

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.2a.1 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.2a.2 acquiring NA and some internal genes from 3C.2a.1 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.2a.1, 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)

viruses during the period of study, leading to co-circulation of variants. The frequency of circulating HA genetic groups was quite variable, with rapidly changing patterns of predominance. Evidence of reassortment events was observed which could be responsible for the rise and predominance of some clades, and might predict the emergence of other variants.

A30 Avian influenza viruses in wild birds: Virus evolution in a multi-host ecosystem

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Wild ducks and gulls are the major reservoirs for avian influenza A viruses (AIVs). The mechanisms that drive AIV evolution are complex at sites where various duck and gull species from multiple flyways breed, winter, or stage. The Republic of Georgia is located at the intersection of three migratory flyways: the Central Asian Flyway, East Asian/East African Flyway, and Black Sea/Mediterranean Flyway. For six consecutive years (2010–6), we collected AIV samples from various duck and gull species that breed, migrate, and overwinter in Georgia. We found substantial subtype diversity of viruses that varied in prevalence from year to year. Low pathogenic (LP)AIV subtypes included H1N1, H2N3, H2N5, H2N7, H3N8, H4N2, H6N2, H7N3, H7N7, H9N1, H9N3, H10N4, H10N7, H11N1, H13N2, H13N6, H13N8, and H16N3, plus two H5N5 and H5N8 highly pathogenic (HP)AIVs belonging to clade 2.3.4.4. Whole-genome phylogenetic trees showed significant host species lineage restriction for nearly all gene segments and significant differences for LPAIVs among different host species in observed reassortment rates, as defined by quantification of phylogenetic incongruence, and in nucleotide diversity. Hemagglutinin clade 2.3.4.4 H5N8 viruses, circulated in Eurasia during 2014–5 did not reassort, but analysis after its subsequent dissemination during 2016–7 revealed reassortment in all gene segments except NP and NS. Some virus lineages appeared to be unrelated to AIVs in wild bird populations in other regions with maintenance of local AIV viruses in Georgia, whereas other lineages showed considerable genetic inter-relationship with viruses circulating in other parts of Eurasia and Africa, despite relative under-sampling in the area.

A31 Diversity change of influenza A (H3N2) strains circulating in Brazil during 2017–8: What to expect in the coming winter?

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The H3N2 subtype of influenza A (H3N2) was the predominant strain during the early months of the 2017 influenza epidemic in Brazil. In Australia, it was responsible for a strong and prolonged 2017 season and reached the Northern hemisphere causing an intense 2017/8 influenza season. Several genetic and antigenic A(H3N2) variants were circulating, which made the decision about which strain to incorporate into the influenza vaccine challenging. For 2018, the WHO selected a new H3N2 strain, A/Singapore/INFIMH-16-0019/2016-like, to replace the strain A/HongKong/4801/2014-like in the Southern Hemisphere trivalent vaccine. The aim of this study was to describe the genetic diversity of influenza A (H3N2) viruses circulating in Brazil between January 2017 and January 2018, checking the match between the vaccines and worldwide circulating strains with the Brazilian influenza strains. Hemagglutinin gene sequencing of the influenza A (H3N2) was

performed, followed by a phylogenetic reconstruction using additional database sequences to define genetic groups and compare with other worldwide circulating strains. We observed a large diversity of H3N2 genetic clusters, including 3C.2a, 3C.2a1, 3C.3a, and their subgroups. During the 2016–7, inter-epidemic and 2017 epidemic period the cluster most frequently detected belonged to clade 3C.2a1 (148/185; 80.0%), a distinct group related to the 2017 vaccine strain A/HongKong/4801/2014-like (3C.2a). However, the genetic profile changed during the study period and in the inter-epidemic season 2017–8 the most commonly detected genetic group was the 3C.2a cluster (43/58; 74.1%). Inside this cluster, the majority (34/43; 79.1%) of strains belonged to a single genetic 3C.2a subgroup 2 (3C.2a2), bearing antigenic substitutions T131K and R142K (site A) and R261Q (site E). The dominance of this 3C.2a2 in the 2017–8 inter-epidemic period in Brazil was similar to the 2017–8 season in Europe and Canada according their surveillance data. The new vaccine strain has five to six antigenic changes in comparison to the predominant 3C.2a2 circulating in South America since September 2017 until now. It is possible that the vaccine mismatch will not protect the population against a majority of circulating strains. Surveillance of the vaccine effectiveness supported by antigenic and serological analysis are necessary to prove this hypothesis. However, this highlights the difficulty of vaccine strain selection and highlights the need for of a universal influenza vaccine.

A32 Genomic surveillance of Zika virus transmission in the Amazonas State, Brazil

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Zika virus (ZIKV) has caused an unprecedented epidemic linked to severe congenital syndromes. Transmission of ZIKV in the Americas was first confirmed in May 2015 in northeast Brazil, though the virus was likely introduced 1–2 years prior to its detection. Manaus, the capital city of the Amazonas State, the largest territory of any state in Brazil and the main economic center in the northern region, reported between 2016 and 2017 more than 2,327 suspected cases of ZIKV infection. To gain insights into the timing, source, and likely route(s) of ZIKV introduction in the Amazonas State, we tracked the virus by sequencing ZIKV genomes from infected patients. Using nanopore sequencing technology, we generated 56 Brazilian ZIKV genomes from Manaus city in the Amazonas state, sampled from human cases. On the basis of available sequences of isolates from the Americas, the Manaus sequences, we analyzed fell within a single strongly supported monophyletic clade (bootstrap support = 99%, posterior support = 1.00) that belongs to the Asian genotype. Genetic analysis suggests the outbreak most likely originated from transmission cycles not previously identified in North Brazil and not from a separate introduction into the Americas. Molecular dating analysis indicates that the outbreak was caused by a single founder strain that is estimated to have arrived in Manaus around February 2015. By analyzing surveillance and genetic data, we discovered that ZIKV moved among transmission zones in Manaus. Geographical analysis further indicates that the Northern part of the Manaus regions has a high transmission potential for ZIKV. Our work illustrates that near-real time genomics in the field can augment traditional approaches to infectious disease surveillance and control. Estimated dates for the international spread of ZIKV from the north region indicate the persistence of the virus transmission in recipient regions. Our study provides an understanding of how ZIKV initiates transmission in new regions.