NVRL

Annual Reference Virology Report 2017

Introduction

Dear Colleagues

We are delighted to present the NVRL Reference Virology Report for 2017. Again, we're slightly later than intended, but hopefully the data contained herein will still prove useful and informative for you and your service users.

As in previous years, the report predominantly covers the calendar year of 2016 for Measles, Mumps, Rubella, Polio and non-polio Enteroviruses, and the 2016-2017 'winter' season for influenza and those viruses causing respiratory and gastroenteric illness. However, in light of the date of publication, and in the interest of timeliness, we have included the 2017 Measles outbreaks in the current report.

New to this year's report are notes on the prevalence of HIV transmitted drug resistance in Ireland, and some sequence data on Hepatitis A and Hepatitis E viruses, which have been topical for different reasons in recent years. These data have been collated with our colleagues in the HPSC. In addition, we have provided a brief summary on our Zika testing figures, as the regional epidemic in the Americas seems to have subsided.

As always, on behalf of all the staff at the NVRL, we would like to sincerely thank all of you for your support over the last year: without you this work would not have been possible. Our primary collaborators are listed in Section 12 (page 32). We apologise in advance to anyone that has been inadvertently omitted.

Dr Suzie Coughlan, Dr Jeff Connell, Deirdre Burke, Dr Niamh O'Flaherty,

Dr Joanne O'Gorman, Dr Cillian De Gascun

January 2018

Executive Summary

- Influenza AH3 subclade 3C.2a1 was the predominant virus in Ireland during the 2016-17 season. Further characterisation at the WHO Collaborating Centres (CC) of this strain confirmed antigenic similarity to the 2016-17 northern hemisphere vaccine strain (A/Hong Kong/4801/2014).
- A significant proportion of circulating influenza AH3 viruses characterized at UCD NVRL during the 2016-17 season belonged to the genetic subgroup 3c.3a (14%). This pattern was distinct from the majority of the rest of Europe where 3C.3a viruses were rarely identified (<1%). The 3C.3a strain was also antigenically similar to the A/Hong Kong/4801/2014 vaccine strain.
- Respiratory Syncytial Virus (RSV) subtypes A and B co-circulated during the 2016-17 season and a high degree of genetic variability was evident within all circulating strains. RSV will continue to be monitored for the coming seasons in order to establish baseline RSV molecular epidemiology data for Ireland.
- Norovirus continues to be the predominant cause of outbreak and sporadic cases of viral gastroenteritis.
- There has been a significant decrease in the number of laboratory confirmed cases of rotavirus infection at UCD NVRL in 2016-17, compared to the 2015-16 seasonal peaks. This can most likely be attributed to the introduction of the rotavirus vaccine for infants of 2 and 4 months of age in December 2016.
- Many diagnostic assays for rotavirus do not distinguish between wildtype and vaccine (Rotarix) strains leading to a possible overestimation of the incidence of rotavirus infection in vaccinated infants. In December 2017, 93% of rotavirus positive cases, in vaccine eligible infants tested at UCD NVRL, were attributed to the vaccine strain. Mild diarrhoea can occur in up to 10% vaccinated infants within 28 days of vaccination.
- During 2017 outbreaks of measles virus infection continued across Europe with large numbers of cases in Romania, Italy and Germany. Measles virus B3 was the predominant genotype in Europe in 2016 and 2017. Two distinct measles outbreaks occurred in the East of Ireland in the last quarter of 2017 with 21 laboratory confirmed cases of genotype B3 associated infection. Infection occurred primarily in unvaccinated individuals, however,

measles infection was also detected in vaccinated individuals with attenuated clinical manifestations.

- There were no cases of Acute Flaccid Paralysis associated with enterovirus infection in 2017.
- Genetic characterisation of non-polio enteroviruses in patients presenting with neurological symptoms in 2016 highlighted a predominance of CV-A6, CV-B1 and CV-B5. These data are now routinely included in the HPSC quarterly reports on Invasive Meningococcal Disease, Bacterial/Viral Meningitis and Haemophilus influenzae in Ireland.
- Enhanced screening for EV-D68 identified 2 children presenting with severe respiratory symptoms.
- Fourteen patients were diagnosed with recent Zika virus infection in 2016, having returned to Ireland from Zika endemic areas, predominantly South and Central America. All patients were symptomatic and the majority were viremic (11/14 patients had detectable RNA in blood).
- The prevalence of transmitted HIV drug resistance among newly diagnosed HIV infected individuals during 2016 was 9.5%.
- Since 2016 there has been a widespread outbreak of Hepatitis A (HAV) amongst MSM in 22 European countries. Three HAV strains have been associated with the outbreak, each of which has been detected in patients diagnosed in Ireland.

Influenza Season 2016-17

Key Messages:

Influenza AH3 subclade 3C.2a1 was the predominant influenza virus in Ireland during the 2016-17 season. WHO Collaborating Centres characterisation of this viral strain confirmed its antigenic similarity to the 2016-17 northern hemisphere AH3N2 vaccine strain (A/Hong Kong/4801/2014)

A significant proportion of circulating influenza AH3 viruses characterized at the NVRL this season belonged to the genetic subgroup 3c.3a (14%). This pattern is distinct (with the exception of Spain) from the rest of Europe where 3C.3a viruses were rarely identified (<1%).

Influenza like illness (ILI) activity in Ireland started earlier than in previous seasons with rates above baseline levels from week 49 (2016) to week 5 (2017). High activity intensity ILI thresholds were surpassed in week 1 (2017) with rates approaching 120 cases/100,000 population [1]. The overall positivity rate for influenza, from sentinel and non-sentinel sources combined, exceeded 75% in week 52 (420/550 influenza RNA positive results). This is significantly higher than peak maximum detection levels in combined EU/EAA data [2] and reflects the high threshold for testing among Irish clinicians and the sensitivity of the influenza assays used.

Influenza AH3 viruses predominated in Ireland, accounting for 92% detections. This reflected activity in the rest of Europe where AH3 represented 87% detections during the 2016/2017 season (Figure 1).

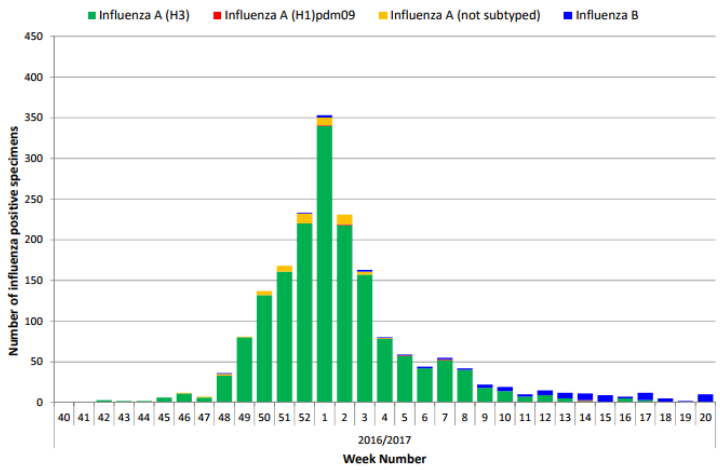


Figure 1: Number of influenza RNA positive specimens: Specimens are from both sentinel and non-sentinel sources and are displayed by influenza type/subtype and by week for the 2016/2017 influenza season

Genetic and Antigenic Characterisation of Influenza viruses

Linda Dunford

Throughout the season (week 40 2016 – week 20 2017), 117 influenza A H3, 1 influenza A H1, 7 influenza B-Yamagata, and 1 influenza B-Victoria viruses were genetically characterised and compared to the appropriate reference and vaccine strains. These data was submitted to the WHO Global Influenza Surveillance and Response System (GISRS) prior to the WHO Consultation and Information Meeting on the Composition of Influenza Virus Vaccines for the Northern Hemisphere 2017/18 [3].

Of particular interest, 14% (n=17) of characterized viruses clustered within the genetic subgroup 3C.3a, represented by A/Switzerland/9715293/2013, and had amino acid substitutions Q197K, S198P and S312N in HA1 antigenic sites B and C (Figure 2). The A/Switzerland/9715293/2013 strain was included in the 2015/2016 northern hemisphere vaccine. 3C.3a viruses were rarely identified in Europe during the season (with the exception of Spain) representing less than 1% of the circulating A H3 viruses characterized across Europe. Specimens that were genetically characterised as 3C.3a were antigenically indistinguishable from the vaccine strain.

The majority of A(H3N2) viruses (72%, n=84/117) clustered in the recently emerged subclade 3C.2a1, a group represented by A/Bolzano/7/2016 and characterised by the hemagglutinin amino acid mutation N171K, often with N121K (49%). Group 3C.2a1 was the dominant strain in Europe during the season. Antigenic characterization, carried out at WHO CC, confirmed that these viruses are antigenically similar to the vaccine strain, 3C.2a. The 3C.2a1 clade is evolving rapidly and several additional amino acid mutations have emerged resulting in a number of clusters within the 3C.2a1 subclade. Ninety-five percent of 3C.2a1 viruses carried the characteristic mutation for 3C.2a1 - N171K – but there was one cluster of 4 viruses that contained an N171**R** substitution: nevertheless, these viruses clustered with the other 3C.2a1 viruses.

Sixteen viruses (14%) fell in the vaccine component clade 3C.2a, represented by A/Hong Kong/4801/2014, which is the strain proposed for the 2017-18 vaccine. The 3C.2a viruses detected in Ireland fell into two clusters – one associated with N144K and one with R261Q mutations.

Influenza A(H1N1)pdm09 was infrequently detected in Ireland during the 2016-17 season. One Influenza A(H1N1)pdm09 virus was characterised and belonged to the 6B.1 genetic clade, represented by A/Michigan/45/2015 (Figure 3). Antigenic characterization data from Europe has found this group to be antigenically indistinguishable from the vaccine component virus

A/California/7/2009. The A/Michigan/45/2015 has been selected for inclusion in the 2017/18 season's vaccine.

Eight influenza B viruses were genetically characterised this season, of which 7 were B-Yamagata lineage and 1 belonged to the B-Victoria lineage. The 7 influenza B-Yamagata viruses clustered in clade 3 represented by B/Phuket/3073/2013. The influenza B-Victoria lineage virus fell into the 1A group represented by B/Brisbane/60/2008, the virus recommended for inclusion in the 2017/2018 vaccine (Figures 4 and 5).

UCD NVRL service for characterisation of influenza viruses:

UCD NVRL is the WHO recognised National Influenza Centre (NIC) for the Republic of Ireland. In order to provide representative national data to WHO CC and to maximise the detection of genetic variants, microbiology departments are encouraged to submit samples for molecular sequencing and antigenic characterisation in the following circumstances:

- influenza positive samples which cannot be subtyped
- influenza A positive samples from patients with a recent travel history in particular to Asia and Eqypt
- fatal cases of influenza infection and cases requiring ITU/HDU admission
- vaccinated patients that subsequently develop infection which results in admission to hospital

This programme has been in operation for many years and UCD NVRL would like to acknowledge and thank our colleagues in clinical microbiology laboratories for the contribution of influenza viral isolates for culture, antigenic, and genetic characterisation. Data from the NVRL influenza surveillance programme are submitted to the ECDC European Surveillance Database (TESSy) and to the WHO Global Influenza Surveillance and Response System (GIRIS) programme.

Please contact Dr Jeff Connell (jeff.connell@ucd.ie) for further details on how to refer specimens to the laboratory.

Figure 2: Maximum Likelihood Phylogenetic Analysis of Influenza A H3 Viruses Detected in Ireland during the 2016/2017 respiratory season. The analysis involved 117 sequences from Irish patients and 30 of this season's recommended reference sequences downloaded from GISAID. Red, Reference and clade representative strains; Green, Irish strains. Branches for clusters of $\geq$ 4 viruses are collapsed and each branch has been annotated with the number of Irish viruses in the cluster and whether or not any of these are ICU patients, Vaccine Failures (VF) or patients who subsequently died. Some amino acids exclusively associated with these clusters are listed.

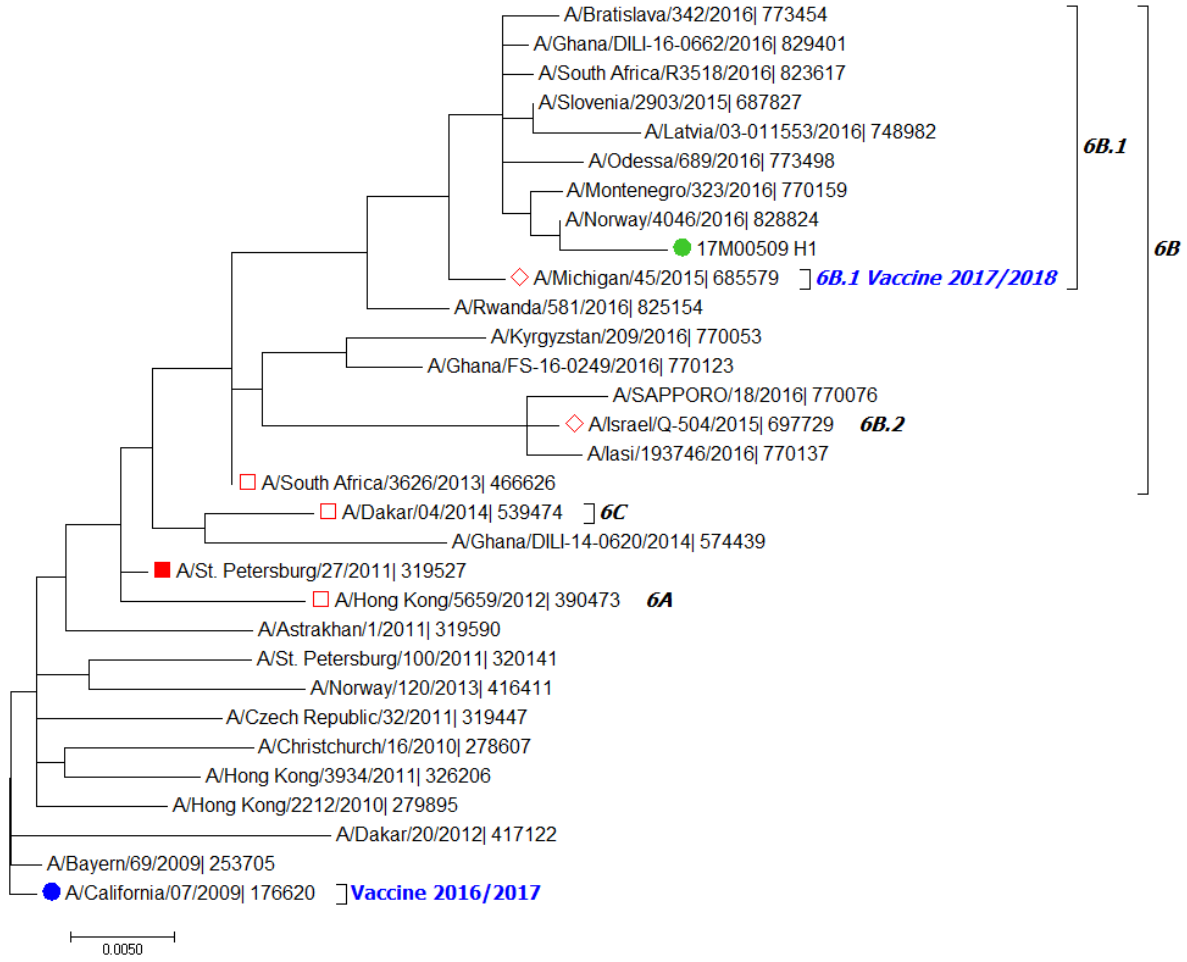


Figure 3: Maximum Likelihood Phylogenetic Analysis of Influenza A H1 strains detected in Ireland during the 2016/2017 season. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model with a discrete Gamma distribution and a proportion of invariable sites. Scale bar indicates the number of substitutions per site. Evolutionary analyses were conducted in MEGA7. Red, Reference and clade representative strains; Green, Irish strain. The vaccine strains are annotated in blue.

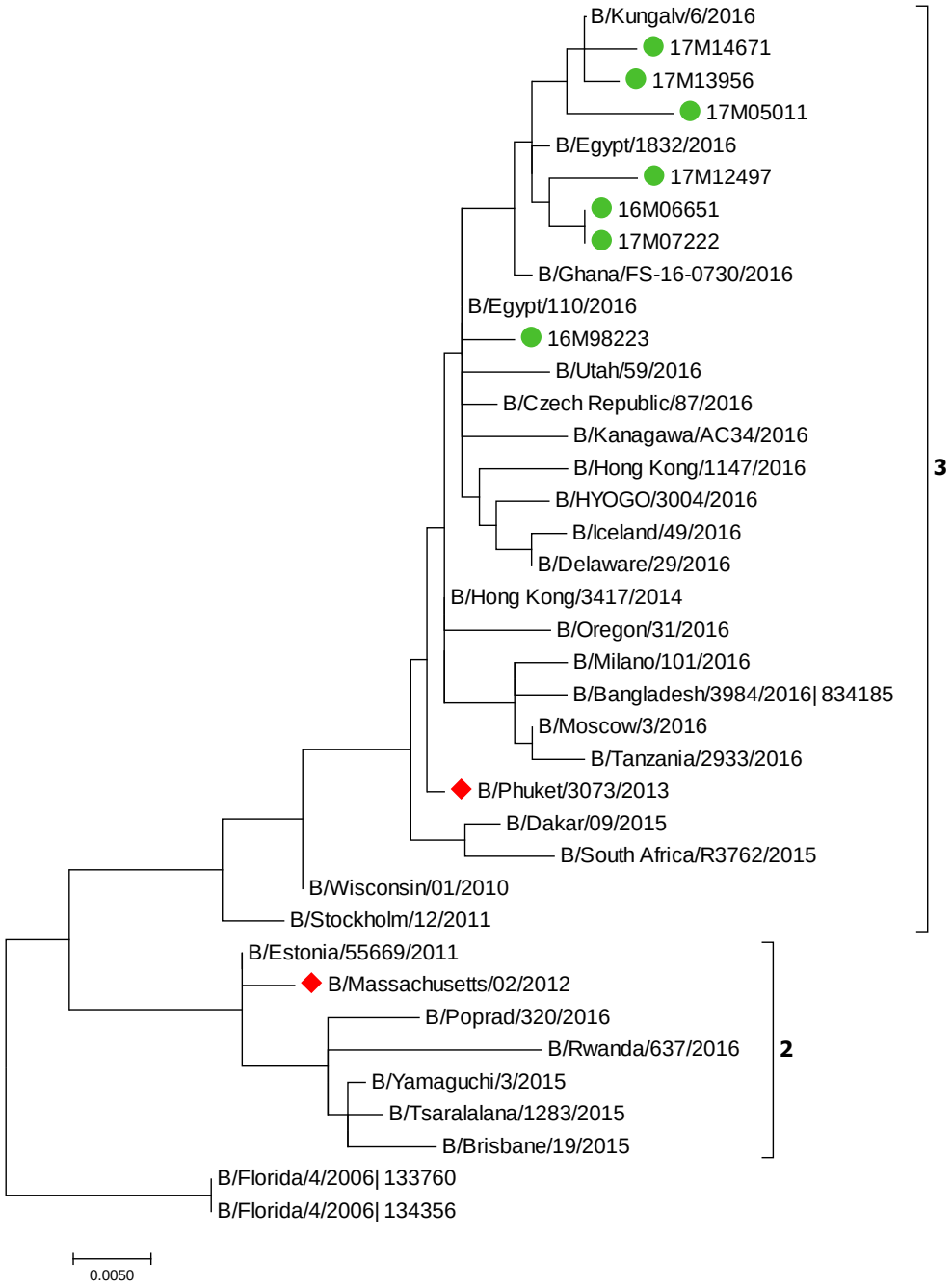


Figure 4: Maximum Likelihood Phylogenetic Analysis of Influenza B Yamagata-lineage viruses detected in Ireland during the 2016/2017 season. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model with a discrete Gamma distribution and a proportion of invariable sites. Scale bar indicates the number of substitutions per site. Evolutionary analyses were conducted in MEGA7. Red, Reference and clade representative strains; Green, Irish strain.

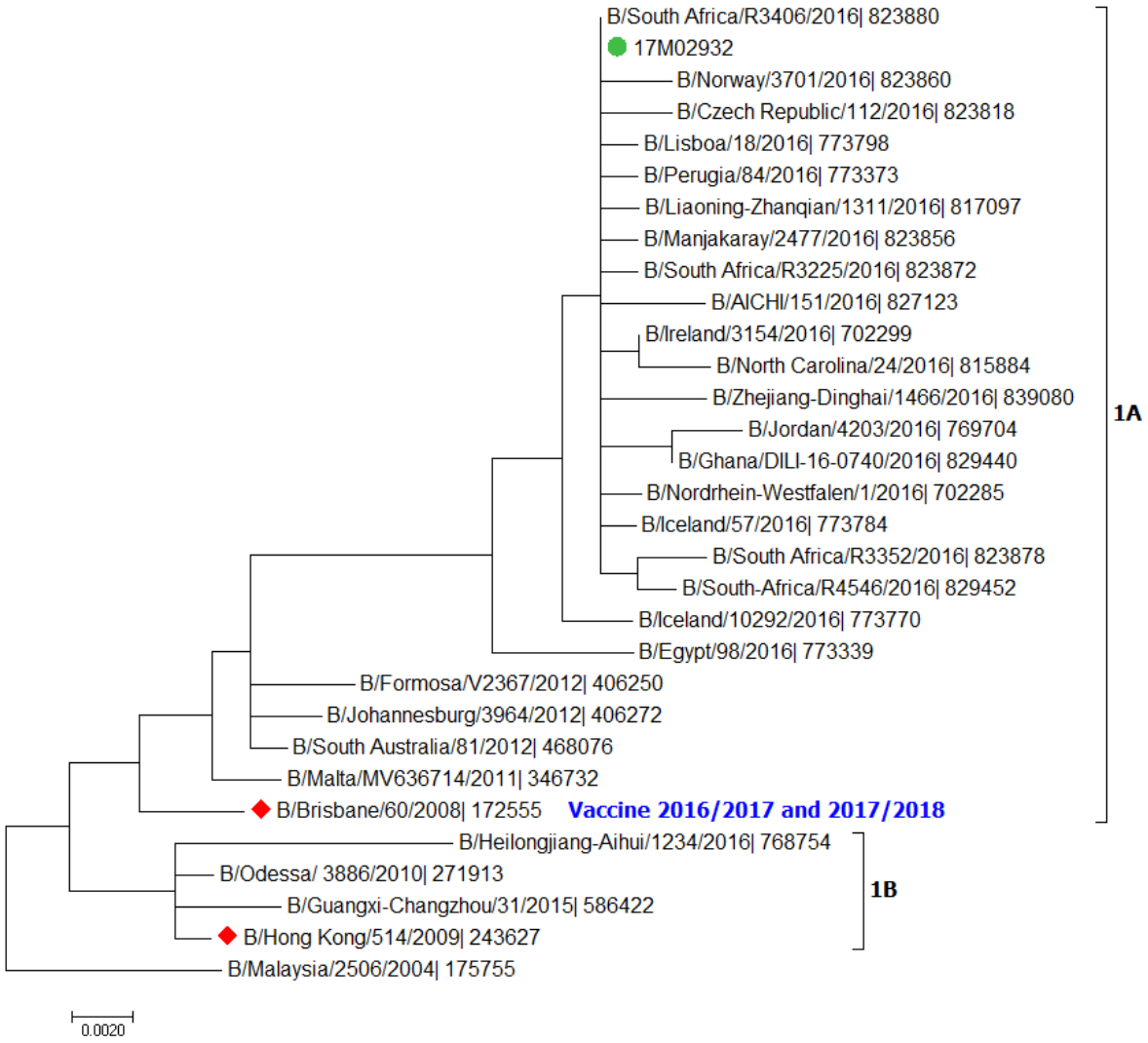


Figure 5: Maximum Likelihood Phylogenetic Analysis of Influenza B Victoria-lineage viruses detected in Ireland during the 2016/2017 season. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model with a discrete Gamma distribution and a proportion of invariable sites. Scale bar indicates the number of substitutions per site. Evolutionary analyses were conducted in MEGA7. Red, Reference and clade representative strains; Green, Irish strain.

Respiratory Syncytial Virus:

Allison Waters and Doireann Waldron O'Loughlin

UCD NVRL notified 1,273 laboratory confirmed RSV infections in the 2016/17 season with a peak of 143 cases diagnosed in week 49 (Figure 6).

Notably, the introduction of a new multiplex respiratory virus diagnostic platform determined that both RSV A and RSV B subytpes were co-circulating in significant numbers throughout the season. Furthermore, preliminary characterisation data has shown significant genetic variability across all RSV strains and subtypes. The WHO and ECDC are hoping to establish European RSV surveillance as part of the overall influenza-like illness (ILI)/ acute respiratory illness (ARI) monitoring programmes. In light of this, RSV will continue to be monitored at the genetic level for the coming seasons in order to establish baseline RSV molecular epidemiology data for Ireland.

Overall, during the respiratory season, 65% of specimens received were positive for at least one viral pathogen. There was a notable increase in the overall positivity rate and the number and diversity of viruses detected, since the introduction of the extended screen in January 2017, particularly with high numbers of human metapneumovirus and parainfluenza virus infections (Figure 4).

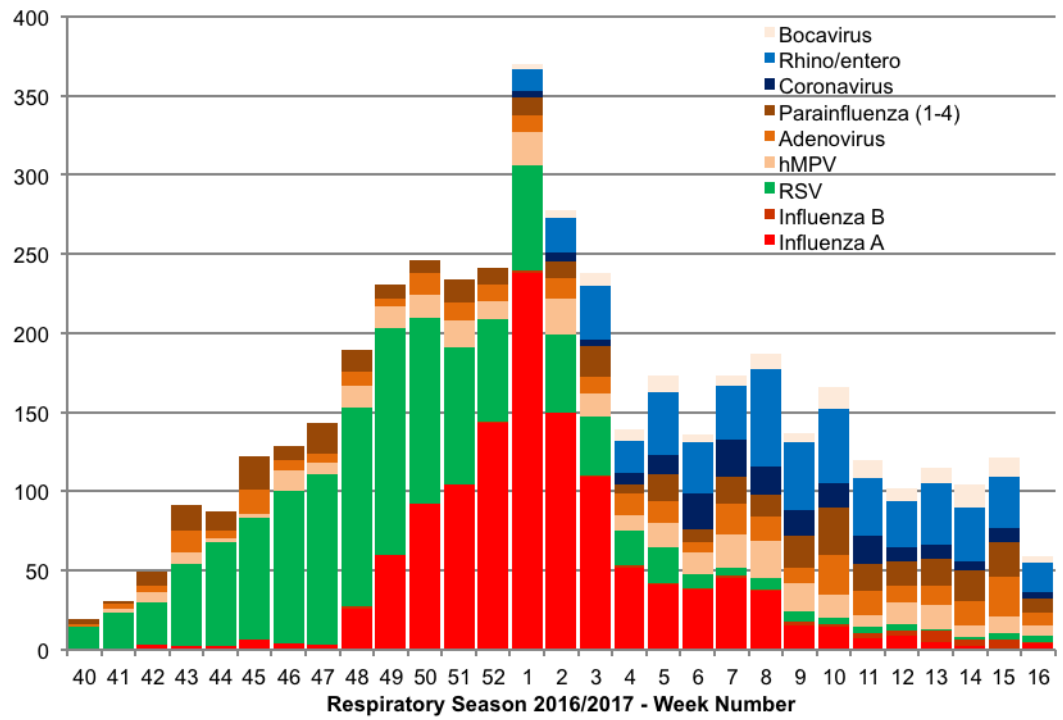


Figure 6: Number of non-sentinel positive specimens detected by the NVRL during the 2016/2017 season. The extended respiratory screen was introduced in week 1 2017. The RSV season peaked in week 49 with a total 143 detections.

Viral Gastroenteritis Season 2016-17

Key Message:

Noroviruses continue to be the predominant cause of outbreak and sporadic cases of viral gastroenteritis among patients tested at the NVRL.

There has been a significant decrease in the number of laboratory confirmed cases of rotavirus infection at UCD NVRL in 2016-17 when compared to the 2015-16 seasonal peaks. This can most likely be attributed to the introduction of the Rotavirus vaccine for infants of 2 and 4 months of age in December 2016.

As in previous years, norovirus (NoV) continues to be the major cause of infectious viral gastroenteritis during the winter season in Ireland. Between July 2016 and June 2017, 57% (n=1,008) of all laboratory confirmed cases of viral gastroenteritis were caused by a NoV strain. The seasonality of the epidemic changed somewhat from the previous season with the peak of activity in the first rather than the second quarter of the year (Figure 7). A significant proportion of notifications in week 2 2017 was associated with a large outbreak of GGI NoV in the North East of the country. In addition to NoV infections, the prevalence of rotavirus (n=488), sapovirus (n=173) and astroviruses (n=104) followed predictable seasonality during the winter months (Figure 8). Cases of enteric adenovirus (n=135) do not appear to follow seasonal trends with constant levels of detection throughout the year.

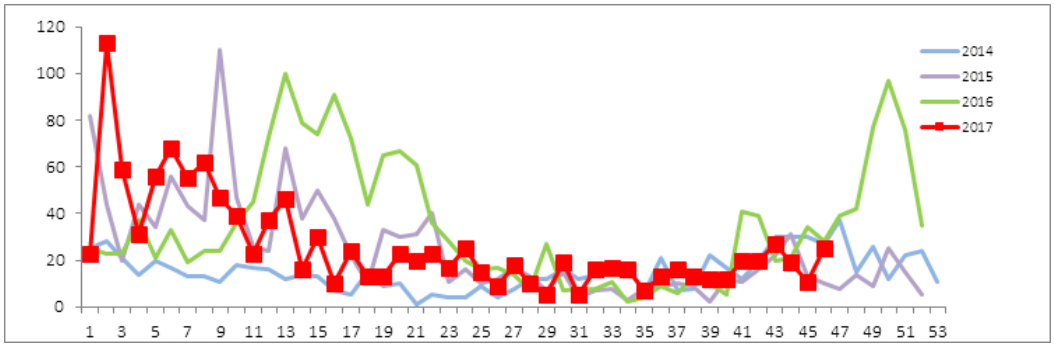


Figure 7: Weekly norovirus notification data from CIDR - 2014 to 2017. Data produced in collaboration with HPSC and is based on the clinical notifications of NoV infection to CIDR. The NVRL would like to acknowledge Fiona Cloak (HPSC) for producing this graph.

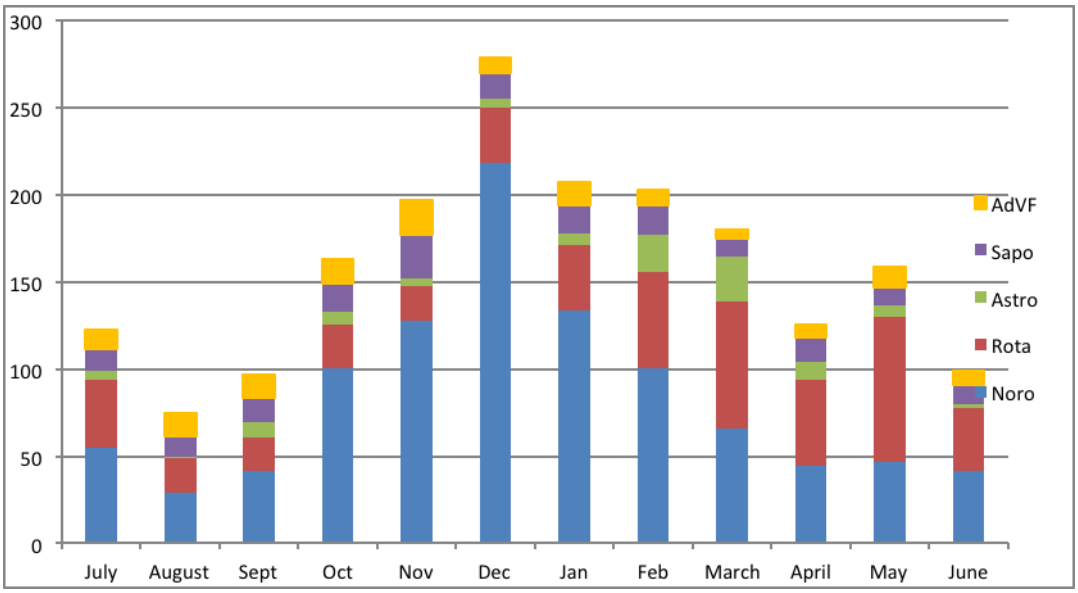


Figure 8: Relative prevalence of viral pathogens associated with gastroenteritis between July 2016 and June 2017. Data shows the number of detectable pathogens in stool samples

NoV RNA was detected in patients of all age groups with the majority of infections in patients over 5 years of age (Figure 9). This is in direct contrast to the other viral pathogens associated with gastroenteritis (rotavirus, astrovirus, sapovirus and enteric adenovirus) which tend to predominate in younger children.

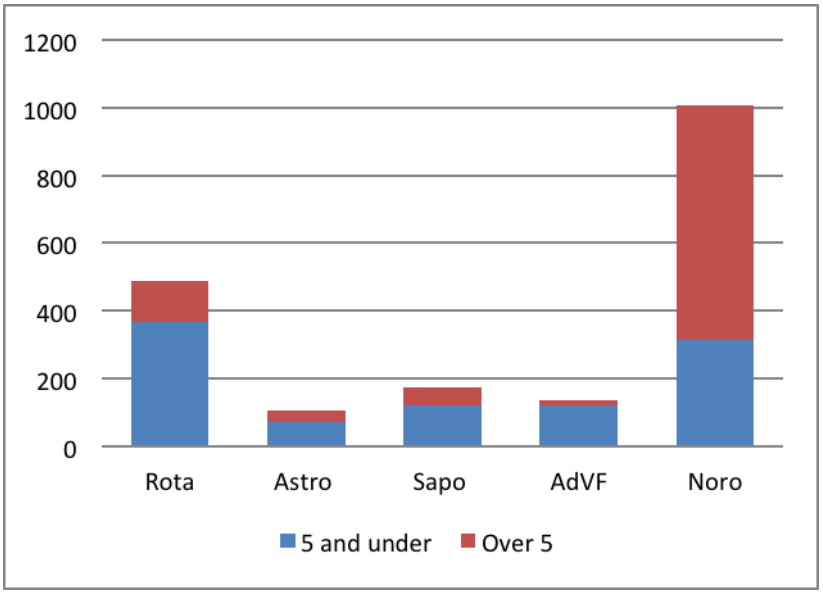


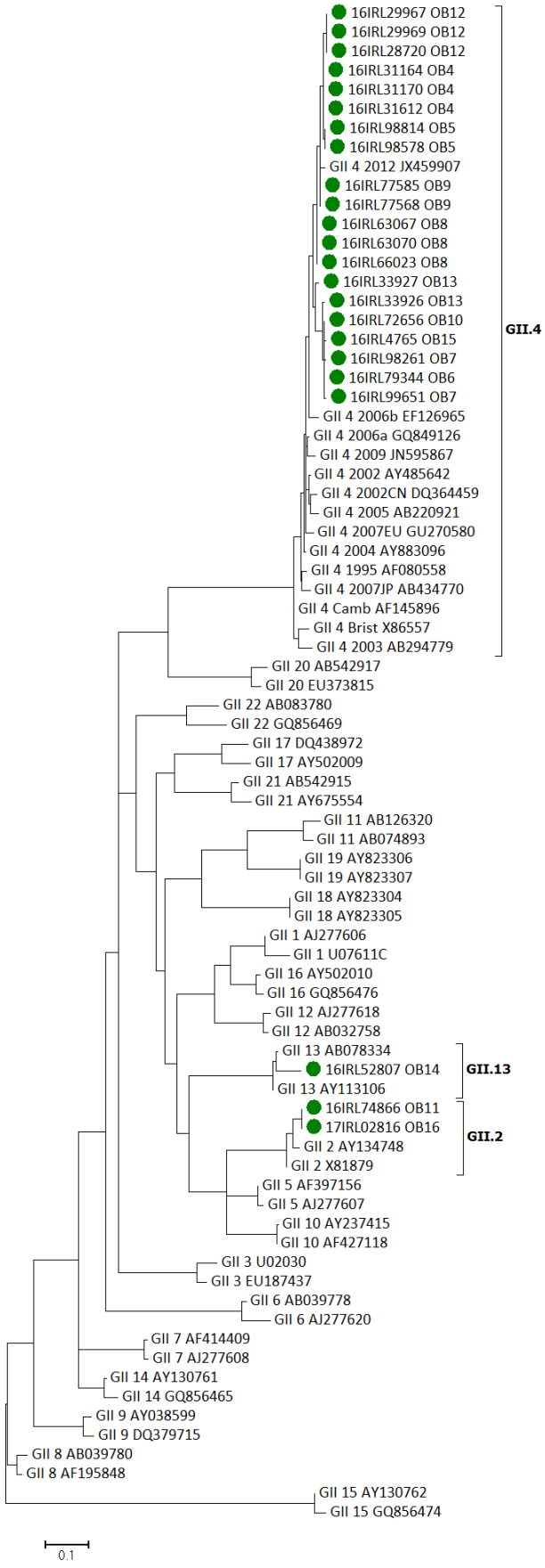
Figure 9: Detection of viral pathogens associated with gastroenteritis in stool samples collected between July 2016 and June 2017 (n = 1,908). Data is analysed in discreet age groups (<5 years and >5 years). The majority of samples were submitted from acute hospitals and residential healthcare facilities.

Genetic characterisation of Noroviruses

Grainne Tuite

UCD NVRL has been analysing nucleotide sequence data from circulating NoV strains in Ireland since 2003. The analysis has allowed an understanding of national and local epidemics of disease and has been valuable during specific outbreak investigations (Figure 10). Limited epidemiological and linked molecular data have been submitted to a NoroNet database hosted by collaborators at the National Institute of Public Health and the Environment in the Netherlands. This database, links clinical-, public health-, and food microbiology laboratories, that are willing to share norovirus molecular and epidemiological data on outbreaks and sporadic cases. The initiative started as EU funded network in 1999, continuing since 2002 as a global NoV surveillance initiative. (www.rivm.nl/mpf/typingtool/norovirus/).

In 2017 the network published the first comprehensive analysis of molecular surveillance NoV data collected between 2005 and 2016 [5]. The study encompassed 16635 sequences with associated epidemiological metadata from 19 countries in Europe, Asia, Oceania, and Africa. The findings highlight the continuous global genetic diversity of noroviruses and document the emergence of the GII.P16-GII.4 Sydney 2012 variant in Europe to quickly become the dominant virus globally. The novel GII.P17-GII.17, first reported in Asia in 2014 has now also been widely detected in some European countries, though not yet in Ireland. The authors emphasis the need for continuous and widespread global surveillance to monitor immune escape, virus evolution by recombination to provide an overview of norovirus epidemiology for future vaccine development and policy decisions.


16IRL29967 OB12
16IRL29969 OB12
16IRL28720 OB12
16IRL31164 OB4
16IRL31170 OB4
16IRL31612 OB4
16IRL98814 OB5
16IRL98578 OB5
GII 4 2012 JX459907
16IRL77585 OB9
16IRL77568 OB9
16IRL63067 OB8
16IRL63070 OB8
16IRL66023 OB8
16IRL33927 OB13
16IRL33926 OB13
16IRL72656 OB10
16IRL4765 OB15
16IRL98261 OB7
16IRL79344 OB6
16IRL99651 OB7
GII 4 2006b EF126965
GII 4 2006a GQ849126
GII 4 2009 JN595867
GII 4 2002 AY485642
GII 4 2002CN DQ364459
GII 4 2005 AB220921
GII 4 2007EU GU270580
GII 4 2004 AY883096
GII 4 1995 AF080558
GII 4 2007JP AB434770
GII 4 Camb AF145896
GII 4 Brist X86557
GII 4 2003 AB294779
GII.4
GII 20 AB542917
GII 20 EU373815
GII 22 AB083780
GII 22 GQ856469
GII 17 DQ438972
GII 17 AY502009
GII 21 AB542915
GII 21 AY675554
GII 11 AB126320
GII 11 AB074893
GII 19 AY823306
GII 19 AY823307
GII 18 AY823304
GII 18 AY823305
GII 1 AJ277606
GII 1 U07611C
GII 16 AY502010
GII 16 GQ856476
GII 12 AJ277618
GII 12 AB032758
GII 13 AB078334
16IRL52807 OB14
GII 13 AY113106
GII.13
16IRL74866 OB11
17IRL02816 OB16
GII 2 AY134748
GII 2 X81879
GII.2
GII 5 AF397156
GII 5 AJ277607
GII 10 AY237415
GII 10 AF427118
GII 3 U02030
GII 3 EU187437
GII 6 AB039778
GII 6 AJ277620
GII 7 AF414409
GII 7 AJ277608
GII 14 AY130761
GII 14 GQ856465
GII 9 AY038599
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0.1

Figure 10 (A)

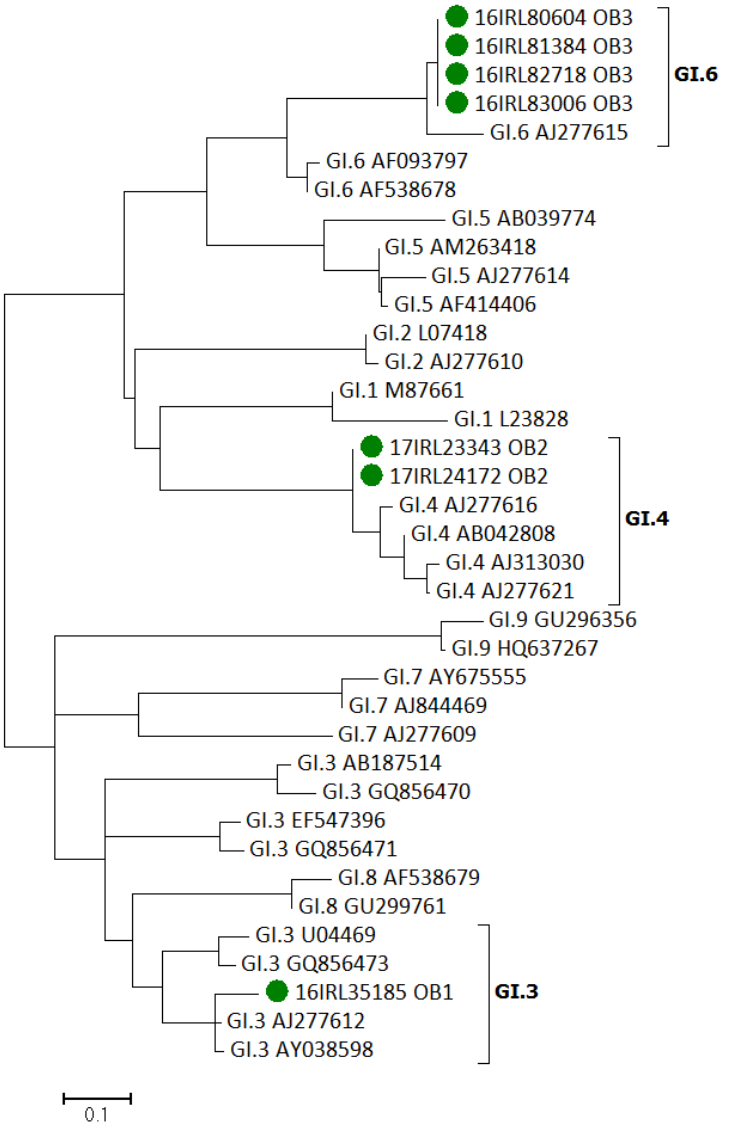

Figure 10 (B)

Figure 10: Maximum Likelihood (ML) Phylogenetic Analyses of Genogroup II (A) and I (B) Noroviruses associated with 16 distinct outbreaks in 2016/2017. Sequences are aligned with a panel of international reference sequences. A Hasegawa-Kishino-Yano model of evolution was used with a discrete Gamma distribution. Scale bar indicates the number of substitutions per site. Evolutionary analysis was conducted in MEGA7. The green dots represent the Irish strains. (A) ML tree was constructed for 246 nucleotide fragments of norovirus genogroup II RdRp ORF2 region of GII viruses (B) ML tree was constructed for 263 nucleotide fragments of norovirus genogroup I RdRp ORF2 region of GI viruses.

Rotavirus (RV) infection 2016-17

Rotarix, a live monolvalent attenuated human type G1P[8] virus vaccine, was introduced by the HSE in December 2016 for babies born on or after October 1st 2016. The vaccine is estimated to be between 82 and 94% efficacious and has resulted in a 77% decline in laboratory confirmed RV cases in England in the first year post introduction of the vaccine there [6]. The impact of vaccination on the prevalence and circulating types of RV in Ireland has not yet been determined. However, incident data from UCD NVRL demonstrate a 46% decrease in the number of laboratory confirmed RV cases between the peak month in 2015-16 (May 2016; n =153 cases) and that in 2016-17 (May 2017; n = 83) (Figure 11). These data are likely to be an underestimate of the actual decrease in RV infection as Rotarix vaccine RNA is also detectable (and indistinguishable from wildtype) in many available commercial and "in-house" assays.

As a result of this, in December UCD NVRL introduced an RV subtyping RT-PCR assay which will discriminate between wildtype RV or the Rotarix vaccine strain. During the first month of testing 93% (n=38) RV strains in suspected clinical cases in vaccine eligible infants were actually the Rotarix vaccine strain.

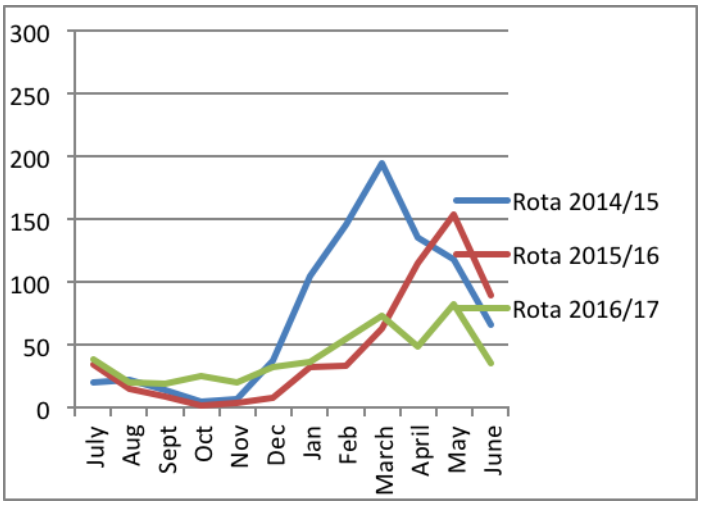

Figure 11: Seasonal Rotavirus detections between July 2014 and June 2017. Absolute numbers of stool samples with Rotavirus RNA detected are presented.

Genetic characterisation of Rotavirus:

Zoe Yandle

Classification of Rotavirus type A viruses is based on structural viral proteins (VP) of which there are at least 35 different VP7 antigens (G-types) and 50 different VP4 antigens (P-types) among which five G–P combinations G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] account for more than 90% of all RV

infections in humans worldwide. Rotarix, has been shown to have cross protection against all of the major types: however, efficacy against emergent RV strains has not been assessed. In Ireland G1P[8] continues to be the dominant strain, although diverse strains have been detected in the small sample popultation assessed by genotyping (Figure 12). The impact of the vaccine on the relative prevalence of rotavirus disease and strain fluctuation will be monitored by UCD NVRL and colleagues at HPSC.

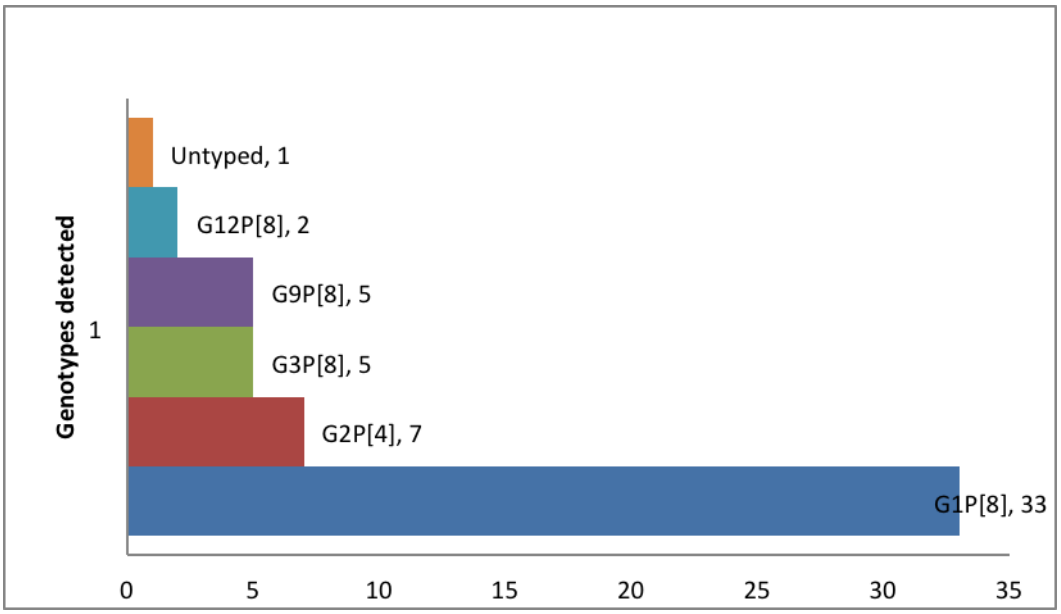

Figure 12: Genotypic analysis of clinical cases of RV infection in Ireland in the 2016-17 season (n=53). Nomenclature and typing methods outlined in WHO manual on rotavirus detection and characterisation methods (http://apps.who.int/iris/bitstream/10665/70122/1/WHO_IVB_08.17_eng.pdf)

Measles and Rubella in Ireland 2016

Jeff Connell and Grainne Tuite

Eliminating measles and rubella is a core goal of the European Vaccine Action Plan 2015–2020 and an important part of WHO Measles and Rubella Global Strategic Plan 2012-2020. UCD NVRL is the accredited WHO Measles/Rubella National Laboratory for diagnosis and viral characterisation and participates in regular WHO proficiency programmes for serological/molecular testing and characterisation of both viruses.

The sixth meeting of WHO European Regional Verification Committee on measles and rubella elimination in June 2017 stated that Ireland has successfully ended rubella transmission and endemic transmission of measles for 24 months. However, between 1st July 2016 and 1st July 2017, there were 10866 cases of measles reported by 30 EU countries and circulating genotypes were B3 and D8. Whereas for the same time period, there were 819 rubella cases, of which 632 were reported from Poland. Therefore Ireland is at continued risk of imported measles and rubella.

Recent measles outbreaks in Ireland have been associated with imported infection followed by endogenous transmission, predominately among unvaccinated individuals. Measles infection continues to cause significant morbidity among infected individuals with hospitalisation rates of up to 40% cases as seen in the 2016 outbreak in the Eastern part of Ireland [7]. There is a well established system for the investigation of suspected measles cases with the urgent collection of samples, particularly oral fluid samples, very soon after rash onset. In conjunction with the sample there is generally good information supplied relating to date of rash onset relative to sample collection and often MMR history. This facilitates the appropriate test selection, rapid diagnosis and subsequent genotyping of cases. However, the investigation for recent rubella infection is not yet comparable with measles.

No case of acute rubella infection was identified by UCD NVRL in 2017. However, a significant number of rubella IgM investigations are performed at post- delivery as they are included in the "TORCH" screen. However, the number of rubella IgM tests performed are unlikely to reflect probable cases of rubella infection with limited or no clinical information and therefore it is not possible to link investigations to clinical notifications of suspected rubella meaning case based investigation is difficult to perform.

As highlighted above, there was a significant number of rubella infections in Europe, especially in countries with close links to Ireland. There is probably significant herd immunity to rubella in Ireland, through the efficacy of the rubella component of the MMR. However, the majority of the acute cases of measles occurred in the unvaccinated cohort and therefore there is potential for rubella infection. Consequently, any case of suspected acute rubella in particular with a recent travel history has to be investigated using both oral fluid and serum samples.

Measles Outbreaks in 2017

Aoife Ronayne and Martha Neary

During the fourth quarter of 2017 two measles outbreaks were reported in the North Dublin regions and then the following week in the North East of the country. No epidemiologic link between outbreaks has yet been identified. This is on the background of ongoing measles outbreaks in EU/EEA member states – most notably in Romania, Italy, Greece and Germany. During the outbreak investigations 169 oral fluid samples, collected using the OraCol collection device, available from the NVRL (https://nvrl.ucd.ie/swabs) or swabs collected from the upper respiratory tract were tested from 154 symptomatic patients. Oral fluid samples were tested for measles RNA using a one-step RT PCR targeting the H and N genes of measles and also for measles IgM as required. Among this group there were 21 laboratory confirmed cases of measles: - 13 from the North Dublin area and 9 from the North East region. The age range of cases was from 1 month to 57 years. Five cases occurred in patients less than 1 year who were not likely to have been vaccinated as the recommendation for MMR vaccination in Ireland is 12 months.

Genotype analysis identified the same measles genotype B3 which were indistinguishable between cases in both outbreaks (Figure 13). In addition to the 21 laboratory confirmed cases, 2 cases of vaccine associated mini-measles illness were identified by genotyping a genotype A vaccine strain. Both cases were in children with a recent history of MMR vaccination. UCD NVRL responded to these outbreaks by increasing frequency of testing provided in order to diagnose and genotype cases. The clinical and laboratory teams were available for advice and actively participated in outbreak meetings convened by the HPSC.

In those cases which were measles RNA negative, additional were performed to identify the causative agent of the rash, HHV-6 DNA, enterovirus RNA, human parechovirus RNA, parvovirus B19 DNA, rubella-specific IgM and rubella RNA. Of note HHV-6 DNA and enterovirus RNA was detected in 64.9% and20.1% of samples, respectively. Parvovirus DNA was detected in only 2 samples (6.3%).

No samples referred for additional testing for, rubella or parechovirus were positive. 52 (72.2%) of the patients who had HHV-6 DNA detected were under 2 years of age, 17 (23.6%) were between the ages of 2 years to 5 years highlighting the importance of testing for HHV-6 DNA during a measles outbreak or investigation of a child with rash illness.

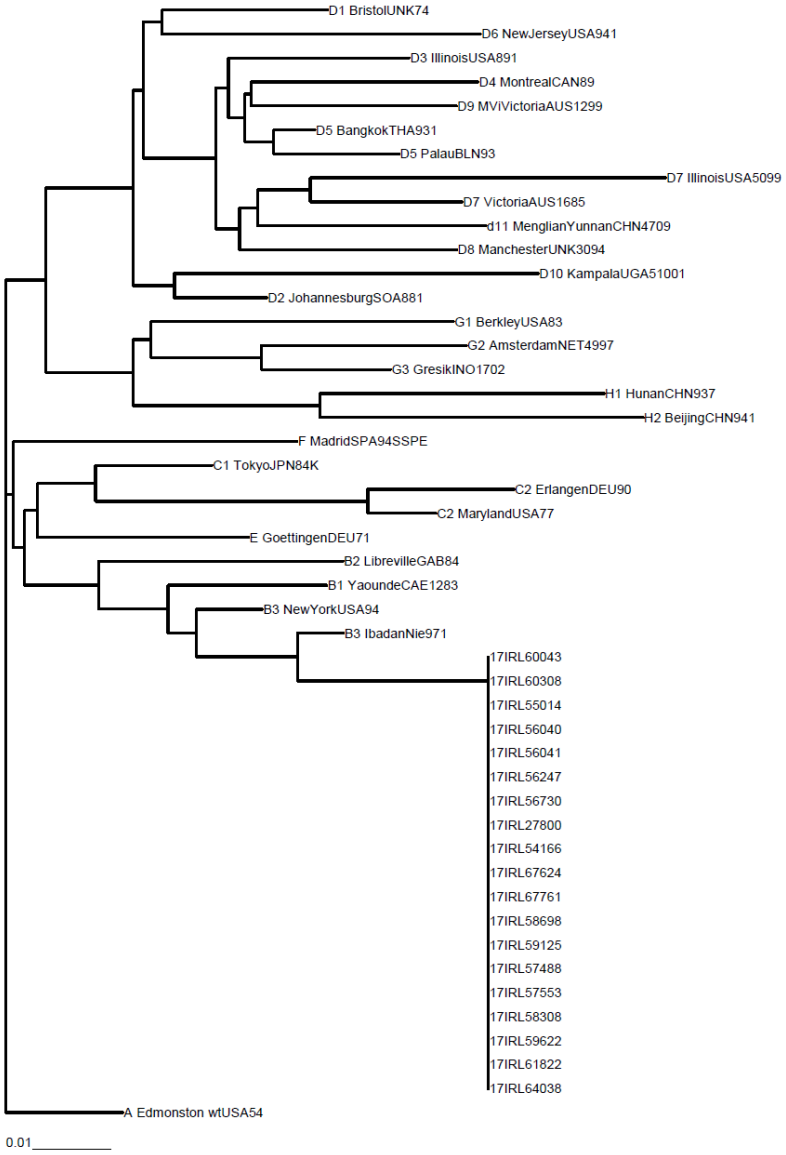

Figure 13. Phylogenetic analysis of the measles 2017 outbreaks attributed to genotype B3 virus This Neighbour Joining tree was constructed using the sequence data generated from the C-terminal 450 nucleotides of the N gene of measles virus. Patient samples (N=19) were analysed alongside WHO reference sequences also containing the specified 450 nucleotides of the N gene of measles. Genotype is assigned based on sequence similarity to the WHO reference sequences which are annotated with the confirmed genotype.

Enterovirus and Picornavirus Surveillance 2016

Key Messages:

Surveillance for polio viruses and non-polio enteroviruses continued at UCD NVRL throughout 2016. Genetic characterisation of non-polio enteroviruses in patients presenting with neurological symptoms highlighted a predominance of CV-A6, CV-B1 and CV-B5.

Enhanced screening for EV-D68 in children presenting with severe respiratory symptoms revealed 2 cases. Four cases of EV-71 subgenogroup C1 were also detected among patients with neurological symptoms.

Poliovirus Surveillance 2016

Jeff Connell and Alison Kelly

The last case of wild poliovirus infection in Ireland was 1984. In 2015, The Global Certification Commission certified that wild poliovirus 2 (WPV2) had been eradicated. Subsequently, polio 2 was removed from the live polio vaccine in 2015. In 2016 there were 37 cases worldwide of wild type polio, restricted to Afganistan, Pakistan and Nigeria. Therefore, to ensure that case of polio virus infection are identified in Ireland, active investigation of Acute Flaccid Paralysis cases for poliovirus occurs in conjunction with enterovirus surveillance.

UCD NVRL is the WHO accredited National Polio Laboratory for the diagnosis and molecular typing of polioviruses. Paediatricians are advised to urgently investigate (and inform HPSC) all children <15 years of age presenting with acute flaccid paralysis but also Guillain-Barre Syndrome, acute myelopathy and peripheral neuropathy. These results are reviewed annually by the National Certification Committee (NCC). In 2016, the NCC reviewed 7 cases of acute paralysis in children, none of which were caused by polio virus. These 7 cases included five Guillain-Barre Syndrome cases and 2 other neurotropic infections, one of which was confirmed as being attributable to enterovirus D68 [9].

In addition, for surveillance purposes 308 faecal samples were cultured and 84 (27.3%) grew enteroviruses which were subsequently types as non-polio enteroviruses. In 2017, UCD NVRL introduced a new laboratory testing algorithm for the rapid characterization of polioviruses identified in tissue culture. This methodology is based on dual stage real-time PCR techniques for intratypic differentiation (ITD) and vaccine-derived poliovirus (VDPV). As a result, ITD results of polioviruses will be available earlier for appropriate programme actions.

Non-Polio Enterovirus Surveillance 2016

Ursula Morley

Classification of enterovirus (EV) into one of the 116 circulating types remains an important priority for the understanding of the genotype-dependent pathogenicity of this family of viruses and in the development of antiviral agents, and vaccine candidates against EV types associated with significant morbidity. Ireland is one of 26 European countries that performs non-polio EV surveillance for clinical cases presenting with neurological conditions [10]. In 2016, UCD NVRL tested 3,517 CSF samples for EV as part of the clinical investigation of suspected meningoencephalitis, of which 234 had detectable EV RNA in CSF (6.7% positivity rate for EV). When throat swab, stool, and other samples are included, 809 samples had detectable EV RNA (11.9% positivity), highlighting the importance of collecting additional specimen types when investigating neurological symptoms, in particular as non-CSF samples are required for viral culture. Enteroviruses were found in all age groups, prevalence was highest in children aged <1 year. Seasonal trends were observed as expected during this period, with EV infections occurring primarily during the summer and autumn months as is seen in other countries.

Also in 2016, EV characterisation was performed on 26.6% (n=215/809) of positive samples which revealed that the majority of sequences belonged to EV species B (62.8%), followed by EV species A (35.8%), and EV species D (1.4%). EVs within group C were not detected in this population of samples tested. The most common EV strains included CV-A6 (n=37), CV-B1 (n=29), CV-B5 (n=34), and Echovirus 6 (n=15) (Figure 14).

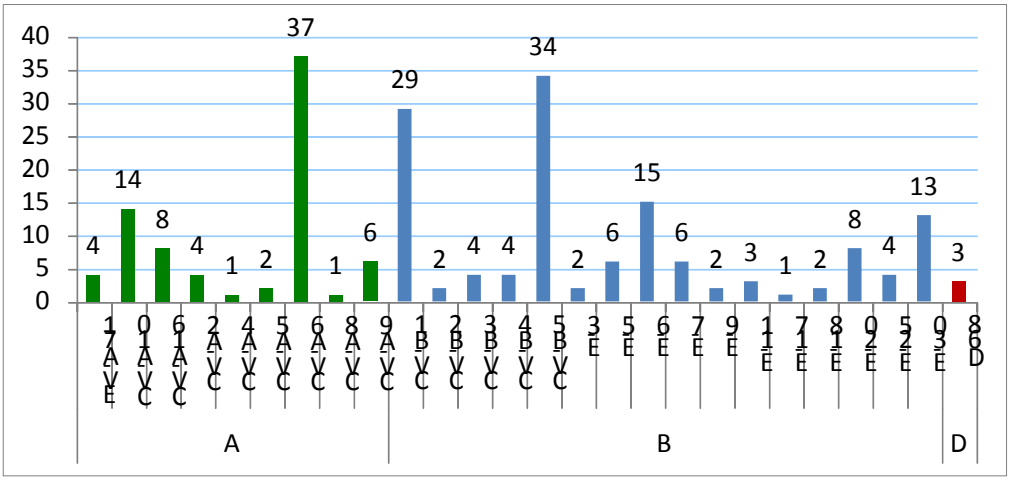

Figure 14. Genetic characterisation of EV strains detected in NVRL during 2016 (n=215). Typing was based on a 350bp fragment of the VP1 gene. Analysis of nucleotide sequences was carried out using the RIVM Enterovirus Genotyping tool (www.rivm.nl/mpf/enterovirus/typingtool).

Enterovirus genotyping results are now routinely included in the HPSC quarterly reports on Invasive Meningococcal Disease, Bacterial/Viral Meningitis and Haemophilus influenzae in Ireland [11] (http://www.hpsc.ie/a-z/vaccinepreventable/bacterialmeningitis/publications/quarterlyreports/2017reports).

In 2016 an upsurge of EV-D68 infection was observed in several European countries, including the Netherlands, Sweden and France, with additional sporadic cases from Germany, Portugal, Italy and the UK [12]. Consequently, the NVRL testing algorithm for patients presenting with respiratory disease was extended to include EV. Targeted typing of the more severe EV positive clinical cases revealed 2 cases of EV D68 among Irish patients. Of note, EV D68 was identified from respiratory and stool specimens respectively rather than from the CSF samples of these patients.

Four cases of EV-71 subgenogroup C1 were detected in Ireland in 2016, however, they were associated with milder symptoms (including meningitis, sepsis and diarrhoea) when compared to encephalitis associated cases in the Catalonian outbreak during the same year [13]. There was an apparent shift from the EV-71 subgenogroup C2 viruses seen in Ireland in 2015 (Figure 15).

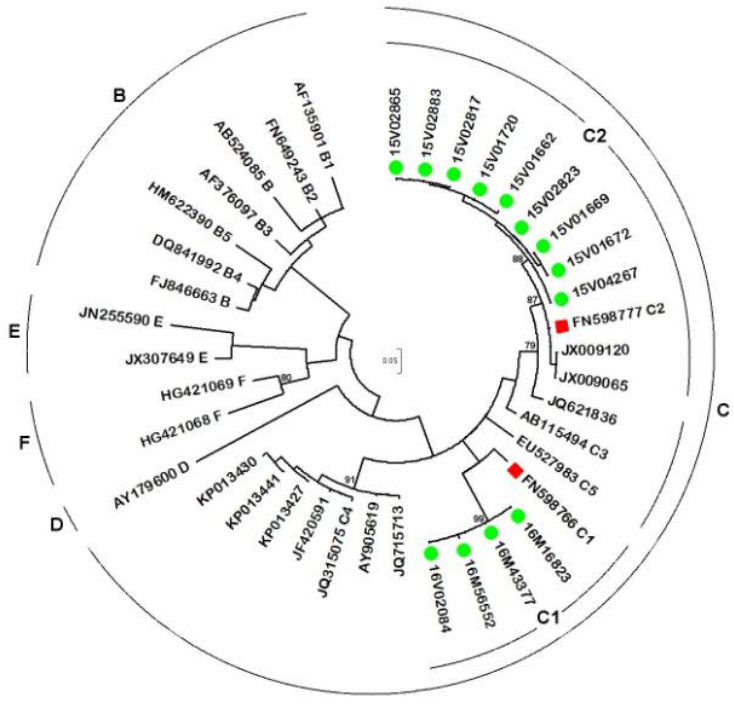

Figure 15. Genetic characterisation of representative EV71 strains detected during 2016 (n=4). Maximum Likelihood tree outlines genetic relationship, based on a fragment on the VP1 gene of EV 71 (B-F) strains. Viruses detected in Irish patients during the 2016 period are within the C1 lineage. EV71 stains detected in 2015 are also displayed and are within the C2 lineage. These subgenogroups are consistent with EV-71 strains circulating across Europe.

Transmission of Drug Resistance among newly diagnosed HIV in Ireland 2016

Key Messages:

The prevalence of transmitted drug resistance among newly diagnosed HIV infected individuals during 2016 was 9.5%.

Martha Neary

Transmitted HIV drug resistance (TDR) occurs when a treatment naïve individual is infected with HIV virus in which resistance associated mutations which compromise efficacy of antiviral agents have already developed. TDR is of significant public health concern as it has implications not only for the individual patient, but also for national treatment policies and first line treatment options.

During 2016, genotypic antiretroviral resistance testing (GART) was performed by the NVRL for 253 patients from the 508 individuals newly diagnosed with HIV, representing 50% of the total cohort. Amongst these, 77 patients had drug resistance associated mutations detected at diagnosis. A comparison of the GART data against the 2009 World Health Organization (WHO) list of surveillance drug resistant mutations (SDRMs) [14] demonstrated that at least one SDRM was detected in 24 presumed treatment naïve individuals, yielding a TDR rate of 9.5%. This is in keeping with previously published findings in Ireland from the period 2004 through 2008 [15] and is not statistically different from the TDR rate of 6.8% [p=0.24] detected in 2015 [16].

Twenty-four SDRMs were detected in these 24 individuals: 15 non-nucleoside reverse transcriptase inhibitors (NNRTI) DRMs; two nucleoside reverse transcriptase inhibitors (NRTI) DRMs; and seven protease inhibitors (PI) DRMS. All patients had resistance to a single drug class only.

Screening for Zika Virus Infections in travellers returning to Ireland in 2016

Key Messages:

Fourteen patients were diagnosed with recent Zika virus infection, having returned to Ireland from Zika endemic areas, predominantly South and Central America. All patients were symptomatic and the majority were viremic (11/14 patients had detectable RNA in blood).

Grainne Tuite and Margaret Duffy

During 2016 the Zika virus epidemic declined significantly in the Americas and the Caribbean. Despite this, ECDC WHO and CDC continue to monitor new scientific evidence and periodically update the assessment of the risk and options for response [17].

Investigation for suspected Zika virus infection is carried out at UCD NVRL in symptomatic patients with a relevant travel history. Serology (IgA, IgM and IgG) and molecular (ZIKA RNA) laboratory tests are currently available to test blood and urine of symptomatic patients. Serological diagnosis of Zika infection in those individuals with a previous history of arboviral infection is complicated due to the level of cross-reactivity observed within the arboviral flaviviruses. In particular, anti-Zika IgM can be absent or detected at very low level in those patient with acute Zika infection but previously infected with another flavivirus arbovirus. In those patients testing for anti-Zika IgA can be beneficial [18].

A total of 14 patients were diagnosed with Zika virus infection at UCD NVRL in 2016, having returned to Ireland from endemic areas, predominantly South and Central America. All patients were symptomatic and the majority were viraemic, with 11/14 patients having detectable RNA in blood. Those that did not have detectable RNA in their blood had submitted samples outside recommended 10-day window post onset of symptoms. Characteristic progression from RNA positivity, to IgM and IgG development was demonstrated in most patients with sequential samples available for testing. However, for a small number of patients IgA detection facilitated diagnosis.

Hepatitis A Outbreaks in Europe mostly affecting MSM cohorts

Key Messages:

Since 2016 there has been a widespread outbreak of Hepatitis A (HAV) amongst MSM in 22 European countries. Three HAV strains have been associated with the outbreak, each of which has been detected in patients diagnosed in Ireland

Jonathan Dean

Outbreaks of Hepatitis A among men who have sex with men (MSM) are well recognised. The main risk factor is related to direct oral-anal contact during sexual acts. Vaccination remains the main method of prevention and control in this context. It has been estimated that a level of >70% immunity among the MSM population would prevent sustained transmission and future outbreaks.

During 2016-17 there has been a widespread outbreak of HAV amongst MSM in 22 European countries [19]. There are three distinct strains of HAV genotype 1A associated with the outbreak: VRD_521_2016; RIVM-HAV16-090; and V16-25801. Between June 2016 and November 2017, 3 813 hepatitis A outbreak-confirmed cases associated with this outbreak have been identified [19] (Figure 16). Since the beginning of the outbreak, the HPSC and NVRL have genetically characterised 19 outbreak-associated cases through enhanced surveillance of HAV cases in Ireland (Figure 17).

As of December 2017, the monthly number of laboratory-confirmed cases of HAV in EU/EAA remains significantly higher than in previous years. A considerable increase in the total number of acute hepatitis A infection in women has also been observed, with outbreak strains also circulating in this group. This indicates that the outbreak has spilled over to the non-MSM population.

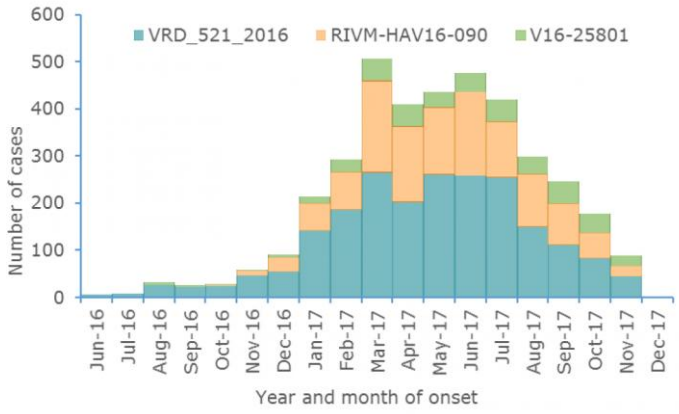

Figure 16: Distribution of hepatitis A outbreak-confirmed cases by month of onset and genetic sequence. Data includes sequences submitted between June 2016 to December 2017 from 22 EU/EEA (n=3 813*) countries including Ireland. Figure reproduced from ECDC Epidemiological update: hepatitis A outbreak in the EU/EEA mostly affecting men who have sex with men.

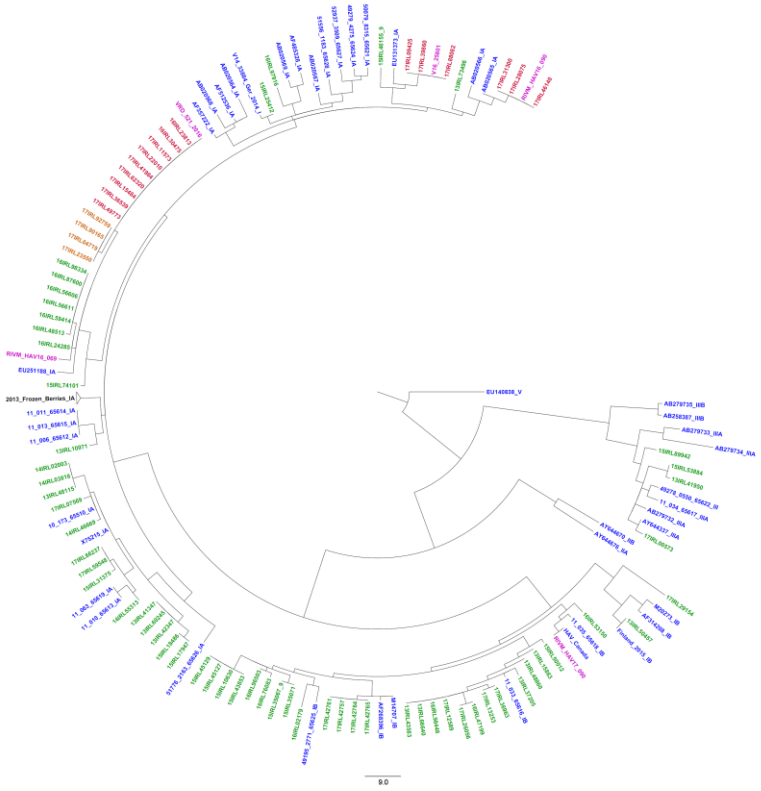

Figure 17: Neighbour joining tree illustrating the variability of HAV sequences generated in the NVRL (green) for 2016 and 2017. MSM outbreak strains VRD_521_2016, V16-25801 and RIVMHA16-090 are shown. Reference sequences, annotated with their Genbank accession number and confirmed genotype, are coloured blue; references coloured purple represent HAV strains currently circulating throughout Europe. Irish sequences matching these outbreak strains are coloured red (or orange for a subgroup bearing a one nucleotide difference from the strain reference). The scale bar indicates an evolutionary distance of 9.0 nucleotide substitutions per site.

Hepatitis E Virus in Ireland 2016

Key Messages:

Hepatitis E virus genotype 3 is the predominant virus associated with infections in Ireland.

Charlene Bennett

In recent years there has been a paradigm shift in our understanding of the epidemiology of Hepatitis E virus (HEV), which is now recognised to be endemic in Europe and likely zoonotic in origin [20]. Hepatitis E virus infection was added to the list of notifiable diseases for Ireland in December 2015.

In 2016, stakeholders in Ireland, including UCD NVRL and the HPSC, joined with ECDC to develop a surveillance system to explore the molecular relationship of HEV strains circulating in Europe in order to better understand the epidemiology of the disease. As part of this initiative the NVRL contributes molecular sequence data to a central database maintained by the National Institute of Public Health and Environment in the Netherlands (RIVM) (HEVNet https://secure.rivm.nl/mpf/database/hev. In 2016, there were 90 notifications of HEV infection in Ireland (Ref). A proportion of these samples were characterised and as in Europe, HEV genotype 3 is the dominant autochthonous strain with a small number of HEV genotype 1 cases associated with travel [21].

UCD NVRL is also leading a Department of Agriculture, Food & the Marine funded research project (FoVIRA Study) to investigate foodborne viral diseases and in particular to determine the association between Hepatitis E viruses in the food chain and those isolated from human infections. The project will be complete in 2019.

Collaborators

UCD NVRL would like to sincerely thank all those who have contributed to the work performed in recent years, including our colleagues in hospitals, clinics, GP practices, public health and in the community throughout the country. We are also extremely grateful to the professional medical societies and organisations, both in Ireland and internationally, who continually provide advice and support and without whose assistance this work would not have been possible.

- University College Dublin (UCD)
- Department of Health
- Health Service Executive (HSE)
- Health Protection Surveillance Centre (HPSC)
- Departments of Public Health
- Irish College of General Practitioners (ICGP)
- Irish Society of Clinical Microbiologists (ISCM)
- Infectious Disease Society of Ireland (IDSI)
- National Immunisation Advisory Committee (NIAC)
- National Polio Certification Committee
- Irish Paediatric Surveillance Unit (IPSU)
- World Health Organisation (WHO)
- European Centre Disease Control (ECDC)
- European Society of Clinical Virology (ESCV)
- Virus Reference Department, PHE Colindale, UK
- WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research, UK
- WHO Global Specialised Poliovirus and Regional Reference Laboratory, National Institute for Biological Standards and Controls (NIBSC), UK
- WHO European Regional Laboratory for Measles/Rubella PHE-VRD, Colindale, UK
- National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
- Rare and Imported Pathogens Laboratory (RIPL) PHE, Porton
- European Network for Diagnostics of Imported Viral Diseases (ENIVD)

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