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Title	Paediatric rotavirus gastroenteritis - regional prevalent serotypes, correlation with disease severity, nosocomial acquisition, viral co-infection and the impact of rotavirus vaccine in one region of Ireland
Author(s)	Barsoum, Zakaria
Publication Date	2019-09-13
Publisher	NUI Galway
Item record	http://hdl.handle.net/10379/15437

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Paediatric Rotavirus Gastroenteritis - Regional Prevalent Serotypes, Correlation with Disease Severity, Nosocomial Acquisition, Viral Co-Infection and the Impact of Rotavirus Vaccine in One Region of Ireland

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Year of submission: 2019

Summary (Abstract):

Background: Rotavirus is the primary cause of gastroenteritis in children worldwide and a leading cause of gastroenteritis in children younger than three years, with a significant burden both globally and in Ireland. Rotavirus vaccine was licensed in 2006, recommended by the World Health Organisation, introduced in Ireland in October 2016. Co-infection with other viruses, in particular adenovirus, may occur in up to one third of cases, associated with severe disease. Nosocomial acquisition of rotavirus gastroenteritis results in a significant economic burden.

Objectives: 1. To determine rotavirus frequency, genotypes, disease severity, viral co infection, nosocomial acquisition, and other viral causes of gastroenteritis in our region.2. To determine the impact of rotavirus vaccine in a single district general hospital regarding admissions, duration of hospital stay, severity of disease and on seasonal characteristics of rotavirus gastroenteritis in a pre and post vaccination period.

Methods: In the post-vaccination year, from November 18th 2016 to November 18th 2017, all children up to 3 years of age who attended the Emergency Department of Mayo University Hospital or were admitted with vomiting and diarrhoea, were recruited and had their stool tested for rotavirus in addition to other enteric disease associated viruses (Adenovirus, Norovirus, Sapovirus and Astrovirus). Each week of the year was studied in relation to the total number of stool samples requested for testing of those viruses, the number of positive stool samples, their calculated median of positive stool samples in two consecutive weeks and their calculated median percentage of positive stool samples in each two consecutive week period.

Results: During the study period, gastroenteritis was the second leading cause of paediatric admissions (16%). Rotavirus gastroenteritis was a leading cause of gastroenteritis and occasionally severe; co- infection with other viruses with more severe gastroenteritis was noted. Rotavirus G1P8 including vaccine strain was the most predominant strain. Rotarix™ G1P8 was only detected among previously vaccinated infants up to six months of age. Compared with the pre vaccination years (2014-2016), the median percentage of reduction of rotavirus +ve stool requests and hospital admissions were high, 48.5% and 73%, respectively. Rotavirus vaccine shortened the duration of rotavirus season in 2016/2017. Nosocomial gastroenteritis was rare.

Conclusion: Rotavirus remains the leading cause of paediatric gastroenteritis. Rotavirus strains are diverse, with the emergence of strains such as G2P4, G4P8, G12P8 and G9P8. Rotavirus co-infection, mainly with adenovirus F, is common. Nosocomial rotavirus is rare and is associated with severe disease. Compared with three pre vaccination years, rotavirus vaccine reduced the total number of gastroenteritis hospital admissions, the total number of rotavirus +ve gastroenteritis cases and shortened the duration of the rotavirus season. Rotavirus vaccine reduced severity of disease, hospital admission rate and a likely reduction in associated health care costs and utilisation.

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Declaration:

I declare that all the work in this thesis is authentic and written by me and that I have not previously been conferred with any medical degree from the National University of Ireland, Galway or any other university based on this work. I performed the role of principal investigator and was responsible for the production of the study protocol. Approval for commencement was granted from the hospital Human Research Ethics Committee at Mayo University Hospital. Following on from this, I was responsible for participant recruitment, organisation of per-protocol investigations, data recording and analysis and reporting of all results.

Acknowledgements:

I acknowledge the National Virus Reference Laboratory's kind contribution to the work including access to their annual report, 2013-2014. I am indebted to Dr Joanne O'Gorman (Consultant Clinical Microbiologist at the National Virus Reference Laboratory, University College Dublin, Deputy Director of the National Virus Reference laboratory-Dublin) who has been fully supportive and encouraging me from the outset to completion of this project. I also extend my cordial gratitude to Professor Martin Cormican (Professor of Bacteriology, NUIG), and Chairperson of my Graduate Research Committee (GRC) who supported the content of my work and monitored my progress over the last three years during the course of the research. I would like also to thank Dr Una Ni Riain (Consultant Clinical Microbiologist-NUIG) who was a valuable member of my GRC with Dr Shyam Pathak (Paediatrics Tutor-NUIG). I higly appreciate the kind guidance and supervision of Dr Edina Moylett (Senior lecturer, paediatrics, NUIG, Consultant Paediatrician at University Hospital Galway) for kind acceptance to be my primary supervisor and

her support from the start of research to the stage of final submission for MD examination at NUIG. I also thank Dr Shomik Sibartie (Consultant Microbiologist, Mayo University Hospital) for support of the content of this research, acting as co supervisor. I thank all my co supervisors. I greatly thank Ms Zoe Yandle (Clinical Scientist at NVRL) for her kind assistance in relation to laboratory investigations. I also acknowledge kind contribution from staff of Mayo University Hospital, Mayo University Hospital Laboratory, Galway University Hospital Laboratory, and colleagues. Finally, I wish to thank Ms Alison Irvine, Northern Ireland Electronic Care Record Project Manager for her kind assistance with the layout of my research. I wish to thank Ms Gloria Avalos (Statistician at NUIG) for her valuable statistical input. I wish also to express my gratitude to all children and carers that chose to contribute to this research. They are all at the heart and forefront of my work, without their help, this research would not have been completed.

Major abbreviations used throughout the text:

- Adenovirus (AdenoV)
- Age (A)
- Astrovirus (AstroV)
- Diarrhoea (D)
- Dual Infection (DI)
- Emergency Department (ED)
- Episode of Infection (EOI)
- Galway University Hospital (GUH)
- Galway University Hospital Laboratory (GUHL)
- Gastroenteritis (GE)
- Genotype (G)
- Intra-venous fluid (IVF)
- Male (M) & Female (F)
- Mayo University Hospital (MUH)
- Median (M)
- Median percentage of Rotavirus (M%RV)
- Mild (M)
- Moderate (Mod)
- National Virus Reference Laboratory (NVRL)
- Norovirus (NoroV)
- Nosocomial gastroenteritis (NGE)
- Nosocomial Rotavirus (NRV)
- Not Applicable (N/A)
- Oral rehydration therapy (ORT)
- Paediatric Ward (PW)
- Peak(P) & Week Peak (WP)
- Rapid Antigen Testing (RAT)
- Rotarix (Rx)
- Rotavirus (RV)
- Rotavirus vaccine (RVVac)
- Sapovirus (SapoV)
- Season (S)
- Senior House Officers (SHOs)
- Total Admissions (TAD)
- Total number of gastroenteritis (TGE)
- Urinary tract infection (UTI)
- Vomiting (V)
- Week (W)
- World Health Organisation (WHO)

Chapter 1

Literature Review

1. Introduction (Summary)

Rotavirus (RV) is the primary cause of gastroenteritis (GE) in children worldwide (1 -3), and the most common pathogen responsible for hospitalisation of Irish children with GE. Nearly all children will experience an episode of RV during the first 5 years of life, notably the most common cause of GE in children younger than three years. Co-infection with other viruses in particular Adenovirus (AdenoV) may occur in up to one third of RV GE cases with the potential for more severe disease. In addition, nosocomial acquisition of RV GE (NRV) results in a significant economic burden both by prolonging the affected child's hospital stay and by that child serving as a reservoir propagating or extending RV infection to other children and therefore adding additional NRV cases.

The burden of RV disease is substantial. There is direct impact of admission costs on hospital budget and direct and indirect societal costs when children are admitted to hospital; days off work being most noteworthy, childcare costs etc. In a systematic analysis of the global burden of diarrhoeal diseases conducted in one study (Troeger *et al*, 2018; *Lancet Infect Dis 18(11)*), diarrhoea was the eighth leading cause of death among all ages (1 655 944 deaths, 95% uncertainty interval [UI] 1 244 073–2 366 552) and the fifth leading cause of death among children younger than 5 years (446 000 deaths, 390 894–504 613). Rotavirus was the leading aetiology for diarrhoeal mortality among children younger than 5 years (128 515 deaths, 105 138–155 133) and among all ages (228 047 deaths, 183 526–292 737).

A RV vaccine was licensed in 2006 and recommended by the World Health Organisation (WHO) for all children. The benefits of RV vaccination in preventing RV GE and its consequences are substantial. For example, in Mexico it is estimated that nationwide use of RotarixTM would prevent approximately 12,000 hospitalisations and 700 deaths from diarrhoea each year, a benefit that greatly outweighs the potential risks. Historically, however the first RV vaccine (RotaShield™) was linked with an increased incidence of intussusception leading to public concerns across the globe regarding RV vaccine safety, and only limited number of European countries adopting RV vaccination in their immunisation programmes. However, in trials which recruited >70,000 participants, prior to registration of RV vaccines, no increased risk of intussusception was observed. Worldwide, therefore a number of countries have adopted this recommendation and implemented vaccination in their paediatric immunisation programmes and RV vaccine (Rotarix™) was added to the primary immunisation schedule in Ireland in 2016. The WHO has recommended that post marketing surveillance for adverse events to continue whenever RV vaccines are introduced into new populations. Such studies are also important to reassure the public concerning vaccine safety, demonstrate to stakeholders that vaccine benefits far outweigh vaccine risks as they continue to monitor, review and put forward future vaccination strategies nationally, and internationally.

Therefore, the objectives of this study are to determine the frequency, severity, and genotype (subtype) distribution of RV infection in a cohort of children in an Irish context by performing a retrospective and prospective study in an era pre- and post vaccine introduction. We sought to also determine NRV incidence rate and its contribution to total cases of positive RV GE infection, as well as co-infection with other pathogens.

The impact of the vaccine upon its introduction in terms of reducing the rate of hospitalisation with GE, and thereof potential reduction to health care utilisation costs, is also investigated.

The remainder of this chapter outlines the relevant literature, and provides an in depth review of diarrhoeal illness in children with particular emphasis on viral (RV)-related infection, and RV vaccination.

2. Viral Gastroenterits (GE)

Acute GE is a clinical syndrome often defined by increased stool frequency (eg, ≥3 loose or watery stools in 24 hours or a number of loose/watery bowel movements that exceeds the child's usual number of daily bowel movements by two or more), with or without vomiting, fever, or abdominal pain (1-4, 115).
Vomiting usually lasts for one to two days and diarrhoea for five to seven days (2, 5,6, 115). In one study from a tertiary care children's hospital between 2006 and 2009, diarrhoea was present in 90%; the median duration of diarrhoea was six days (interquartile range 3 to 14); the median maximum number of stools per day was six (interquartile range 4 to 10). Vomiting was present in 56%; the median duration of vomiting was four days (interquartile range two to six); the median maximum number of episodes of emesis per day was three (interquartile range two to five). Fever (>38.3°C) was present in 42% (5). Acute viral GE may be complicated by dehydration, hypoglycaemia (1-4, 115-116). Acute viral GE occurs throughout the year, with a fall (autumn) and winter predominance (5, 7-9).

2.1 Rotavirus (RV)

Group A RV (RVA) is recognised as the most common aetiological agent of acute diarrhoeal disease in children (10). RV is a double stranded RNA virus with two outer capsid proteins which are used to classify RV into Glycoprotein (G) and protease sensitive types (P) respectively (10). RV GE usually occurs in children between six months and two years of age (11-12), occuring in the fall and winter in temperate climates and throughout the year in tropical climates.

2.1.1 RV Predominant Serotypes leading to Severe Disease

In one Irish study conducted from 2006 to 2008, the most common RV strain type was G1P8, and the emergent global G9-type was identified in both years. RVA strain type G2P4, previously reported in Ireland in 1999, was also detected. Genotypes G2 and G3 in combination with P4 were detected in 2006–2007 only (10). There was also an emergence of strain types including G3P4, G9P4, G2P[4þ8] and G2G4P[8] in one study (10). Notably, during that study G4, was not detected as a single infection, but was found in 8 co-infection combinations with G2G4P[8] (n=1), G1G4P[8] (n=6) and G4G9P[8] (n=1) (10).

In recent studies in Ireland, G9P8 has been detected at levels of 4.1–4.2% (13). In a study in southern Ireland between 2006 and 2009, G4 was found in combination with G1þG3P [8] in two mixed infection samples (14). This decrease in the frequency of detection of G4 strains has been observed in other studies carried out in Spain and China (10, 14-15). Worldwide, the emergence of serotype G9 as an epidemiologically important strain had raised concerns for RV vaccine development, calling into question the vaccine’s ability to induce heterotypic protection against the molecularly diverse circulating G9

strains, but both Rotarix™ and Rotateq™ RV vaccines have been found to be similarly effective against human G9 RVAs (10, 16-17).

Another recent study in the south of Ireland demonstrated that G1P8 (70.7%) and G3P8 (12.1%) were the prevalent genotypes responsible for GE cases between 2006 and 2009 (10, 13). Globally, the main human RVA G and P types circulating are G1-G4, G9 and P[8], P[4], P[6], with the majority of RV studies reporting G1P8 as the dominant strain (11, 18-20). Worldwide, RVA is the main cause of childhood diarrhoea and is responsible for over 100 million cases of GE annually (21). The results indicate that the common RV strain, G1P8, has remained dominant in Ireland since surveillance began in 1995.

Globally, G1P8 is the predominant strain, representing over 70% of RV infections in North America, Europe, and Australia (10, 19, 22). Up until 2015/16 RV G1P8 strain was the most prevalent strain year on year, ranging from 61% (2007/08) to 29% (2014/15), in 2015/16, G1P8 strain was the fourth most prevalent strain (13%) (22). G9P8 is becoming increasingly detected across Europe both in countries with and without routine RV vaccination, was the most prevalent strain in 2015/16 (34%) (22). G9P8 strain is widely circulating across Europe and was detected in 21% of typed samples in Germany in 2015/16 (22).

The diversity of circulating human RVA genotypes has been well documented in the absence of vaccination, with human genotypes fluctuating both geographically and temporally. G2P4 became the predominant strain in the UK since the introduction of RV vaccine in 2013 and was detected in 45% of typed samples in 2015/16 (10, 13, 22, 23). There are six RV genotypes circulating widely (G1P8, G2P4, G3P8, G4P8, G9P8, G12P8) and were isolated

in 96% from typed samples across Europe in 2015/16 as single RV strain infection (22). G12P8 is predominant in Italy (37%) and Spain (44%) (22).

In June 2014, the NVRL in Ireland introduced real-time (RT-) PCR assays for frontline screening of all specimens received for viral GE investigations. Between July 2014 and June 2015, The NVRL tested 11,500 stool specimens for viruses associated with GE. Of these, 27.8% (n=3,206) tested positive for at least one viral pathogen. RV was the principal pathogen identified in paediatric patients, detected in 15.8% of specimens tested from patients aged ≤5 years; followed by NoroV (13.2%), Sapov (5.9%), AstroV (5.2%) and group F adenoviruses (3.5%) (24).

Although the majority of the severe disease burden is located in developing countries, RV is estimated to cause 45% of hospitalisations and 20% of emergency department (ED) attendances for AGE in children < 5 years of age (25, 26). In addition, RV is estimated to cause approximately 80 000 general practice (GP) consultations and 750 000 diarrhoea episodes each year in the UK (26).

In a Swedish study that recruited 604 children <5 years of age from three geographical areas, who were admitted to hospital with RV-induced GE, RV G1P8 was most prevalent in all regions (77%), while the most varied pattern was observed in the western region, with G1P8 observed in 61%, G4P8 in 13%, G9P8 in 10%, G2P4 in 8%, and G3P8 in 8% of the children (27).

RVG9P8 may have a huge burden and was confirmed to be the cause of four deaths in children ≤ three years of age during an outbreak of diarrhoea in the Solomon islands in 2015 (28).

Nosocomial RV (NRV) infections are mainly associated with infants up to 5 months of age, RV was found to be the major aetiologic agent of paediatric nosocomial diarrhoea (31-87%)

(29). In the Swedish study that recruited 604 children <5 years of age from three geographical areas, admitted to hospital with RV-induced GE, 49 of 604 (8.1%) fulfilled the criteria for nosocomial infection (27).

2.1.2 GE Viral Co-infection

Mixed viral infections are not uncommon, but the clinical significance of coinfection with multiple viruses is unclear. However, some studies have shown that co- infection with other viruses can lead to severe disease (30-34). GE co-infection seems to be common in young infants (34-36). In a 2014 literature review of studies using polymerase chain reaction to detect viral GE pathogens, the prevalence of mixed infections in children with symptoms of GE ranged from 5.7 to 17% (37). In an American study conducted from October 2008 to September 2009, fairly high percentages (13.1%) of the patients with AGE were infected with multiple viruses. Most mixed infections included NoroV, identified in 21% of fecal specimens from patients in this study population (34, 38). Combinations of NoroV-AstroV and NoroV-AdenoV were most frequently identified as co-infections (30-31, 34, 38-39).

3. Impact of RV Vaccine

Two RVA vaccines are currently licensed in Ireland: Rotarix™ and RotaTeq™, introduced in the national immunisation schedule, in December 2016 (Rotarix ™). Implementing a universal RVA vaccination programme in Ireland may prevent approximately 2,000 GP visits, 3,280 ED attendances and 2,490 hospitalizations (10, 40). One RV vaccine is a two dose schedule (Rotarix ™, GSK) and the other a three dose schedule (Rotateq, Sanofi Pasteur MSD).

The RV vaccine being used as part of the HSE programme in Ireland is Rotarix™ (GSK). All children born in Ireland on or after October 1, 2016 were due to be given RV oral vaccine at 2 and 4 months of age; no RV vaccine should be given to a baby after 8 months of age due to possible risk of intussusception. A systematic review of the published literature was conducted in one study to examine the effectiveness and impact of RV vaccines in Europe following the first eight years of routine use (41). Across Europe, vaccine effectiveness against RV-related healthcare utilisation ranged from 68% to 98%, consistent with efficacy data from clinical trials. Reductions in RV hospitalisations ranged from 65% to 84%, consistent with findings from post-marketing studies from the US and Latin America. There is a significant public health benefit of RV vaccination in Europe with evidence to support implementation of universal RV vaccination in all European countries (41).

In two German studies, RV-vaccination was estimated to provide high protection against symptomatic RV-infection (Vaccine Effectiveness (VE)=96%; 95% credibility interval (CI): 91-99%) that remains at its maximum level for three years (95% CI: 1.43-5.80 years) and is fully waned after twelve years based on predictions of RV vaccine effectiveness made within a Bayesian framework (42). A Bayesian framework utilised adaptive Markov Chain Monte Carlo inference to compute the predictive distribution of RV-incidence after achieving high vaccination coverage with the introduction of routine vaccination (42).

At population level, routine vaccination at 90% coverage is predicted to reduce symptomatic RV-incidence among children aged <5 years by 84% (95% prediction interval (PI): (71-90%) including a 2.5% decrease due to herd protection (42). In areas attaining vaccine coverage of 64%, RV-related hospital admissions of 0 and 1 year old children decreased by 60%

compared with 19% reduction in the low vaccination coverage area (43).

In a third German study, data from 5 randomised controlled trials demonstrated a high efficacy of RV vaccines in preventing severe RV-associated GE (91%) and hospitalisation (92%) in settings comparable to Germany. Post-marketing observational studies confirmed these findings. In several countries, impact studies suggest that age groups not eligible for vaccination might also benefit from herd effects and demonstrated a decrease in the number of nosocomial RV infections after RV vaccine introduction (44).

In Finland where universal RV vaccination was introduced in September 2009, with exclusive use of the pentavalent human-bovine reassortant RV vaccine RotaTeq® and following a vaccination schedule at 2, 3 and 5 months of age, severe RV GE requiring hospitalisation was virtually eliminated in vaccine eligible children in the 3 years following implementation of universal RotaTeq vaccination (45).

In Australia, following the introduction of RV vaccine, there was 75% reduction in RV GE-associated hospitalisations and an 87% reduction in nosocomial RV infection. The peak incidence was lower and delayed compared with pre vaccination years. The reduction in RV events was observed not only in children younger than two years of age who were eligible for vaccination but also in older children consistent with herd immunisation (46, 47).

In 2010, and due to a quality problem identified in the vaccine manufacturing due to the detection of circovirus, the RV vaccination was withheld in Spain during a 5-month period. One study aimed to evaluate the impact that this sudden cease had on acute RV GE hospitalisations. An increase in RA GE hospitalisation was observed in parallel to the drop in vaccine

coverage. That was the first reverse evidence of RV vaccine impact (48).

RV vaccine may not give absolute protection against RV GE. However, it has been shown to reduce severe GE (44). RV vaccine may reduce hospital admissions with GE and subsequently reducing associated health care costs and utilisation. This was shown in several studies worldwide (30, 32, 42-48, 50). RV vaccine may also shorten the duration of RV season (50).

A systematic review of all publicly available data from RotaTeq™ vaccine-effectiveness and vaccination-impact studies in the USA, Europe and Australia between 2006 and February 2010 was undertaken in one study (50). Depending on the population studied, effectiveness of up to 100% (95% confidence interval 85-100%) associated with decreased hospitalisations for RV GE was seen. Vaccination impact studies demonstrated that the burden of RV GE has been reduced significantly since the introduction of RV vaccination. Evidence included reductions in healthcare utilisation due to RVGE (hospitalisations and emergency department visits reduced by up to 90%), reductions in the magnitude and duration of the RV season as assessed by laboratory testing for RV, and the possible induction of herd immunity (50).

Currently, two vaccines, RotaRix™ and RotaTeq®, have been licensed for use in many countries throughout the world following comprehensive safety and efficiency trials. Monitoring their effectiveness after licensure has confirmed that their incorporation into early childhood vaccination schedules can significantly prevent severe RV diarrhoea, which would have resulted in hospitalisations, emergency room visits or increased diarrhoea-related mortality. Although the efficacy of both vaccines is lower at approximately 40-59% in developing countries, their use could significantly reduce the mortality

associated with RV disease that is concentrated in these countries (51).

Whereas continued surveillance in the post vaccination era in the United States has shown that RV G12P8 is predominant with 70% prevelance due to a possible impact of RV vaccine on circulating RV strains (52), surveillance in Australia has shown dominance of G12P8 in states using Rotateq® and G2P4 and equine like G3P8 in states using Rotarix™ (53).

RV may cause seizure through neurotoxicity, neurotransmitter dysregulation or direct central nervous system infection and RV vaccine may have a positive protective impact on RV related seizure by preventing break of immune tolerance caused by the exacerbated inflammatory effect of RV disease (54).

Transmission of vaccine virus has not been well studied; it appears to occur more frequently among recipients of Rotarix ™ than RotaTeq (55); however, this rarely results in symptoms. In a randomised trial, in which one twin in each of 100 twin pairs received two doses of Rotarix™ and the other twin received placebo, transmission of vaccine virus occurred in 15 of 80 evaluable cases (18.8%) but was not associated with symptomatic GE (56). Such asymptomatic transmission may contribute to community (herd) protection (55). RV shedding in the stool peaks within approximately seven days of administration of vaccine and is most common after the first dose (57-59). Vaccine strain can be the cause of GE in these cases but one cannot be certain about this conclusion; as other causes of GE cannot be ruled out and the presence of vaccine strain may just be a co-incidental finding or just a shedding of vaccine strain with no direct causal relationship to GE (22). Older children who are not eligible for RV vaccine may benefit from vaccine herd protection (22, 42-44, 47, 49, 50).

RV vaccine has also a positive impact reducing RV related hospital admissions (26, 33, 41- 48, 50). Total attendance to ED with GE was reduced due to RV vaccine (26, 50).

Many studies worldwide have shown the positive effect of RV vaccine in terms of reducing the total number of presentation with GE (22, 26, 30, 42-47, 50). RV vaccine has been shown to reduce the total number of positive stool requests in many studies worldwide (30, 45-47, 60-61).

Across Europe and worldwide, following its introduction, RV vaccine has led to a significant reduction of RV GE associated disease (46-47, 60-61, 117), not only for the age group eligible for RV vaccine but also for children born before (46-47, 49).

4. Adenovirus

Adenoviruses (AdenoV) have a double-stranded DNA genome of approximately 35 kb surrounded by a non-enveloped icosahedron with fiber-like projections from each of the 12 vertices (62). Adenoviruses are a family of viruses that are an important cause of febrile illnesses in young children. They are most frequently associated with upper respiratory tract syndromes, such as pharyngitis or coryza, but can also cause pneumonia. Less commonly, adenoviruses cause gastrointestinal, ophthalmologic, genitourinary, and neurologic diseases. Most adenoviral diseases are self-limiting, although fatal infections can occur in immunocompromised hosts and occasionally in healthy children and adults.

Over 50 human AdenoV serotypes have been identified based upon antigenic determinants detected by viral neutralisation assay. Serotypes are further classified into six subgroups, A to F, based upon differences in patterns of hemagglutination (63).

Detection of AdenoV can be made by electron microscopy, specific AdenoV antigen assays or PCR assays (63).

In young children, 5 to 10 % of acute diarrhoeal illnesses are caused by the subgroup F adenoviruses types 40 and 41. AdenoV A 31 was reported to cause infantile GE. However, its association with infantile GE is not firmly established as compared with AdenoV F (63-64).

Other gastrointestinal complications of AdenoV, especially with subgroup C type 5 may include Hepatitis A especially in immunocompromised children (65). In addition, lower serotype adenoviruses have been associated with mesenteric adenitis, which may mimic appendicitis and occasionally cause intussusception. (66)

Enteric adenoviruses also may be a cause of nosocomial infection, as suggested by one report of 14 cases of enteric AdenoV-related diarrhoea in hospitalised infants over a three-month period (67).

AdenoV occurs throughout the year, with a peak in the summer (68-70).

5. Norovirus

Noroviruses (NoroV) were first identified as viral causes of GE in an outbreak in Norwalk, Ohio, and were previously referred to as the Norwalk-like viruses (71). Caliciviruses contain a single-stranded RNA genome and have a relatively simple structure, containing one major (VP1) and one minor (VP2) capsid protein (72).

Noroviruses are subdivided into at least seven genogroups based upon sequence homology (73, 74). Genogroups GI, GII, and GIV include human pathogens, and multiple genotypes are recognised within each genogroup (74).

Most epidemics of NoroV infection globally have been associated, until recently, with the emergence of novel genotype II.4 strains; these viruses have been associated with higher hospitalisation and death rates than other NoroV genotypes, as would be expected from an emerging strain (75-79). Two GII.4 variants were responsible for outbreaks of GE in Australia and New Zealand from 2005 to 2006 (80). Subsequently, one of these strains was also linked to approximately one-fourth of the outbreaks reported in the United Kingdom. In 2012, GII.4 Sydney strain (named for the location in Australia where it was first isolated) replaced GII.4 New Orleans as the predominant strain in the United States (81). The proportion of outbreaks attributed to this new strain increased from 19 to 58% between September and December 2012.

In 2014, a GII.17 variant emerged in Japan and has spread worldwide (82-85). Its clinical presentation is indistinguishable from that of previously predominating strains. In 2016, GII.4 genotypes remain highly predominant in the United States and most of the world.

NoroV GE occurs in people of all ages, occurs year-round, with a peak in autumn and winter (5, 7-9, 68-70, 86).

6. Sapovirus

Sapovirus (SapoV) is one of the two human caliciviruses that cause acute GE and have a worldwide distribution (NoroV is the second) (87).

SapoV is a single-stranded RNA genome and have a relatively simple structure, containing one major (VP1) and one minor (VP2) capsid protein (88). Sapoviruses were first identified as viral causes of GE in Japan in 1977 and were previously referred to as the SapoV (89-90). Sapoviruses are differentiated from

noroviruses on the basis of differences in antigens and genome organisation.

Sapoviruses are divided into Geno groups GI to GV; all but GIII are associated with human infection. Frequent genomic recombination contributes to the genetic diversity of these viruses (88, 91). The relative frequency of sapoviruses as a cause of acute GE in children is increasing (up to 10%) in regions where child vaccination rates for RV are high (92).

SapoV traditionally circulated at a low prevalence in Europe, linked to sporadic cases of GE. However, in recent years the epidemiology of the virus has changed and the prevalence of both sporadic cases and associated outbreaks of SapoV has increased significantly, possibly linked to the emergence of a new genotype (93). To date, limited information has been available regarding SapoV in Ireland. The NVRL detected SapoV in 3.4% of all specimens tested (n=390/11,510) from July 2014 to June 2015, with SapoV infections comprising 12% of all laboratory confirmed cases of GE (24). Although the majority of these infections occurred in paediatric patients, infection in adults was observed and both laboratory-confirmed outbreaks of SapoV occurred in nursing home settings and long-term facilities (90). SapoV GE mainly affects infants and toddlers (68, 94-95). SapoV occurs year-round, with the highest proportion from March through July (5, 7-9, 68-70).

7. Astrovirus

Astrovirus (AstroV) was first discovered in 1975 using electron microscopes following an outbreak of diarrhoea in humans (96). AstroV have a star-like appearance with five or six points. Human Astroviruses have been shown in numerous studies to be an important cause of GE in young children worldwide (97).

Their name is derived from the Greek word "astron" meaning star. They are non-enveloped RNA viruses with cubic capsids, approximately 28–35 nm in diameter (98).

The main symptoms are diarrhoea, followed by nausea, vomiting, fever, malaise and abdominal pain. Some research studies have shown that the incubation period of the disease is approximately three to four days. Diagnosis can be made by electron microscopy, enzyme-immunoassay (ELISA), immunofluorescence, and polymerase chain reaction for detecting virus particle, antigens or viral nucleic acid in the stools of infected people (99). A method using real-time RT-PCR, which can detect all human AstroV genotypes, has been reported (100).

AstroV causes endemic childhood diarrhoea; worldwide, it is responsible for 3 to 9% of diarrhoeal illness (101-102). Transmission is primarily person-to-person via the faecal-oral route and also via contaminated food and water (102-103). In temperate regions, there is a peak in infection during winter months; in tropical regions, infection occurs most frequently during rainy seasons (104-105).

AstroV is responsible for 4 to 7% of diarrhoeal illness in childcare centres and in the community (106) and has been associated with nosocomial disease in up to 16% of cases (107-108). AstroV GE occurs primarily in children younger than four years of age; AstroV GE usually occurs in the winter months (5, 7-9, 68-70, 109). Nosocomial infection with AstroV was reported in other studies (107-108).

8. Sensitivity & Specificity of Virus Detecting Methods

Detection of virus antigen using real time reverse transcription-polymerase chain reaction (RT-PCR) is more sensitive and specific than RAT and comprised of amplification of certain regions of the genome of the virus followed by

identification of the genotype by fragment size analysis using electrophoresis (110-112). RAT - as RV detecting tool - is more specific than sensitive (higher specificity and lesser sensitivity) (113-114), a quick and easy tool to detect virus antigen in stool samples using immunochromatography and may be useful for investigating and screening group A RV during outbreaks of food-borne and person-to-person transmitted GE (113). This rapid diagnostic test is easy to perform at the bedside, as it takes only 20 minutes to reach a diagnosis with a simple procedure, and does not require special equipment (113). Of 71 samples that were positive for RV by RT-PCR, 69, 68 and 63 were also recognised by RAT kits, indicating 97.2, 95.8 and 88.7% sensitivity for RAT kits, with only one false positive result in one of the three RAT kits (specificity up to 100%) (113).

When comparing the sensitivity of RAT for group A RV detection, it is clearly demonstrated that among various immunochromatography kits, sensitivity and specificity for group A RV infection were a bit different. In addition, it was observed that several RV genotypes G1, G3 and G9 were reacted with these kits. Therefore, genotype variations of RV may not be a problem for false negative results (113). RT-PCR assay was found to be specific to RV and broadly reactive to RV genogroups 1–4, 9, 10 and 12 (112). Specificity testing did not identify any cross-reactivity of the assay with a panel of 36 non-RV enteric virus specimens (112).

Highly sensitive and specific methods such as one-step RT-PCR are still required for true diagnosis of viral GE following clinical suspicion of GE and GE associated complications and for RV vaccine efficacy trials (110-112, 115-117).

9. Summary and Research Objectives

RV is the primary cause of GE in children worldwide and the most common pathogen responsible for hospitalisation of Irish children with GE. The burden of RV disease is substantial. A RV vaccine was licensed in 2006 and recommended by the World Health Organisation. The potential benefits of RV vaccination in preventing RV GE and its consequences are substantial. RV vaccine (Rotarix™) was added to the primary immunisation schedule in Ireland in 2016. The WHO has recommended that post-marketing surveillance for adverse events should continue whenever these vaccines are introduced into new populations. It is also important to reassure the public concerning vaccine safety, and benefits versus risks and to advise key stakeholders to monitor, review and put forward future vaccination strategies including RV vaccine, at national as well as international levels.

Therefore, the objectives in this study were to perform a retrospective and prospective study examining some of these issues, in a pre-and post vaccine era in an Irish context (district regional hospital). Specficially the aims were to:

- Determine the frequency, genotype spectrum, seasonal characteristics, and severity of RV infection.
- Determine NRV incidence rate and its contribution to total cases of positive RV GE infection, AND co-infection rate with other pathogens.
- Determine the the impact of the vaccine introduction in terms of reducing the rate of hospitalisation with GE; thereof also potential reductions to health care utilisation costs.

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Chapter 2
Methodology

2.1 Period of prospective recruitment: November 18th 2016 to November 18th 2017. Additional review of retrospective data for years 2014-2016.

2.2 Inclusion criteria

1-All children up to 3 years old that attended the Emergency Department (ED) or were admitted to hospital with vomiting and diarrhoea (loose stool) or diarrhoeal symptoms.
2-All children on the paediatric ward who developed diarrhoea 3 days (72 hours) after their admission, possible nosocomial RV (NRV).
3-All children up to 3 years old readmitted to the paediatric ward within 48 hours following recent discharge, possible NRV.

2.3 Exclusion criteria

1-Parents not willing to allow their child to participate or those who chose to opt out of the research at any time.
2- Children presenting with chronic diarrhoea due to other disease e.g., immunodeficiency or inflammatory bowel disease.
3-Children presenting with the same diagnosis within a 48-hour period. One child with GE was transferred to another hospital to be admitted there and was excluded, as the child did not stay more than one night at MUH. In total, four children were excluded.

2.4 Definitions

2.4.1 The median (M) positive stool samples for GE related viral pathogen (RV, AdenoV, NoroV, SapoV and AstroV) in two consecutive weeks was defined as the total number of positive stool samples for this virus in two consecutive weeks divided by two, the calculated median percentage of positive stool samples for GE related viral pathogen in each two consecutive week period was defined as the mean or the median of positive stool samples for this virus in two consecutive weeks divided by the total number of stool samples tested for this viral infection in these two consecutive weeks.

2.4.2 A 'week' was defined in this study as week starting from 0900 AM each Monday to 0900 AM on the following Monday, throughout the whole year, and for the whole period of the study.

A 'week peak' was defined as the week with the highest number of confirmed positive stool samples with GE related viral infection.

2.4.3 Onset of GE related virus Season: any two consecutive weeks in any month when the median percentage of positive stool samples for this virus was 10% or more.

2.4.4 Peak of GE related virus Season: any two consecutive weeks in any month when the median percentage of stool samples confirmed to be positive for this viral infection was the highest percentage.

2.4.5 End of GE related virus Season: any two consecutive weeks in any month with the median percentage of stool samples confirmed to be positive for this viral infection was less than 10%.

2.4.6 A short episode of GE related virus infection (EOI): Any short period of any virus infection not longer than three weeks with a rapid onset and a rapid end, with a median percentage of

positive infection of less than 10% in the immediate two consecutive weeks following the first week of the onset of this viral infection. A short episode of viral infection has no ‘peak’ of infection.

2.5 Research Dynamics

Information leaflets (see appendix 1), were given to parents of children contributing to the research, explaining the purpose of the research study. Consent was obtained and parents were given the right to choose to withdraw or opt out of the research at any time.

A certificate of ethical approval for the research study was obtained from the Clinical Research Ethical Committee at Mayo University Hospital (MUH) before study commencement.

Stool samples were split into two on receipt to the local laboratory at MUH. One half of the stool samples were sent to Galway University Hospital (GUH) as routinely done and the other half were stored frozen at MUH laboratory.

The results of the samples processed at MUH were followed up and recorded. RV positive results were identified and their matching samples, already stored frozen at MUH, were sent in batches to the National Virus Reference Laboratory (NVRL) in Dublin to be tested for:

A- Multiple pathogen panel of viruses (RV, Adeno F, SapoV, Astro V and NoroV).

B- RV genotype.

The method of testing used in NVRL to detect viruses was by real time PCR of viral antigens (1-6). All samples received at the

NVRL for the investigation of viral GE were extracted on the Roche MagNA Pure 96 as per manufacturer's protocol. Samples were tested by real-time RT-PCR for the detection of RV, Rotarix™-derived RV, NoroV GI, NoroV GII, SapoV, AstroV, and AdenoV F (1–6). Testing and analysis were performed on the Applied Biosystems ABI 7500 Fast (NoroV, Rotarix™) or ABI Viia7 (RV, AdenoVF, SapoV, AstroV) instrument and analysed using the ABI software version, 2.3 (NoroV, Rotarix™) or version 2.1.1 (RV, AdenoVF, SapoV, AstroV) respectively. Samples identified as wild-type RV positive were genotyped. The RV genotyping method comprised of amplification of the VP7 and VP4 regions of the genome followed by identification of genotype by fragment size analysis using electrophoresis (7).

Samples that were tested negative for RV at MUH were also identified. Their other halves, already stored frozen at MUH, were sent in quarterly batches to the NVRL for further testing via multiple pathogens panel.

At GUH lab, testing included RV and AdenoV. The method of RV testing is by Rapid Antigen Strip testing. AdenoV is detected by Pan AdenoV testing that can detect AdenoV type (A-F).

Infants who were vaccinated and their stool tested positive for RV were included. The stool samples from these children were tested in the NVRL for multiple pathogens panels and were genotyped to determine if a wild type or a vaccine strain was present with or without dual infection. All RV positive stool samples were tested in the NVRL for vaccine strain whether or not infants received RV vaccine by real time (RT) PCR testing to differentiate between a wild and a vaccine strain and was used for the first time in Ireland in December 2017 immediately after our studt finished.

Every week of each year was studied in relation to the total number of stool samples requested for viral testing, the number of positive stool samples, the calculated mean (or median) of positive stool samples for viral GE in two consecutive weeks, the calculated median percentage of positive stool samples for viruses in each two consecutive week period.

A specific referral request form was enclosed with all the stool samples sent to the NVRL indicating Date of Birth (DOB) of patients, gender and the outcome of testing at GUH.

All results were sent confidentially and pseudo-anonymously using DOB to identify patients.

Positive results of RV and other viruses were reported to all parents based on their request. Bacterial pathogens were beyond the scope of this RV research and were not included in this study.

Appendix 1

Information leaflet & Consent form

Dear Parents,

Rotavirus (RV) is the commonest cause for diarrhoea (loose stool) in childhood. RV can lead to excessive water loss and weakness of your child. RV commonly affects children younger than three years of age. There is a significant financial burden associated with RV infection both to you and the state.

RV vaccine has been introduced as part of Irish childhood immunisation programme to protect your child from RV disease.

This study will look at the frequency of rotavirus and other viruses that can cause diarrhoeal disease in children in our community. The study will look at how the vaccine will work. A stool sample is part of routine clinical care of all children presenting with loose stool. There will be no other extra tests and no blood testing will be required. For the study to be conducted, I need to collect a stool sample from your child to be tested for rotavirus and other viruses.

Once results are available, I will notify you if you wish.

All information about your child will be kept confidential.

You can choose to opt out of this research at any time.

Thank you for your co-operation.

Dr. Zakaria Barsoum

Consultant Paediatrician (Primary investigator)

Mayo University Hospital

Are you willing to participate in this study and allow us to obtain a stool sample from your child for rotavirus and other virus testing?

Please circle your answer.

Please print your name, date and sign.

1-Yes

2-No

Name: _____

Signature: _____

Date: _____/_____/_____

2.6 Assessment of Severity of GE

Internationally recognised Vesikari Scoring System (see appendix 2) was used to assess disease severity (8). Vesikari clinical scoring system was applied to all children presenting with GE to our regional hospital- on a daily basis for the whole period of the study.

Senior House Officers (SHOs) were given training on the method of assessment of GE severity using Vesikari scoring system. They were provided with copies of the scoring system. Where possible, the scoring system was checked again on the following day to ensure that the scoring process was conducted effectively.

Based on Vesikari Scoring System, GE was classified into mild, moderate and severe forms. Hospital admission in our study was defined as a period of at least one night stay.

A number of parameters were used in Visikari scoring systems (See Appendix 2; Table below).

Any score below seven was defined as mild GE, scores between seven and ten were defined as moderate GE, equal to or higher than 11 were defined as severe GE. No scores were given for any parameter that was not present or was not applicable (N/A) at the time of assessment. The highest score was 20.

Appendix 2

Vesikari Clinical Severity Scoring System

Date of 1st dose RV Vaccine (if applicable):

Date of 2nd dose RV Vaccine (if applicable):

Parameter	**Score**		
	1	**2**	**3**
Diarrhoea			
Maximum number of stools/day	1-3	4-5	6 or more
Diarrhoea Duration (Days)	1-4	5	6 or more
Vomiting			
Maximum number vomiting episodes/day	1	2-4	5 or more
Vomiting Duration (Days)	1	2	3
Temperature	37.1-38.4	38.5-38.9	39 or more
Dehydration	N/A	1-5%	6% or more
Treatment	ORT therapy	Hospitalisation or IV hydration	N/A
Total score			
Severity Category	Mild	Moderate	Severe
	<7	7-10	11 or more
Severity score			

2.7 Data Management

Data related to hospital outcome of disease were collected confidentially using electronic hospital laboratory system. Data were verified on a case to case basis against the following: patient name, age, date of birth (DOB), hospital number, date of hospital admission, date of hospital discharge, clinical scoring of severity, duration of hospital stay, date of stool sample and result of testing including nosocomial acquisition of RV GE infection where applicable, onset of diarrhoea after 72 hours following to hospital admission, date of hospital re- admission with diarrhoea within 48 hours following to hospital discharge.

Data were collected prospectively for 12 months from November 18th 2016 to November 18th 2017. Date of introduction of RV vaccine was recorded. RV (Rotarix™ vaccine) was introduced in Ireland on the first of October 2016, was scheduled to be given to all babies born in Ireland from the first of October 2016. Two doses were to be given to babies at two and four months of age. Comparative analysis was conducted comparing three pre vaccination years (2014,2015, 2016) and one post vaccination year (2017) in relation to the impact that the vaccine may have on RV GE seasonality, severity, nosocomial acquisition of RV infection and any possible protective indirect effect on non-vaccinated children (umbrella cover or herd protection effect).

Data related to vaccine coverage rate or uptake rate following vaccine introduction were obtained in collaboration with Mayo Immunisation Office. Data were collected regarding the two doses of vaccine, the total number of children vaccinated and the total number of infants eligible for RV vaccine in Co Mayo, Ireland.

In our study, data are presented in relation to total number of children with gastroenteritis (TGE), expressed as a percentage

of the total number of paediatric admissions (TGE:TAD), total number of children up to three years of age with GE (TGE≤3Y), expressed as a percentage (%) of the total number of children with GE (TGE≤3Y:TGE), total number of admissions with GE in children up to three years old (TAD GE≤3Y), as a percentage in relation to the total number of paediatric admissions (TAD) and percentage of GE in children up to three years old as a proportion of the total number of paediatric attendances to the ED department (TGE≤3Y:TED).

Data are also presented in relation to GE related length of hospital stay, clinical spectrum of GE; vomiting (V): number per day and duration in days; diarrhoea (D): number per day and duration in days; temperature: 37.1°-38.4°, 38.5°-38.9° and >38.9°; management: Oral Rehydration Therapy (ORT), Hospitalisation and Intravenous Fluid Therapy (IVF).

Data are presented in relation to viruses associated with GE: RV and its genotypes, NoroV, AdenoV F, AstroV and SapoV in children ≤ three years of age, presented to MUH, from November 18 th 2016 to 18 November 18 th 2017, based on the outcome of multiple viral pathogens testing performed in the National Virus Reference Laboratory (NVRL) in Dublin-Ireland.

Data are presented in relation to viruses associated with GE: RV and its genotypes, NoroV, AdenoV F, AstroV and SapoV, viral season, total (T) number of viral GE cases, age of patient in years (Y) or months (M) or weeks (W), gender, RV vaccine (RVVac) given and at what age, week peak (WP) of cases of GE, week (W) of onset of GE, and Dual infection (DI) with more than one virus causing GE.

RV related data are presented in relation to RV predominant genotypes, RV genotypes including vaccine strain (Rotarix™) correlation to disease severity, nosocomial acquisition of RV

(NRV), length of hospital stay, RV negative GE in RV vaccinated infants and RV positive GE in RV vaccinated infants and age at onset of GE in RV vaccinated infants.

Data are also presented in relation to RV and AdenoV detecting method: Rapid Antigen Testing (RAT) in Galway University Hospital laboratory (GUHL) Versus (PCR) testing of virus in the NVRL, sensitivity and specificity of RAT during the study period.

Data are presented in relation to the impact of RV vaccine concerning: a) vaccine effect on RV season in terms of number of seasons in one year, b) onset and duration of season, comparing data from the immediate three pre vaccination years (2014-2016), c) effect of RV vaccine on total number of RV stool requests comparing pre- and post RV vaccination years and the effect of RV vaccine on the total number of RV GE positive stool samples for the same period of analysis. Discriptive statistics using Chi Square test compared proportions of RV detected and not detected pre and post vaccination and proportions of GE admitted and not admitted cases for the same period of analysis.

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Chapter 3: Results I

The results in this chapter are based on the objectives to determine GE frequency, disease severity, viral co infection and nosocomial acquisition of GE in our region. In this results chapter are data regarding:

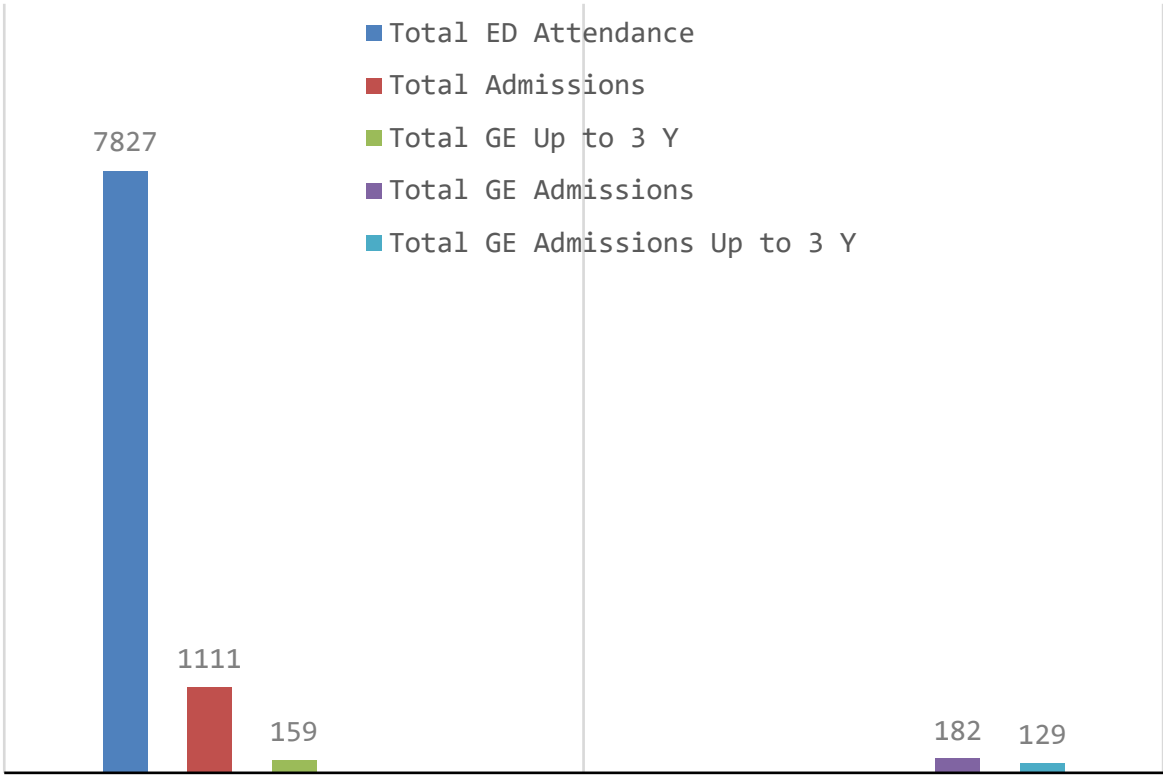
- The paediatric admission rate, viral GE admission rate and respective clinical details at Mayo University Hospital (MUH) during the post-RV vaccination period 2016-2017.
- The principle enteric viral pathogens detected in the post-RV vaccine period including seasonal variations, and age distributions at MUH post-vaccine (2016-2017).
- Specific clinical and epidemiological details regarding RV and other viral pathogens detected at MUH post- RV vaccine.
- The types, seasonal variation, and age distributions of the principle enteric viral pathogens in Ireland pre- RV vaccine (2014-2016).
- Appendices of Chapter 3:
    - Sensitivity specificity data analysis defining accuracy of viral detection methods for two most common pathogens in the post-vaccine era (Rotavirus and Adenovirus).
    - Supplemental (Suporting) Data Tables

1. Viral GE-related admissions, clinical presentation and management

1.1 GE-related admission rate

During the prospective (post-vaccine) study period (Nov. 18th 2016 to Nov 18th 2017), almost 8,000 children presented to the ED at MUH; 1,111 were admitted, 55% male. Respiratory diagnoses were the lead cause of admissions (41%). GE was the second leading cause of paediatric admissions (182), 71% ≤ 3 years old. Total number of children ≤ 3 years of age who attended to ED with GE was 159(2% of the total number of children attending the ED, which was 7,827 (Figure 1). Nosocomial GE (NGE) was only reported in 2 cases (1.1%): one case was a 10 month old female with complex cardiac problems; no viral pathogen was detected; case was of moderate severity and remained hospitalised for 3 weeks; case was detected during the fourth week of April. The second case was a 25 month old male with I-cell disease; case was severe, RV G2P4 was detected; hospitalised for 4 weeks, detected during the 3rd week of July. Viral illness was detected in 110 patients (9%); UTI in 42 children (4%); other causes for admission noted in 343 patients (30%).

Figure 1 Paediatric Attendance-MUH



1.2 GE: Length of Hospital Stay

During the prospective study period (post-vaccination), 128 patients were admitted, one readmitted. Expectedly, the majority of paediatric GE cases (68%) stayed between 1-2 days, 23% between 2-3 days, and 9% more than 4 days. The greatest number of children admitted with GE in one month was noted in May and June; 18 and 15 respectively. The least number of children admitted with GE in one month was noted in September.

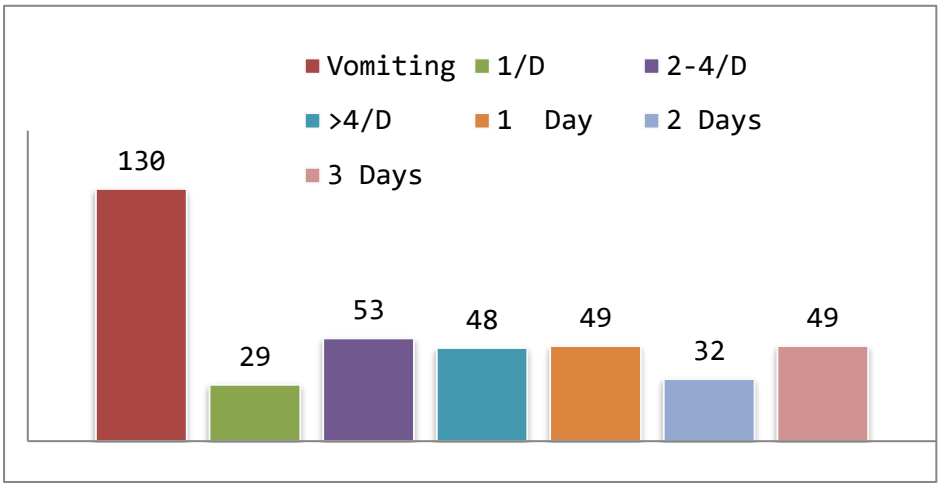
1.3 GE: Clinical Spectrum

During the prospective (post-vaccine) study period, 159 cases of GE were detected. Clinical scoring was not applicable in one patient due to a language barrier. Therefore, 158 cases were

studied for clinical spectrum using Visikari clinical scoring system; 90 cases were severe.

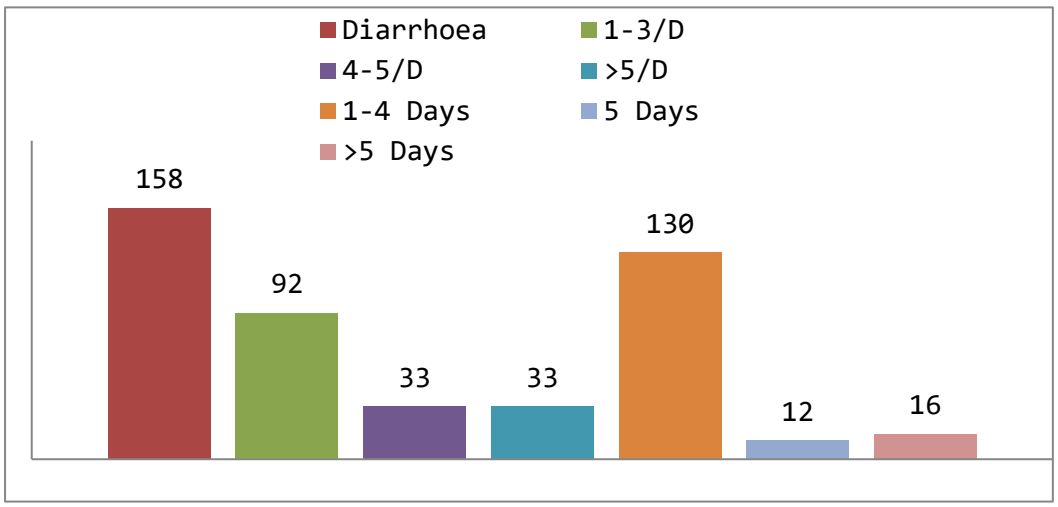
Vomiting (Figure 2) was a predominant feature of paediatric GE, noted in (82%), with a duration mostly between one to three days (62%). The median % of the frequency of vomiting was more than four per day (30%).

Figure 2: Vomiting- Number/Day & Duration in Days



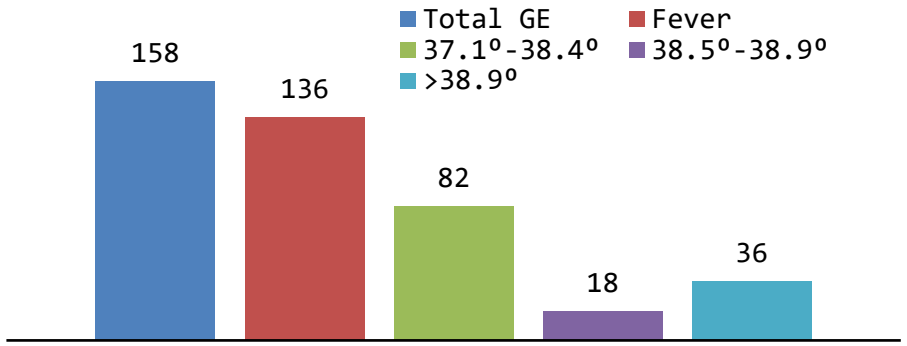
Diarrhoea was noted in all 158 children. The majority of children had diarrhoea for a period between one to four days (82%); 58% of children had episodes of diarrhoea between one to three times per day (Figure 3).

Figure 3 Diarrhoea-Number/Day & Duration in Days



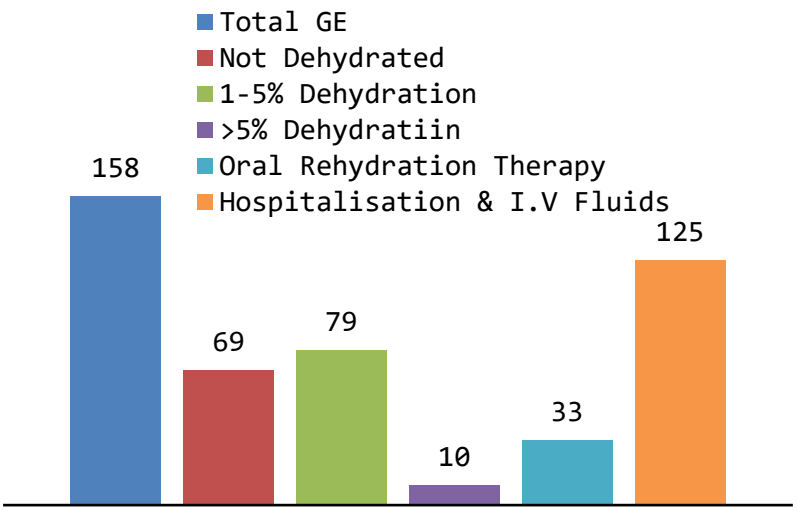
Fever was noted in 86% of children with GE; >38.5°C was present in 34% (Figure 4).

Figure 4: Fever



Half of children were mildly dehydrated at presentation; 69 patients were not dehydrated (44%). Greater than 5% dehydration was detected in ten patients (6%). Oral Rehydration Therapy (ORT) was only successful in the management of GE in 21%. IVF were administered to the remaining 79% of children (Figure 5).

Figure 5: Management



1.4 Seasonal variation

The fourth week of June was the week peak of the year, during which seven cases of GE presented, all admitted. Four were male, followed by the second week of April, the first week of May, the first week of August and the fourth week of December (six patients in each month). The largest number of presentations with GE was noted in May (20 cases) followed by December and June (18 cases each) with the largest number of severe GE noted in June (12 cases) followed by December and May (11 cases each) (Figures 6 & 7). In total, 90 cases were severe (57%); 57 moderate (36%); 11 mild (7%).

Figure 6: GE- Monthly Figures

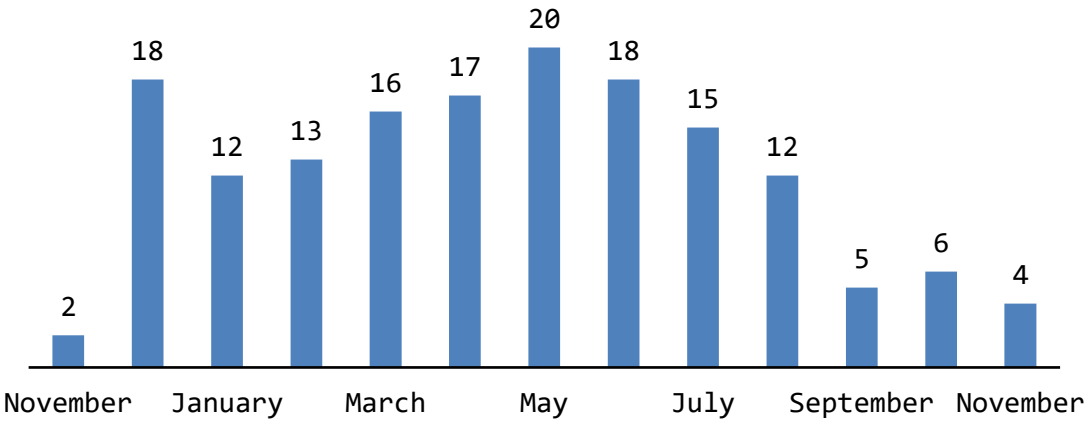
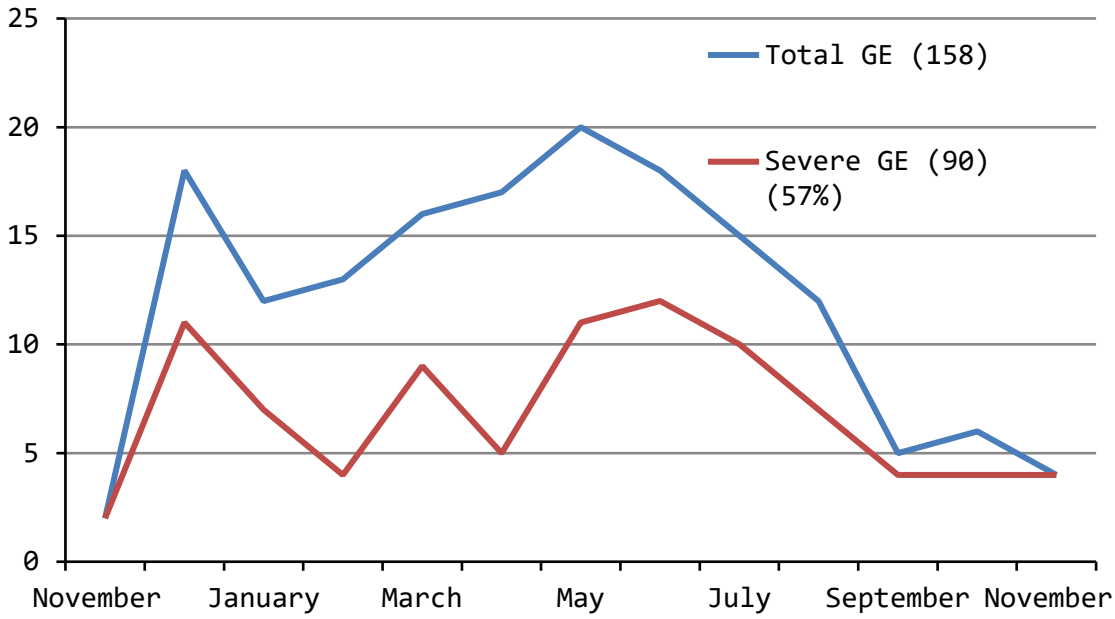


Figure 7: GE-Severity



1.5 Discussion

Acute GE harbours a significant global burden. (6). Acute viral GE may be complicated by dehydration (7) as shown in our study (56%). Our study supports the findings of other studies that

vomiting usually lasts for one to two days (2, 8-10) and that diarrhoea is the most predominant feature of GE (8). Fever (>38.3°C) was present in 42% (8), whereas in or study, fever was noted in 34%. Acute viral GE occurs throughout the year, with a fall and winter predominance (8, 11-13) and seasonal variability of GE was also noted in our study.

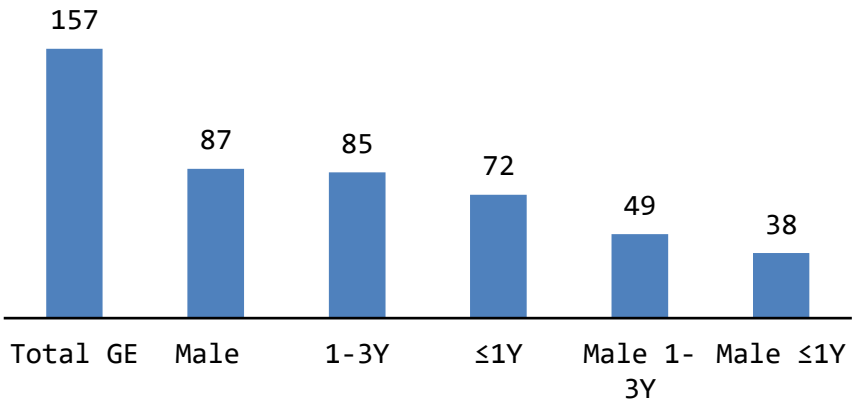
2. Viral Gastroenteritis (2016-2017)

Below are data describing the distribution and details of the specific viral GE pathogens detected during the prospective study period (Nov.18th 2016- Nov 18th 2017) at MUH. This is followed by national data from the NVRL in the preceeding 2 years for comparison.

2.1 Number of viral GE cases, age & gender distribution (2016-2017)

157 patients were diagnosed with viral GE, after excluding two females who presented twice during the study period with GE; 87 were male (55%); 85 patients were between 1 to 3 years of age (54%) of whom 49 were male (56% of the total male number) and 36 were female (51%); 72 were age ≤ 1 year (46%), of whom 38 patients were male (44% of the total male number) and 34 patients were female (49%)(Figure 8). The median age at presentation was 15 months. The 2 female patients who presented twice with GE did not contract the same viral pathogen more than once. Nine samples were not tested in the NVRL as the samples were either missing or mislabelled. They were excluded from RV and other viruses testing.

Figure 8: All GE - Age & Gender (2016-2017)



The total number of stool samples tested for RV and other viruses was 150. The total number of stool samples (patients) tested +ve for a single infection with one of the viruses causing GE was 90 (60%). 19 infants received RV vaccine prior to their first presentation with GE (12%), of whom ten were male. Data in relation to vaccinated infants will be discussed in Chapters 4 and 5.

2.2 Viral Pathogens

2.2.1. RV was the leading cause of GE (37 patients, 24.6%) followed by human AdenoV F (19 patients, 13%), NoroV (18 patients, 12%), SapoV (9 patients, 6%) and AstroV (7 patients, 5%), also distributed by age and gender (Figures 9 & 10).

Figure 9: Specific types of viral GE

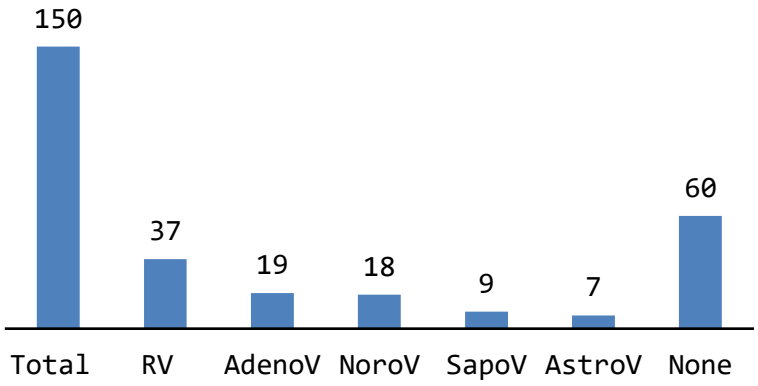
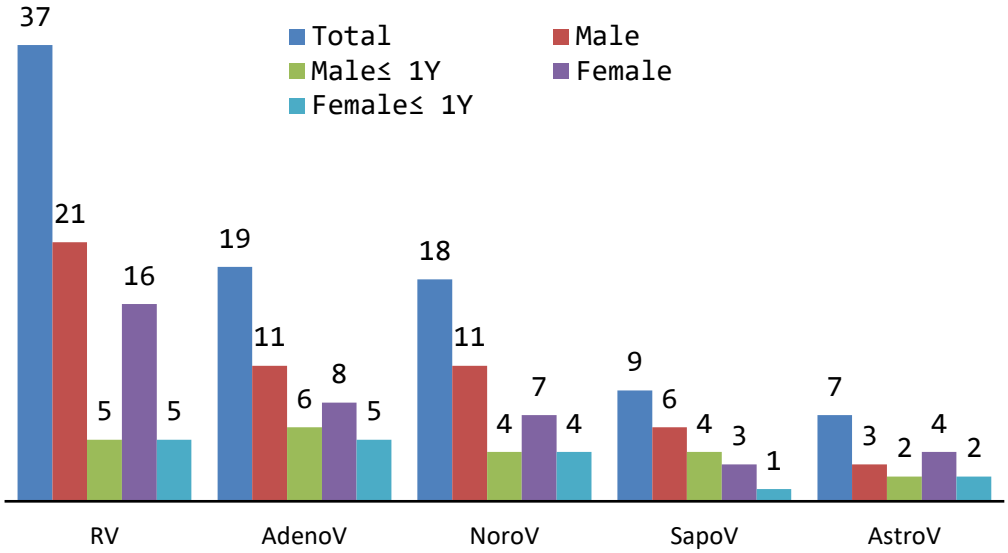


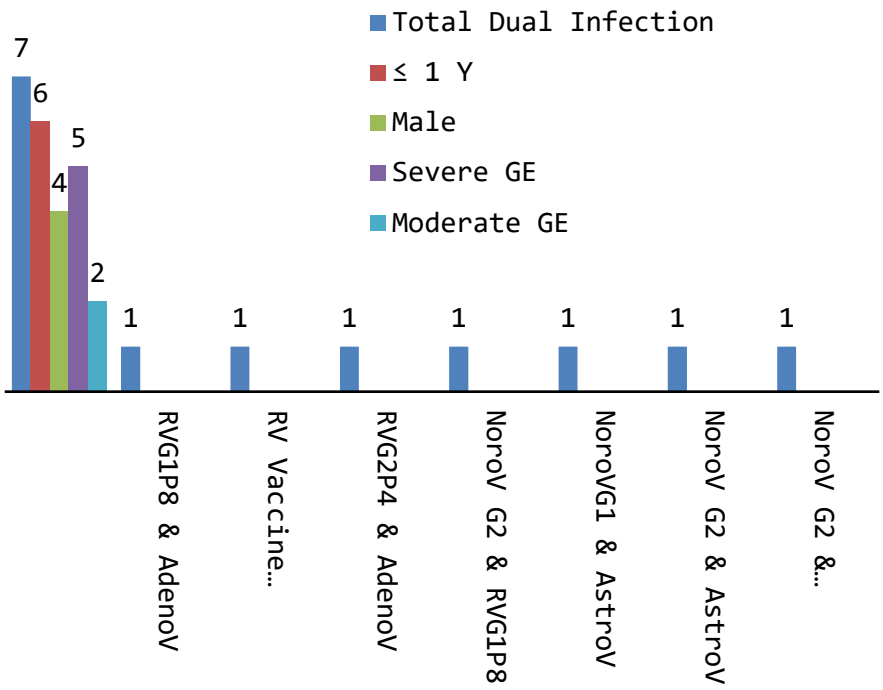
Figure 10: Specific Viral GE pathogens (Gender, Age)



2.2.2. *Dual infection*

Dual infection was detected in seven patients (5%) (Figure 11). Four were male (57%), Six were ≤1 year of age. One patient had a triple infection. Mixed viral infections are not uncommon, but the clinical significance of co- infection with multiple viruses is unclear. However, other studies have shown that co- infection with other viruses can lead to severe disease (15-19). In our study, five patients with GE co-infection with other viruses had a severe disease (71%). GE co- infection seems to be common in young infants (19-21). Similarly, in our study, six patients out of seven patients with GE co infection were one year old or younger (86%).

Figure 11: Dual viral GE infection



2.2.3 Discussion

Although the numbers are small, mixed infection in 2016-2017 revealed interesting findings. In a 2014 literature review of studies using PCR to detect viral GE pathogens, the prevalence of mixed infections in children with symptoms of GE ranged from 5.7 to 17% (22). Similarly, in our study, GE co-infection with other viruses was detected in seven (5%) of all patients or stool samples tested for multiple viral pathogens panel in the NVRL. In an American study conducted from October 2008 to September 2009, high percentages (13.1%) of the patients with AGE were infected with multiple viruses. Most mixed infections included NoroV, identified in 21% in this study population (19, 23).

Combinations of NoroV-AstroV and NoroV-AdenoV were most frequently identified as coinfections (15-16, 19, 23-24). Similarly, in our study, both NoroV and RV co-infection with other viruses were common, and each of them was detected in mixed infection with other viruses in four (4/7) cases (57%), of

which 50% of NoroV GE co-infection was due to AstroV and 50% of RV GE co-infection was due to AdenoV F. RV Co-infection in our study was detected in 11% of cases of RV GE, in particular with AdenoV F in 50% of cases. RV co-infection was associated with GE related hospital admission in 75% of RV GE (three out of four cases) and with prolonged duration of hospital stay, longer than two days in 66% of cases.

NoroV GE was confirmed in mixed infection with other viruses in 22% (4 cases) of all cases of NoroV GE. Co-infection was confirmed in 29% of AstroV GE (2 cases), of which NoroV G1 & NoroV G2 were confirmed (one strain in each case). SapoV Co-infection with other viruses was noted in two cases (22%) of SapoV GE. AdenoV Co-infection with other viruses was noted in three cases (16%) of AdenoV GE. RV was confirmed in almost 11% of all cases with AdenoV GE.

Co-infection with RVG1P8 (Rotarix ™) or RV vaccine strain was detected in a six month male, severe GE; vaccine strain was detected about 16 days following RV vaccine second dose. Triple infection of GE may also occur and can be associated with severe disease. In our study, triple infection with NoroVG2, AdenoV F and SapoV was detected in one male, nine months of age, with severe GE. Case was detected in the fourth week of June.

2.3 Viral GE: National Data (July 2014 – June 2015)

During 2014/2015, 11,510 faecal specimens were tested at the National Virus Reference Laboratory (NVRL), Ireland, for the five primary viral agents associated with GE. Of these, 27.8% (n=3,206) tested positive for at least one viral pathogen. As expected, the predominant virus detected was NoroV (n=1479) of which 89% were genogroup II (GII), followed by RV (n=870), SapoV (n=390), AstroV (n=291) and group F adenoviruses (n=176; Figures 12-13) (kind permission, NVRL).

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

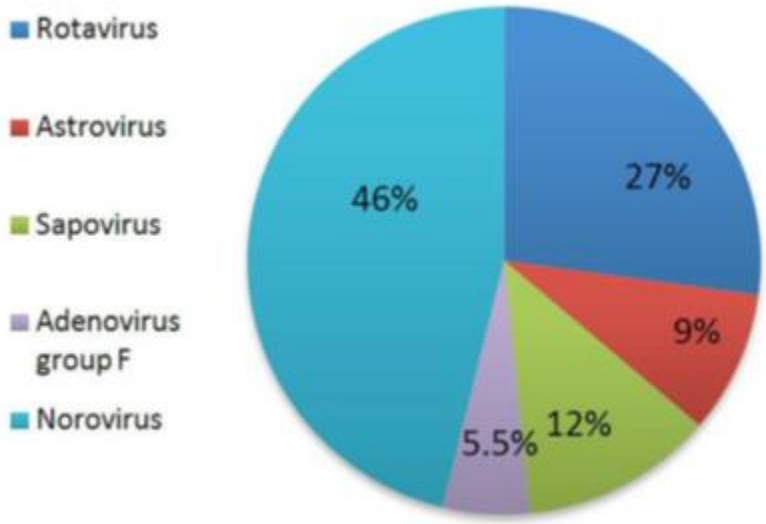


Figure note: *the method of testing for virus at the NVRL is outlined in Appendices of this Chapter Appendix 3(i).*

RV was the principal pathogen identified in paediatric patients, detected in 15.8% of specimens tested from patients aged ≤ 5 years; followed by NoroV (13.2%), SapoV (5.9%), AstroV (5.2%) and group F adenoviruses (3.5%; Figure 13). The high levels of NoroV infection in paediatric patients have been previously reported by the NVRL and others, and are a reminder that NoroV should always be considered in paediatric diarrhoeal illness. In fact, should the RV vaccine be introduced, the likelihood is that NoroV would quickly become the predominant virus associated with paediatric, as well as adult GE. Figures 12, 13 and 14 summarise male to female ratio and age group of RV and other viral pathogens that were detected during the NVRL 2014-2015 study period.

Figure 13: Viral GE agents detected at the NVRL from July 2014 – June 2015, n=3206

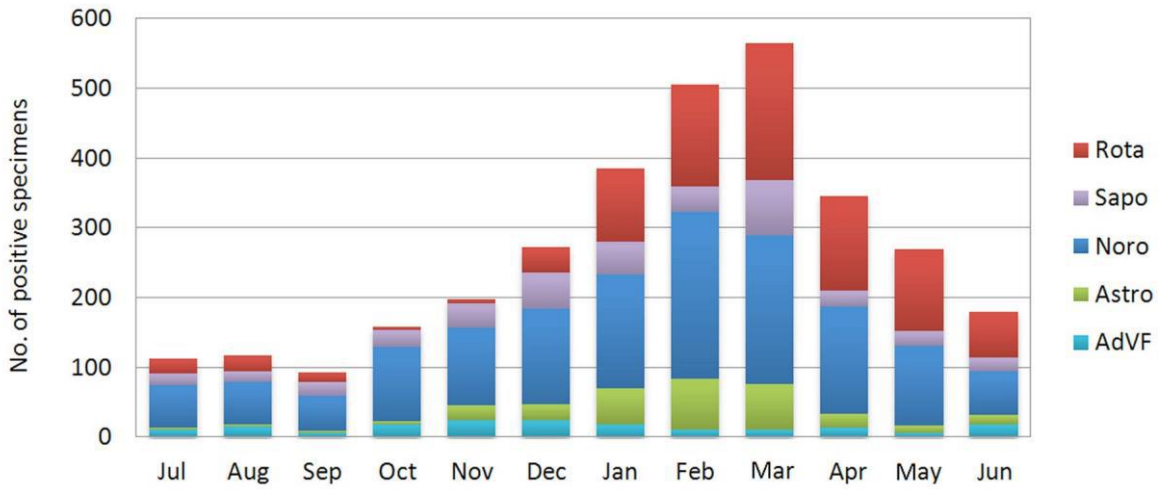
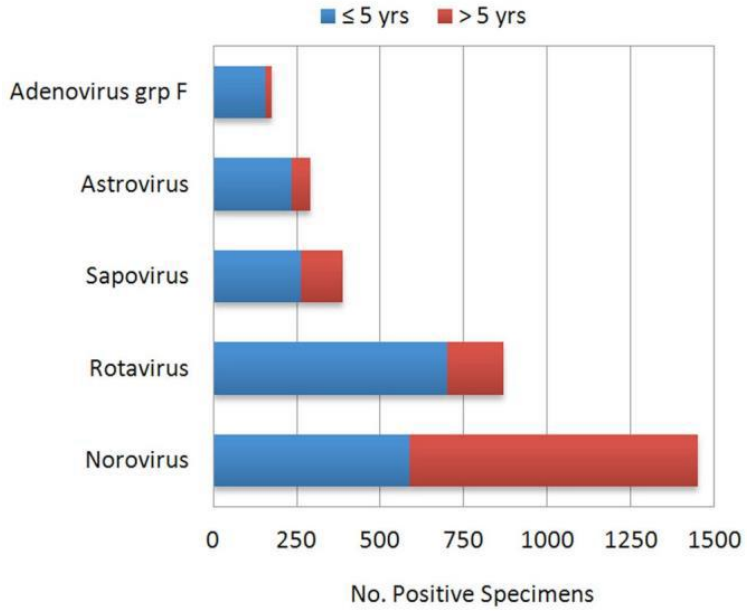


Figure 14: Age demographic associated with viral agents of GE detected in the NVRL from July 14 - June 15, n=3,206



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, a category of individuals who may benefit from the herd protection generated following the introduction of the RV

vaccine. However, this is frequently under-reported as many countries/laboratories do not screen for RV infection in adults (14).

2.4 Discussion:

The data in this section represent one region of Ireland (Co. Mayo), in the year following the introduction of RV vaccine (2016/2017) and shows RV remained the predominant cause of GE in the first year following to RV vaccine. Other viruses such as NoroV and AdenoV are expected to become predominant causes of GE in the years to come due to the anticipated effect of RV vaccine in reducing the number of RV GE not only in infants eligible for RV vaccine but also in older children due to the possible effect of RV vaccine herd protection and will require monitoring over the coming years.

The frequency of the different viruses detected for 2016-2017 is similar to the data acquired from a study in the NVRL in Ireland (July 2014-June 2015). However, NVRL recruited children up to age 5 years, with more NoroV GE cases and less adenovF GE cases detected compared with our study. More or less similar percentages of SapoV GE and AstroV GE cases were noted in both our study and NVRL study (6% & 5%) respectively.

3. Specific viral Pathogens

Rotavirus is the major pathogen being studied in this thesis. However this virus is discussed in more detail, particulary in terms of trends pre and post vaccine, and the impact of vaccination in **Chapters 4 & 5.**

3.1. AdenoV GE

3.1.1. 19 stool samples tested positive for AdenovF (13%). Three cases received RV vaccine prior to presentation to hospital with GE. Twelve were male (63%); 12 were ≤ one year of age (63%); 14 severe (74%), five moderate (26%) (Figure 15). Associated respiratory symptoms were noted in seven cases (37%).

Figure 15. Adenovirus GE

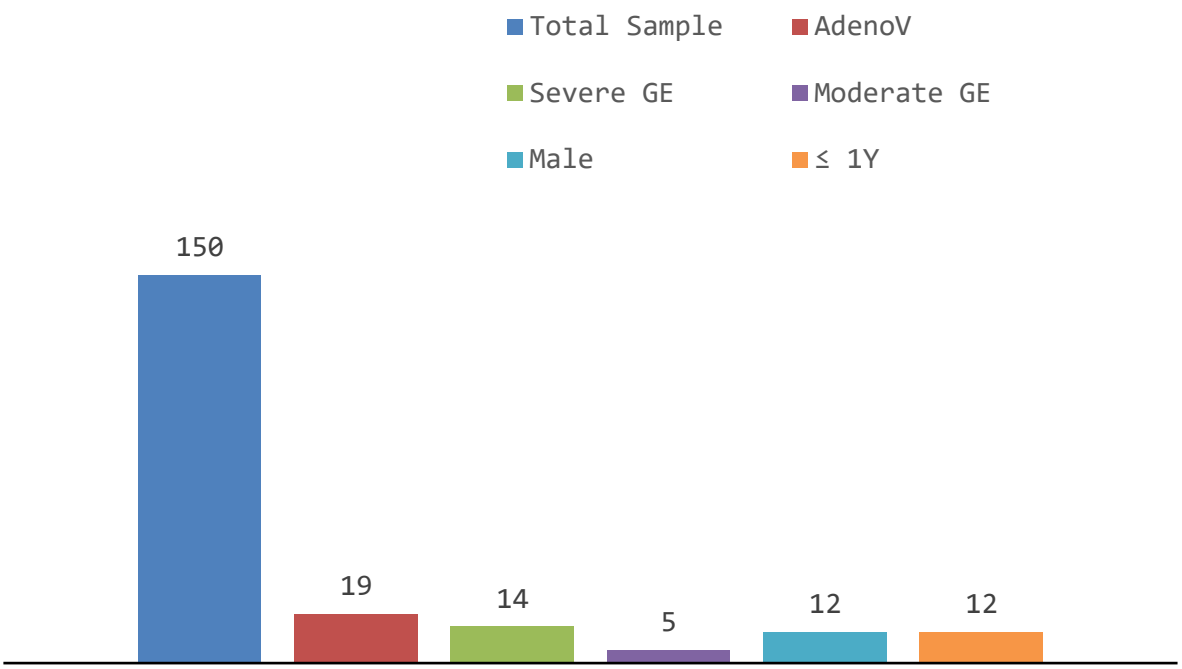


Figure footnote: *the method of lab testing for adenovirus is outlined in Appendix 3(ii).*

3.1.2 AdenoV Season

Three seasons of AdenoV GE and six very short episodes of AdenoV infection were noted during 2016/2017. Onset, Peak, Median percentage of AdenoV infection, End of season and short episodes of AdenoV GE are shown in Apppendix table 3 & figures 16 & 17.

There may be either a seventh short episode of AdenoV infection or a fourth season starting in the first and second week of

November 2017. A high rate of infection with a median percentage of positive AdenoV infection of 50% and continued throughout the second and third week of November with a median percentage of positive AdenoV infection of (16.6%). Our study stopped at that pre-determined date and no further stool samples were sent for analysis. Therefore, we are not certain about a new onset of a fourth season or a short seventh episode of AdenoV infection starting from the first week of November 2017.

3.1.3. Discussion

Adenoviruses have a worldwide distribution, and infections occur throughout the year, adenoviruses causes 5 to 10% of all febrile illnesses in infants and young children (25). Enteric adenoviruses also may be a cause of nosocomial infection (26). No nosocomial AdenoV GE cases were detected in our study. AdenoV GE predominantly affects children younger than four years (27, 28). In our study, AdenoV was the second predominant virus leading to GE following RV and was confirmed in 13% of all cases of GE. The second week of April (W2/April) was AdenoV week peak during which 16% of all cases of AdenoV GE were confirmed (50% of the total samples requested for viral testing on that week). AdenoV can lead to severe disease in the majority of patients. In our study 74% of cases with AdenoV GE were associated with severe disease.

AdenoV occurs throughout the year, with a peak in the summer (8, 11-13, 27, 29-30). Our study demonstrated that there were three definite seasons of AdenoV GE and six definite short episodes of AdenoV infection noted throughout 2016/2017. AdenoV season started in February, April and again in September with a higest peak in September (33%). We acknowledge the limitation of a small sample size of one geographic location, both can lead to an overestimation of the analysis.The duration of each AdenoV

season was generally short (three to four weeks). Episodes of very brief periods of infection with AdenoV without a peak of infection were noted throughout the year and these lasted for two to three weeks. Though very short, a high rate of infection with AdenoV was noted.

Figure 16. AdenoV season (S): Onset, Peak, End & Median % of Infection

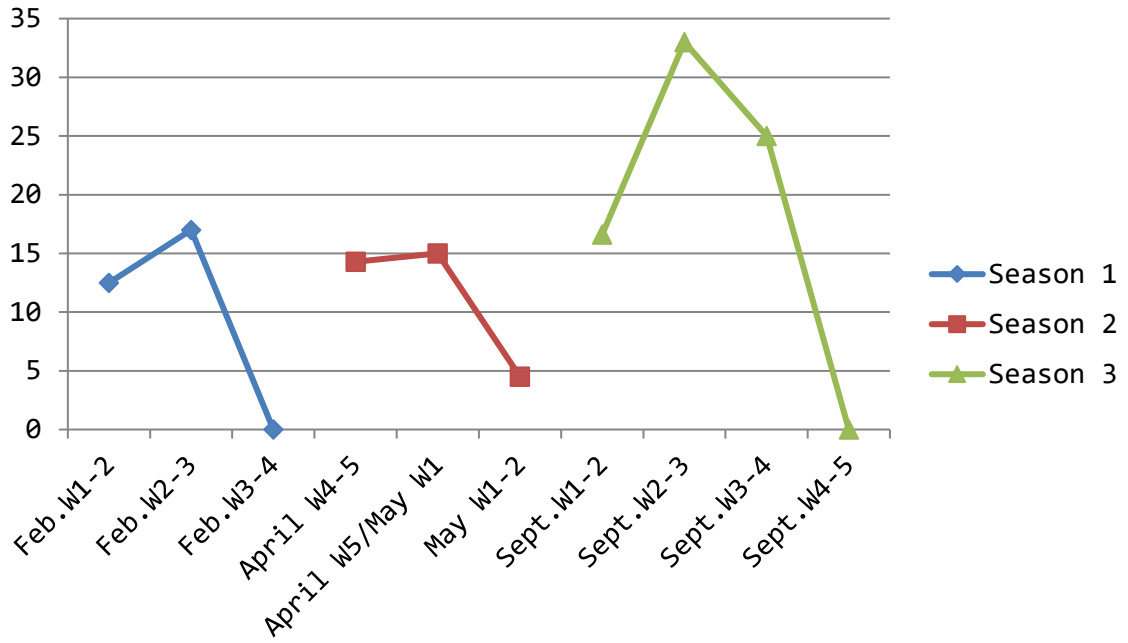
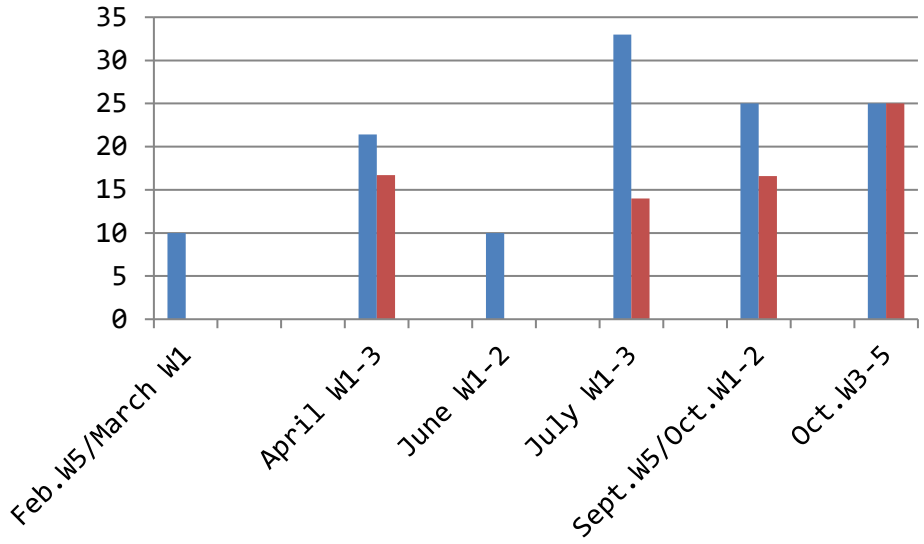


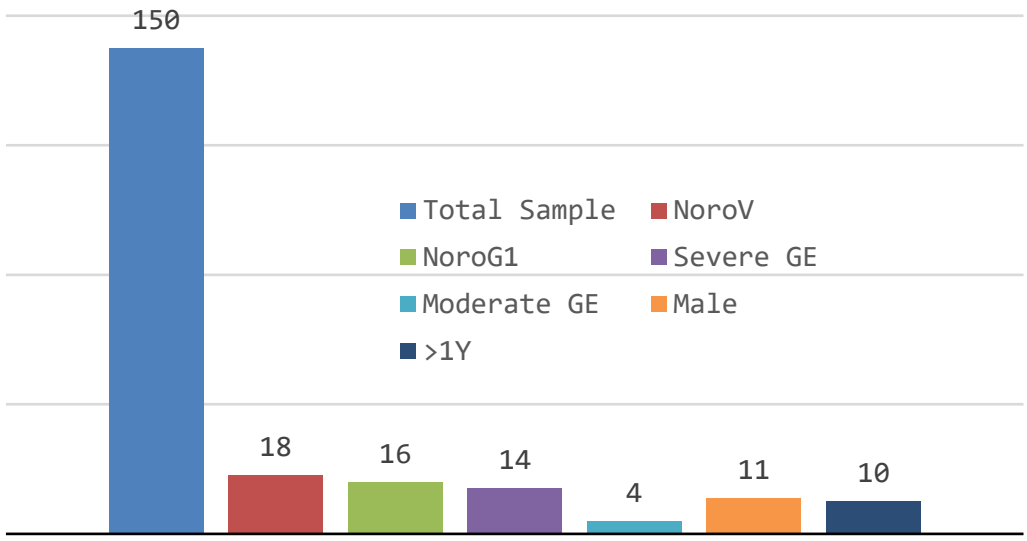
Figure 17. Short episodes of AdenoV infection & Median % of Infection



3.2. NoroV GE

3.2.1. NoroV was the third most predominant virus leading to GE following RV and AdenoV and was confirmed in eighteen (12%) of all cases of GE (Figure 18). Two cases received RV vaccine prior to presentation to hospital with GE, (61%) were male, 56% of cases were older than one year of age. NoroV can lead to severe disease in the majority of patients. In our study, 78% of cases with norovirus GE were associated with severe disease. No nosocomial disease was detected during our study. The fifth week of January was the week peak of NoroV GE infection during which three cases were confirmed (17% of the total number of confirmed NoroV GE in the season), and 100% of all stool samples tested for viruses on that week.

Figure 18. NoroV GE



3.2.2. NoroV Season

Three seasons of NoroV GE and four short episode of NoroV infection were noted during 2016/2017. Onset, Peak, Median percentage of NoroV infection, End of season and short episodes of NoroV GE are shown in Appendix table 4, figures 19 & 20.

3.2.3 Discussion

NoroV can lead to severe disease, NoroV G2 is the predominant strain worldwide (31- 37). In our study NoroV G2 was isolated in 16 cases (89%) of cases, the majority were severe GE (14 cases) (78%), Four moderate (22%). There were no mild cases with NoroV GE.

NoroV GE occurs in people of all ages, occurs year-round, with a peak in the fall and winter (8, 11-13, 24, 27-30). Our study demonstrated that there were three seasons of NoroV GE and four very short episodes of NoroV infection noted in 2016/2017. NoroV season started in December and again in March and late September with peaks noted in December, March and September (50%) which reflects possible over analysis due to our limited sample size in our region. The duration of NoroV Season was generally short (three to four weeks). Episodes of very brief periods of infection with NoroV were noted throughout the year (November, January and May) and these lasted for two to three weeks. Though they were very short, a high rate of infection with NoroV was noted.

Figure 19: NoroV season(S) - Onset, Peak, End & Median % of Infection

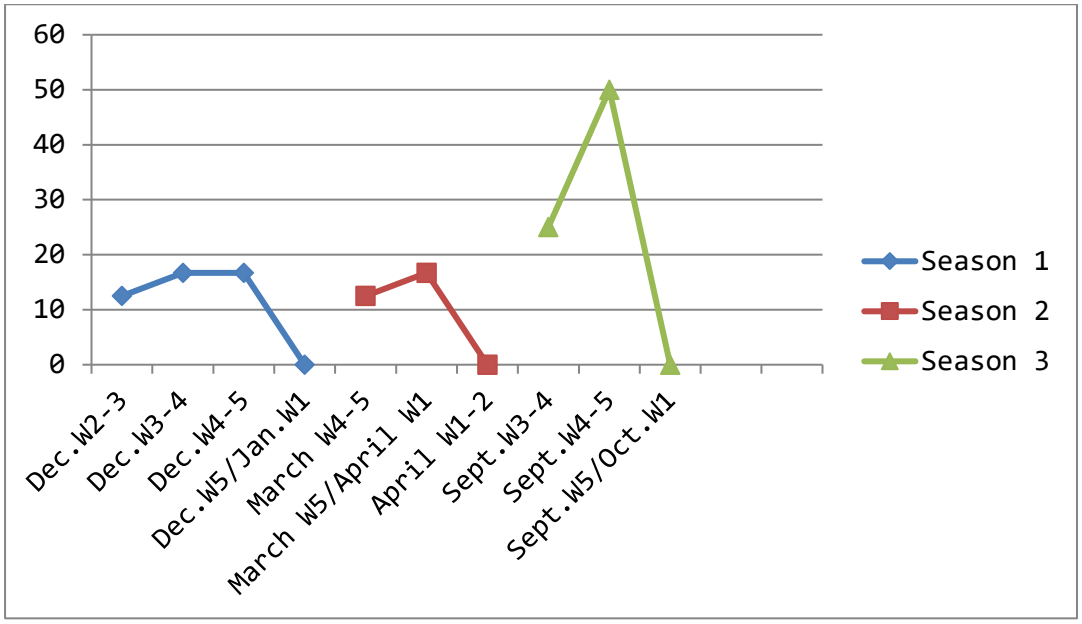
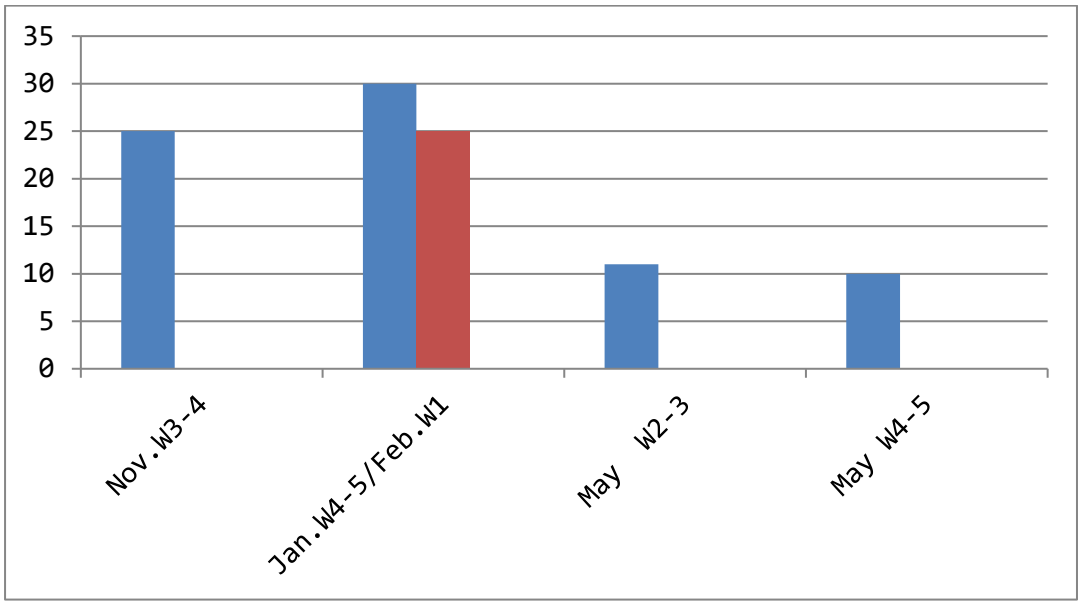


Figure 20: Short episodes of NoroV infection- Median % of Infection



3.3 SapoV GE

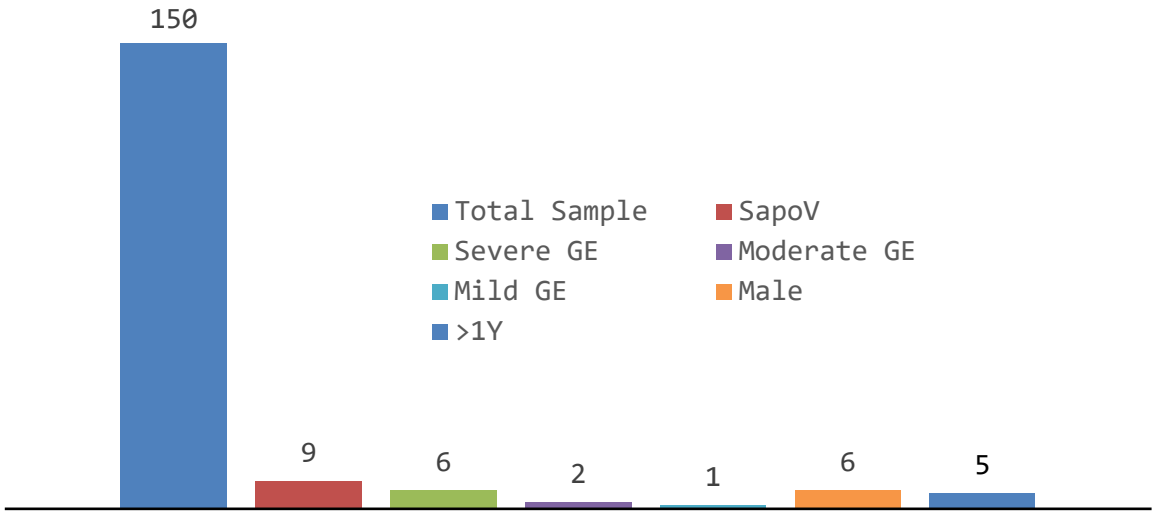
3.3.1 SapoV Season

Our study demonstrated that one definite season of SapoV GE and two short episodes of SapoV infection were noted in 2016/2017, with a possible second season or a third short episode of SapoV infection starting in the second and third week of November 2017 with a median percentage of positive SapoV infection of 16.7%. Neither were confirmed given that the study ended on the third week of November. Onset, peak, median percentage of SapoV infection, end of season and short episodes of SapoV GE are shown in Appendix table 5, figures 22 & 23.

SapoV occurs year-round, with the highest proportion from March through July (12-18). In our study, SapoV GE season commenced in late November and early December with a peak in December of 12.5%. The duration of SapoV season was five weeks. Episodes of very brief periods of infection with SapoV were noted throughout the year (Late April, May, August) each of these episodes lasted two weeks. A possible third short episode of infection with SapoV or a possible second SapoV season commencing in the second

and third week of November 2017 was noted, with a median percentage of positive SapoV infection of (16.7%).

Figure 21: SapoV GE



3.3.2. Discussion

SapoV GE mainly affects infants and toddlers (27, 38-39). In our study (67%) of Sapov GE were male and 56% of SapoV GE in our study occurred in infants. SapoV GE was confirmed in nine (6%) of all cases of GE, as also shown in other studies (14, 40). Two cases received RV vaccine prior to presentation to hospital with GE.

SapoV occurs year-round, with the highest proportion from March through July (8, 11-13, 27, 29-30). In our study, the first week of May was SapoV GE week peak; two cases of SapoV GE were detected (22% of the total SapoV GE cases) and 66% of the total stool samples requested for viral testing on that week. The majority of our cases were severe in nature (6 cases).

Figure 22: SapoV season- Onset, Peak, End & Median % of Infection

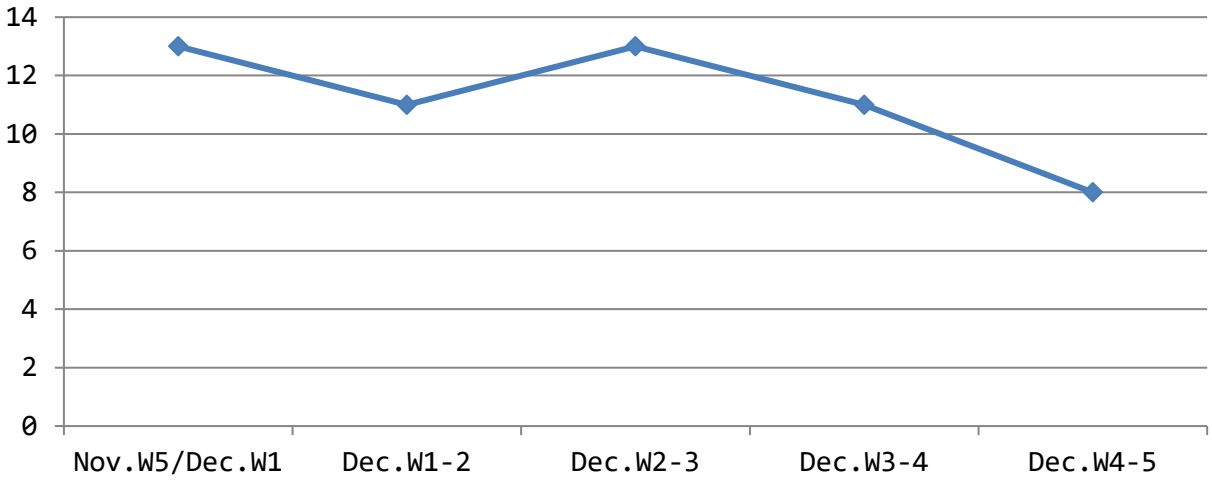
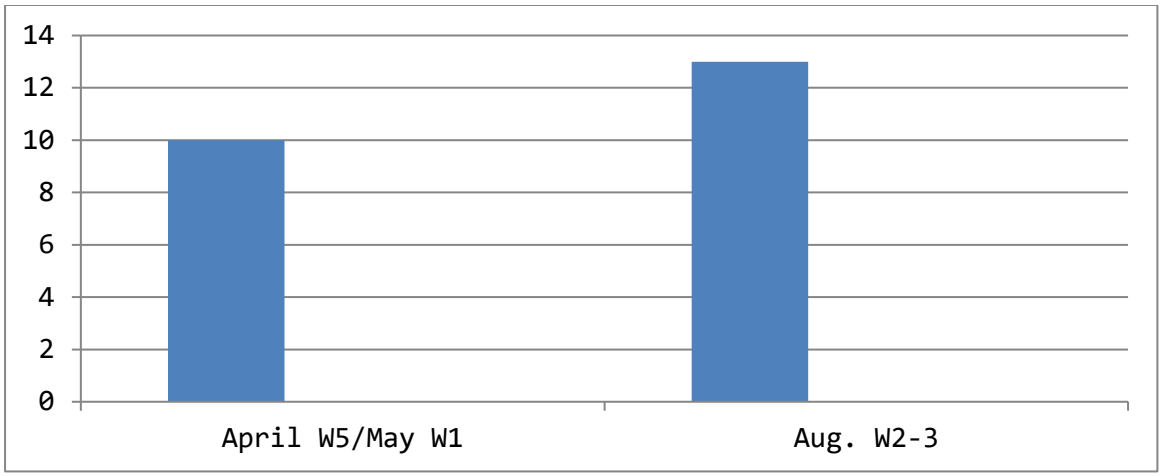


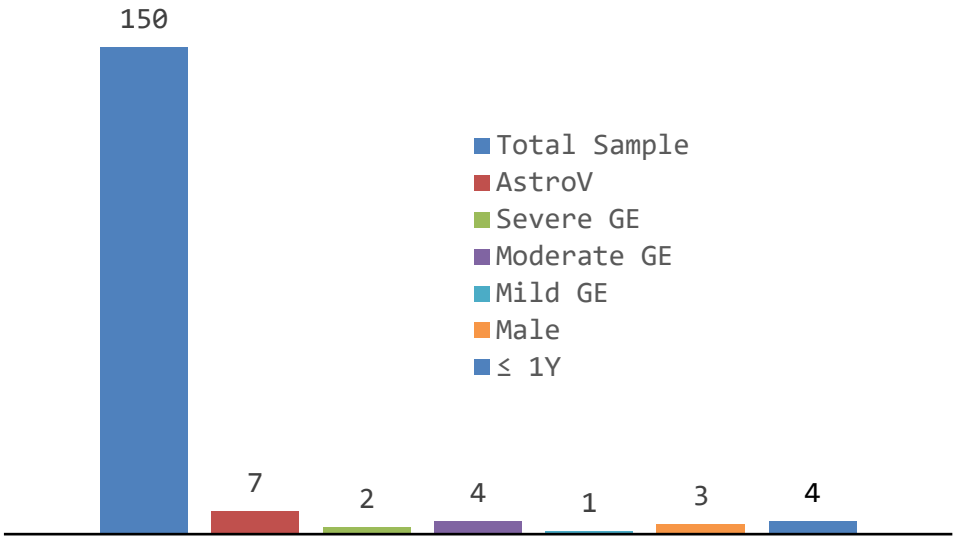
Figure 23: Short episodes of SapoV infection & Median % of Infection



3.4 Astrov GE

3.4.1. AstroV was confirmed in seven (5%) of all cases of GE, nearly similar to other studies (14, 39, 41), female (57%), 57% infants (Figure 24). Two cases received RV vaccine prior to presentation to hospital with GE. No week peak of AstroV GE infection was detected in our group of children in our region of Ireland. The majority of cases were moderate GE (57%), 29% severe and 14% mild. Unlike reports of nosocomial infection with AstroV in other studies (42-43), AstroV was not associated with nosocomial acquisition in our study.

Figure 24. AstroV GE



3.4.2 AstroV Season

Our study demonstrated that there was only one season of AstroV GE and four very short episodes of infection with AstroV (EOI) were noted during 2016/2017. Onset, peak, median percentage of AstroV infection, end of season and short episodes of AstroV GE are shown in Appendix table 6, figures (25 & 26).

AstroV GE usually occurs throughout the year with a winter predominance. AstroV was predominantly detected from November

through May (8, 11-13, 27, 29-30, 44). AstroV GE season started in late January and early February with a peak of 16.7%. The duration of AstroV season was short (four weeks). Episodes of very brief periods of infection with AstroV were noted throughout the year (March, May, June) and each of these episodes lasted for two weeks.

Figure 25. AstroV season- Onset, Peak, End & Median % of Infection

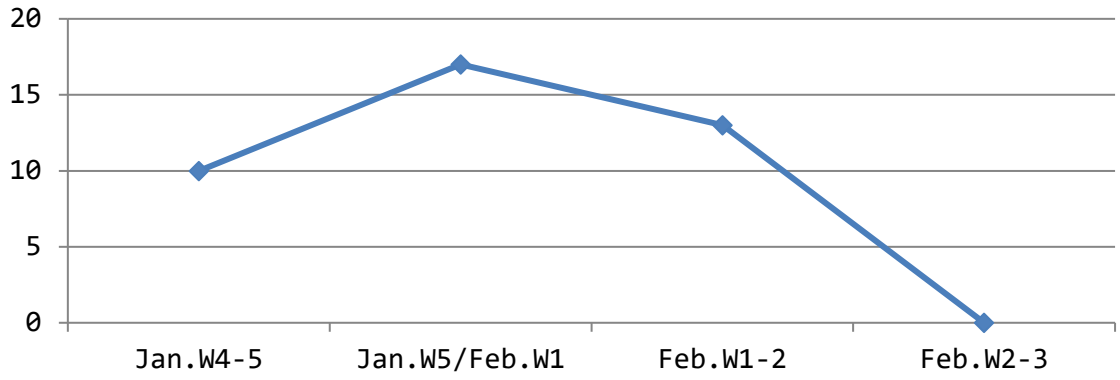
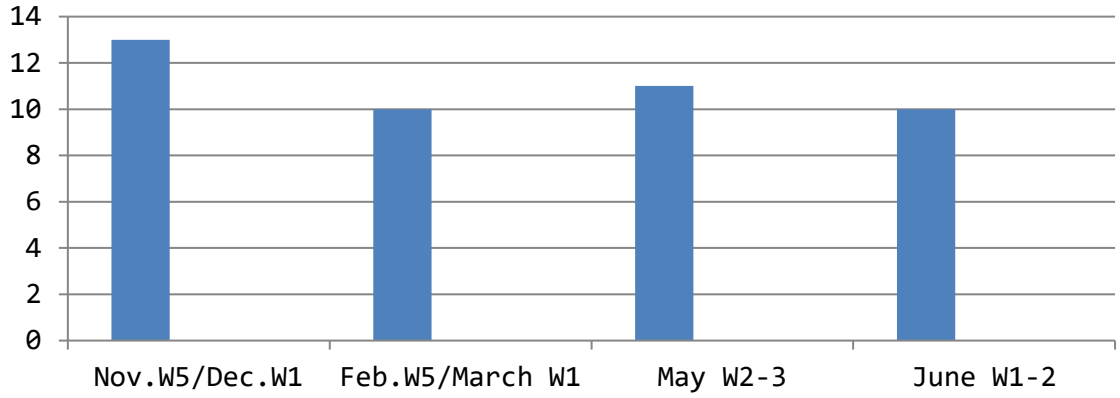


Figure 26. Short episodes of AstroV infection & Median % of Infection



APPENDICES of Chapter 3

Appendix 3(i): National Data 2014-2016: Method of Testing:

In June 2014, the NVRL introduced real-time (RT) PCR assays for frontline screening of all specimens received for viral GE investigations. Previously, the NVRL screened specimens from patients >5 years old with NoroV-specific real-time RT-PCR and, as the other primary viral aetiological agents associated with GE 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.

Appendix 3(ii): Virus Detection Methods for AV post-vaccine era

These data assessed Rapid Antigen Testing (RAT) in Galway University Hospital laboratory (GUHL) Versus PCR testing of Virus in the National Virus Reference Laboratory (NVRL), Sensitivity and Specificity of RAT (Tables 1 and 2).

AdenoV Detection Method (Tables 1-2) 2016-2017

AdenoV is detected by RAT in GUHL and by PCR testing of virus in the NVR. During the study period, 144 stool samples were tested in both GUHL and the NVRL for AdenoV and other viruses. One sample was inhibitory to AdenoV due to interference with enzyme marker and was excluded; 143 stool samples were valid for analysis, 15 were confirmed Adenov +ve in GUHL, 17 were confirmed Adenov +ve in the NVRL. PCR testing is more sensitive and specific than RAT; two samples were false negative for AdenoV (Adenov F-ve) in GUHL, 128

samples were confirmed AdenoV-ve in GUHL, 126 were confirmed AdenoV –ve in the NVRL; two samples were false positive for AdenoV (Adenov F+ve) in GUHL.

Sensitivity of RAT (*=1-B)* (B=false -ve rate) was (88%). Specificity of (RAT) (1-α) (α=false +ve rate) was (98.5%).

In conclusion, our study demonstrated that RAT –as an Adenov detecting tool had higher specificity with lower sensitivity.

Table 1. AdenoV Detecting Method: RAT in GUHL versus PCR detection of Virus in the NVRL

Month	Test	Number of Confirmed AdenoV +ve cases	Number of false -ve cases (GUHL)	Number of confirmed AdenoV–ve cases	Number of False+ve cases (GUHL)
Nov 18-30	RAT(GUH)	0	0	2	0
	PCR(NVRL)	0		2	
December	RAT(GUH)	0	0	18	0
	PCR(NVRL)	0		18	
January	RAT(GUH)	0	0	12	0
	PCR(NVRL)	0		12	
February	RAT(GUH)	1	0	11	0
	PCR(NVRL)	1		11	
March	RAT(GUH)	2	0	13	0
	PCR(NVRL)	2		13	
April	RAT(GUH)	3	2	14	
	PCR(NVRL)	5		12	0
May	RAT(GUH)	1		18	
	PCR(NVRL)	1	0	18	0
June	RAT(GUH)	4	0	11	2
	PCR(NVRL)	2		13	
July	RAT(GUH)	2	1	11	0
	PCR(NVRL)	3		10	
August	RAT(GUH)	0	0	10	0
	PCR(NVRL)	0		10	
Sept	RAT(GUH)	1	0	2	0
	PCR(NVRL)	1		2	
October	RAT(GUH)	1	1	4	0
	PCR(NVRL)	2		3	
Nov 1-18	RAT(GUH)	0	0	2	0
	PCR(NVRL)	0		2	
Total stool samples tested			**143**		

Table 2. AdenoV Detection Method: RAT in GUHL Versus PCR testing of Virus in the NVRL - Sensitivity & Specificity of RAT

	Total AdenoV +ve	Total AdenoV False-ve	Sensitivity of RAT (1-B) B= false -ve rate	Total AdenoV -ve	Total AdenoV False+ve	Specificity of RAT (1-α) α=false +ve rate)
RAT GUH	15	2	88%	128	2	98.5%
PCR NVRL	17			126		

Table 3. AdenoV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total AdenoV Request	Adeno V +ve	Median Adeno +ve	Median +ve AdenoV%	Notes	Duration Of Season or (EOI)
Nov 18-30	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Nov Dec	W5/W1	4	0	0	0		
December	W1-2	9	0	0	0		
	W2-3	8	0	0	0		
	W3-4	9	0	0	0		
	W4-5	6	0	0	0		
Dec/ Jan	W5/W1	0	0	0	0		
January	W1-2	2	0	0	0		
	W2-3	7	0	0	0		
	W3-4	7	0	0	0		
	W4-5	5	0	0	0		
Jan/ Feb	W5/W1	6	0	0	0		
February	W1-2	4	1	0.5	12.5	Onset Season 1	
	W2-3	3	1	0.5	16.7	Peak Season 1	3 Weeks
	W3-4	6	0	0	0	End Season 1	
	W4-5	6	0	0	0		
Feb/ March	W5/W1	5	1	0.5	10	1st(EOI)	2 Weeks
March	W1-2	8	1	0.5	6.3		
	W2-3	9	1	0.5	5.6		
	W3-4	6	1	0.5	8.3		
	W4-5	4	0	0	0		
March/ April	W5/W1	3	0	0	0		
April	W1-2	7	3	1.5	21.4	2nd EOI	3 Weeks
	W2-3	9	3	1.5	16.7		
	W3-4	6	0	0	0		
	W4-5	7	2	1	14.3	Onset S2	
April/ May	W5/W1	10	3	1.5	15	Peak S2	3 Weeks
May	W1-2	11	1	0.5	4.5	End S 2	

Month	Week (W)	Total AdenoV Request	Adeno V +ve	Median Adeno +ve	Median +ve AdenoV%	Notes	Duration Of Season or (EOI)
	W2-3	9	0	0	0		
	W3-4	8	0	0	0		
	W4-5	5	0	0	0		
May/ June	W5/W1	2	0	0	0		
June	W1-2	5	1	0.5	10	3rd. EOI	2Weeks
	W2-3	9	1	0.5	5.6		
	W3-4	11	1	0.5	4.5		
	W4-5	6	1	0.5	8		
June/ July	W5/W1	0	0	0	0		
July	W1-2	3	2	1	33	4th. EOI	3 Weeks
	W2-3	7	2	1	14		
	W3-4	8	1	0.5	6		
	W4-5	6	1	0.5	8		
July/ August	W5/W1	7	0	0	0		
August	W1-2	9	0	0	0		
	W2-3	4	0	0	0		
	W3-4	1	0	0	0		
	W4-5	2	0	0	0		
Aug/ Sept	W5/ W1	2	0	0	0		
Sept	W1-2	3	1	0.5	16.6	Onset S3	Duration of S3 4 Weeks
	W2-3	3	2	1	33	Peak S3	
	W3-4	2	1	0.5	25	S3	
	W4-5	1	0	0	0	End S3	
Sept/ Oct	W5/W1	2	1	0.5	25	5Th EOI)	3Weeks
October	W1-2	3	1	0.5	16.6		
	W2-3	1	0	0	0		
	W3-4	2	1	0.5	25	6th.EOI	3 Weeks
	W4-5	2	1	0.5	25		
Oct/ Nov	W5Oct/W 1 Nov.	0	0	0	0		
Nov 1- 18	W1-W2	1	1	0.5	50	?7th.EOI or ?(S)	??
	W2-W3	3	1	0.5	16.6		

Table 4. NoroV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total NoroV (R)	NoroV +ve	Median NoroV +Ve	Median +ve NoroV%	Notes	Duration Of Season or (EOI)
Nov 18-30	W3-4	2	1	0.5	25	1st EOI	2 Weeks
	W4-5	1	0	0	0		
Nov/ Dec	W5/W1	4	0	0	0		
Dec	W1-2	9	1	0.5	5.6		
	W2-3	8	2	1	12.5	Onset S1	Duration of Season 1 4 Weeks
	W3-4	9	3	1.5	16.7	Peak S1	
	W4-5	6	2	1	16.7		
Dec/ Jan	W5/W1	0	0	0	0	End of Season 1	

Month	Week (W)	Total NoroV (R)	NoroV +ve	Median NoroV +Ve	Median +ve NoroV%	Notes	Duration Of Season or (EOI)
January	W1-2	2	0	0	0		
	W2-3	7	1	0.5	7.1		
	W3-4	7	1	0.5	7.1		
	W4-5	5	3	1.5	30	2nd. EOI	
Jan/ Feb	W5/ W1	6	3	1.5	25		Duration 3 Weeks
Feb	W1-2	4	0	0	0		
	W2-3	3	0	0	0		
	W3-4	6	1	0.5	8.3		
	W4-5	6	1	0.5	8.3		
Feb/ March	W5/W1	5	0	0	0		
March	W1-2	8	1	0.5	6.3		
	W2-3	9	1	0.5	5.6		
	W3-4	6	0	0	0		
	W4-5	4	1	0.5	12.5	Onset S2	Duration
March/ April	W5/ W1	3	1	0.5	16.7	Peak S 2	3 weeks
April	W1-2	7	0	0	0	End S2	
	W2-3	9	0	0	0		
	W3-4	6	0	0	0		
	W4-5	7	0	0	0		
April/ May	W5 Apr./ W1 May	10	0	0	0		
May	W1-2	11	2	1	9		
	W2-3	9	2	1	11	3rd. EOI	2 Weeks
	W3-4	8	1	0.5	6		
	W4-5	5	1	0.5	10	4th. EOI	2 Weeks
May/ June	W5/W1	2	0	0	0		
June	W1-2	5	0	0	0		
	W2-3	9	0	0	0		
	W3-4	11	1	0.5	4.5		
	W4-5	6	1	0.5	8		
June/ July	W5/W1	0	0	0	0		
July	W1-2	3	0	0	0		
	W2-3	7	0	0	0		
	W3-4	8	0	0	0		
	W4-5	6	1	0.5	8		
July/ August	W5/W1	7	1	0.5	7		
August	W1-2	9	0	0	0		
	W2-3	4	0	0	0		
	W3-4	1	0	0	0		
	W4-5	2	0	0	0		
Aug/ Sept	W5/W1	2	0	0	0		
Sept	W1-2	3	0	0	0		
	W2-3	3	0	0	0		
	W3-4	2	1	0.5	25	Onset S3	Duration
	W4-5	1	1	0.5	50	Peak S3	3 Weeks

Month	Week (W)	Total NoroV (R)	NoroV +ve	Median NoroV +Ve	Median +ve NoroV%	Notes	Duration Of Season or (EOI)
Sept/ Oct	W5/ W1	2	0	0	0	End S3	
Oct	W1-2	3	0	0	0		
	W2-3	1	0	0	0		
	W3-4	2	0	0	0		
	W4-5	2	0	0	0		
Oct/ Nov	W5/W1	0	0	0	0		
Nov	W1-2	1	0	0	0		
1-18	W2-3	3	0	0	0		

Table 5. SapoV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total SapoV Request	SapoV +ve	Median	Median +ve SapoV%	Notes	Duration Of Season or(EOI)
Nov 18-30	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Nov/Dec	W5/W1	4	1	0.5	12.5	Onset S1	
December	W1-2	9	2	1	11.1	S1	Duration of Season 1 5 Weeks
	W2-3	8	2	1	12.5	Peak S1	
	W3-4	9	2	1	11.1	S1	
	W4-5	6	1	0.5	8.3	End S1	
Dec/Jan	W5/W1	0	0	0	0		
January	W1-2	2	0	0	0		
	W2-3	7	0	0	0		
	W3-4	7	0	0	0		
	W4-5	5	0	0	0		
Jan/Feb	W5/W1	6	0	0	0		
Feb	W1-2	4	0	0	0		
	W2-3	3	0	0	0		
	W3-4	6	0	0	0		
	W4-5	6	0	0	0		
Feb/ March	W5/W1	5	0	0	0		
March	W1-2	8	0	0	0		
	W2-3	9	0	0	0		
	W3-4	6	0	0	0		
	W4-5	4	0	0	0		
March/ April	W5/W1	3	0	0	0		
April	W1-2	7	0	0	0		
	W2-3	9	0	0	0		
	W3-4	6	0	0	0		
	W4-5	7	0	0	0		
April/ May	W5/W1	10	2	1	10	1st.EOI	2 weeks
May	W1-2	11	2	1	9		
	W2-3	9	0	0	0		
	W3-4	8	0	0	0		
	W4-5	5	0	0	0		
May/	W5/W1	2	0	0	0		

Month	Week (W)	Total SapoV Request	SapoV +ve	Median	Median +ve SapoV%	Notes	Duration Of Season or(EOI)
June							
June	W1-2	5	0	0	0		
	W2-3	9	0	0	0		
	W3-4	11	1	0.5	4.5		
	W4-5	6	1	0.5	8		
June/ July	W5/W1	0	0	0	0		
July	W1-2	3	0	0	0		
	W2-3	7	0	0	0		
	W3-4	8	0	0	0		
	W4-5	6	0	0	0		
July/ August	W5/W1	7	0	0	0		
August	W1-2	9	1	0.5	5.6		
	W2-3	4	1	0.5	12.5	2nd.EOI	2 Weeks
	W3-4	1	0	0	0		
	W4-5	2	0	0	0		
Aug/ Sept	W5/W1	2	0	0	0		
Sept	W1-2	3	0	0	0		
	W2-3	3	0	0	0		
	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Sept/ October	W5/W1	2	0	0	0		
October	W1-2	3	0	0	0		
	W2-3	1	0	0	0		
	W3-4	2	0	0	0		
	W4-5	2	0	0	0		
Oct/Nov	W5/W1	0	0	0	0		
Nov 1-18	W1-W2	1	0	0	0		
	W2-W3	3	1	0.5	16.7	??S2 or 3rd. EOI	

Table 6. AstroV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total AstroV (R)	AstroV +ve	Median AstroV +ve	Median +ve AstroV%	Notes	Duration Of Season or (EOI)
Nov 18-30	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Nov/Dec	W5/W1	4	1	0.5	12.5	1st.EOI	2 Weeks
Deceber	W1-2	9	1	0.5	5.6		
	W2-3	8	0	0	0		
	W3-4	9	0	0	0		
	W4-5	6	0	0	0		
Dec/Jan	W5/W1	0	0	0	0		
January 2017	W1-2	2	0	0	0		
	W2-3	7	0	0	0		

Month	Week (W)	Total AstroV (R)	AstroV +ve	Median AstroV +ve	Median +ve AstroV%	Notes	Duration Of Season or (EOI)
	W3-4	7	0	0	0		
	W4-5	5	1	0.5	10	Onset S 1	Duration of Season 1 4 Weeks
Jan/ February	W5/W1	6	2	1	16.7	Peak S 1	
February	W1-2	4	1	0.5	12.5	S1	
	W2-3	3	0	0	0	End S1	
	W3-4	6	0	0	0		
	W4-5	6	1	0.5	8.3		
February /March	W5/W1	5	1	0.5	10	2nd.EOI	2 Weeks
March	W1-2	8	0	0	0		
	W2-3	9	0	0	0		
	W3-4	6	0	0	0		
	W4-5	4	0	0	0		
March/ April	W5/W1	3	0	0	0		
April	W1-2	7	0	0	0		
	W2-3	9	0	0	0		
	W3-4	6	0	0	0		
	W4-5	7	0	0	0		
April/ May	W5/W1	10	0	0	0		
May	W1-2	11	1	0.5	4.5		
	W2-3	9	2	1	11	3rd.EOI	2 Weeks
	W3-4	8	1	0.5	6.3		
	W4-5	5	0	0	0		
May/ June	W5/W1	2	0	0	0		
June	W1-2	5	1	0.5	10	4Th EOI	2 Weeks
	W2-3	9	1	0.5	5.6		
	W3-4	11	0	0	0		
	W4-5	6	0	0	0		
June/ July	W5/W1	0	0	0	0		
July	W1-2	3	0	0	0		
	W2-3	7	0	0	0		
	W3-4	8	0	0	0		
	W4-5	6	0	0	0		
July/ August	W5/W1	7	0	0	0		
August	W1-2	9	0	0	0		
	W2-3	4	0	0	0		
	W3-4	1	0	0	0		
	W4-5	2	0	0	0		
August/ Sept	W5/W1	2	0	0	0		
Sept	W1-2	3	0	0	0		
	W2-3	3	0	0	0		
	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Sept/ October	W5/W1	2	0	0	0		
October	W1-2	3	0	0	0		

Month	Week (W)	Total AstroV (R)	AstroV +ve	Median AstroV +ve	Median +ve AstroV%	Notes	Duration Of Season or (EOI)
	W2-3	1	0	0	0		
	W3-4	2	0	0	0		
	W4-5	2	0	0	0		
October/ November	W5/W1	0	0	0	0		
Nov 1-18	W1-W2	1	0	0	0		
	W2-W3	3	0	0	0		

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Chapter 4: Results II

Rotavirus epidemiology Pre and Post Vaccine

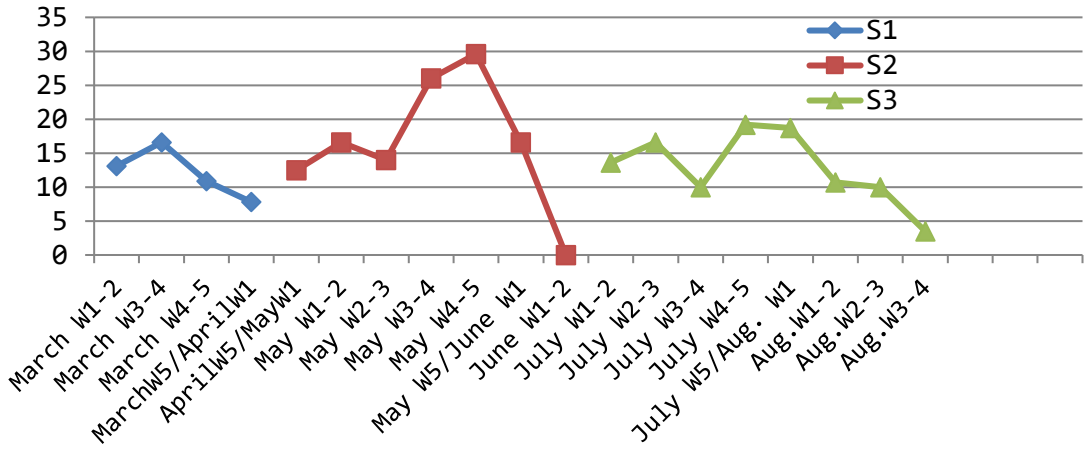
1. RV Season: Pre vaccination (2014-2016)

The RV Season was studied for three consecutive years prior to the introduction of RV vaccine in Ireland October 2016. Retrospective analysis was from January 2014 to November 18th 2016, (date of the start of prospective analysis) on a weekly basis until the study ended (18th November 2017). RV vaccine in Ireland became available in December 2016, the start of analysis of RV season (S) for 2016/2017.

1.1. 2014 Season

515 stool samples were requested; 269 were male (52%), 70 were RV positive (RV+ve)(13.6%). Three seasons of RV GE and one short episode of RV infection were noted. Onset, peak, median percentage, end of season and short episodes of RV GE are shown in Appendix Tables 1 & 4 and Figure 1. One short episode of RV infection started during the 5th week of September and the 1st week of October with a median percentage of +ve RV infection of 13.6%, lasted 2 weeks and ended immediately in the 2 consecutive weeks following the 1st week of the onset of this short episode of RV infection (1st and 2nd week of October) with a median percentage of RV+ve infection of 0%.

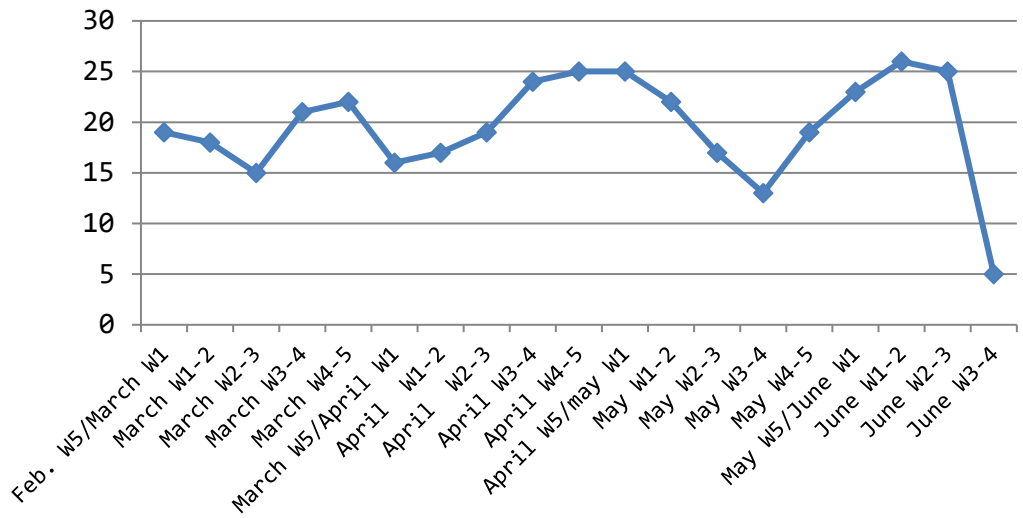
Figure 1. 2014 RV Season: Onset, Peak, End & Median & of Infection



1.2. 2015 Season

586 stool samples were requested, 301 were male (51%). 118 samples were RV positive (20%). One long season of RV and one short episode of RV infection were detected. Onset, peak, median percentage of RV infection, and end of season is shown in Appendix Tables 2, 4 and figure 2. One short episode of RV infection commenced in the third and the fourth week of December with a median percentage of positive RV infection of 12.5%, lasted two weeks and ended immediately in the two consecutive weeks following the first week of onset of this short episode of RV infection (4th and 5th week of December), with a median percentage of RV positive infection of 6.3%.

Figure 2. 2015 RV season- Onset, Peak, End & Median % of Infection



1.3. RV Season 2016 (January 1 to December 1)

During 2016, 430 stool samples were requested; 235 were male (54.6%); 68 samples were RV positive (15.8%). Three seasons of RV GE and three short episodes of RV infection were noted in 2016. Onset, peak, median percentage of RV infection, end of season and short episodes of RV GE are shown in Appendix Tables 3 and 4 and figures 3 and 4.

Figure 3. 2016 RV Season- Onset, Peak, End & Median % of Infection

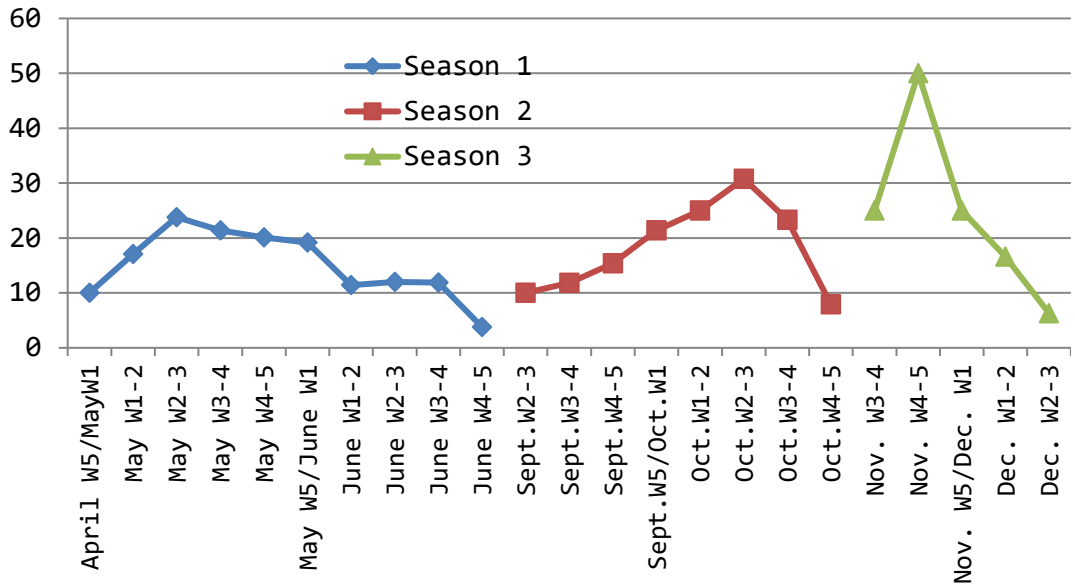
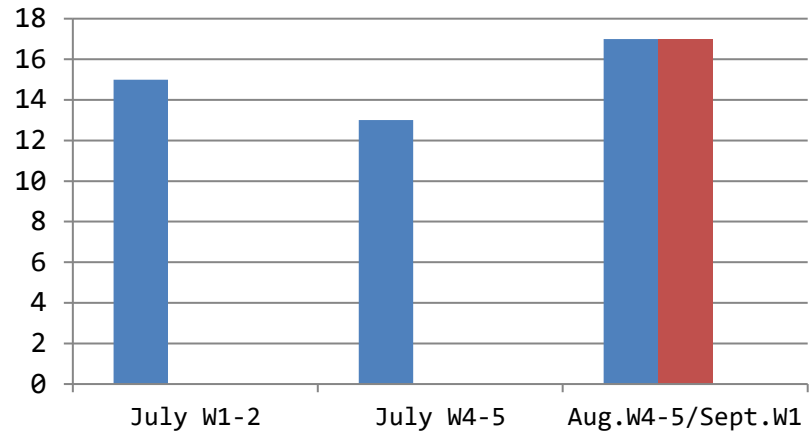


Figure 4. 2016-Short episodes of RV infection & Median % of Infection



1.4 RV Season Post Vaccination (December 2016 to November 2017)

During 2017, 148 stool samples were requested; 80 were male (54%); 36 samples were RV positive (24.3%). Four seasons of RV GE and five short episodes of RV infection were noted. Onset, peak, median percentage of RV infection, end of season and short episodes of RV GE are shown in Appendix table 5 and figures 5-6.

Figure 5. RV seasons-Post vaccination year 2016/2017- Onset, Peak, End & Median Percentage of Infection

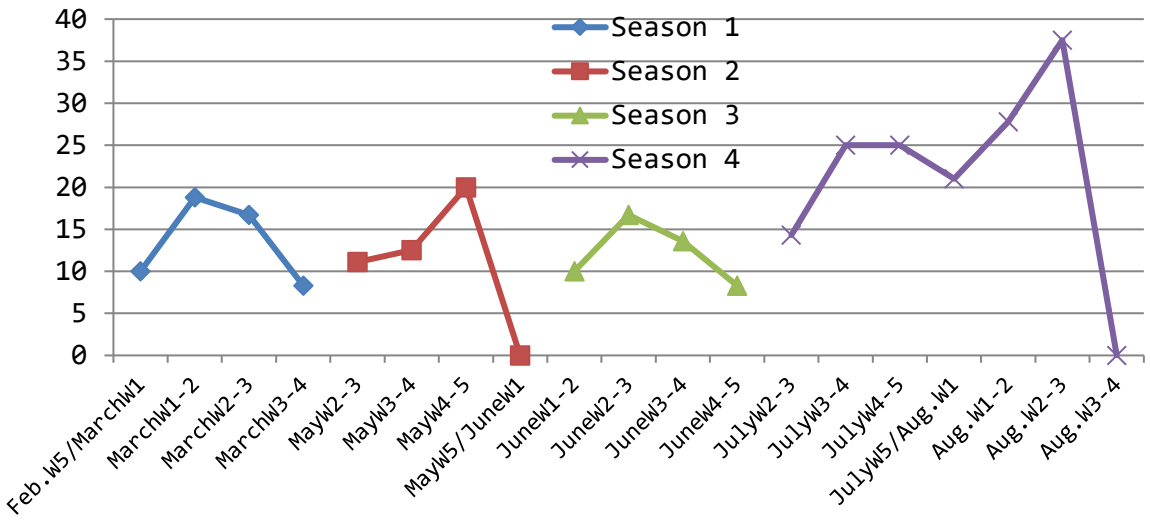
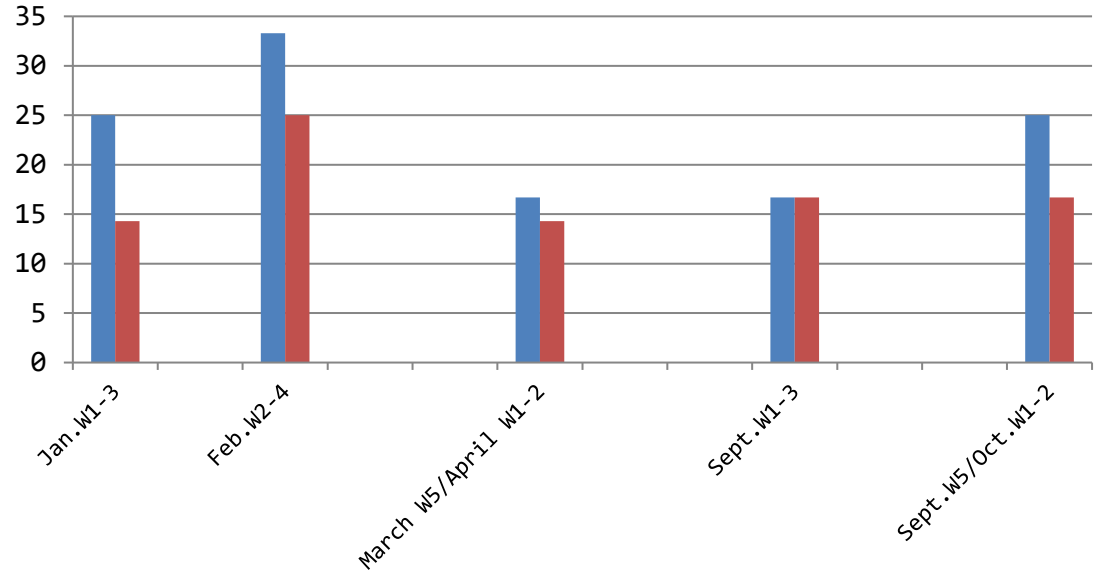


Figure 6. 2017-Short episodes of RV infection & Median % of Infection



1.5 RV genotype data prospective study period (2016-2017)

This data determined rotavirus strains, frequency, predominance and trends in severity. In total 150 samples were tested for RV and other viruses; thirty-seven stool samples tested positive for RV (24.6%) of which one sample was not typable (G??P8). The total number of stool samples that were genotyped for RV was 36(24%); 20

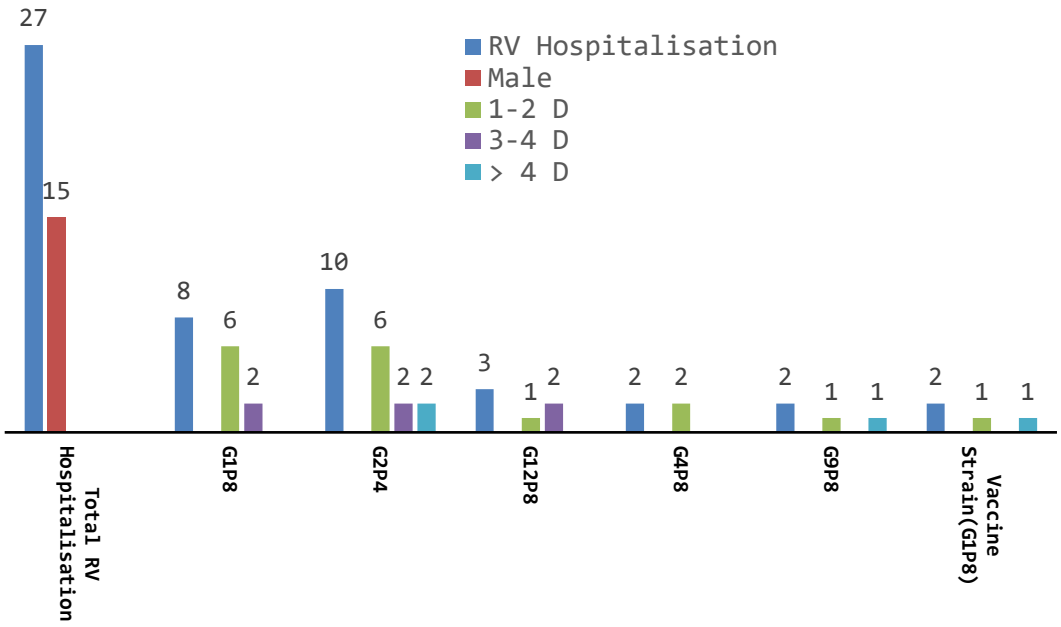
were male (56%), 25 were older than one year of age (69%). Only one single strain of RV was detected in all cases (100%). Majority of cases were severe (29 cases, 78%), five moderate (14%), three mild GE (8%).

RV G1P8: 17 patients (47%), 13 were older than one year of age (76%) and 12 were male (71%). Twelve cases were categorised as severe GE (71%), three mild with two moderate.
RVG1P8 (Rotarix ™ or Vaccine strain): six cases of 17 patients (35%).Three were mild, two severe and one was moderate GE.
RV G2P4: 11 patients (31%). Nine were ≤ one year of age (82%); Seven females (64%). Ten cases were severe GE (91%), one case was moderate GE. Nosocomial RV (RVG2P4) was confirmed in one case, 25 month old male (I-cell disease). Case was severe. Detected third week of July.
RV G4P8: Detected in three patients (8%). Two were older than one year of age (67%), three females (100%). Three cases were severe GE (100%). Nosocomial RV (RVG4P8) was not detected.
RV G12P8: Detected in three patients (8%), all older than one year of age, all male. One case was severe, others moderate. Nosocomial RV (RVG12P8) was not detected.
RV G9P8: Two patients (6%), all were older than 1 year of age, one male, all severe GE. Nosocomial RV (RVG9P8) was not detected.

1.6 RV GE Hospitalisation and Genotype correlation

During the study period, 128 children ≤3 years of age were admitted with GE, 27 patients were confirmed positive for RV (21%); 15 male (56%). Length of hospital stay was between one to two days in 17 cases (63%), three to four days in six cases (22%) and >four days in four cases (15%). All patients were tested for RV. The following describe genotype detection rates (Figure 7).

Figure 7. RV GE Hospitalisation and Genotype Correlation



RV G1P8 including vaccine strain: ten cases (37%), hospital admission one to two days (70%).Vaccine strain was isolated in two patients, hospital stay 1-2 days in one patient.

RVG2P4: ten cases (37%), hospital admission 1-2 days (60%).

RVG12P8: three cases (11%), hospital admission 1-2 days (67%).

RVG4P8: two cases (7%), hospital admission one to two days (50%).

RVG9P8: two cases (7%). One case stayed in hospital more than four days (50%) and required admission to intensive care unit (ICU).

1.7 Discussion

RV GE usually occurs in children between six months and two years of age, occurs in autumn and winter in temperate climates and a leading cause of paediatric GE (1-9). In our study, the second week of August was RV week peak with the highest rate of RV positive infection (75%, three of four cases). RV remained the leading cause of GE in children ≤ three years old, in the first year following RV vaccine introduction followed by human AdenoV F, similar to the findings of the NVRL in Ireland (July 2014 to June 2015). However, the NVRL recruited children up to five years old in their study,

with more NoroV GE cases and less AdenovF GE cases detected compared with our study (10).

Data here demonstrated that RV remains a leading cause of GE and GE related hospital admission (21%), compared with all other viruses, in our region of Ireland almost one year following the introduction of RV vaccine, followed by AdenoV (14%), NoroV (12%), AstroV (5%) and SapoV (4%). In the Swedish study that recruited 604 children <5 years of age from three geographical areas, admitted to hospital with RV-induced GE, RV G1P8 was most prevalent in all regions (77%). The most varied pattern was observed in the western region, with G1P8 observed in 61%, G4P8 in 13%, G9P8 in 10%, G2P4 in 8%, and G3P8 in 8% of the children (19). In our study that recruited 150 patients ≤ 3 years of age, RVG1P8 & RVG2P4 were the two commonest strains of RV related hospital admission with GE. Each strain was isolated in 37% of cases. RVG9P8 & RVG4P8 were rare (7% each) however, their burden was severe, both in terms of prolonged duration of hospital stay and in terms of clinical severity.

RVG1P8 is the predominant strain of RV associated with severe GE in children three years of age or younger worldwide (11-15). In our study, RVG1P8 was confirmed in 47% of all cases of RV GE in our region in the west of Ireland. Our study has also demonstrated the diversity of RV strains in one geographical region of Ireland, with the emergence of strains such as G2P4, G4P8, G12P8 and G9P8, as shown in other studies (11, 15-17). RVG2P4 was the second most common strain detected in 31% of RV GE cases.

Unlike other studies that showed that RVG4, may not be detected as a single infection, but was found in 8 co-infection combinations with G2G4P[8], G1G4P[8] and G4G9P[8] (11), RV G4 was only found in single combination with (P8) in our study. No combination of more than one RV strain was detected in our study.

RV was associated with severe GE in the majority of cases (78% of all cases of RV GE). RVG4P8 & RVG9P8 were associated with severe disease in all RV GE cases (100%). RVG2P4 was associated with severe GE in 91% of cases. RVG9P8 was associated with the most severe clinical form of GE in a 19-month-old boy; with severe electrolyte imbalance, encephalopathy and seizures requiring ICU.

Up until 2015/16 RV G1P8 strain was the most prevalent strain year on year across Europe, ranging from 61% (2007/08) to 29% (2014/15), in 2015/16, G1P8 strain was the fourth most prevalent strain (13%) (15). G9P8 is becoming increasingly detected across Europe both in countries with and without routine RV vaccination, was the most prevalent strain in 2015/16 (34%) (15).

Although the majority of the severe disease burden is located in developing countries, RV is estimated to cause approximately 80 000 general practice (GP) consultations and 750 000 diarrhoea episodes each year in the UK (21, 23). In addition, 45% of hospitalisations and 20% ED attendances for AGE in children < 5 years of age are attributable to RV (22, 23). RVG9P8, although a rare strain, its burden is severe, RVG9P8 was confirmed to be the cause of four deaths in children ≤ three years of age during an outbreak of diarrhoea in the Solomon islands in 2015 (20).

Nosocomial RV (NRV) infections are mainly associated with infants up to 5 months of age. RV was found to be the major etiologic agent of pediatric nosocomial diarrhea (31-87%) (18). In a Swedish study that recruited 604 children <5 years of age from three geographical areas, admitted to hospital with RV-induced GE, 49 of 604 (8.1%) fulfilled the criteria for nosocomial infection (19). In our study, surprisingly, NRV was rare and was detected in only one case, among 150 samples that were tested for RV (0.7%). NRVG2P4 was detected in the third week of July in a male with an underlying I-cell disease, with admitted for 25 days and developed severe GE. The low rate of NRV in our study may be attributed to a small sample size over a

short period of study, limited by recruiting patients from only one geographic area in Ireland. Adhering to strict local policies of hygienic measures in our unit may also have a positive effect in reducing the rate of NRV infection.

More research is required in the post RV vaccination era for a number of years to follow the pattern of geographical and temporal fluctuation of RV strains and whether or not other viruses will become a leading cause of hospital admission with GE, due to the possible effect of RV vaccine.

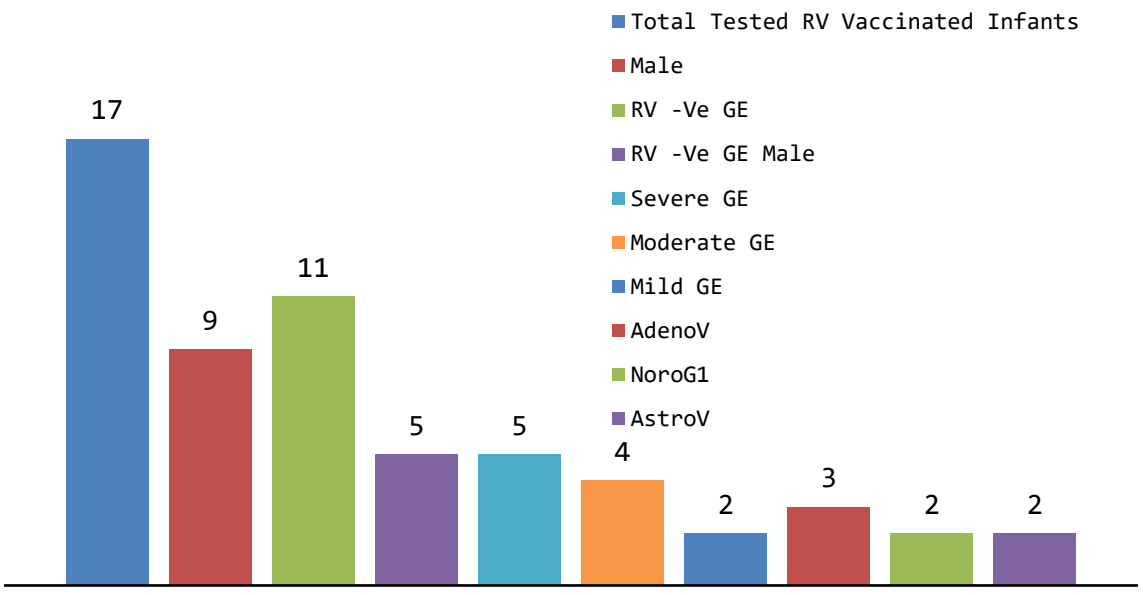
2. GE in RV Vaccinated Infants Post vaccine (2016-2017)

One-hundred and fifty stool samples were tested for RV and other viruses in the NVRL. Nineteen infants received first and/or second dose of RV vaccine (13%). Ten were male. Two patients were vaccinated but were not tested in the NVRL and were therefore excluded; 17 were tested. Detection methods are reviewed in Appendix 4(i).

2.1 RV negative GE in Vaccinated Infants

Eleven cases of RV -ve GE (65%) were detected in RV vaccinated infants; five males (45%). The median age at presentation was five months. Three patients were still eligible for RV vaccine second dose (18%), five cases were moderate GE (45.5%), four severe (36.4%) and two mild (18.1%). Onset of illness following first or second RV vaccine dose was variable from three to twenty-two weeks. No viral pathogen was detected in five cases (45%), AdenovF detected in three cases (27%), NorovG1 detected in two cases (18%) and AstroV was detected in two cases (18%).(Table 6 Appendix, Figure 8).

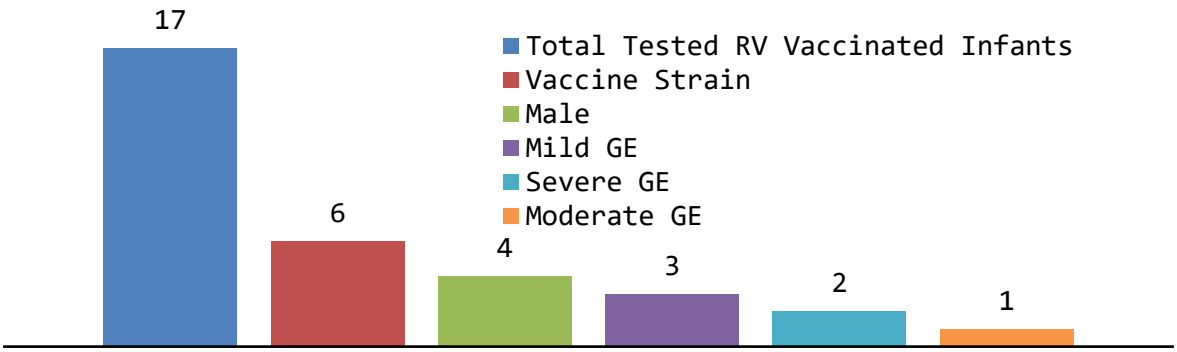
Figure 8. RV –Ve GE in RV Vaccinated Infants



2.2 RV positive GE in Vaccinated Infants

RV +ve GE were detected in six RV vaccinated infants (35%); five were still eligible for RV vaccine second dose, four male (67%); three cases of mild GE (50%), two severe (33%) and one moderate (17%)(Figure 9). The median age at presentation was 13 weeks. Onset of illness following first or second RV vaccine dose was variable from ten days to seven weeks. RVG1P8 (Rotarix ™) (Vaccine strain) was detected in all cases. RV vaccine strain was never isolated or detected in stool of any non RV vaccinated case of GE (129 cases)(Table 7, Appendix).

Figure 9. RV +Ve (Vaccine Strain) in RV Vaccinated Infants



2.3 Discussion

Transmission of vaccine virus has not been well studied. Viral transmission appears to occur more frequently among recipients of Rotarix ™ than RotaTeq (24); however, this rarely results in symptoms. In a randomised trial, in which one twin in each of 100 twin pairs received two doses of Rotarix™ and the other twin received placebo, transmission of vaccine virus occurred in 15 of 80 evaluable cases (18.8%) but was not associated with symptomatic GE (25). Such asymptomatic transmission may contribute to community (herd) immunity (24). RV shedding in the stool peaks within

approximately seven days of administration and is most common after the first dose (26 - 28).

Our study demonstrated that RV vaccinated infants may still present with GE and that infants may continue to shed vaccine strain (Rotarix™) in stool for a period up to 7 weeks following their first dose of RV vaccine. Rotarix ™ (RVG1P8) (vaccine strain) was detected in all cases (6 infants) who presented with GE during the study period (almost one year following RV vaccine introduction in Ireland). The majority shed vaccine strain after their first Rotarix™ (5 patients). This vaccine strain can be the cause of GE in these cases but one cannot be certain about this conclusion as other causes of GE cannot be ruled out and the presence of vaccine strain may just be a co-incidental finding or just a shedding of vaccine strain with no direct causal relationship to GE (15).

RV vaccine may not give absolute protection against RV GE. However, it has been shown to reduce severe GE (29). In our study, 50% of cases with RV GE in RV vaccinated infants presented with mild form of GE. Our study also demonstrated that RV-ve GE in RV vaccinated infants was much more encountered than RV +ve GE in this group of infants (65% versus 35%). This may -in part- reflect the efficacy of RV vaccine in reducing the risk of RV GE. However, other viruses may become more predominant due to the possible effect of RV vaccine in reducing the rate of RV infection and possible herd protection. In this study, infection with AdenoV, for example, was detected in 27% of RV -ve GE in vaccinated infants, NorovG1 was detected in 18% and similarly, AstroV was detected in 18%.

Appendix to Chapter 4

Table 1. 2014 RV Season & Short Episode of Infection (EOI)

Month	Week (W)	Total RV Request	RV +ve	Median RV+ve	Median +ve RV%	Notes	Duration Of Season or (EOI)
January	W1-2	14	0	0	0		
	W2-3	13	0	0	0		
	W3-4	7	0	0	0		
	W4-5	13	0	0	0		
Jan/Feb	W5/W1	9	0	0	0		
Februray	W1-2	11	0	0	0		
	W2-3	26	1	0.5	1.9		
	W3-4	23	1	0.5	2.1		
	W4-5	20	0	0	0		
Feb/ March	W5/W1	12	0	0	0		
March	W1-2	21	4	2	9.5		
	W2-3	38	10	5	13.1	Onset S1	Season 1
	W3-4	30	10	5	16.6	Peak S1	
	W4-5	32	7	3.5	10.9	S1	4 Weeks
March/ April	W5/W1	32	5	2.5	7.8	End S1	
April	W1-2	19	2	1	5.2		
	W2-3	15	2	1	6		
	W3-4	22	4	2	9		
	W4-5	21	4	2	9.5		
April/ May	W5/W1	8	2	1	12.5	Onset S2	Season 2
May	W1-2	12	4	2	16.6	S 2	
	W2-3	21	6	3	14		
	W3-4	25	13	6.5	26		7 Weeks
	W4-5	27	16	8	29.6	Peak S2	
May/ June	W5/W1	15	5	2.5	16.6	S 2	
June	W1-2	10	0	0	0	End S2	
	W2-3	14	0	0	0		
	W3-4	21	2	1	4.7		
	W4-5	13	2	1	7.6		
June/ July	W5/W1	6	0	0	0		
July	W1-2	11	3	1.5	13.6	Onset S3	Season 3
	W2-3	9	3	1.5	16.6	S 3	
	W3-4	10	2	1	10		
	W4-5	13	5	2.5	19.2	Peak S3	
July/ August	W5/W1	8	3	1.5	18.7	S 3	8 Weeks
August	W1-2	14	3	1.5	10.7		
	W2-3	20	4	2	10		
	W3-4	14	1	0.5	3.5	End S 3	
	W4-5	16	1	0.5	3.1		
Aug/Sept	W5/W1	14	1	0.5	3.5		
Sept	W1-2	18	0	0	0		
	W2-3	16	0	0	0		
	W3-4	13	0	0	0		
	W4-5	16	3	1.5	9.6		

Month	Week (W)	Total RV Request	RV +ve	Median RV+ve	Median +ve RV%	Notes	Duration Of Season or (EOI)
Sept/ October	W5/W1	11	3	1.5	13.6	1st EOI	2 Weeks
October	W1-2	10	0	0	0		
	W2-3	12	0	0	0		
	W3-4	15	0	0	0		
	W4-5	11	0	0	0		
Oct/Nov	W5/W1	3	0	0	0		
November	W1-2	12	0	0	0		
	W2-3	27	0	0	0		
	W3-4	28	1	0.5	1.8		
	W4-5	23	1	0.5	2.1		
Nov/Dec	W5/W1	20	0	0	0		
December	W1-2	23	0	0	0		
	W2-3	28	1	0.5	1.8		
	W3-4	27	3	1.5	5.5		
	W4-5	23	2	1	4.3		

Table 2. 2015 RV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total RV (R)	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season
January	W1-2	24	2	1	4.1		
	W2-3	30	2	1	3.3		
	W3-4	19	3	1.5	7.9		
	W4-5	20	2	1	5		
Jan/Feb	W5/W1	25	3	1.5	6		
Feb	W1-2	28	5	2.5	8.9		
	W2-3	26	2	1	3.8		
	W3-4	20	1	0.5	2.5		
	W4-5	8	1	0.5	6.2		
Feb/ March	W5/W1	18	7	3.5	19.4	Onset S 1	Long Season1 Duration 19 weeks (>3 months)
March	W1-2	30	11	5.5	18.3	Long S 1	
	W2-3	23	7	3.5	15.2		
	W3-4	31	13	6.5	20.9		
	W4-5	27	12	6	22.2		
March/ April	W5/W1	16	5	2.5	15.6		
April	W1-2	29	10	5	17.2		
	W2-3	31	12	6	19.3		
	W3-4	17	8	4	23.5		
	W4-5	16	8	4	25	Peak of Long S 1	
April/ May	W5/W1	12	6	3	25		
May	W1-2	18	8	4	22.2	Long S 1	
	W2-3	26	9	4.5	17.3		
	W3-4	27	7	3.5	13		
	W4-5	27	10	5	18.5		
May/ June	W5/W1	35	16	8	22.9		
June	W1-2	31	16	8	25.8	2nd. Peak S 1	
	W2-3	12	6	3	25		

Month	Week (W)	Total RV (R)	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season
	W3-4	10	1	0.5	5	End S 1	
	W4-5	6	0	0	0		
June/ July	W5/W1	11	2	1	9		
July	W1-2	22	4	2	9		
	W2-3	18	2	1	5.6		
	W3-4	14	1	0.5	3.6		
	W4-5	9	1	0.5	5.6		
July/ August	W5 July/W1 Aug.	0	0	0	0		
August	W1-2	11	1	0.5	4.5		
	W2-3	22	4	2	9		
	W3-4	26	3	1.5	5.8		
	W4-5	20	0	0	0		
Aug/ Sept	W5/W1	10	1	0.5	5		
Sept	W1-2	11	2	1	9		
	W2-3	12	1	0.5	4.2		
	W3-4	20	1	0.5	2.5		
	W4-5	18	1	0.5	2.8		
Sept/ October	W5/W1	12	0	0	0		
October	W1-2	19	1	0.5	2.6		
	W2-3	17	1	0.5	2.9		
	W3-4	12	0	0	0		
	W4-5	17	0	0	0		
Oct/ Nov	W5/W1	17	1	0.5	2.9		
Nov	W1-2	20	1	0.5	2.5		
	W2-3	18	0	0	0		
	W3-4	11	0	0	0		
	W4-5	19	0	0	0		
Nov/Dec	W5/W1	27	0	0	0		
Dec	W1-2	37	2	1	2.7		
	W2-3	33	5	2.5	7.6		
	W3-4	16	4	2	12.5	1st EOI	2weeks
	W4-5	16	2	1	6.3		

Table 3. 2016 (Jan. to Dec 1st) RV Season (S) & Short Episode of Infection (EOI)

Month	Week (W)	Total RV Request	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season or(EOI)
January	W1-2	14	0	0	0		
	W2-3	31	1	0.5	1.6		
	W3-4	27	1	0.5	1.9		
	W4-5	18	0	0	0		
Jan/Feb	W5/W1	24	0	0	0		
Februry	W1-2	27	0	0	0		
	W2-3	26	0	0	0		
	W3-4	23	0	0	0		
	W4-5	15	1	0.5	3.3		

Month	Week (W)	Total RV Request	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season or(EOI)
Feb/Mar	W5/W1	16	2	1	6.2		
March	W1-2	16	1	0.5	3.1		
	W2-3	9	0	0	0		
	W3-4	8	0	0	0		
	W4-5	11	0	0	0		
March/ April	W5/W1	9	0	0	0		
April	W1-2	11	0	0	0		
	W2-3	12	0	0	0		
	W3-4	13	1	0.5	3.8		
	W4-5	16	1	0.5	3.1		
April/ May	W5/W1	30	6	3	10	Onset S 1	Duration of a long Season 1 10 weeks
May	W1-2	35	12	6	17.1	S 1	
	W2-3	21	10	5	23.8	Peak S1	
	W3-4	21	9	4.5	21.4	S 1	
	W4-5	17	7	3.5	20.1		
May/ June	W5/W1	13	5	2.5	19.2		
June	W1-2	22	5	2.5	11.4		
	W2-3	25	6	3	12		
	W3-4	21	5	2.5	11.9		
	W4-5	13	1	0.5	3.8	End S 1	
June/ July	W5 June/ W1 July	4	0	0	0		
July	W1-2	10	3	1.5	15	1st EOI	2 weeks
	W2-3	17	3	1.5	8.8		
	W3-4	11	1	0.5	4.5		
	W4-5	12	3	1.5	12.5	2nd EOI	2 weeks
July/ August	W5 July W1 Aug	16	2	1	6.2		
August	W1-2	18	0	0	0		
	W2-3	18	1	0.5	2.8		
	W3-4	14	2	1	7.1		
	W4-5	9	3	1.5	16.7	3rd EOI	3 weeks
August/ Sept	W5/W1	6	2	1	16.7		
Sept	W1-2	7	0	0	0		
	W2-3	15	3	1.5	10	Onset S 2	Duration of Season 2 8 weeks
	W3-4	17	4	2	11.8	S 2	
	W4-5	13	4	2	15.4		
Sept/ October	W5/W1	7	3	1.5	21.4		
October	W1-2	6	3	1.5	25		
	W2-3	13	8	4	30.8	Peak S 2	
	W3-4	15	7	3.5	23.3	S 2	
	W4-5	19	3	1.5	7.9	End S 2	
Oct/Nov	W5/W1	17	1	0.5	2.9		
Nov	W1-2	16	2	1	6.3		
	W2-3	21	2	1	4.8		
	W3-4	2	1	0.5	25	Onset S3	
	W4-5	1	1	0.5	50	Peak S3	
Nov/Dec	W5-W1	4	2	1	25	S 3	

Table 4. 2014-2016 (Pre Vaccination years)- RV Season (S) & Short Episodes of Infection (EOI), Median Percentage of RV Positive Infection (M%RV), Duration (D) in Weeks (W)

Year	Seasons (S)		Onset Of season & (M% RV)	Peak of season & (M% RV)	End of season & (M% RV)	(D) of season	RV(EOI) & (M% RV)			(D) of (RV) EOI
2014	3	S1	March (W2-W3) 13.1%	March (W3-W4) 16.6%	March (W5)-April (W1) 7.8%	4 (W)	1		Sept. W5-Oct. (W1) 13.6%	2 (W)
		S2	April (W5)-May (W1) 12.5%	May (W4-W5) 29.6%	June (W1-W2) 0%	7 (W)				
		S3	July (W1-W2) 13.6%	July (W4-W5) 19.2%	August (W3-W4) 3.5%	8 (W)				
2015	1	Long S1	Feb. (W5)-March (W1) 19.4%	April (W4-W5)& W5 April-May (W1) 25% each 2^{nd}., Peak: June (W1-W2) 25.8% June (W2-W3) 25%	June(W3-W4) 5%	19 (W)	1		Dec. (W3-W4) 12.5%	2 (W)
2016 to Dec 1	3	S1	April (W5)-May (W1) 10%	May (W2-W3) 23.8%	June (W4-W5) 3.8%	10 (W)	3	1	July (W1-W2) 15%	2 (W)
								2	July (W4-W5) 12.5%	2 (W)
		S2	Sept (W2-W3) 10%	Oct. (W2-W3) 30.8%	Oct. (W4-W5) 7.9%	8 (W)		3	Aug. (W4-W5)-Sept (W1) 16.7% 3 (W)	
		S3	Nov. (W3-W4) 25%	Nov.(W4-W5) 50%	Dec. (W2-W3) 6.3%	5 (W)				

Table 5. RV Season in Post Vaccination Year from December 2016 to November 18 2017 & Short Episode of Infection (EOI)

Month	Week (W)	Total RV Request	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season or(EOI)
Dec 2016	W1-2	9	3	1.5	16.6	S3 (Cont.)	5 weeks
	W2-3	8	1	0.5	6.3	End S3	
	W3-4	9	1	0.5	5.6		
	W4-5	6	1	0.5	8.3		
Dec/ Jan 2017	W5/W1	0	0	0	0		
Jan 2017	W1-2	2	1	0.5	25	1st EOI	3 weeks
	W2-3	7	2	1	14.3		
	W3-4	7	1	0.5	7.1		
	W4-5	5	0	0	0		
Jan/Feb	W5/W1	6	0	0	0		
February	W1-2	4	0	0	0		
	W2-3	3	2	1	33.3	2nd EOI	3 weeks
	W3-4	6	3	1.5	25		
	W4-5	6	1	0.5	8.3		
Februray /March	W5/W1	5	1	0.5	10	Onset S 1	4 weeks
March	W1-2	8	3	1.5	18.8	Peak S 1	
	W2-3	9	3	1.5	16.7	S 2	
	W3-4	6	1	0.5	8.3	End S 1	
	W4-5	4	0	0	0		
March/ April	W5/W1	3	1	0.5	16.7	3rd EOI	3 weeks
April	W1-2	7	2	1	14.3		
	W2-3	9	1	0.5	5.6		
	W3-4	6	0	0	0		
	W4-5	7	1	0.5	7.1		
April/ May	W5/W1	10	1	0.5	5		
May	W1-2	11	2	1	9.1		
	W2-3	9	2	1	11.1	Onset S 2	4 weeks
	W3-4	8	2	1	12.5	S 2	
	W4-5	5	2	1	20	Peak S 2	
May/ June	W5/W1	2	0	0	0	End S 2	
June	W1-2	5	1	0.5	10	Onset S 3	4 Weeks
	W2-3	9	3	1.5	16.7	Peak S 3	
	W3-4	11	3	1.5	13.6	S 3	
	W4-5	6	1	0.5	8.3	End S 3	
June/ July	W5/W1	0	0	0	0		
July	W1-2	3	0	0	0		
	W2-3	7	2	1	14.3	Onset S 4	7 Weeks
	W3-4	8	4	2	25	1st. Peak S4	
	W4-5	6	3	1.5	25		
July/ August	W5/W1	7	3	1.5	21	S 4	
August	W1-2	9	5	2.5	27.8		
	W2-3	4	3	1.5	37.5	2nd.Peak	

Month	Week (W)	Total RV Request	RV +ve	Median	Median +ve RV%	Notes	Duration Of Season or(EOI)
						S 4	
	W3-4	1	0	0	0	End S 4	
	W4-5	2	0	0	0		
August/ Sept	W5/W1	2	0	0	0		
Sept	W1-2	3	1	0.5	16.7	4th. EOI	3 Weeks
	W2-3	3	1	0.5	16.7		
	W3-4	2	0	0	0		
	W4-5	1	0	0	0		
Sept/ October	W5/W1	2	1	0.5	25	5th. EOI	3 Weeks
October	W1-2	3	1	0.5	16.7		
	W2-3	1	0	0	0		
	W3-4	2	0	0	0		
	W4-5	2	0	0	0		
Octobr/ November	W5/W1	0	0	0	0		
Nov 1-18	W1-W2	1	0	0	0		
	W2-W3	3	0	0	0		

Table 6. RV Negative (-Ve) GE in RV Vaccinated Infants

Month	G	Age at onset	Viral Pathogen	RV vaccine 1	RV vaccine 2	Onset after RV vaccine	Week of Onset (W)	Severity
April	M	5 M	AdenoF	1	1	5 Weeks	W5	Moderate
May	M	6 M	Noro G1& AstroV	1	0	15 Weeks	W2	Moderate
	M	5 M	AstroV	1	1	6 Weeks	W3	Mild
June	F	13 week	Excluded Not Tested	1	0	4 Weeks	W1	Moderate
	M	7 M	Excluded Not Tested	1	1	12 Weeks	W4	Severe
July	F	5 M	None	1	1	6 Weeks	W2	Mild
	F	7 M	None	1	1	11 Weeks	W3	Moderate
Aug	F	8 M	None	1	1	14 Weeks	W2	Moderate
	F	6 M	None	1	1	5 Weeks	W5	Moderate
Sept	F	10 M	NoroG1	1	1	22 Weeks	W4	Severe
Oct	F	7 M	AdenovF	1	1	12 Weeks	W4	Severe
	M	3 M	None	1	0	4 Weeks	W4	Severe
Nov 1-18	M	3 M	AdenovF	1	0	3 Weeks	W2	Severe
Gender (G): Male (M), Female (F)								

Table 7. RV Positive (+Ve) Vaccine Strain in RV Vaccinated Infants–

Month	G	RV strain,Age at Onset	RV vaccine1	RV vaccine2	Onset after RV vaccine	Week (W)	Severity
January 2017	F	**Rotarix™, 14 weeks**	1	0	5 Weeks	W2	Mild
February	M	**Rotarix™ 3 Month**	1	0	4 Weeks	W4	Moderate
March	M	**Rotarix™, 4 Month**	1	0	7 Weeks	W3	Mild
May	F	**Rotarix™, 11 weeks**	1	0	3 Weeks	W4	Mild
	M	**Rotarix™, 9 weeks**	1	0	10 days	W4	Severe
August	M	**Rotarix™ &SapoV, 6 Month**	1	1	2 Weeks	W2	Severe
Gender (G): Male (M), Female (F); Age at Onset							

Appendix 4(i): Virus Detection Methods for RV post-vaccine

These data assessed Rapid Antigen Testing (RAT) in Galway University Hospital laboratory (GUHL) Versus PCR testing of Virus in the National Virus Reference Laboratory (NVRL), Sensitivity and Specificity of RAT (Tables 9 **and** 10). RV is detected by RAT in GUHL and by PCR testing in NVRL. From November 18th 2016 to November 18th 2017, 144 stool samples were tested in both GUHL and NVRL for RV and other viruses.

Twenty-seven samples were confirmed RV +ve in GUHL; 36 samples were confirmed RV +ve in the NVRL. Ten samples were false negative for RV RV F-ve in GUHL.

One hundred and seventeen samples were confirmed negative RV-ve in GUHL; 108 samples were confirmed negative RV-ve in the NVRL. Sensitivity of RAT (*1-B)* (B = false -ve rate) was 72%. Specificity of RAT (1-α) (α = false +ve rate) was 99%. PCR is more sensitive and specific than RAT (30-32). Our study has shown similar results. RAT is ratified as more specific than sensitive in detecting RV (33). Our study demonstrated that RAT - as RV detecting tool- was more specific than sensitive (higher specificity and lesser sensitivity)

as shown in other similar studies (33-35). Although the rapid test is able to quickly give results, our study found that it has a high false negative rate. Thus, other highly sensitive methods such as one-step RT-PCR are still required for true diagnosis (34-35).

Table 8. RV Detecting Method: RAT in GUHL Versus PCR testing of Virus in The NVRL

Month	Test	Number of Confirmed RV +ve cases	Number of false –ve cases (GUHL)	Number of confirmed RV –ve cases	Number of false +ve cases (GUHL)
Nov 18-30	RAT(GUHL)	1	0	1	0
	PCR(NVRL)	1		1	
December	RAT(GUHL)	4	0	14	0
	PCR(NVRL)	4		14	
January	RAT(GUHL)	1	1	11	0
	PCR(NVRL)	2		10	
February	RAT(GUHL)	2	1	10	0
	PCR(NVRL)	3		9	
March	RAT(GUHL)	2	2	14	0
	PCR(NVRL)	4		12	
April	RAT(GUHL)	2		15	0
	PCR(NVRL)	3	1	14	
May	RAT(GUHL)	2	2	17	0
	PCR(NVRL)	4		15	
June	RAT(GUHL)	5	0	10	1
	PCR(NVRL)	4		11	
July	RAT(GUHL)	4	1	9	0
	PCR (NVRL)	5		8	
August	RAT(GUHL)	3	1	7	0
	PCR(NVRL)	4		6	
September	RAT(GUHL)	1	0	2	0
	PCR(NVRL)	1		2	
October	RAT(GUHL)	0	1	5	0
	PCR(NVRL)	1		4	
Nov 1-18	RAT(GUHL)	0	0	2	0
	PCR(NVRL)	0		2	
Total (144)	**RAT(GUHl)**	**27**	**10**	**117**	**1**
	PCR(NVRL)	**36**		**108**	

Table 9. RV Detecting Method: Sensitivity and Specificity of (RAT)

	Total RV +ve	Total RV False -ve	Sensitivity of RAT (1-B) B=false -ve rate	Total RV -ve	Total RV False +ve	Specificity of RAT (1-α) α =false +ve rate)
RAT GUHL	**27**	**10**	**72%**	**117**	**1**	**99%**
PCR NVRL	**36**			**108**		

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Chapter 5: Results III
Impact of RV Vaccine

Seasonal variations are first outlined followed by effect on RV testing and infection for comparisons.

1.1 RV season Pre vaccination (2014 to Dec 2016)

The longest season was noted in 2015 and lasted 19 weeks, with two long and high peaks of positive RV infection.The highest peak of RV positive infection was 30.8% and was noted in October 2016 upon the introduction of RV vaccine in Ireland and prior to the first dose of vaccination that was due in December 2016. The first RV vaccine was to be given at two month of age to all babies born in Ireland since October 2016. The RV season started early in February and March in 2014 and 2015, the RV season onset was delayed until April in 2016. Short episodes of RV infection were noted during the three seasons (2014-2016). The highest number of these short episodes was detected in 2016, with three episodes of RV infection detected.

1.2 RV season Post Vaccination (Dec 2016-Nov 18th 2017)

Four seasons of RV GE and five short episodes of RV infection were noted in 2016/2017 from Dec 2016 to November 18th 2017. Onset, peak, median percentage of RV infection, end of season and short episodes of RV GE are shown previously in Chapter 4 (Table 5 of Appendix and figures 5-6 in Chapter 4).

Monthly figures showing reduced total presentation with GE and peak of RV GE during post vaccination year 2016/2017 in contrast to the pre vaccination years (2014-2016) are shown in figures 1 & 2. Further statistical comparisons are described below.

Figure 1. Pre Vaccination Data (2014-2016): Monthly Figures

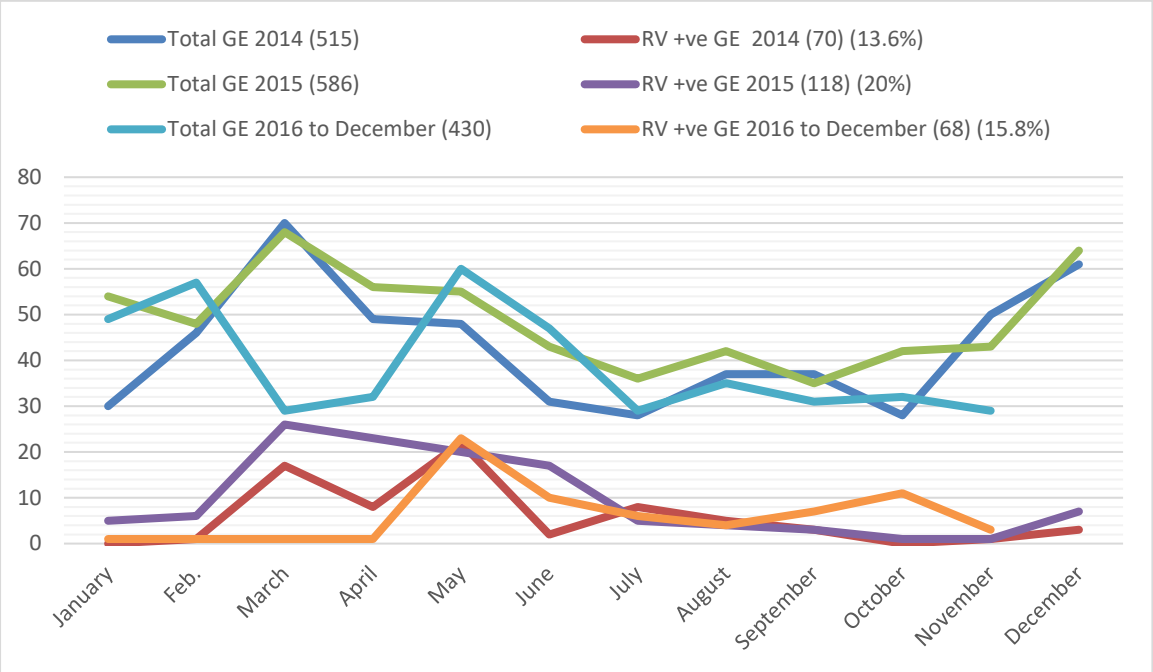
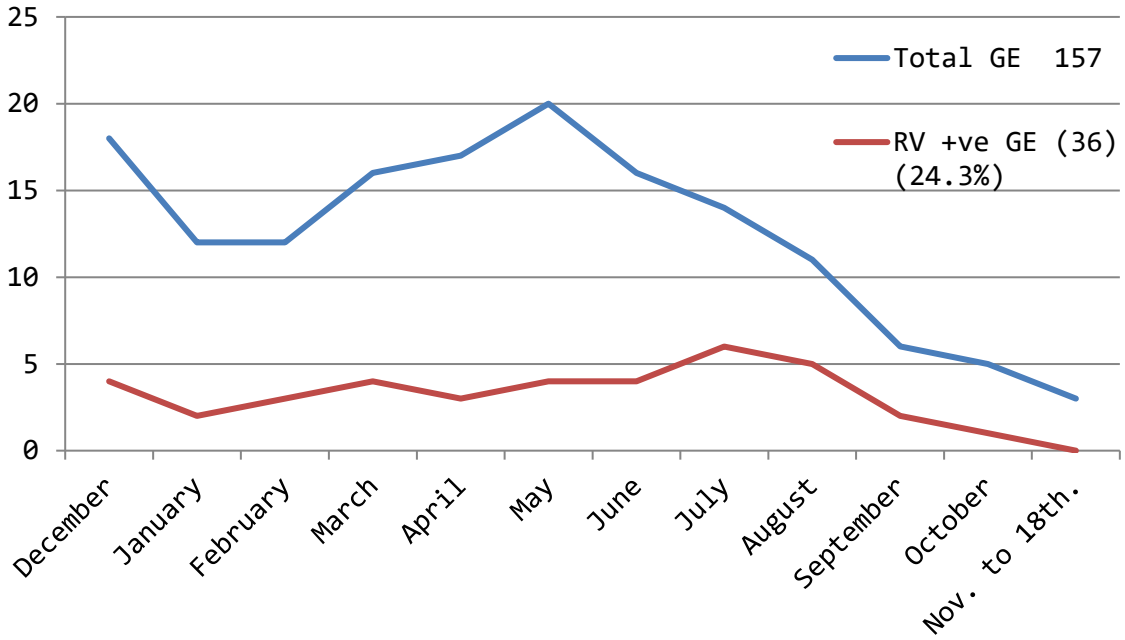


Figure 2. Post Vaccination Data- Monthly Figures



1.3 Pre versus Post Vaccine comparisons

1.3.1. From 2014 to December 2016 prior to when RV vaccine was first administered in Ireland (pre vaccination years), 515, 586 and 430 RV stool samples were requested respectively. The percentage of RV positive stool samples in those years were (13.6%), (20%) and (15.8%) respectively. From December 2016-2017, 148 stool samples were requested, RV positive (24.3%) (Table 1, Figure 3).

The total number of RV positive stool samples or requests, post vaccination dropped significantly compared with pre vaccination years. Reduction in the percentage of the total number of positive RV stool samples of 48.5%, 69% and 47%, compared with pre vaccination years, with a median percentage of reduction of positive RV stool requests of 48.5%, similar to other studies (11, 16-17, 20-21). The total number of RV stool requests from the PW or hospital admissions due to GE, dropped to 128 post vaccination compared with 483, 556 and 391 in pre vaccination years with a percentage of reduction of GE related hospital admissions of about 73%, 77% and 67% and a median percentage of reduction of GE related hospital admission of 73%, similar to other studies worldwide (9-10, 12-17, 19, 22).

The total number of stool requests from ED dropped to 29 compared with 32, 30 and 39 in the pre vaccination years with a percentage of reduction of about 9%, 3% and 26% and a median percentage of reduction of ED presentation with GE of 9%, similar to other studies (9, 19). These figures are likely attributed to the efficacy of RV vaccine in terms of reducing the number of presentations with GE to ED, PW and subsequently reducing hospital admissions and health care utilisation as shown in other similar studies worldwide (9-17, 19-22).

Table 1. RV stool requests, & admission rates Pre-vaccination (2014-Dec 2016) and Post-vaccination (Dec 2016 to Nov 2017)

	2014	2015	2016 to December	2016 (From December to Nov.18th 2017)
Total number of RV stool requests	515 (Median)	586	430	157 9 excluded from (RV) testing Total tested (148)
% of reduction of RV stool requests from (PW & ED)	(69.5%) (Median %)	(73%)	(65.6%)	
Total number of Positive RV stool	70(13.6%) (Median)	118(20%)	68(15.8%)	36/148 (24.3%)
% of reduction in RV+ve stool requests	(48.5%) (Median %)	(69%)	(47%)	
Total number of RV stool requestson PW (Hospital Admissions)	483 (Median)	556	391	128
% of Reduction of Hospital Admission	(73%) (Median %)	(77%)	(67%)	
Total number of RV stool requests (ED)	32	30	39	29
% of reduction of RV stool requests (ED)	(9.3%) (Median %)	(3.3%)	(25.6%)	
Outcome from PW (Hospital Admissions) and ED (Emergency Department)				

Figure 3. 2014-2016 versus Post Vaccination (2016/2017) RV request and GE hospitalisations

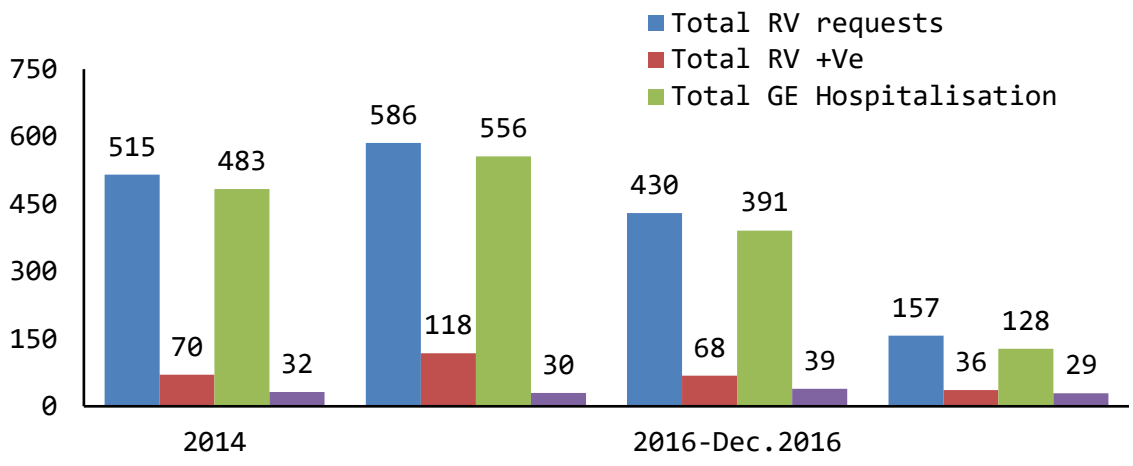


Table 2. Compared Proportions of RV infection, Pre and post vaccination-Chi Square test

	RV detection rate		
	Detected	**Not Detected**	**p-value***
Pre Vaccination	256 (16.7%)	1,275 (83.3%)	0.020
Post Vaccination	36 (24.3%)	112 (75.7%)	
**p-value results from Pearson ChiSquare* (χ^2=5.4, df=1) RV (rotavirus)			

A statistically significant difference in the proportion of detection of RV between pre and post vaccination status was identified. However, it merits highlighting that the percentage of detection was greater in the post-vaccination group, possibly due to the smaller sample size (only one year post vaccination), that which is less than 10% of the pre-vaccination cohort. Sampling variability may have played a role. The significant difference is not as expected likely due to the small sample size of the Post vaccination group.

Table 3. RV yearly compared proportions of infection pre-vaccine versus post vaccine-Chi Square test

	RV		
	Detected	**Not Detected**	**p-value***
2014	70 (13.6%)	445 (86.4%)	**0.003**
2015	118 (20.1%)	468 (79.9%)	
2016	68 (15.8%)	362 (84.2%)	
Post vaccination	36 (24.3%)	112 (75.7%)	
**p-value results from Pearson Chi Square*			

For GE-related admission rates, a statistically significant lower proportion of children were admitted to the hospital after the RV vaccine was introduced 128 (81.5%). Admissions rate (%) due to GE infection pre-vaccination was 11.9% higher than admissions during the post vaccination months (Table 4).

Table 4. GE-related compared proportions of admissions pre-vaccine versus post vaccine-Chi Square test

	Admitted	Not Admitted	p-value*
Pre-vaccination	1,430 (93.4%)	101 (6.6%)	< 0.0001
Post Vaccination	128 (81.5%)	29 (18.5%)	
**p-value results from Pearson Chi Square* (χ^2=28.24, df=1)			

1.4 Discussion

The data presented in this chapter demonstrate that the total number of requested stool samples for RV testing has significantly decreased during the post RV vaccination year, both from Paediatric Ward (PW), reflecting hospital admissions and from the ED. Our study demonstrated that the total number of stool samples requested for RV testing in children presenting with GE, both from PW and ED significantly decreased to 159. This is seen as a significant reduction of the total number of presentations with GE compared with pre vaccination years. Our study supports the findings of many studies worldwide showing the effect of RV vaccine in terms of reducing the total number of presentations with GE (8-17, 19).

The total number of RV positive stool samples or requests, post vaccination dropped significantly compared with pre vaccination years. Another significant reduction in the percentage of the total number of positive RV stool samples compared with pre vaccination years, with a median percentage of reduction of positive RV stool requests of 48.5%, similar to other studies (11, 16-17, 20-21).

The total number of RV stool requests from the PW or hospital admissions due to GE dropped compared to pre vaccination years with a median percentage of reduction of GE related hospital admission of 73%, similar to other studies worldwide (9-10, 12-17, 19, 22).

The total number of stool requests from ED has also dropped to 29 compared with 32, 30 and 39 in the pre vaccination years with a percentage of reduction of about 9%, 3% and 26% and a median percentage of reduction of ED presentation with GE of 9%, similar to other studies (9, 19). These figures are likely attributed to the efficacy of RV vaccine in terms of reducing the number of presentations with GE to ED, PW and subsequently reducing hospital admissions and health care utilisation as shown in other similar studies worldwide (9-17, 19-22).

Chapter 6

Conclusion and Discussion

Our study demonstrated a number of interesting findings regarding the epidemiology of RV and other enteric viral pathogens, as well as the short-term impact of RV vaccination, in a district general hospital in the West of Ireland. This study was the first study to evaluate RV diversity of genotypes, predominant genotypes, correlation with disease severity and co infection with other viruses in the post vaccination year, in Ireland.

As seen in **Chapter 3**, RV remains the leading cause of GE (24.6%) and GE related hospital admission (21%) with G1P8 being the commonest strain as included in Rotarix ™ vaccine. The diversity of other rare strains with its burden on disease severity was also noted such as RV G9P8. The majority of cases with RV G2P4, G4P8 were associated with severe GE. We also showed for the first time in a regional context, the distribution of other causes of viral GE (AdenoV, NoroV, AstroV and SapoV) in terms of seasonal and disease characterstics in both isolated and mixed infection. In **Chapter 3**, data presented set the contextual framework for further focused study of RV and the impact of vaccination described in subsequent **Chapters 4 and 5**, respectively. In determining the gastroenteritis admission rates for the hospital and the principle enteric viral pathogens, we saw RV as the predominant enteric viral pathogen, in the pre-vaccination (including nationally) and post vaccination eras, serving as baseline epidemiological data at the onset of RV vaccine roll out in Ireland.

In **Chapter 4** the focus on RV disease epidemiology in the pre and post vaccination era noted several observations. RV occurs in autumn and winter in temperate climates and throughout the year in tropical climates (1-7). Seasonal trend of RV was variable in our study where

RV GE occured during summer and throughout the year (various short episodes) in the pre- and post vaccination period.

RV vaccine may delay the onset of RV infection (8) and reduce the magnitude or seasonal peak of infection (9). Following vaccination in 2013 in the UK, RV season in 2014/2015 started late in April and May in 2016 in contrast to the years preceeding vaccine introduction when the peak occurred in March (8). During the post RV vaccination year (Dec 2016 to Nov 18th 2017) in our study, four seasons of RV GE and five short episodes of RV infection were noted and RV season started in late February-early March, almost similar to the onset of RV season in 2014 and 2015. There appeared to be no difference or effect noted in terms of RV vaccine efficacy in delaying the onset of RV season or reducing the peak of RV infection; high peaks of RV infection were noted in the immediate one post vaccination year. We acknowledge the limitation of this observation due to a limited sample size in a small geographical location resulting in the possibile over estimation of data effects. Additional research for years in the post vaccination era to include larger geographical locations and data from GE cases within primary care units will enable further observation of the possible effect of RV vaccine on seasonal trends of RV.

We determined that thus far G1P8 remains a predominant RV strain in Ireland both before and after the administration of RV vaccine. However, attributing any future change in the pattern of molecularly determined circulating strains in the post vaccination era to the impact of RV vaccine needs to be considered cautiously as this may reflect natural temporal variation in genotype distribution (8), requiring research for years in the post vaccination era.

As outlined **in Chapter 5** the direct impact of RV vaccine was apparent, as total number of requested stool samples for RV testing reflecting hospital admissions and the total number of presentations with GE and RV +ve stool samples compared with pre vaccination years

dropped significantly. These findings likely attributed to the efficacy of RV vaccine in terms of reducing the number of presentations with GE to ED and ward and subsequently reducing hospital admissions and health care utilisation. These findings are similar to those seen in other similar studies worldwide (9-17, 19-22).

RV vaccine may shorten the duration of RV season (9) as seen in in this study. In **Chapters 4 & 5** we determined the impact of RV vaccine in terms of RV testing, positivity rates and GE-related admissions. We also saw seasonal differences, where all four RV seasons in the post vaccination year (Dec 2016 to Nov 2017) were short and not extending beyond seven weeks in contrast to pre vaccination (2014-2016) when most of the seasons were long. We acknowledge the limitation of not having collecting data earlier than 2014 or longer than 1 year post vaccine for further extrapolation of this trend.

RV vaccine may reduce hospital admissions with GE and subsequently reduce associated health care costs and utilisation. The latter have been demonstrated in several studies worldwide (9-10, 11-17). Our study supports these findings where 67% of RV +ve GE cases in RV vaccinated infants did not require hospital admission. Only one case was severe and was admitted to hospital for a period longer than four days. Since the first dose of RV vaccine administration on December 1 2016, 84 of 317 children aged 1-3 years presented to hospital with GE (i.e 26% of the total number of all hospital presentations of children for this age group) during the study period. This group of children were ineligible for RV vaccine but may have benefitted from herd protection (8-10, 12, 15, 17-18). Additional research is required to study the effect of RV vaccine herd protection affecting children older than one year. In our study, RV vaccine strain was not isolated from the stool of non-vaccinated cases of GE (129 cases) which may raise questions about herd protection. However, our study was conducted for only one year

following RV vaccine introduction, so additional prospective study may address this question.

Vaccination uptake rate varies across Europe (8) and efficacy studies require high vaccine uptake rates. In Germany, routine vaccination at 90% coverage was predicted to reduce symptomatic RV-incidence among children aged <5 years by 84% (95% prediction interval (PI): (71-90%) including a 2.5% decrease due to herd protection (10). In areas attaining vaccine coverage of 64%, RV-related hospital admissions of 0 and 1 year old children decreased by 60% compared with 19% reduction in the low vaccination coverage area (12). Recent figures from the UK show high uptake rates for first dose (94%) and second dose (90%) in 2016 (8). Recent figures of RV vaccine uptake from the Co. Mayo Immunisation Office revealed that 2,410 infants in this region were administered RV vaccine during the study period, with a high uptake rate of vaccine first dose (94.2%) and second dose (92.8%). Those figures are based on vaccination claim forms submitted for payment by GPs, therefore we assume that figures of RV vaccine uptake rates will increase.

We saw from data in **Chapter 4,** that RV vaccine strain was isolated from only 6 vaccinated infants and only 19 patients presented to our local hospital with diarrhoeal illness or GE following their first and/or second dose of RV vaccine (0.8 % of all RV vaccinated infants in Co Mayo in Ireland during the study period). The latter may serve as a marker of RV vaccine efficacy in terms of reducing the number of cases of GE in our region. However, we cannot assume that all 2,410 children who received RV vaccine would have presented to our hospital if they developed GE; a number may have presented to GPs or managed at home. Therefore, the exact figure of vaccination failure or vaccine efficacy is difficult to estimate. Moreover, the isolation of (Rotarix™) or vaccine strain in the stool of patients following vaccination may not necessarily be the cause of their presentation with GE. Future research is warranted to include GE

cases in primary care and more extensive geographical locations, to better study the vaccination failure rate of RV vaccine.

This study was the first study undertaken to evaluate the impact of RV vaccine immediately following vaccine introduction in 2016 for one year during the post vaccination period in one region of Ireland. Our study was the first to evaluate RV vaccine effect on: seasonal trends of RV, RV GE severity, hospital admissions with GE comparing data from the immediate three pre vaccination years (2014-2016). Our research has proven the efficacy of RV vaccine in reducing GE severity, hospital admissions with GE, total number of ED attendance with GE and health care utilisation.

Our study was also the first study in Ireland to isolate vaccine strain (Rotarix ™), highlight its genotype (G1P8) separating it from other wild strain RV G1P8, prove vaccine strain correlation with mild to moderate GE in the majority of cases and to demonestrate the absence of vaccine strain isolation in non vaccinated cases. Shedding of vaccine strain in our study was noted over a wide range of days (10 days to 7 weeks) following the first dose of RV vaccine in the majority of cases. Shedding was noted in 6 of 17 RV vaccinated infants (35%), 5 following the first dose of RV vaccine. While the numbers are small, these findings are important as they show the potential for horizontal transmission of vaccine strain with the potential risk of infecting immunocompromised household contacts of vaccinated infants, and open up avenues for future research.

Though the numbers of patients studied is limited to examine for intussusception risk, no cases of intussusception following first or second dose of RV vaccine were noted. However, post marketing surveillance for this adverse event should continue after RV vaccine was introduced into the Irish populations to ensure vaccine safety and further reassure the public.

This study was the first study to evaluate RV (and other enteric viral pathogen) diversity of genotypes, predominant genotypes, correlation with disease severity and co infection with other viruses in the post vaccination year, in Ireland and the short term impact of RV vaccination in the Mayo region of Ireland. Additional research is required, in larger geographical locations with greater sample size, to better evaluate fluctuation of seasonal trends of viral GE in the post RV vaccination era. It is also important to follow the pattern of geographical and temporal fluctuation of RV strains and other viruses in Ireland. Our study is a novel start point opening the door to future research. We hope that this study will provide a reference point for further researchers and the Department of Health as they continue to propose policies and strategies to monitor review and evaluate the impact of RV vaccine in Ireland for the best interest of children.

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