UCD NVRL
Annual
Reference Virology
Report 2015

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2. INTRODUCTION

Dear Colleagues

We are delighted to be able to present the second annual NVRL Reference Virology Report, and hope that you will find the data contained herein both interesting and informative.

With the West African Ebola outbreak, the ongoing circulation of MERS Coronavirus in the Middle East, and the emergence of Enterovirus D68 in the US, the last year has been an interesting, albeit busy, one for the NVRL and I would like to take the opportunity to acknowledge the hard work and dedication of our staff.

Whilst the overall format of this year's report remains the same as last year, we have included an Executive Summary for the first time. In addition, there has been some evolution and new additions in the viral contents of the report.

Whilst influenza retains pride of place, mumps has usurped measles in the MMR section; the norovirus section now includes data from our first year's experience with a multiplex molecular viral gastroenteritis screen (that includes rotavirus, sapovirus, astrovirus, and enteric adenovirus); similarly, the poliovirus section has been expanded to include more data on non-polio enteroviruses (NPEV).

The inclusion of NPEV is in keeping with the recognition internationally that enhanced enterovirus surveillance will be required in what will hopefully soon be a polio free world. Indeed, the WHO, CDC, and UNICEF has recently published Guidelines on this subject.

As in last year's report, we would again like to stress that all of the collated data presented in these pages ultimately belong to you and your patients. As such, if you're interested in analysing burden of disease data, or infection patterns in your own institution, please get in touch. In addition, if you are considering validating new tests or platforms in the diagnosis of viral infections, the NVRL would be very happy to help however we can. We would also welcome any comments, questions, or feedback in relation to this report.

Finally, 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: our colleagues in hospitals and in community clinics; GP practices and primary care centres; public health; and professional medical societies and organisations, both in Ireland and internationally. Without your assistance, advice, and commitment to sharing clinical knowledge, this work would not have been possible. Our primary collaborators are listed in Section 12 (page 28). We apologise in advance to anyone that has been inadvertently omitted.

Dr Jeff Connell, Deirdre Burke, Dr Joanne O'Gorman, Dr Cillian De Gascun

September 2015

3. EXECUTIVE SUMMARY

Influenza

- Influenza A(H3N2) was the predominant virus circulating in Ireland in 2014/2015 season with influenza B virus detections increasing following the peak of activity in February and influenza A(H1N1)pdm09 viruses circulating at low levels throughout the season.
- A major concern in the 2014/2015 influenza season was the reduced vaccine effectiveness (VE) which was widely reported across Europe and the northern hemisphere. The lower levels of VE were associated with significant antigenic drift of the dominant circulating A(H3N2) viruses (subgroup 3c.2a) from the vaccine strain (A/Texas/50/2012).
- Seventy-five percent of the A(H3N2) viruses genetically characterised belonged to the 3C.2a subgroup, genetically distinct from the vaccine strain.
- Good concordance was observed between the circulating influenza B (predominantly Yamagata lineage) and influenza A(H1N1)pdm09 (clade 6B) viruses and the vaccine strains.
- Frontline respiratory testing at the NVRL for 2015/2016 will include influenza A and B and influenza A characterisation, RSV, hMPV, adenovirus and parainfluenza viruses types 1 - 4.

Measles, Rubella and Mumps

- In 2014, 252 measles IgM investigations were performed in the NVRL, of which 23 (9.1%) tested positive. Measles RNA investigations were performed on 123 specimens, of which 21 (17.1%) were measles RNA positive. Genetic characterisation identified measles genotypes D8 and B3.
- Rubella activity remains extremely low in Ireland. However, as high levels of infections were reported across parts of Europe, it is important to remain vigilant in monitoring for rubella infections.
- A significant increase in mumps virus infection (MuV) occurred during this time, predominantly associated with outbreaks in schools and colleges. The increase did not appear to be associated with a new genetic variant, as all investigated outbreaks were found to be associated with genotype G5, the predominant strain in Europe and the US and endemic in Ireland since 2006.
- From 2015, oral fluid specimens sent to the NVRL for investigation of acute MuV infection will initially be tested for MuV RNA using RT-PCR and then reflex tested for MuV IgM.

Poliovirus and Enteroviruses

- During 2014, poliovirus emerged and spread in countries in which it had been previously eliminated causing WHO to declare a “public health emergency of international concern”.
- In 2014, the number of Acute Flaccid Paralysis (AFP) notifications in Ireland met the expected rate of reporting (one case of non-polio AFP/100,000 children under 15 years of age), with 10 cases notified to the HPSC. However, in the majority of cases, specimens were not collected in accordance with the investigation of AFP guidelines. A review determined that none of the cases were associated with poliovirus.
- During 2014, a wide variety of enterovirus (EV) types were identified by serotyping cell culture isolates from stool specimens, including coxsackie viruses (CV) A16, B2, B4 and B5, echoviruses 3, 7, 9, 11, 22, 25 and

30 and EV 71. Further molecular characterisation techniques were used to investigate viruses of interest, including EV D68, EV 71 and echo 30.

- Following a recent announcement by the WHO that they will shortly cease provision of the polio- and non-polio typing anti-sera, the NVRL will transition to a new WHO approved screening strategy for polioviruses. The introduction of molecular techniques in the classification of enteroviruses should increase the sensitivity of genotyping, significantly reduce the number of enterovirus 'untypeable' results and, in conjunction with this, detect a wider variety of enterovirus genotypes.

Viral Gastroenteritis

- In June 2014, the NVRL replaced its Electron Microcopy (EM) service with a more sensitive molecular-based screen for front line screening of viral agents of gastroenteritis resulting in enhanced detection rates of the five primary pathogens associated with the disease.
- Norovirus (NoV) remains the primary causative agent of gastroenteritis in both outbreaks of AIG, and in individuals aged > 5 years. Of note, however, was the variety of pathogens identified in this older age category. In fact, 30% of laboratory confirmed infections in this age group were associated with rotavirus (RV), sapovirus, astrovirus and group F adenoviruses.
- The RT-PCR screen enabled the identification of sapovirus in a further 13.3% (n=2/15) of outbreaks of non-NoV associated AIG. In previous years, it's possible the causative agent associated with these outbreaks would not have been identified.
- As observed in previous seasons, GII.4, more specifically GII.4 Sydney 2012, was the predominant variant of NoV circulating in Ireland in the 2014/2015 season, although a variety of other variants were also detected including GI.3, GII.1 GII.3 and GII.6 strains.
- The recently described novel NoV variant GII.17 was not detected in Ireland this season: of note, it has been reported that some commercial NoV specific immunoassays are markedly less sensitive in detection of GII.17 compared to GII.4

Rotavirus

- WHO has recommended that all countries introduce RV vaccine into their immunisation strategies and, to facilitate this, should develop a robust and representative laboratory based genotypic surveillance scheme to monitor any resulting evolution in the genotypic profile of RV. The NVRL is currently introducing a RV genotyping service, which can address this requirement.
- Preliminary RV genotyping identified 3 circulating variants, G1P[8], G9P[8] and G4P[8], all strains that are commonly reported in Europe.

4. INFLUENZA

Background

The NVRL is the designated World Health Organisation (WHO) National Influenza Centre (NIC) for the Republic of Ireland and, as such, is required to provide virus isolates for influenza vaccine strain characterisation, risk assessment, and antiviral susceptibility monitoring. Laboratory confirmed cases of influenza are notified to the Computerised Infectious Disease Reporting system (CIDR) throughout the season [1]. Aggregate virological and epidemiological data are uploaded to a central database, The European Surveillance System (TESSy) at the European Centre for Disease Control (ECDC). Genetic and antigenic data from influenza isolates is shared with the WHO Global Influenza Surveillance and Respiratory System (GISRS) through TESSy and Global Initiative on Sharing All Influenza Data (GISAID) and the NVRL also contributes to the International Monitoring of Vaccine Effectiveness (I-MOVE) study [2].

Respiratory Virus Infections in Ireland Oct 2014 – May 2015

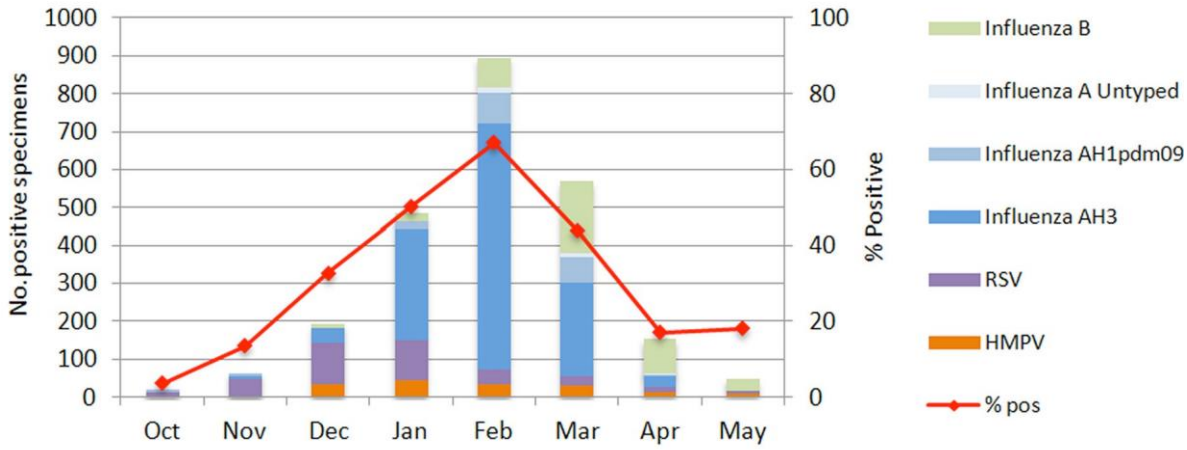
For the 2014/2015 influenza season the NVRL frontline respiratory screen was Influenza A, Influenza B, Influenza A characterisation (H1/H3), respiratory syncytial virus (RSV) and human metapneumovirus (hMPV).

A total of 8,639 specimens was analysed at the NVRL during the 2014/2015 influenza season (October 2014 - May 2015). Twenty-nine percent (n=2,527) of clinical specimens tested were positive for at least one respiratory virus, with the majority of these (77.5%; n=1,959) positive for either influenza A or B (Figure 1). Influenza infections peaked in February/March 2015. RSV infection peaked earlier in December and January and the numbers of hMPV infection detected remained consistent throughout the winter months (Figure 1).

Overall, the 2014/2015 influenza season was dominated by A H3 viruses (67%), whereas influenza B viruses (22%) predominated towards the end of the season and low levels of AH1pdm09 (9.4%) circulating throughout the season (Figure1, Page 7). However, of the influenza A infections detected, A(H3N2) viruses (88%) predominated over A(H1N1)pdm09 viruses (12%) in both the sentinel and non-sentinel specimens tested.

Influenza vaccine effectiveness was low, which was associated with the mismatch between the circulating strain of A(H3N2) and the A(H3N2) component of the vaccine [3]. This was raised by the NVRL as a potential issue during the 2013/2014 season when 21.3% of A(H3N2) viruses isolated demonstrated low reactivity with antibodies specifically raised against the vaccine strain, A/Texas/50/2012. However, despite this the A/Texas/50/2012 variant was chosen again as the candidate vaccine virus for the 2014/2015 season.

Figure 1: Specimens collected from sentinel and non-sentinel sites testing positive for respiratory viruses in the 2014/2015 "respiratory" season



Influenza Typing

Overall, during the 2014/2015 "respiratory" season, a total of 132 influenza positives (7%) was characterised genetically and/or antigenically. These data were compared with the influenza vaccine strains for the season: Influenza A(H1N1)pdm09: A/California/7/2009; Influenza A(H3N2): A/Texas/50/2012; and Influenza B (Yamagata lineage): B/Massachusetts/02/2012.

Influenza A(H3N2)

Genetic characterisation of representative circulating Influenza A(H3N2) viruses (n=49) in Ireland identified that the HA genes fell into genetic group 3C and clustered into 3 genetic subgroups (Figure 2, Page 8).

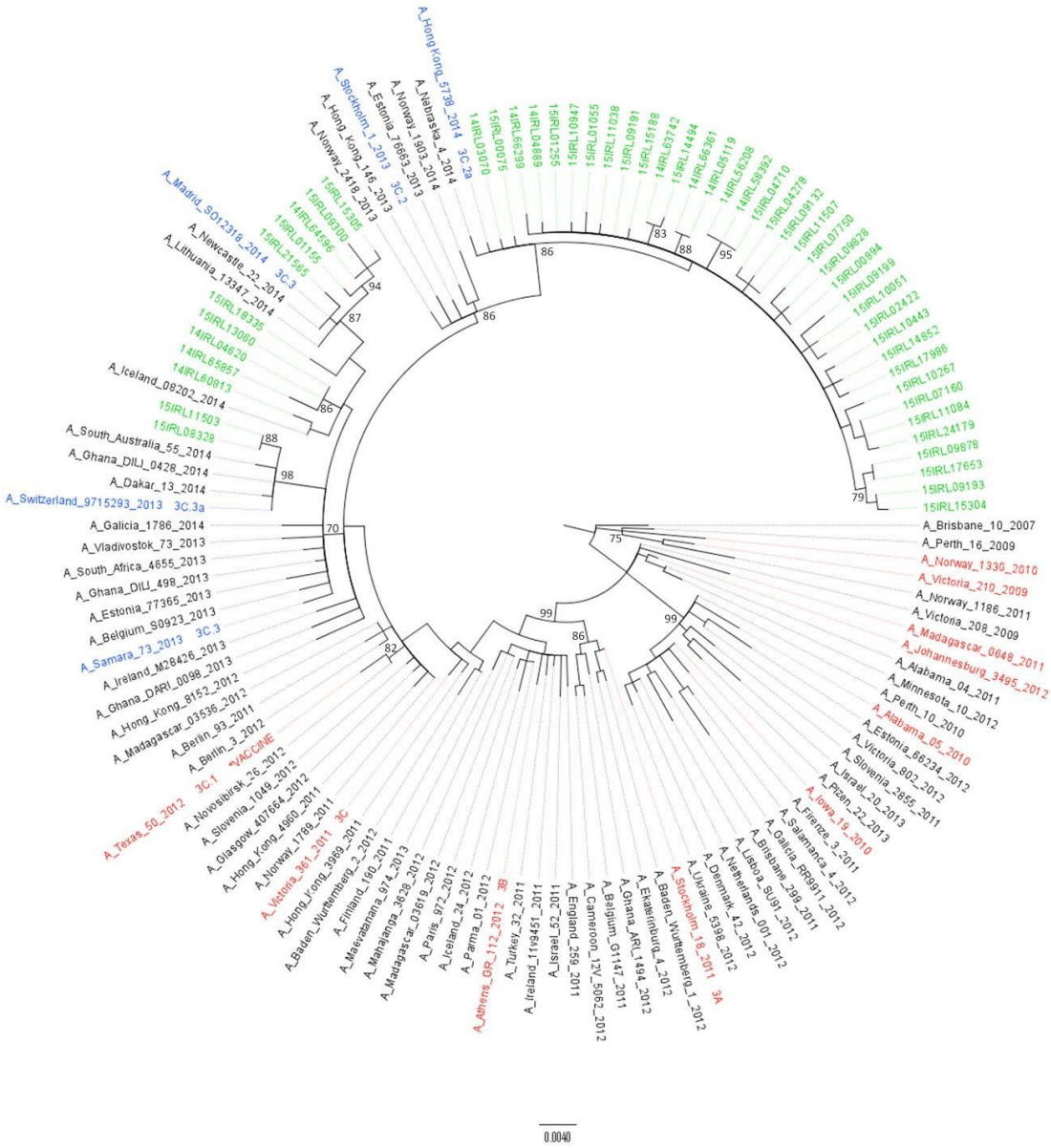
Seventy-five percent (n=36) of A(H3N2) viruses characterised clustered in subgroup 3C.2a, represented by A/Hong Kong/5738/2014, a variant associated with vaccine failure. In addition, two other subgroups were identified: 3C, represented by A/Samara/73/2013 (n=11) and 3C.3a, represented by A/Switzerland/9715293/2013 (n=2).

Based on these and other European data, the vaccine for the northern hemisphere for 2015/2016 season will be modified to include an A/Switzerland/9715293/2013 (H3N2)-like virus.

Unfortunately, this year the circulating A H3 viruses were difficult to characterise antigenically by Haemagglutination Inhibition (HAI) assay as these viruses did not adequately agglutinate red blood cells. However, the vast majority of these viruses was subjected to genetic analysis and belonged to genetic subgroup 3C.2a. The inability to utilize HAI to characterise this variant was widely reported across Europe this season.

Figure 2: Maximum Likelihood phylogenetic tree of Influenza A(H3N2) strains detected in Ireland during the

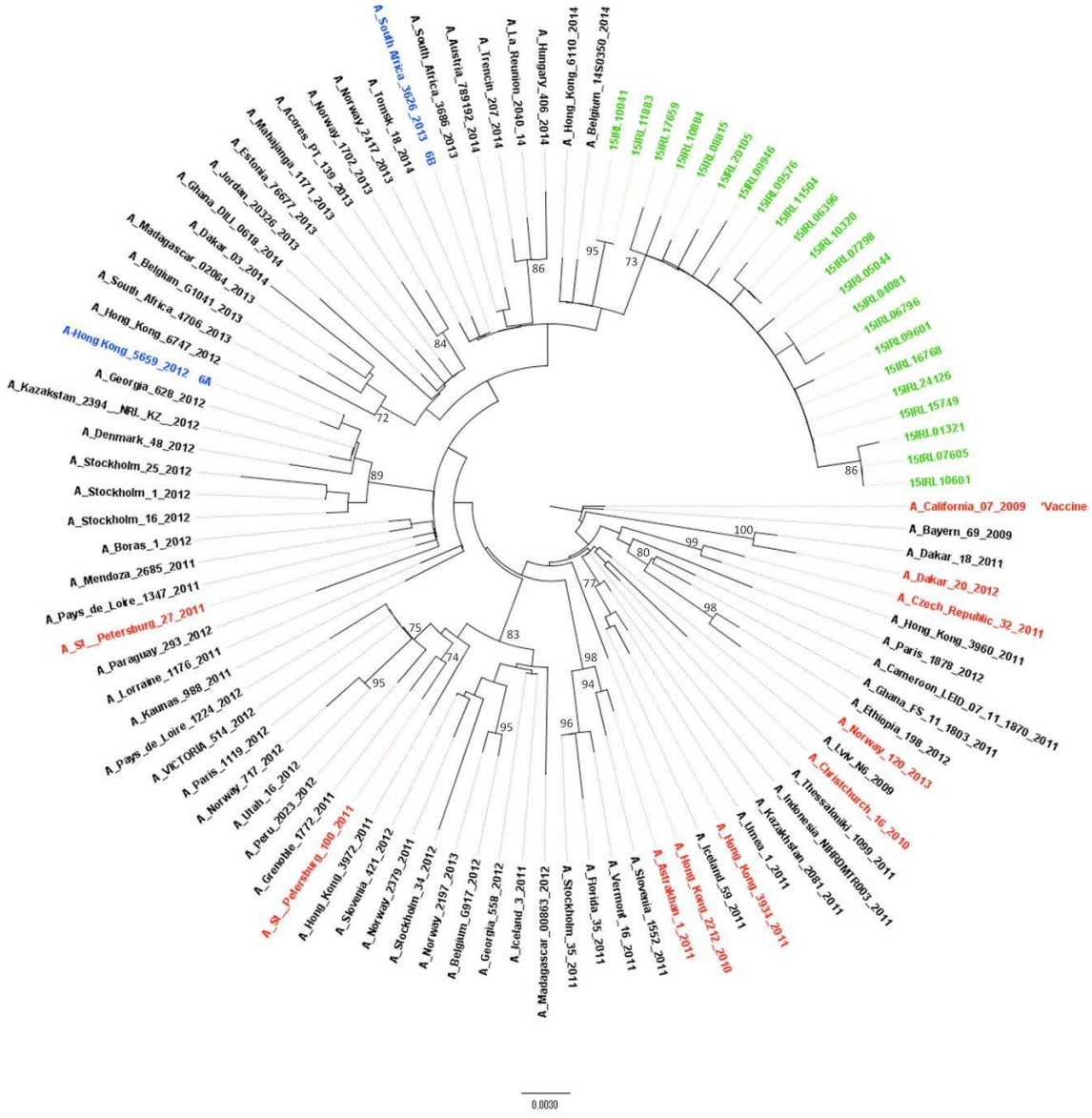
2013/2014 season. Red, Reference strain; *, vaccine strains; Blue, Clade representative; Green, Irish strains. Bootstrap values >70% are represented. Scale bar indicates nucleotide substitutions per site.



Influenza A(H1N1)pdm09

All Influenza A(H1N1) strains characterised were similar to the vaccine strain, and consequently, the majority showed good reactivity with antisera raised against A/California/7/2009. Sequence and phylogenetic analysis of representative circulating A(H1N1)pdm09 viruses (n=22) demonstrated that the HA genes all belonged to genetic subgroup 6B, with the representative strain A/South Africa/3626/2013. The Irish data were consistent with international results and as such, A/California/7/2009 remains the recommended influenza strain for the 2015/2016 vaccine.

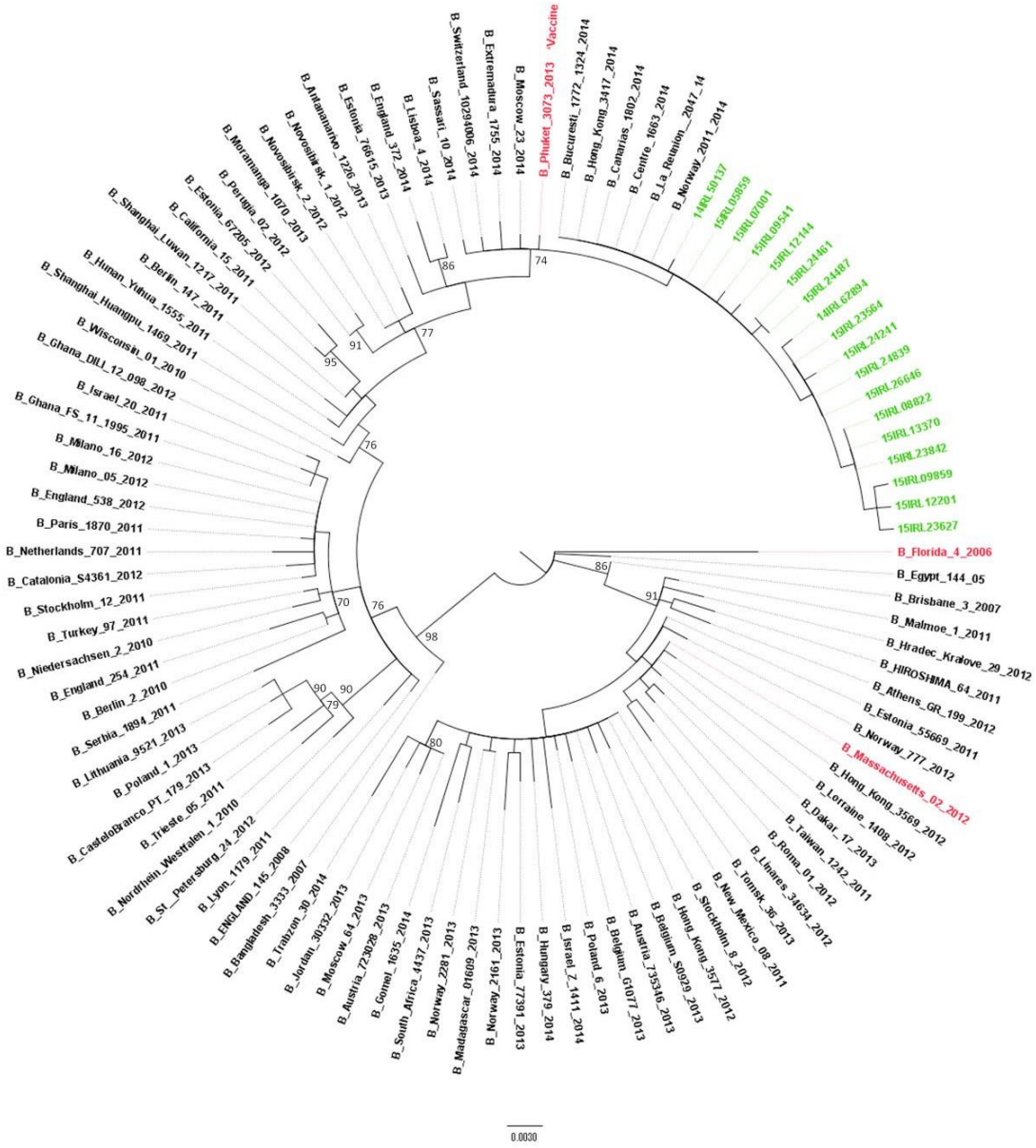
Figure 3: Maximum Likelihood phylogenetic tree of Influenza A(H1N1)pdm09 strains detected in Ireland during the 2014/2015 season. Red, Reference strain; *, vaccine strains; Blue, Clade representative; Green, Irish strains



Influenza B

The vast majority (90%) of characterised influenza B viruses detected in Ireland was B/Yamagata lineage. Antigenic characterisation determined that all viruses reacted with antisera raised against B/Massachusetts/02/2012, the clade 2 virus included in the 2014–15 northern hemisphere vaccine. Genetic characterisation of the Irish B/Yamagata-lineage viruses demonstrated that all viruses clustered in genetic clade 3 with B/Phuket/3073/2013, which is recommended for inclusion in the northern hemisphere 2015-16 vaccine. In addition, 2 B/Victoria lineage viruses were identified with both belonging to Clade 1A, represented by B/Brisbane/60/2008, a variant which has been in circulation for a number of seasons.

Figure 4: Maximum Likelihood phylogenetic tree of Influenza B/Yamagata lineage variants detected in Ireland during the 2013/2014 season. Red, Reference strain; *, vaccine strains; Blue, Clade representative; Green, Irish strains



5. MEASLES

In 2014, 252 measles IgM investigations were performed in the NVRL, of which 23 (9.1%) tested positive. Measles RNA investigations were performed on 123 specimens, of which 21 (17.1%) were measles RNA positive. Measles virus culture was not utilized for any patients.

Genetic characterisation of measles was performed on viruses from 7 specimens, all of which were submitted to MeaNS (WHO's Measles Nucleotide Surveillance database) for phylogeny comparison with viruses in other countries. Of the 7 sequences generated, 4 were genotype D8 and 3 were genotype B3. The genotype D8 cases were all associated with an outbreak in the Southwest of Ireland and were most closely associated with MVs/Frankfurt Main.DEU/17.11 strain. The genotype B3 cases all had a recent history of travel to Japan or the Philippines and were most closely associated with the MVi/Harare.ZWE/38.09 strain.

6. RUBELLA

There is no evidence for the endemic transmission of rubella infection in Ireland. Nonetheless, levels of rubella infection remains high in many European countries, particularly Poland in which 96.5% of all rubella cases in Europe in 2014 occurred, and therefore there remains a significant risk of importation of rubella to Ireland [4].

In 2014, 1,780 rubella IgM investigations were performed in the NVRL, predominantly associated with ante-natal and post-natal investigation. Only two specimens (0.11%) were IgM positive. No clinical or vaccination information was available on these cases and no follow up specimens were received, and therefore no conclusions can be drawn on whether or not these constituted genuine cases. Rubella virus was not isolated (in culture) from any specimens and none were suitable for genotyping. Please note Rubella RNA testing is available at the NVRL if clinically indicated.

The vast majority of rubella IgM investigations are linked to screening during pregnancy or post partum testing of a baby, as a component of the "TORCH" screen. They are not specifically linked to a rubella-like rash illness and consequently the large number of IgM investigations performed does not reflect a good surveillance system for rubella infection. It is advisable to test for the presence of rubella virus only when there is a definite risk of infection and to rapidly follow up patients with positive rubella IgM results to determine if it is a true case.

7. MUMPS

Background

Two doses of a mumps-containing vaccine are recommended to protect against mumps virus. However, an increase in incidence and outbreaks of acute mumps virus (MuV) infection since 2004, frequently seen in schools and universities and often in highly vaccinated populations, has raised concerns regarding: i) waning immunity in adolescence and young adulthood; ii) suboptimal herd immunity in high risk settings such as schools; and iii) the potential for reduced vaccine effectiveness due to differences between circulating genotypes and the variant used in the vaccine [5,6].

There are 12 known MuV genotypes that have been confirmed by WHO, designated A-N (excluding E and M). In 2012, WHO proposed an updated mumps nomenclature which excluded genotypes E and M following extensive phylogenetic analyses which determined genotype E to be more closely related to genotype C and genotype M to cluster with genotype K [7,8].

The virus strain incorporated into the MMR vaccine is the genotype A Jeryl Lynn strain [7,8]. Different genotypes have been found to co-circulate within one region at the same time; however, since the MuV resurgence in 2004, genotype G has been the predominant strain circulating in Europe and North America [8,9].

Laboratory confirmation of acute MuV infection typically involves testing serum or oral fluid specimens for MuV-specific IgM antibodies and detection of MuV IgG to indicate previous infection or vaccination. MuV IgM may be undetectable in oral fluid specimens collected in the first 7 days of illness, whereas MuV RNA is detectable from onset of symptoms until approximately 8-10 days later. MuV IgM can be detected in serum specimens from approximately 2-3 days post onset.

Serological diagnosis of recent mumps infection can be problematic in those individuals who have received either 1 or 2 doses of vaccine. In these cases, the IgM response may be attenuated or absent and the IgG is boosted, so the profile suggests past infection rather than recent mumps infection.

Therefore, there is an increasing reliance on the use of RT-PCR for the detection of MuV RNA in oral fluid specimens. In 2015, the NVRL changed its testing algorithm to reflect this and now all oral fluid specimens sent for investigation of acute MuV infection will first be screened for MuV RNA by RT-PCR and then reflex tested (if RNA negative) for MuV IgM. Of note, oral fluid swabs for the investigation of suspected MuV cases are available from the NVRL

Mumps in Ireland

A notable increase in MuV infections and outbreaks was identified in 2014, with the HPSC reporting that more than double the number of MuV notifications were made in 2014 compared to 2013 [10]. The increased circulation in MuV appeared to be associated primarily with schools, colleges and universities and coincided with the beginning of the academic year [10].

During 2014, 1,524 specimens (1,219 sera, 191 oral fluids and 114 other specimen types including CSF, swabs, blood, urine etc.) were received at the NVRL for MuV investigations. Of 1,358 specimens tested for MuV IgM, 196 (14.4%) were positive. A further 122 specimens tested weakly reactive for

Mumps in Ireland (continued)

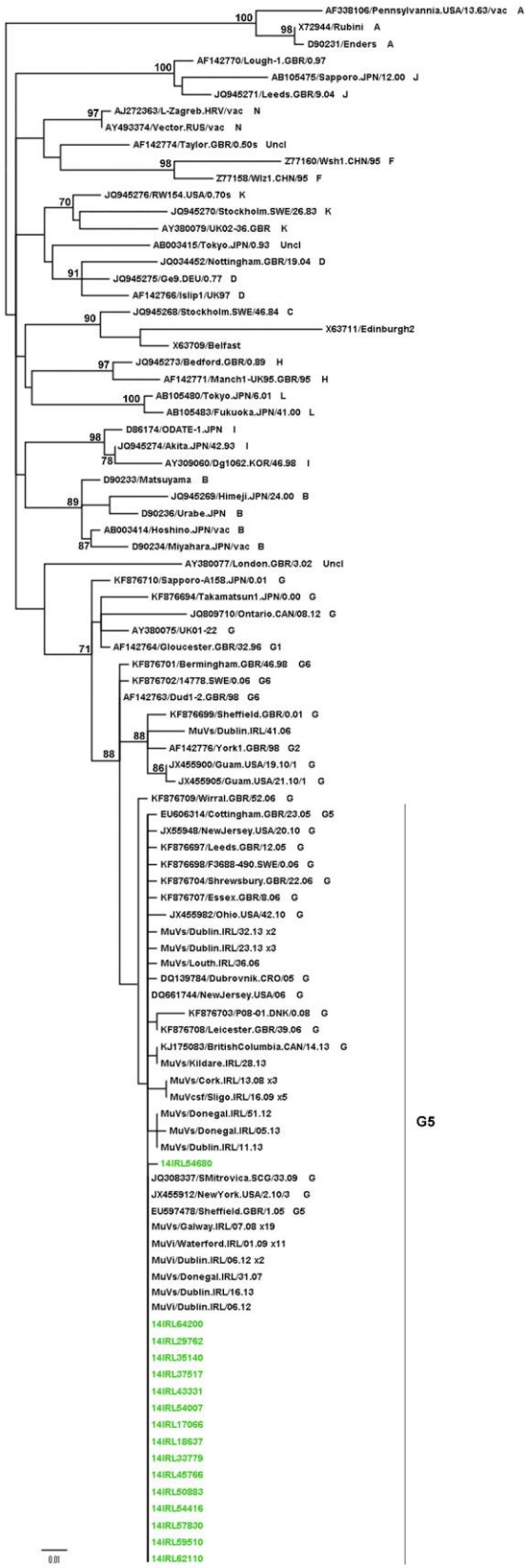
MuV IgM (9%). In addition, MuV RNA was detected in 69 of 233 (29.6%) specimens using RT-PCR. The burden of infection remains predominantly with young adults, with 62.5% (n=165/264) of positive specimens received from individuals aged 15-24 and a median age of infection of 20 years (range: 1-78 years). MuV infections were detected in all regions of the country and, as previously reported, occurred predominantly in males at a ratio of 1.6:1 [9].

Throughout 2014, 24 CSF specimens from suspected acute mumps infections were tested by RT-PCR. MuV RNA was detected in 12.5% (n=3/24) of these cases. Incidentally, IgG data indicated that 75% of specimens tested (n=768/1,029) were already MuV IgG positive, as described above, with a further 13.9% (n=143/1,029) testing weakly reactive for MuV IgG.

The SH genes of 16 MuV RNA positive specimens, representing many of the outbreaks in schools, colleges and universities across the country, were genetically characterised. All MuV outbreaks investigated were exclusively associated with genotype G5 (Figure 5, Page 14). In addition, the vaccine strain, genotype A, was identified in two individuals who had recently been vaccinated.

Although genotype G5 remains the predominant strain circulating in Ireland over the past decade [9], it is imperative that MuV surveillance is routinely performed to facilitate analysis of transmission events and monitor any upsurge in MuV infection levels for the introduction of any new/differing strains.

Figure 5: Maximum Likelihood phylogenetic tree of mumps viruses associated with mumps outbreaks in Ireland during 2014. Green represents the Irish strains analysed in 2014. Reference sequences used are annotated with Genbank accession codes and the genotype, where available. Bootstrap values over 70% are represented on the tree.



8. ENTEROVIRUSES

8.1. POLIOVIRUS

International Update

During 2014, poliovirus emerged and spread in countries in which it had been previously eliminated. The WHO declared this to be a "public health emergency of international concern" and recommended a coordinated international response to halt the spread of the disease and raise immunity [11]. In 2014, there were 359 cases of wild polio virus (WPV) infection globally, 340 of which occurred in countries in which WPV is still endemic [12]. Currently, Pakistan and Afghanistan are exporting WPV, Nigeria and Somalia are infected but not thought to be exporting WPV, and Cameroon, Equatorial Guinea, Ethiopia, Iraq, Israel and Syrian Arab Republic are considered to be no longer infected but remain vulnerable to international spread [11].

Ireland, along with the rest of Europe, was declared poliovirus free in 2002. The recent spread of WPV is a reminder not to become complacent in our surveillance of AFP to ensure we detect any possible importations to Ireland.

Laboratory Update

In March 2015, the WHO declared that they will shortly cease provision of the polio- and non-polio typing anti-sera and that all laboratories in the WHO European Polio Laboratory Network will transition to a new diagnostic algorithm for the isolation and molecular characterisation of poliovirus (and other enteroviruses) from stool specimens by April 2016. The new algorithm aims to significantly improve the speed of diagnosis.

Polio Results for Ireland 2014

In 2014, 10 cases of Acute Flaccid Paralysis (AFP) were notified to the HPSC but on review none was found to be associated with poliovirus. The required 2 stool specimens were received at the NVRL for virological investigations of AFP from 6 notified cases. However, only three of these cases had specimens sent in accordance with the investigation of AFP guidelines [13], that is, two stool specimens collected at least 24 hours apart and within the requisite 14 days of paralysis. None of the specimens tested positive for poliovirus.

Although, for the first time in many years, the number of notifications of AFP in 2014 reached the expected threshold of reporting (one case of non-polio AFP for every 100,000 children under-15 years of age), the appropriate specimens were not submitted in the majority of cases. Clinicians are strongly recommended to consider polio virus infection in the initial diagnosis and are urged to send the requisite two faecal specimens, collected 24-48 hours apart, from all cases of AFP in children under 15 and to notify these cases to the HPSC.

Timely laboratory investigation of all suspected cases is recommended by the WHO to ensure that virus importations are detected and that Europe retains its polio-free status.

8.2. NON-POLIO ENTEROVIRUSES

Background

The NVRL characterises non-poliovirus enterovirus (NPEV) isolates, both for clinical investigation and as a component of its WHO poliovirus commitments. Enteroviruses (EV) are an extremely genetically diverse group of viruses belonging to the Picornaviridae family. There are 4 species, or genogroups, associated with human infection – EV groups A to D – each of which comprises a wide variety of genotypes/serotypes.

EV infection is associated with a variety of clinical manifestations including asceptic meningitis, encephalitis, hand foot and mouth disease, gasteroenteritis and respiratory disease. While all 4 groups are linked with numerous and varied clinical presentations, certain genotypes tend to have a more defined infection profile and present with similar clinical manifestations in the majority of patients.

An EV molecular genotyping assay is now being introduced at the NVRL which will enable NPEV characterisation directly from clinical specimens (including stool and other specimen types) in addition to cell culture isolates.

Enterovirus Typing in Ireland in 2014

In 2014, 488 stool specimens were cultured for enteroviruses (EV), yielding 61 (12.5%) positive results. Group A, B and D viruses – including Coxsackie viruses (CV) A16, B2, B4 and B5, echoviruses 3, 7, 9, 11, 22, 25 and 30 and EV 71 – were detected using the traditional serotyping methodology. Group B EV were the most commonly detected, specifically CVB5 (19.7%), echovirus 11 (14.8%) and echovirus 30 (14.8%).

Five EV were untypeable by traditional methods: however, this is not unusual, as serotyping techniques may be unable to characterise all – particularly novel – EV strains, an issue which should be largely resolved with the introduction of the molecular characterisation methods.

Enterovirus RNA Testing

Enterovirus RNA testing was performed on 5,344 specimens in 2014, with the majority cerebrospinal fluid (CSF) specimens (n=3,352). In total, enterovirus RNA was detected in 11.2% of specimens (n=597/5,344); specifically, in 8.9% of CSF specimens (n=300/3,352), 25.3% faecal specimens (n=122/483), 14.4% of swabs tested (n=137/950), and 3.6% of the other specimen types. This represents a marked increase on 2013, in which 192 CSF specimens tested positive for enterovirus RNA.

Of note, 277 faecal specimens were tested in parallel by viral culture and RT-PCR; 56 tested positive by both methods, 46 were RNA positive but culture negative and the remainder were negative by both methods. This increased sensitivity of the RT-PCR is further evidence to support the introduction of molecular techniques for enterovirus characterisation.

It is hoped that the new genotyping method may allow for characterisation directly from CSF in clinically relevant cases.

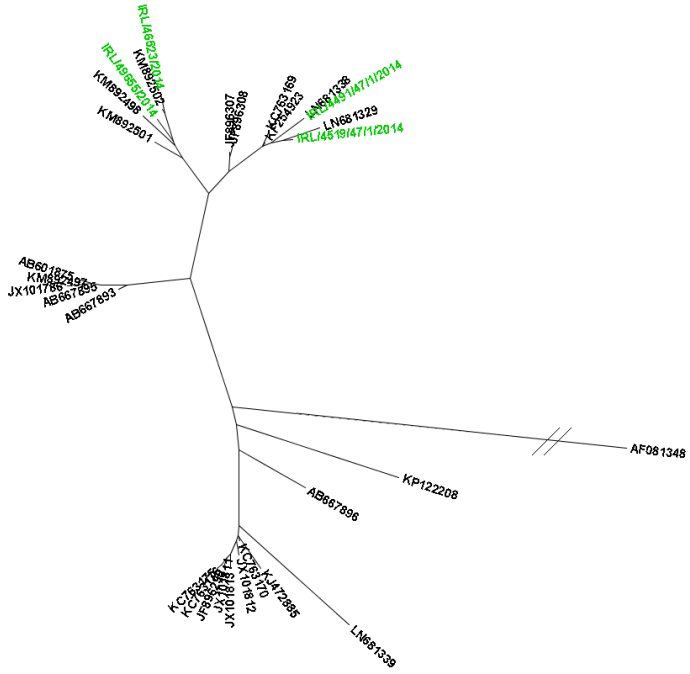
EV Developments in 2014

Enterovirus D68

During the summer of 2014 unprecedented levels of enterovirus-D68 (EV-D68) were reported across the United States in association with severe respiratory disease and an increased incidence of AFP [14,15]. An initiative was launched by ECDC in collaboration with the European Society for Clinical Virology (ESCV) to specifically screen respiratory specimens received between July and December 2014 for EV-D68. The study confirmed that EV-D68 was circulating across Europe, but with a moderate disease burden and different pathogenic profile compared to the North-American epidemic [16]. Specifically in Ireland, four cases were detected during the study period. The emergence of enterovirus D68 as a possible cause of AFP supports the requirement for a robust surveillance system for AFP.

Phylogenetic analysis demonstrated that all 4 EV-D68 sequences clustered in clade B, similar to the majority of strains detected in Europe and the U.S (Figure 8). A link has not yet been established between viral genetic variation and associated virulence.

Figure 8: Maximum Likelihood phylogenetic tree of enterovirus D68 variants detected in Ireland during 2014. Green represents the Irish strains. Reference sequences used are annotated with Genbank accession codes.

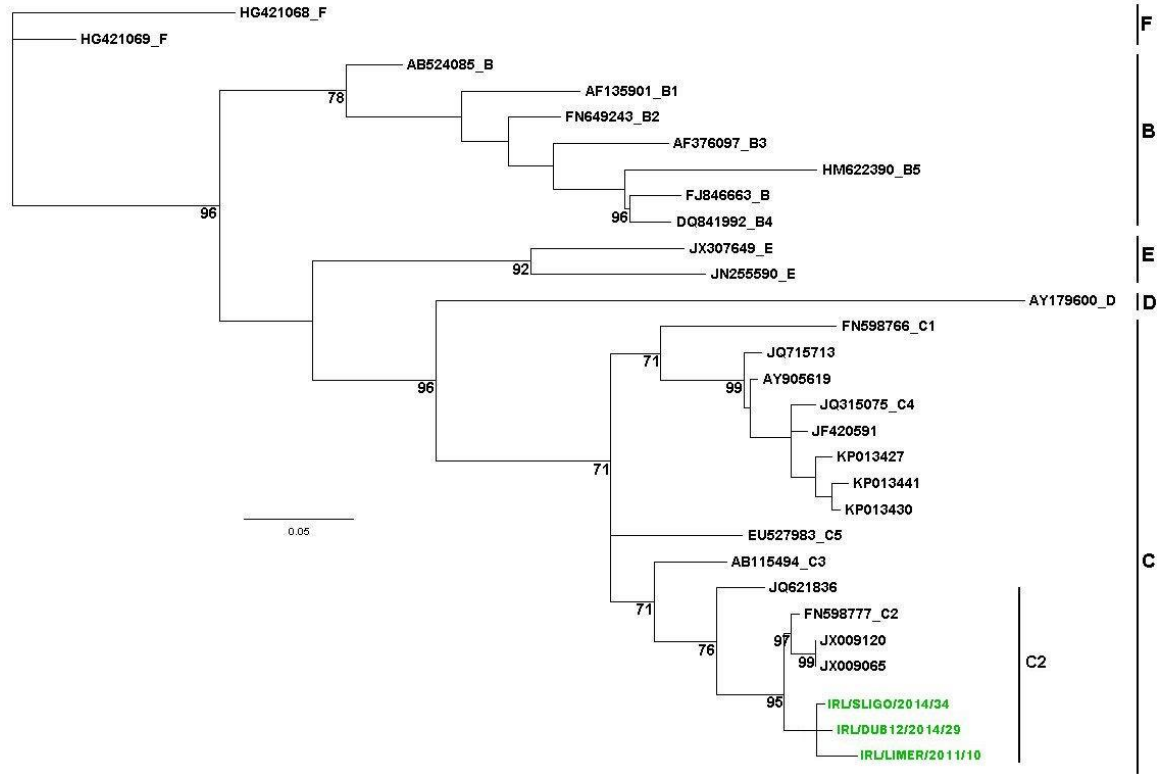


Enterovirus A71

Enterovirus A71 (EV-A71) is a primary pathogen associated with hand, foot and mouth disease (HFMD) and, in addition, has also been implicated in severe disease involving the central nervous system. Three strains detected in 2014 were characterised and all clustered in clade C2, alongside a sequence detected in 2011 (Figure 7).

These findings are consistent with other circulating strains detected across Europe. A neurotropic subgenotype C4 virus recently emerged in Europe which remains as of yet undetected in our studies. However, the significant burden of illness associated with this variant means that continued surveillance for EV-A71 is prudent.

Figure 7: Maximum Likelihood phylogenetic tree of enterovirus 71 variants detected in Ireland during 2014. Green represents the Irish strains. Reference sequences used are annotated with Genbank accession codes. Bootstrap values over 70% are represented on the tree.

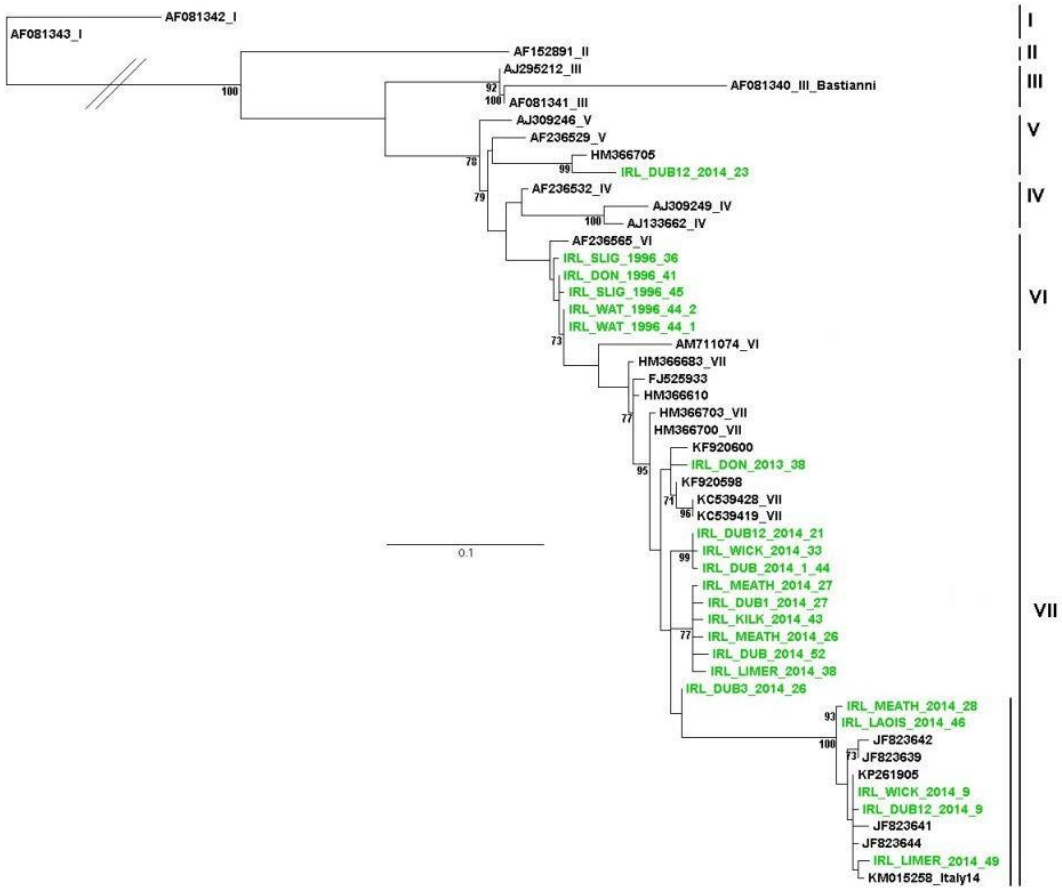


Aseptic Meningitis and Echovirus 30 (EV30)

In recent years, the HPSC has described an increase in notifications of aseptic meningitis [17]. Previous reports in Europe and other regions worldwide have described an association between the emergence of a new variant of echovirus 30 and outbreaks of aseptic meningitis. Consequently, Irish EV30 strains (n=16) detected at the NVRL in 2014 were phylogenetically analysed and 4 different genetic clusters were identified (Figure 6, Page 19).

This is the first 'snapshot' of EV30 genetic surveillance for Ireland and continued monitoring is crucial for understanding the role genetic variation plays in disease severity and outbreaks.

Figure 6: Maximum Likelihood phylogenetic tree of echovirus 30 variants detected in Ireland during 2014. Green represents the Irish strains. Reference sequences used are annotated with Genbank accession codes. Bootstrap values over 70% are represented on the tree.

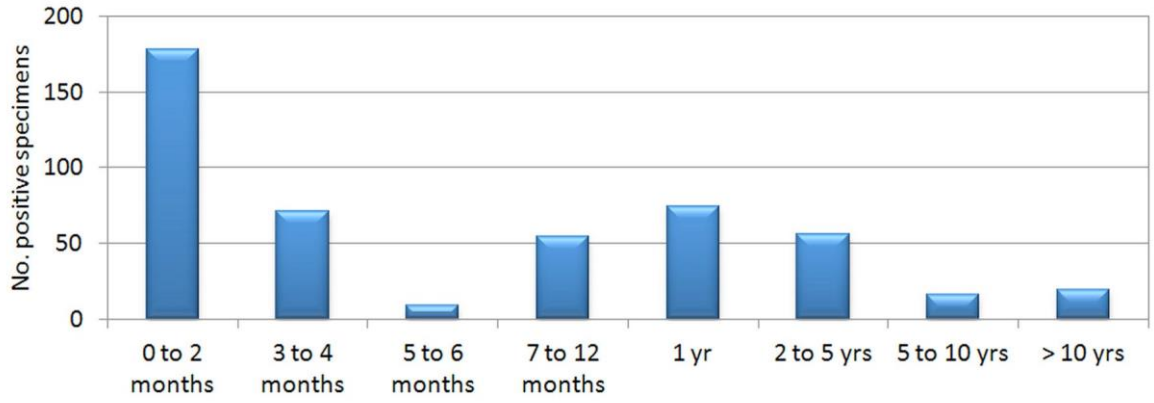


8.3. A 10 YEAR REVIEW OF ENTEROVIRUS SURVEILLANCE

The impending change in WHO enterovirus (EV) characterisation methods, and the new 2015 surveillance guidelines prompted a review of EV serotypes characterised at the NVRL over the previous 10 years. From 2005 to 2014 inclusive, a total of 8,997 specimens was analysed, of which 5.1% (n=459) were EV culture positive.

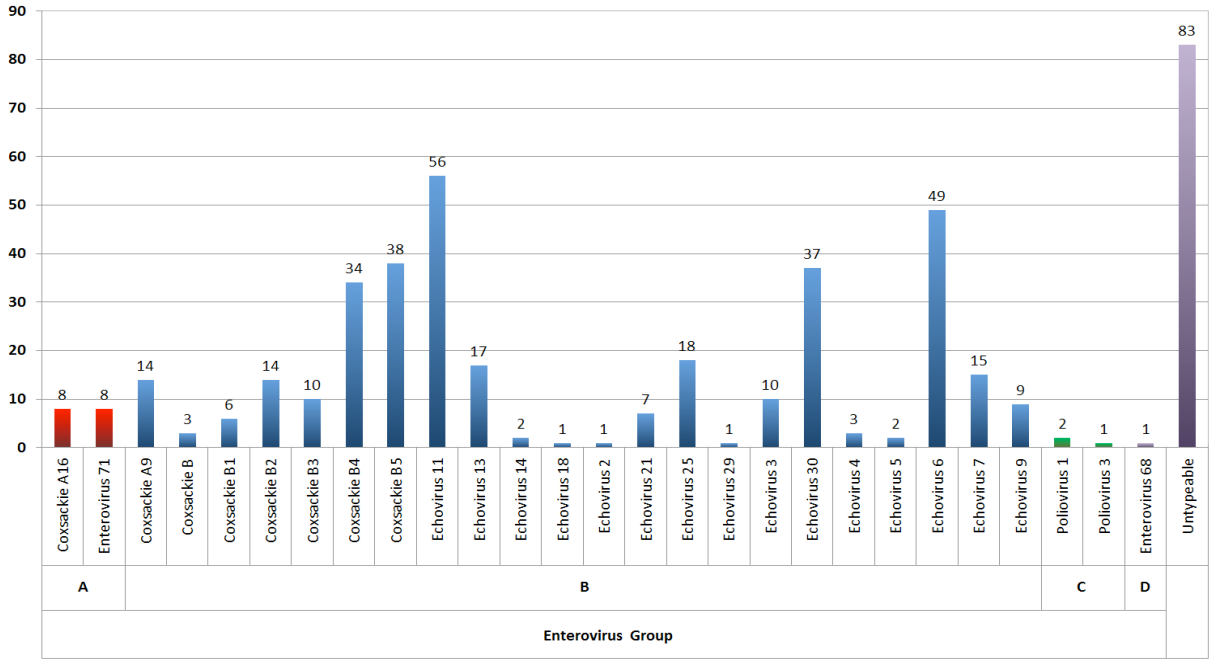
Overall, EV predominantly affected young children with 52.1% of all specimens analysed received from children less than 1 year of age. Notably, the majority of specimens positive for EV was received from children less than 2 months of age (37.3%; Figure 9, Page 20).

Figure 9: Age demographic associated with enterovirus infection in specimens tested at the NVRL from 2005 – 2014.



EV serotypes detected over the 10-year period are presented in Figure 10. EV-B serotypes were the most common, with echoviruses -6, -11 and -30, and CV –B4 and –B5 the most prevalent types detected. All of these serotypes are associated with viral meningitis and encephalitis, and echoviruses are responsible for a number of documented outbreaks in Europe and worldwide. EV-A71 and CV-A16, which belong to EV group A, are common causes of hand, foot and mouth disease. However, EV 71 is also a significant cause of viral meningitis and meningo-encephalitis.

Figure 10: Enterovirus serotypes detected at the NVRL from 2005 to 2014



Eighty three non-polio isolates could not be typed using anti-sera. However, as mentioned above, the introduction of molecular-based genotyping at the NVRL should help resolve the majority of untypeable EV results in the future.

9. GASTROENTERIC VIRAL PATHOGENS

9.1. GASTROENTERITIS VIRAL SCREEN

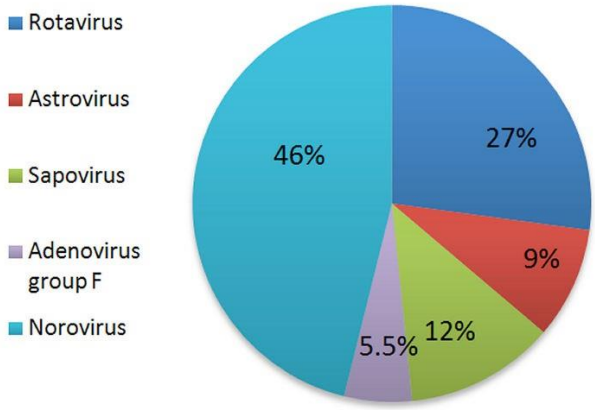
Background

The principal viral agents associated with acute gastroenteritis are human caliciviruses, including genogroup I and II noroviruses (NoV) and sapovirus, rotavirus (RV), astrovirus and human group F adenoviruses (serotypes 40 and 41) [18]. NoV is the principal pathogen in both paediatric and adult diarrhoeal disease and is responsible for approximately 80% of acute infectious gastroenteritis (AIG) outbreaks [19-22]. NoV and RV infections are notifiable in Ireland, with surveillance performed by the Departments of Public Health (DPH) and the Health Protection Surveillance Centre (HPSC).

Viruses Associated with Gastroenteritis at the NVRL July 2014 – June 2015

In June 2014, the NVRL introduced real-time (RT-) PCR assays for frontline screening of all specimens received for viral gastroenteritis investigations. Previously, the NVRL screened specimens from patients > 5 years old with NoV-specific real-time RT-PCR and, as the other primary viral aetiological agents associated with gastroenteritis are predominantly identified in young children, specimens from patients ≤ 5 years were screened using electron microscopy (EM). EM is a rapid, catch-all method but it is significantly less sensitive than molecular techniques and not suitable for large-scale screening. However, EM is still available on request for the investigation of atypical cases.

Figure 11 : Viruses associated with gastroenteritis detected at the NVRL from July 2014 – June 2015 (n=3 206)

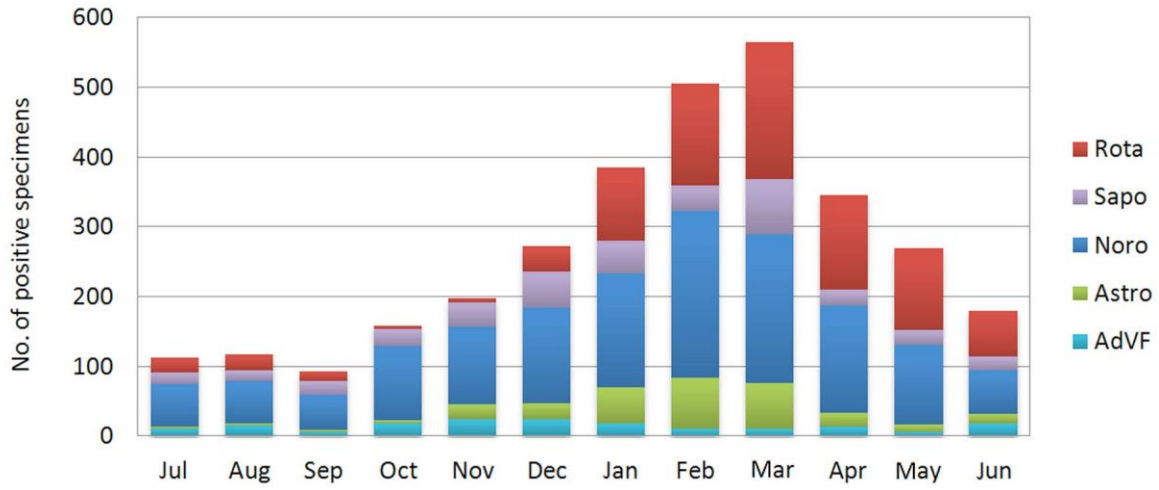


During 2014/2015, 11,510 faecal specimens were tested for the five primary viral agents associated with gastroenteritis. Of these, 27.8% (n=3,206) tested positive for at least one viral pathogen. As expected, the predominant virus detected was NoV (n=1,479) of which 89% were genogroup II (GII), followed by RV (n=870), sapovirus (n=390), astrovirus (n=291) and group F adenoviruses (n=176; Figure 11).

Between July 2014 and June 2015, 193 outbreaks of AIG were notified to CIDR [23]. The NVRL received specimens from 44% of the notified outbreaks with NoV detected in 82% of the specimens (n=69; Figure 12). Of note, as a result of the introduction of the gastroenteric viral RT-PCR screen, the NVRL's diagnostic gap in the identification of the aetiological agents associated with AIG outbreaks was certainly diminished. Sapovirus was identified as the pathogen associated with 2 outbreaks, representing 13.3% of outbreaks of non-NoV associated

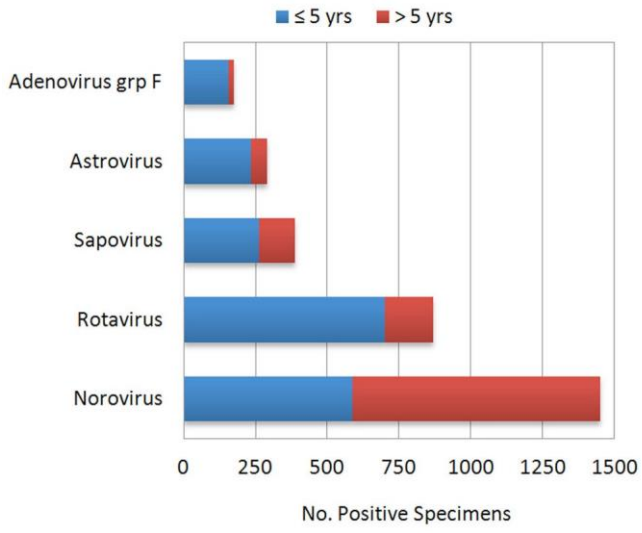
AIG. Viral gastroenteritis is traditionally seasonal with the bulk of infections occurring in the winter and spring months. In the 2014/2015 season, NoV and astrovirus infections peaked in February, whereas RV and sapovirus peaked slightly later in March. Enteric adenovirus infections are the exception and were observed at low levels throughout the year (Figure 12).

Figure 12: Viral gastroenteritis agents detected at the NVRL from July 2014 – June 2015, n=3206



RV was the principal pathogen identified in paediatric patients, detected in 15.8% of specimens tested from patients aged ≤ 5 years; followed by NoV (13.2%), sapovirus (5.9%), astrovirus (5.2%) and group F adenoviruses (3.5%; Figure 13). The high levels of NoV infection in paediatric patients have been previously reported by the NVRL and others, and are a reminder that NoV should always be considered in paediatric diarrhoeal illness [19]. In fact, should the rotavirus vaccine be introduced, the likelihood is that NoV would quickly become the predominant virus associated with paediatric, as well as adult gastroenteritis.

Figure 13: Age demographic associated with viral agents of gastroenteritis detected at the NVRL from July 14 - June 15, n=3,206



Of note, while NoV was the principal viral pathogen detected in specimens from individuals > 5 years of age (n=863), 30% of the laboratory confirmed infections in this age category were associated with other viral agents: 168 specimens were positive for RV, 127 for sapovirus, 57 for astrovirus, and 17 for group F adenoviruses (Figure 13). This represents a notable increase in the detection of viral pathogens as these infections would have remained largely undiagnosed in previous years.

In the >5 year old age category, RV infections were detected in 2.5% of specimens received, with 54% of these infections (n=91) in patients >60 years old. Although RV infection is generally considered a paediatric disease, it is known to occur in adults [24-26], a category of individuals who may benefit from the herd immunity generated following the introduction of the rotavirus vaccine. However, it is frequently under-reported as many countries/laboratories do not screen for RV infection in adults.

Sapovirus traditionally circulated at a low prevalence in Europe, linked to sporadic cases of gastroenteritis. However, in recent years the epidemiology of the virus has changed and the prevalence of both sporadic cases and associated outbreaks of sapovirus has increased significantly, possibly linked to the emergence of a new genotype [27]. To date, limited information has been available regarding sapovirus in Ireland. The NVRL detected sapovirus in 3.4% of all specimens tested (n=390/11,510), with sapovirus infections comprising 12% of all laboratory confirmed cases of gastroenteritis. Although the majority of these infections occurred in paediatric patients, infection in adults was observed and both laboratory-confirmed outbreaks of sapovirus occurred in nursing home settings.

A high number of dual infections were identified (n=293/3,206, 9.1%), with the majority in patients ≤5 years of age. In addition, there were 9 cases of triple infections, all in patients aged < 3. The most common dual infection identified was NoV with RV (n=67). A limitation of this information is the lack of accompanying clinical data. Although numerous pathogens may have been detected in one specimen, it is difficult to ascertain which is the primary pathogen associated with the presenting symptoms or if multiple infections were associated with the acute gastroenteritis.

9.2. NOROVIRUS GENETIC CHARACTERISATION

International Update

NoVs exist in 7 known genogroups (GI-GVII), of which 3 (GI, GII and GIV) can cause infections in humans. GII is the most common, accounting for approximately 90% of NoV infections and 70-80% of NoV-associated outbreaks worldwide [20,21,28]. Since the mid 1990s, GII.4 has been the predominant strain circulating in Ireland and globally [20-22,28]. However, recently alerts have been issued regarding the emergence of a novel variant of GII.P17 (GII.17 Kawasaki 2014) in parts of Asia, including China and Japan [29,30].

The new variant has been associated with an increase in outbreaks of AIG and has quickly replaced GII.4 Sydney 2012 as the dominant strain in these regions [31]. GII.17 NoV has been circulating since at least 1978, detected in environmental samples and low numbers of sporadic cases and outbreaks of gastroenteritis worldwide [31]. Indeed, GII.17 was identified in Ireland in influent wastewater in 2010 [32]. However, to date, the new variant GII.17 has been identified in only a limited number of clinical cases outside of the affected regions in Asia. In recent years, the emergence of new variants of NoV has been associated with a rapid rise to dominance of the new type and increased NoV activity, such as occurred following the emergence of GII.4 [21].

Noroviruses in Ireland

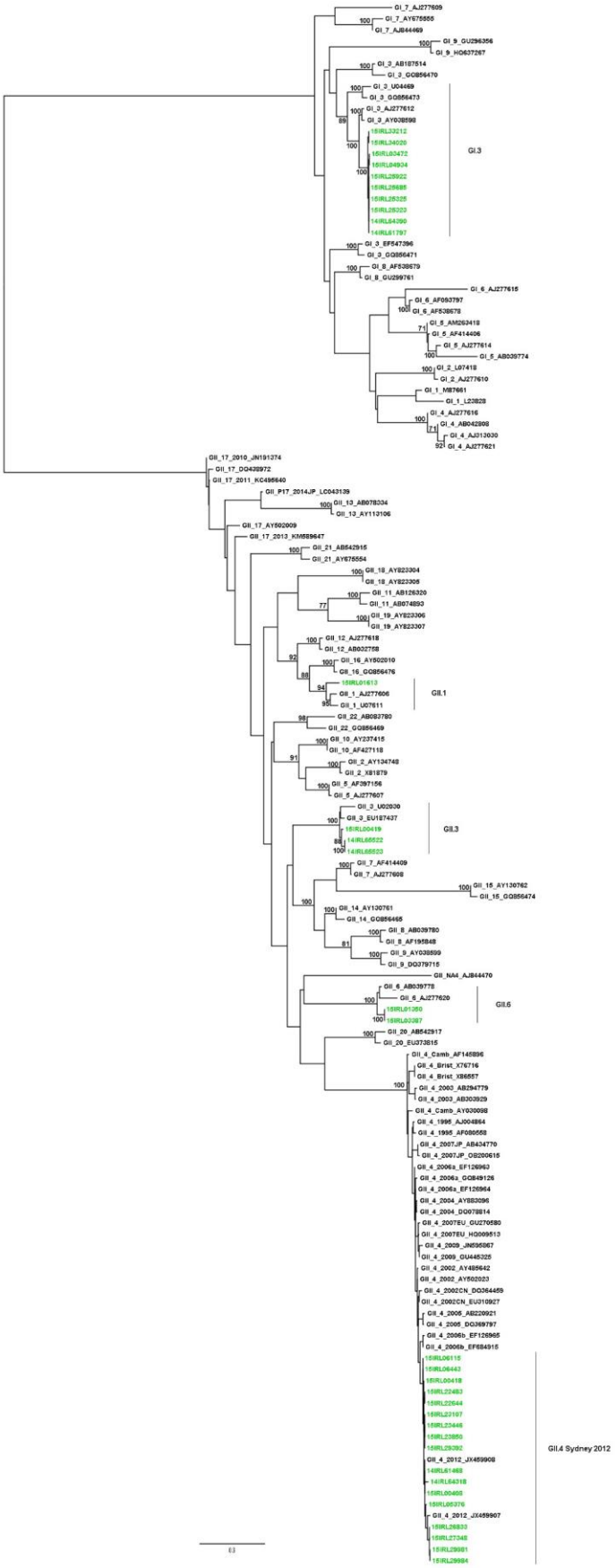
Thirty-three representative NoV positive specimens were chosen for genetic characterisation in the NVRL based on geographic distribution, age and outbreak severity. This included specimens from 17% of laboratory-confirmed notified outbreaks (2 GI, 9 GII) and a further 12 specimens (4 GI, 8 GII) representing non-notified outbreaks and sporadic cases of interest. Sequence and phylogenetic analysis identified viruses clustering with GI.3, GII.1 GII.3, GII.4 and GII.6 strains (Figure 15, Page 25). As in previous years, GII.4 (GII.4 Sydney 2012), remains the most prevalent circulating strain.

Of note, GII.17 was not detected in any outbreaks or specimens investigated at the NVRL this season. However, there is a need for physicians and public health and surveillance systems to be vigilant in the coming winter season for detection of this new strain and any possible associated increase in NoV activity.

Of note, it has been reported that some commercial NoV specific immunoassays and point of care tests might not detect the new GII.17 variant. Indeed, a recent study of 4 commercial immunoassays found them to be markedly less sensitive in detection of GII.17 compared to GII.4 [33].

The NoV assays in the NVRL are capable of detecting the novel variant, so if you have any concerns about outbreaks of AIG in which a pathogen cannot be identified, and in which there is a strong clinical suspicion of NoV, please don't hesitate to refer samples to the NVRL for further testing.

Figure 15: Maximum likelihood phylogenetic tree of Genogroup I and II NoV sequences (n=33) associated with outbreaks of NoV in Ireland in 2014. Irish strains are highlighted in green. Bootstrap values >70% are represented.



9.3. ROTAVIRUS

RV is the most significant cause of paediatric diarrhoea worldwide. RV, a member of the family *Reoviridae,* is classified into eight different species/groups A-H [34], with group A RVs responsible for the vast majority of human infections. RVs (not unlike influenza) have a dual classification system and are further classified into P and G types based on the properties of their outer capsid proteins, VP4 and VP7, respectively. There are at least 27 G and 35 P recognised genotypes, with several G/P combinations described [35]. The most commonly reported genotypes associated with human disease are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], accounting for approximately 90% of RV infections worldwide [36-38].

In 2009, WHO recommended the inclusion of RV vaccination into all national infant immunisation programmes [39]. Currently licensed vaccines, Rotarix® (a human-attenuated G1P[8] vaccine) and RotaTeq® (a pentavalent human-bovine reassortant vaccine), are both thought to elicit a heterotypic immune response in vaccinated individuals. To date, 77 countries have successfully integrated rotavirus vaccination in their national immunisation strategies [40-42]. Substantial reductions in RV associated illness and a decrease in related hospitalisations and healthcare visits of up to 84% have been reported in European countries in which RV vaccination has been implemented [42]. As the number of European countries successfully implementing RV vaccination strategies increases, it is likely that it will also be adopted into the Irish infant immunisation schedule. The WHO recommends that countries with RV vaccination programmes should develop robust surveillance systems to monitor the impact of vaccination on viral epidemiology [43].

The NVRL is currently introducing a RV characterisation service, which will be operated in accordance with WHO recommendations. Genotyping can be utilised to monitor current circulating strains of RV and any resulting genetic evolution or change in the epidemiology of viruses that may occur if RV vaccination were included in the national immunisation programme in Ireland.

As part of the validation process, a pilot study was performed at the NVRL characterising a selection of representative RV positive specimens from patients of differing ages and geographical regions throughout the 2014/2015 winter season in Ireland (n=18). Genotyping analyses identified 3 circulating RV variants, G1P[8], G9P[8] and G4P[8], all commonly reported in Europe [36]. In fact, 50% of specimens (n=9) characterised were identified as G1P[8], the most prevalent genotype reported in Europe in recent years [44,45]. Genotypes G9P[8] and G4P[8] were identified in the remaining 28% (n=5) and 22% (n=4) of specimens investigated. These results represent preliminary investigations and will be expanded on throughout the coming winter season.

10. HEPATITIS A VIRUS

Hepatitis A virus (HAV) is a *Hepatovirus* within the *Picornaviridae* family with an RNA genome of 7,500 nucleotides. Analysis of the capsid protein VP1 has led to the identification of six HAV genotypes, of which genotypes I, II and III (divided into subtypes A and B) cause human infection. Genotype 1 is the most prevalent worldwide, with 1A more frequently identified than 1B.

The NVRL offers a real time RT-PCR for the detection of HAV RNA, which can be useful to confirm serology results in acute HAV infection. In addition, molecular genotyping of HAV can be performed on request. This genetic characterisation is important in the identification and investigation of HAV outbreaks. The NVRL contributes HAV sequence data to the global hepatitis A surveillance system HAVNET which is coordinated by the Dutch National Institute for Public Health and the Environment (RIVM).

In 2014, molecular sequencing of six HAV cases was undertaken. This was significantly fewer than 2013, when 37 cases were characterised as part of an investigation into international foodborne HAV outbreaks [46,47]. All six cases from 2014 were identified as genotype 1A and no links to new international outbreaks were identified.

11. FUTURE DEVELOPMENTS

Hepatitis E Virus (HEV)

A Hepatitis E genotyping assay is currently in development at the NVRL. The introduction of this assay will lead to a greater understanding of the molecular epidemiology of autochthonous HEV infection in Ireland.

Respiratory Viruses

In light of the increasing interest in Human Rhinovirus infection, particularly Rhinovirus C, we are currently developing a Rhinovirus typing service which we hope will be introduced in the year ahead.

12. COLLABORATORS

The NVRL would like to sincerely thank all those who have contributed to the work performed at the NVRL 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. Our primary collaborators include:

- University College Dublin (UCD)
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- 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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