24 Phylogeographic analysis of hepatitis A virus in Russia

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Hepatitis A virus (HAV) is a positive-stranded RNA virus, a member of Picornaviridae, and a representative of genus Hepatovirus. It is unique among picornaviruses with regards to its hepatotropism, structure, and life cycle. HAV is spread via the fecal-oral route as a non-enveloped particle, while, in the blood the virus circulates in an envelope formed from the host cell membrane. HAV causes acute hepatitis in adults and is usually asymptomatic in children <6 years of age. The clinical features include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice, all of which usually last >2 months. There is no evidence of chronic liver disease or persistent infection following acute infection. Due to its mode of transmission, HAV prevails in areas with low hygiene standards but does not give rise to epidemics because most people are infected at an early age and derive a life-long immunity. Thus, HAV infection has more impact on countries with higher socio-economical level where it is mostly registered as an outbreak in adults, which is the case in Russia. One feature distinguishing HAV from other picornaviruses is its remarkably slow mutation rate. HAV genotyping is typically carried out using highly variable regions VP1/2A and 2C/3A. Recently, it was shown that resolution provided by short fragments is not enough for reliable results. Unfortunately, previous research in HAV phylogeography was carried out only on these short sequences and did not include Russia or CIS territories. HAV comprises six genotypes, of which I and III are most frequent in humans and are both divided into A and B subgenotypes. Preliminary phylodynamic analysis of 80 highly variable region sequences (carried out by A. Neverov) has shown a particular pattern of geographical distribution of HAV genotypes in Russia. There are only two subgenotypes widely spread: IA predominates in the European part of Russia, and IIIA is found mainly in the Asian part. However, the history of HAV spread in Russia remains unclear. We hypothesized that IIIA subgenotype originated from India, while IA subgenotype came later from Europe and is still expanding. The Central Research Institute of Epidemiology kindly provided us with the unique collection of HAV isolates obtained from more than 30 subjects of the Russian Federation, as well as a number of isolates obtained from CIS countries. Samples (>500 isolates) were collected from 1999 to 2015 and characterized by one or both of the two most variable fragments of HAV genome (VP1/2A and 2C regions). The dataset includes 145 unique sequences of 2C/3A region, length ~650 bp, and 243 sequences of VP1/2A region, length ~400 bp. For each sample, date and location of collection are indicated. Whole-genome sequences of HAV from GenBank database were also used. They were aligned with MUSCLE, and the target 2C/3A and VP1/2A fragments were extracted. Partial HAV sequences from GenBank were not added to the analysis due to too little overlap with our sequences. Initial phylogeographic analysis was carried out in BEAST. Results were checked with the Tracer program, and the Spread3 package was used to visualize the results of the phylogeographic analysis in continuous space [16]. The BEAST output supports the hypothesis that IIIA subgenotype originated from India, whereas the situation with the IA subgenotype remains unclear. The reason for this might be either poor sampling of the Mediterranean area and Middle East in our analysis or low precision provided with variable fragments. The next step is to obtain full-genome sequences of approximately 100 of our samples to increase resolution and make use of hundreds of partial sequences of HAV genomes available in GenBank.

A25 Impact of polymorphism in the hepatitis B surface gene on human leukocyte antigen (HLA) class II

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There is still no cure for chronic hepatitis B virus infection (CHBV), a major cause of liver cancers and related malignancies. Elucidating the role of CD4+ T-helper cells in activating immunological responses that clear antigenic peptides during primary HBV infection holds a potential strategy for developing potent vaccines. Since the strength of CD4+ T cell responses is dictated by binding of viral epitopes to class-II human leukocyte antigens (HLAs), we hypothesize that the quality of immunological responses in CHBV patients is influenced by host genetics and HBV genotypes. Here, ninety-two non-recombinant complete HBV surface-gene proteins (PreS1/S) from Botswana were sequenced (genotype A 44(47.8%); D 48(52.2%)) and 15-mer binding epitopes restricted to nine HLA-class II molecules (DRB5/1) were mapped *in silico*. The HLAs used have high population coverage in Botswana. The total predicted epitopes per HLA were 94-(genotype A) and 105-(genotype D) for PreS1, 42 (A and D) for PreS2, and 105 (A and D) for S. Epitope densities (binding peptides to total epitopes) were 3 per cent and 6 per cent (PreS1A&D), 4 per cent and 2 per cent (PreS2A&D), and 23 per cent and 22 per cent (S1A&D). SA&D proteins had most polytopes: CPGYRWMCLRRFII66-81, PGYRWMCLRRFIIF67-82, GYRWMCLRRFIIFL68-83, and YRWMCLRRFIIFLF69-84 binding to 5 (55.6%) HLAs (DRB1*0101/0701/1101/1501 and DRB5*0101) used. HLA-DRB*0101 bound the most epitopes, and the least were bound by HLA-DRB*0302/0701/0401 for both genotypes. PreS1D polytope: PAFRANTANPDWDFN32-46 binds to DRB1*0101/0401/1302 and PreS2 polytopes: TAFHQALQDPRVRG6-19 and AFHQALQDPRVRGL7-20 bind to DRB1*010/1501 alleles. Non-synonymous mutations impair peptide-HLA binding when assessed as combinations of > 2. The least active HLAs may be associated with CHBV and vice-versa for HBV clearance, thus the algorithm may be used to predict HBV prognosis for different haplotypes. The results favor the use of epitopes from S protein as broad genotype vaccine. This study highlights the need to explore further the mechanisms of PreS1 and its effect on the immune system.

A26 Molecular epidemiology of hepatitis E virus in Ireland 2016

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Foodborne viruses such as hepatitis E virus (HEV) pose an increasing risk to public health and to confidence in Irish food. Hepatitis E has been acknowledged as a significant pathogen of likely zoonotic transmission, with pork products and shellfish being implicated as potential sources. The European Food Safety Authority has recommended that systematic strain typing of viruses in humans, animals, and food commodities is needed to improve understanding of etiological agents and foodborne transmission pathways, in particular for HEV. The dominant autochthonous genotype of HEV in Europe is genotype 3, thought to be associated with consumption of contaminated food, specifically pork products. However, little is known about the epidemiology of HEV in Ireland. In 2016, HEV became a notifiable disease in Ireland. Following this, as part of the Department of Agriculture, Food and the Marine-funded FoVIRA study, the molecular epidemiology of HEV in Irish clinical samples has been characterized for the first time. HEV RNA-positive clinical

specimens from 2016 were genetically characterized ($n = 14$). A 450 nucleotide fragment of the ORF2 region of the HEV genome was amplified, with contiguous sequence assembly performed using DNA Lasergene v14. Sequences were aligned with ClustalW implemented in Bioedit v7.1.9 and compared to reference strains from GenBank. A maximum likelihood phylogenetic tree was constructed using the Hasegawa–Kishino–Yano model and a discrete gamma distribution to model evolutionary rate distances between sites. Evolutionary analyses were conducted in MEGA7. Statistical support was provided by bootstrapping with 1,000 replicates. Fourteen strains belonged to genotype 3 and were classified as the following subtypes: 3c ($n = 7$), 3e ($n = 4$), 3f ($n = 1$), 3 untyped ($n = 1$), and 1 untyped. Phylogenetic analysis showed the formation of two distinct clusters of genotype 3:3abchij and 3efg, with strong bootstrap support. A genotype 1 was detected and found to be associated with travel. Data generated from this research will contribute to a risk exposure assessment and will be used to identify potential control points and risk mitigation measures for viral foodborne pathogens. This study will provide a unique opportunity to build national capability in the area of food testing within Irish public laboratories.

A27 Whole genome characterization of influenza D viruses detected in cattle herds in northern Italy between 2015 and 2017

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Influenza D virus (IDV) is a new viral genus identified within the Orthomyxoviridae family, showing 50 per cent amino acid identity with human influenza C virus. Similar to human influenza viruses of the C genus, IDV also harbors 7 genomic segments and uses 9-O-acetylated sialic acids as cell receptors. This newly emerged virus exhibits a broad host range and is capable of infecting swine, cattle, sheep, goats, ferrets, and guinea pigs. In Italy, IDV was first detected in archived samples collected between 2014 and 2015 from cattle and swine in the Po Valley area. Here, we report the genetic characterization of IDV viruses detected in an extensive area of northern Italy, namely Veneto, Lombardy, and Piedmont, through passive surveillance between September 2015 and October 2017. A total of 482 samples, including nasal swabs, lungs, and bronchoalveolar lavage fluid, collected from 309 cattle farms were tested. Thirty cattle herds turned out to be positive, for a total of 40 samples positive by Real Time RT-PCR targeting the PB2 gene. Representative IDV positive swabs were sequenced on an Illumina Miseq platform, and phylogenetic analyses were performed for each genome segment. The analyses of the seven gene segments demonstrated that the viruses identified in the north of Italy clearly grouped within a genetic cluster of IDV sequences previously described in Italy and in the USA, thus suggesting a common origin for these viruses. Interestingly, the IDVs identified in Italy presented a low similarity (96.1% to 98.8% for the seven gene segments) to the French IDVs, which is the only other European country where this pathogen has been identified and characterized so far. The wide IDV host range and the ability of this virus to reassort are a matter of concern. Results of this study indicate that IDV is extensively circulating among bovine herds in Northern Italy and suggest a potential role of IDV in the bovine respiratory disease complex, highlighting the need to perform surveillance on an ongoing basis to track its spread and evolution.

A28 Spatial spread of highly pathogenic avian influenza A (H5N8) virus in Italy, 2017–8

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In winter 2016–7 the highly pathogenic avian influenza (HPAI) virus, H5N8 subtype, clade 2.3.4.4 group B, circulated extensively both in wild and domestic birds in Europe. Northern Italy was hit by three epidemic waves: the first in January–May 2017, the second in July–December 2017, and the latest in March 2018. To genetically characterize the viruses circulating in Italy we used the Illumina MiSeq platform to sequence the complete genome of representative viruses from each infected farm, for a total of 86 cases in poultry and 17 in wild birds. Maximum likelihood phylogenetic analyses performed using PhyML version 3.1 identified multiple viral introductions of distinct genotypes of HPAI H5N8 viruses in Italy at the beginning of the epidemic (January–February 2017). During the second epidemic wave a single genetic group originating from the virus A/wild duck/Poland/82A/2016 seemed to have been selected, further evolving into two different clusters, namely Italy-A and Italy-B. We identified four clusters of secondary outbreaks, the largest being the epidemic in the province of Brescia between October 2017 and March 2018, which had affected 26 farms. Evolutionary and phylogeographic analyses performed with the BEAST v1.8.4 package (applying a Bayesian Markov chain Monte Carlo approach, using a constant size coalescent tree prior and a SRD06 model of nucleotide substitution) indicated that different viral introductions had probably occurred through migratory birds from West Russia, Siberia, Central and East Europe. The discrete and continuous phylogeographic analyses showed that group Italy-A had probably emerged between February and April 2017 in the province of Mantua and had then spread eastwards, circulating in the Veneto region and eastern Lombardy; on the contrary, Italy-B had arisen between March and July 2017 in the central part of Lombardy and had spread westwards, circulating in the western part of Lombardy, Emilia Romagna, and Piedmont regions. This study was instrumental to reconstruct the virus dissemination routes and indicated that wild and domestic birds from Lombardy most likely represented the key source for the re-emergence and spread of the HPAI virus during the second and the third epidemic waves. This key spatial information will help to define appropriate disease control strategies.

A29 Genetic heterogeneity of influenza A (H3N2) viruses in the United Kingdom, 2016–8

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For the last four influenza seasons in the UK, genetic characterization of seasonal influenza viruses has shifted from single hemagglutinin (HA) and neuraminidase (NA) genes to whole genome (WG) analysis, allowing for better insight into the evolutionary dynamics of this virus. Sequences (WG or HA/NA) were obtained from >900A (H3N2) viruses sampled in the UK during influenza seasons 2016/7 and 2017/8 and the inter-seasonal period. Viral RNA was extracted from clinical samples and amplified using a multi-segment RT-PCR. Amplicons were sequenced using Nextera library preparation for Illumina MiSeq sequencing. Sequence data ????were processed using BAM-SAM tools and PHE in-house scripts. Phylogenetic analysis of the HA gene indicates that they belong to genetic group 3C.2a, which has circulated since 2014. Season 2016/7 was characterized by the emergence of cluster 3C.2a.1; further genetic heterogeneity was seen with 6 new subclusters within 3C.2a and 3C.2a.1, with predominance of those characterized by amino acid changes N121K and S144K (3C.2a) and N121K, N171K, I406K, G484E (3C.2a.1). The NA genes clustered with a similar topology to the HA. Season 2017/8 was characterized by persistence of some clades from previous season with further diversification. Three of the 3C.2a clusters continued to circulate, with predominance of clade showing T131K, R142K, and R261Q (clade 3C.2a.2). The majority of HA sequences in 3C.2a1 fall into a new subcluster which has become predominant within this subgroup, with amino acid changes E62G, K92R, and T135K (3C.2a.1b). The topology of NA and internal gene trees showed evidence of reassortment events occurring at some point between the two seasons, with group 3C.2a2 acquiring NA and some internal genes from 3C.2a1 lineage viruses. The predominance of this group during 2017–8 might be due to fitness advantage related to the new genetic constellation. Emerging viruses from group 3C.3a also have acquired genes from lineage 3C.2a1, which could be the reason for their increased frequency to 20 per cent by the end of season 2017–8. Molecular epidemiology indicates emerging genetic diversity in A(H3N2)