**Changing molecular epidemiology of rotavirus infection after introduction of monovalent rotavirus vaccination in Scotland**

**Running title:** Genotyping of rotavirus strains from Scotland

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**Abstract**

**Background:** Rotaviruses (RV) are the leading cause of severe gastroenteritis in children less than five years of age worldwide. Rotarix®, a live attenuated monovalent vaccine containing a RV strain of G1P[8] specificity has been included in the childhood immunisation schedule from June 2013 in Scotland. This study aimed to characterise the prevalent RV strains in Scotland before and after the introduction of the RV vaccine.

**Methods:** RV positive faecal samples from Scottish regional virology laboratories covering the years 2012-2015 were genotyped. Viral RNA was extracted from faecal suspensions. VP7 and VP4 gene specific primers were used for multiplex hemi-nested PCRs and sequencing. Mann-Whitney U test and Chi-square test were used for statistical comparison.

**Results:** There was a decrease in RV positive samples from the regional virology laboratories from 7409 samples in the pre-vaccination years (2009-2013) to 760 in 2014-15, with an annual reduction of RV infections by 74.4% (RR-3.95; 95%-CI, 3.53-4.42,p<0.001). 362 samples from the pre-vaccination period and 278 samples from the post-vaccination were genotyped. There was a drop in prevalence of G1P[8] strains (72.1%, 95%-CI, 67.42-76.33 to 15%, 95%-CI, 11.38-19.79) after introduction of the vaccine. In the post-vaccination period G2P[4] was the dominant strain in Scotland (21.9%,95%-CI, 17.48-27.17) with increase in G9P[8] (12.9%, 95%-CI, 9.50-7.41), G12P[8] (12.2 %, 95-CI, 8.89-16.60) and G3P[8] (11.9 %, 95%-CI, 8.58-16.20) infections. Phylogenetic analysis of the VP7 and VP4 genes showed no major differences between the pre and post-vaccination G1P[8] strains.

**Conclusion:** This lab based surveillance study shows significant reduction in reported RV cases and a shift in proportion from G1P[8] to G2P[4] strains after introduction of RV vaccination in Scotland. This genotyping data from a subset of the total reported RV cases will be used to ascertain cross protection against strains and identify vaccine induced RV strain shifts in the years to come.

**Key Words:** Rotavirus, genotyping, rotavirus vaccine, Rotarix®, Scotland

**1. Introduction**

Rotaviruses (RV) are the most common aetiological agents of acute gastroenteritis in children less than 5 years of age [1]. In the last decade, successful licensure and documented efficacy of two live oral vaccines, Rotarix® (GlaxoSmithKline Biologicals), a live attenuated vaccine containing a RV strain of G1P[8] specificity and Rota Teq® (Merck & Co, Inc) a pentavalent RV vaccine have raised the hopes of achieving worldwide control of this viral infection [2]. However, as of September 2016 only 84 countries had incorporated RV vaccines in their national programmes with an estimated global coverage of just 23 % [3, 4]. These vaccines have been found to be efficacious in many countries with reductions in morbidity and mortality related to RV associated diarrhoea [5]. In 2009, the World Health Organisation recommended that immunisation against RV should be included in all national immunisation programs [6]. The Global Alliance for Vaccines and Immunisation (GAVI) along with UNICEF has seized the initiative by successfully negotiating a decrease in procurement prices of both these vaccines so that their benefit can filter to children in developing countries and prevent an estimated 2.46 million childhood deaths in the next two decades [7].

Rotavirus infection has significantly impacted health services in the UK, with an estimated 750,000 episodes of diarrhoea, 80,000 general practitioner (GP) consultations and 14,300 hospital admissions every year [8, 9]. The total cost of rotavirus infection in the UK had been estimated to be about £25 million in 2008 with more than 80 % of the cost borne by patients [10] .To combat this major public health problem, Rotarix® was included in the childhood vaccination schedule in June 2013 in the UK, as a two dose schedule at two and three months of age [11]. The efficacy of this vaccine has already been documented with a 67 % decrease in laboratory confirmed RV infection in England and Wales [12]. There was a 77 % decline in documented RV infection in infants and a 26 % reduction in all cause admission due to acute gastroenteritis in hospitals in 2013-2014, compared to the pre-vaccination era [13]. More recently in 2015, certain regions in the UK have documented a 92 % decrease in laboratory confirmed RV infections compared to the pre-vaccine periods [14].

The Global Rotavirus Information and Surveillance Bulletin of WHO had estimated in 2010 that the G1P[8] genotype was the most frequent detected genotype globally in about 31 % of cases reported [15]. It has been postulated that introduction of vaccines may significantly alter the epidemiology of circulating rotavirus strains in the coming years, though both vaccines have been found to confer a good level of cross protection against heterotypic strains [16] . However, there has been no global shift in genotypic patterns, after a decade of vaccine use [17]. Post-vaccination surveillance studies in some countries, especially those using the Rotarix® vaccine have reported increase in prevalence of some genotypes (G2 and G3) in contrast to the pre-vaccination era [17-19].

In this backdrop, local understanding of circulating RV strains before and after introduction of this vaccine is of paramount importance. Baseline epidemiological data pertaining to circulating strains of RV is lacking in Scotland. This report of rotavirus strains from reporting laboratories from various regions in Scotland encompasses two RV seasons prior to the introduction of the vaccine and two seasons after. This will help in studying the effectiveness of the vaccine programme in Scotland and also document change in the molecular epidemiology of RV strains after introduction of the vaccine programme. The epidemiological assessment in Scotland is unique as this will reflect the uniform response of a large naïve population of children to a single type of RV vaccine administered efficiently through the National Health Service.

**2. Methods**

**2.1 Study design**

The study aimed to compare the molecular epidemiology of rotavirus infections before and after introduction of the RV vaccine in Scotland. The national surveillance data for laboratory confirmed RV infections is part of the Electronic Communication of Surveillance in Scotland (ECOSS [20]. This surveillance system is Scotland wide and captures all laboratory confirmations of RV positive specimens from every NHS diagnostic lab in Scotland.

The regional Scottish virology laboratories (n= 6) were requested by Health Protection Scotland (HPS) to retain RV positive faecal samples from the RV season prior to the start of the programme to obtain a baseline of the circulating strains and subsequently to retain samples for two years post vaccine to monitor for impact. Participating laboratories submitted lists of the samples retained along with standard data fields, when available, to HPS. The study was conducted using anonymised samples and was in accordance with HPS clinical governance procedures to monitor the efficacy and safety of vaccination programmes.

**2.2 Sample Selection**

The spread of contributing laboratories ensured that the samples were geographically representative of the Scottish population. There was no selection of samples by the study team and all samples which were retained by the laboratories both pre and post introduction of the vaccine were included in the study. The total number of samples included in the pre- vaccination genotyping was 30% of the total rotavirus positive samples reported to HPS between 2012 and May 2013 (387/1302) with a similar age breakdown to correctly represent the entire group of subjects. The total number of samples included in the post- vaccination genotyping was 21% of the total rotavirus positive samples reported to HPS in the two years post vaccination (from June 2013 until the end of June 2015; 278/1322) with a similar age breakdown.

**2.3. Surveillance sites and sample collection**

Laboratory confirmed RV positive faecal samples, faecal suspensions or nucleic acid extracts from various regional Scottish virology laboratories were transported to Department of Virology at Aberdeen Royal Infirmary. They were then anonymised and handed over to the University of Aberdeen, Gastrointestinal Research Laboratory for genotyping. For the pre-vaccination RV genotyping study a total of 387 samples were received from Aberdeen (n=98), Dundee (n=29), Inverness (n=24), Glasgow (n=36), Glasgow paediatric, Yorkhill (n=54), and from Edinburgh (n=146) (Figure 1). The post-vaccination RV genotyping study included 278 samples received from Aberdeen (n=28), Dundee (n=44), Inverness (n=25), Glasgow (n=48), Glasgow paediatric, Yorkhill (n=31), Edinburgh (n=96) and from Fife (n=6) (Figure 1).

The data of circulating RV strains in Scotland between the years 2006-2011, previously published in the context of the European rotavirus surveillance was made available from Public Health England (PHE) and was used as a historical control to compare with the current pre and post-vaccination data set [21].

**2.4. RNA extraction and cDNA synthesis**

Viral RNA was extracted from RV positive faecal suspension using guanidine isothiocyanate-silica, according to the method described by Boom et al [22]. Reverse transcription was performed using random primers to generate complementary DNA (cDNA) from the extracted RNA [23].

**2.5. Genotyping PCRs**

Genotyping of the rotavirus-positive samples was done using hemi-nested multiplex RT-PCR as previously described [24-26]. Both VP7 and VP4 genotyping was done using cDNA as template for the first round PCR and previously published primers to yield amplicon sizes of 881 bp and 663 bp respectively. The VP7 gene specific second-round genotyping PCR was a multiplex PCR and incorporated the VP7R and the G-type-specific primers G1-G4, G8-G10 and G12. The second round VP4 gene specific PCR included primers specific for genotypes P[4], P[6], P[8], P[9]-P[11] and the primer VP4F.

**2.6. Sequencing**

Sequencing of the VP7 and VP4 genes were done using cDNA as template using specific primers. Sequences generated were initially analysed with Chromas Lite 2.1.1 (Technelysium, Australia). The nucleotide sequences of the VP7 and VP4 genes were compared with sequences available in the NCBI (National Centre for Biotechnology Information) GenBank database using BLAST (Basic Local Alignment Search Tool) program. Multiple alignments were performed using the Clustal W algorithm and phylogenetic trees were constructed with MEGA7, applying the Maximum-Likelihood method, using the substitution models suggested by the MEGA7 model test for each tree. Sequences from this study have been submitted to the GenBank database (KX467644-KX467685).

**2.7. Data Analysis**

The median age between pre and post vaccination cases were compared using Mann Whitney U test. Chi-square test for proportions was used to compare the pre-vaccination to the post-vaccination age groups, and sample source. P values of ≤ 0.05 were considered statistically significant. Mid-year population estimates were obtained by year and age from Information Services Division, Scotland (<http://www.isdscotland.org/Products-and-Services/GPD-Support/Population/Estimates/>). Annual incidence rates were calculated using a pooled mean of the total number of pre and post vaccine lab reports reported to HPS via ECOSS and rate ratios (RR) were calculated. Pre-vaccination period was defined as 2009-2013 and post-vaccination from 2014-15. 95% confidence intervals for proportions were calculated using the Wilson method. Statistical analysis was performed using SPSS 21 (IBM Corp; Armonk, NY) software.

**3. Results**

**3.1. Demographics of RV cases**

A total of 387 samples were collected in the pre-vaccination study and 278 samples in the post-vaccination period. The median age was 1.08 years (0.92 to 2.0 years) in the pre-vaccination period and significantly higher at 1.95 years (0.47 to 3.2 years) in the post-vaccination period (Mann Whitney U test, p=0.003). There was a significant shift in proportions towards older children increasing from 10.6 % of samples in the pre-vaccination years to 20.0 % in the post-vaccination period and a reduction amongst age groups of 36.0 % to 32.7 % in children aged < 1 year and 53.5 % to 47.3% in those aged 1-4 years (Chi-square, p=0.007). Whilst no significant difference in proportion of samples from community and hospital samples were noted in the pre and post-vaccination time periods (Chi-square, p=0.078), a small decline in the proportion among hospitalised samples may indicate a milder presentation in the post-vaccination group.

**3.2. Prevalence and seasonality of RV infection**

Figure 2 outlines the prevalence of RV infection in Scotland based on laboratory samples received between the years 2009-2015. A distinct seasonal distribution was noted in the pre-vaccination years with a sharp rise in reports in February which peaked in April. There was a decrease in the number of laboratory confirmed RV cases in 2014-16 as depicted in Figure 2. The total number of laboratory samples positive for RV in the years 2009-2013 was 7409 and 760 in 2014-15 accounting for an average annual reduction of RV infections by 74.4 % (RR , 3.95; 95% CI, 3.53, 4.42, p<0.001) (Table1). The proportion of positive samples for infants < 1 year and children aged 1-4 years also decreased by 64.7 % and 75.4 % respectively (RR, 2.83, 95% CI, 2.36, 3.40, p<0.001 and RR, 4.07, 95% CI, 3.52, 4.70, p<0.001). Annual incidence rates were calculated and rate ratios are reported in Table 1. Laboratory reports of RV infection are available for 2016 and continue to show substantial reduction in the third year following the introduction of the vaccine (Figure 2). Additionally, no seasonality was noted in RV infections in the post-vaccination seasons.

**3. 3. RV genotyping distribution pre-vaccination**

The distribution of the various rotavirus strains circulating in Scotland prior to the introduction of the RV vaccine is summarized in Table 2. Before the introduction of the RV vaccine, the commonest strain was G1P[8] accounting for 72.1 % (95 % confidence interval [CI], 67.42-76.33) of cases. The other strains that were identified were G2P[4] in 7.2 % (95 % CI, 5.05-10.26), G4P[8] in 6.9 % (95 % CI, 4.84-9.96), G9P[8] in 3.4 % (95 % CI, 1.97-5.66 ) and G3P[8] in a further 2.3 % (95 % CI, 1.23-4.36) of cases. Single cases with either G12P[8] or G9P[4] were also found (0.26 %, 95% CI 0.05-1.45, respectively). Mixed infections were seen in 5.4 % (95 % CI, 3.58-8.15) of cases (Table 2). The predominant RV strain was G1P[8] in all the regions studied (Figure 3). The rate of mixed infections was higher in Edinburgh (11 %, 95 % CI, 5.92% to 16.08%) as compared to the other regions (5.4 % overall, 95 % CI, 3.17% to 7.69%). The rate of identification of G2P[4] was higher in the Dundee and Glasgow centres (13.8 % each respectively 95 % CI, 1.24% to 26.34% and 2.53% to 25.05%).

**3.4. RV genotyping distribution post-vaccination**

The most common strain identified after the introduction of the RV vaccine was G2P[4] accounting for 21.9 % (95% CI, 17.48-27.17) of cases followed by G1P[8] (15.1 %, 95% CI, 11.38-19.79), G9P[8] (12.9 %, 95% CI, 9.50-17.41), G12P[8] (12.2 %, 95% CI, 8.89-16.60) and G3P[8] (11.9 %, 95% CI, 8.58-16.20) (Table 2). The other strains identified were G4P[8] in 5 % (95% CI, 3.02-8.27) cases, G1P[4] in 1.4 % (95% CI, 0.56-3.64) cases, G9P[4] in 1.1 % (95% CI, 0.37-3.12) cases and G12P[4] in a further 1.4 % (95% CI, 0.56-3.64) of cases. Two cases of G10P[4] (0.72 %, 95% CI, 0.20-2.58) were also identified in this study. Mixed infections were seen in 16.2 % (95% CI, 12.32-20.97) of cases. A comparative analysis of the pre and post vaccination scenario in various individual centres in Scotland is summarized in Figure 3. Every participating centre showed a decline in G1P[8] RV infection and complete absence of this genotype was noted in Inverness and Yorkhill. The predominant strain in the post vaccine period in Aberdeen and Edinburgh was G12P[8] (29 % [95% CI, 12.19-45.81] and 17 % [95% CI, 9.49-24.51] respectively) but G2P[4] in Yorkhill (55 %,95% CI, 37.49-72.51), Glasgow (33 %, 95% CI, 19.7-46.3) and Fife (33 %, 95% CI, 4.62-70.62). Inverness showed a predominance of the G3P[8] strain (36 %, 95% CI, 17.18-54.82). Only in the Dundee region, G1P[8] continued to be the dominant strain accounting for 41 % (95% CI, 26.47-55.53) of all cases.

The pre and post-vaccination RV genotyping data from the current study was compared with the historical data of circulating RV strains in Scotland between the years 2006-2011 (Figure 4). There were no changes in pattern noted for the pre-vaccination data, with G1P[8] being the commonest RV strain in both the time periods. G9P[8] and G12P[8] were detected more frequently in the years between 2006-2011.

**3.5. Sequence analysis of the VP7 and VP4 encoded gene of the RV strains**

A subset of 21 G1P[8] RV strains from the pre-vaccination (n=8) and the post-vaccination cohort (n=13) were selected for nucleotide sequencing and phylogenetic assessment. The latter group included 3 strains isolated from infants (2-3 months old) who had diarrhoea within 6-15 days after receiving the first dose of Rotarix® vaccination identified through the vaccine surveillance network setup by HPS.

Nucleotide sequencing and phylogenetic analysis confirmed that all 21 RV strains selected for sequencing were G1P[8] strains as previously determined by genotyping PCR. For the VP7 gene analysis, the nucleotide sequence identity of the 18 strains ranged from 99-100% with other G1 strains detected globally with closest identity to G1 strains detected in Italy and India. All 18 strains clustered in the G1-lineage I of the VP7 gene phylogenetic tree (Figure 5A). There were no major differences identified between the pre and post vaccination G1 strains for the VP7 gene as they clustered closely together. The three post vaccination, potential vaccine derived strains shared a 100% homology with the Rotarix® vaccine strain and clustered in G1-lineage II of the phylogenetic tree along with the Rotarix® reference sequences downloaded from GenBank. The phylogenetic tree for the VP4 gene showed a similar picture with all 18 strains clustering together in P[8]- Lineage III with 99-100% homology at the nucleotide level with other P[8] strains reported worldwide and with closest nucleotide identity to Italian and Indian P[8] strains (Figure 5B). Conversely, the three post vaccine strains were found to be in P[8]-Lineage I with 100% similarity to the Rotarix® vaccine strain.

**4. Discussion**

There is a paucity of published data regarding circulating RV strains in Scotland and this retrospective analysis is the first of its kind in the last decade [27]. The immediate effectiveness of the introduction of the Rotarix® vaccine in Scotland is highlighted in this study by a significant annual reduction (74.4%) in laboratory reports of confirmed RV infection in the two seasons following its introduction as opposed to the previous years. This reduction was also noted for both the children < 1 year and those between 1-4 years suggesting the possibility of herd immunity. The other striking feature of the post-vaccination genotyping data is the change noted in the prevalence of the G1P[8] strain across all Scottish centres, dropping from a preponderant proportion of 72.1% to 15.1%. There was a parallel increase of G2P[4] infections from 7.2% to 21.9%.

The efficacy of Rotarix® against the homotypic G1P[8] strains has been documented to be 90-97 % in Europe and the US where it was the dominant strain [28, 29]. More importantly, this decrease was sustained in the US up to seven years after the introduction of RV vaccination in 2006 [30]. This has immense significance in Scotland, where G1P[8] was the predominant strain in the pre-vaccine RV seasons. The national laboratory reporting system in the US also noted the gradual erosion of seasonal peaks of RV infection in the post-vaccine seasons as documented in our study [30]. A systematic review of the European experience has also shown that vaccination resulted in a reduction of RV related hospitalizations by 65-84 % [31]. More specifically in Belgium where RV vaccines were introduced in 2006, there was a 50 to 61% reduction of laboratory reporting of RV infection in the post-vaccine seasons as compared to the pre-vaccine period [32, 33]. The other suggestion of the efficacy of the vaccine in Scotland is the significant increase in age of the patients affected by RV diarrhoea in the post vaccine period with a fifth of patients affected being more than 5 years of age. This could also be a relative increase in proportion of older children as the efficacy was more evident in the younger cohort of children. This effect will be discerned only in the early phase of the vaccination programme as vaccine induced protection will surpass natural protection in the years to come.

The comparative analysis of rotavirus strains before and after introduction of Rotarix® in this study gives a snapshot of the impact of vaccination on circulating strains in Scotland. . This reduction is also documented from the previous assessment of 2006-11 which acted as a historical control for this study (Figure 4). It is important to note that in the paediatric hospital of Yorkhill in Glasgow there were no documented cases of G1P[8] infection at all after introduction of the vaccine. This paediatric hospital admits children with severe RV infection and their laboratory processes samples obtained from these patients. This change in local RV epidemiology could suggest greater reduction of severe RV infections by the G1P[8] strain necessitating admission to the hospital.

Equally, it is very important to look at the figures for other viral strains but their proportional increase may be a mere reflection of the reduction of the dominant G1P[8] strain. This could be the partial explanation for the rise in G2P[4] cases noted across all centres in Scotland in the post-vaccination analysis. A similar shift in prevalence towards the fully heterotypic G2P[4] RV strain has been noted in the first two years after introduction of Rotarix® in Belgium, Brazil and in particular states in Australia where this vaccine was used [34-36]. The protective efficacy of the Rotarix® vaccine against G2P[4] strains, which is completely heterotypic from the vaccine strain, has been documented to be between 39-58 % in clinical trials as opposed to 80 % against those strains which are fully or partially heterotypic [37, 38]. Real life data from Belgium suggests that this efficacy against G2P[4] strains may be higher than that reported in clinical trials at around 85% [28]. This strain needs to be closely monitored in the subsequent seasons across all regions in Scotland.

On the other hand, G3P[8], G9P[8] and G12P[8] strains were infrequently detected in the pre-vaccine surveys but were detected in greater absolute numbers in the post-vaccine year. There was also a rise in mixed rotavirus infections in the post-vaccine period. Mixed infections are a common feature in developing countries where the burden of disease is higher and the risk of acquisition of multiple strains is increased but there are a few newer reports suggesting an increased prevalence in developed countries recently [39, 40]. It is also quite interesting to note the regional differences of these strains with G12P[8] increasing in Aberdeen and Edinburgh, G9P[8] being a dominant strain in Fife and G3P[8] prominent in Inverness, although the number of cases was very low to make a studied conclusion. This has not been reliably documented in a large global meta-analysis of post-vaccine RV strains, which did not report any outbreaks of rotavirus infection with heterotypic strains in older children who would have been previously immunized from naturally acquired RV infections [41].

The sequencing and phylogenetic analysis of a sub group of G1P[8] strains before and after the introduction of the vaccine has not shown any major shift, with all the strains clustering together in Lineage I for the VP7 gene and Lineage II for the VP4 gene. This needs to be monitored over subsequent seasons to ensure that immune pressure of the vaccine is not leading to escape mutants. It is also important to note that due to the live nature of the Rotarix vaccine, diarrhoea is a putative side effect. This has been proven by the homology of three strains isolated from infants who had diarrhoea soon after administration of the vaccine.

There were a few limitations to our study as samples were acquired as part of a public health surveillance programme from regional virology laboratories. Only a subset of the entire group of patients were included for genotypic analysis which may have introduced a putative selection bias. On account of this study design, the clinical data of children affected and the severity and nature of their disease could not be ascertained. This could have given a more accurate idea about the true benefit of the vaccine. Moreover, test reports to rule out other co-existing bacterial infections were not available in this group of children. The change in proportion of genotypes from a G1P[8] predominance in the pre-vaccine period to the G2P[4] strains after the introduction of the Rotarix vaccine is a surrogate indicator and needs to be validated by documenting a decrease in the absolute numbers of the former strain in the whole community to exactly gauge the impact of the vaccine.

This molecular, epidemiological data from Scotland is unique as it offers an opportunity to study the effect of a single type of RV vaccine in a large naïve paediatric population in whom the vaccine is effectively administered through the National Health Service. It also opens up a useful avenue to study the emergence of other RV genotypes in this population to understand the true nature of the immune pressure exerted by the vaccine strain against other circulating wild type strains.

**Authors’ contribution to the manuscript**

IM, HM, AH, ASP, CC & GLH conceived this study and contributed to design of this study. IM, SB, HM, AH, ASP, MIG & GLH contributed to data acquisition. IM, HM, ASP, MIG & GLH contributed to data analysis. IM and HM drafted the manuscript. All authors critically revised the manuscript for important intellectual content and approved the final version.

**Acknowledgement**

The study was funded by the Health Protection Scotland. The authors thank the Scottish regional virology departments of Aberdeen, Dundee, Inverness, Fife, Glasgow, Glasgow paediatric (Yorkhill) and Edinburgh for their participation in this study and providing the RV positive faecal samples.

**Conflict of interests**

The authors have no conflict of interest.

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**Figure legends**

**Figure 1:** Summary of rotavirus positive samples received from regional virology laboratories of Scotland for genotyping

**Figure 2:** Laboratory reports of rotavirus in Scotland to HPS, five year average (2009-2013), 2014, 2015 and 2016.

**Figure 3:** Comparison of rotavirus genotyping data from Scotland pre (2012- May 2013) and post (June 2013 - 2015) introduction of the rotavirus vaccine.

**Figure 4:** Comparison of RV genotyping data from Scotland over last ten years.

**Figure 5:** Phylogenetic tree based on the partial VP7 (A) and VP4 (B) nucleotide sequences of 21 G1P[8] rotavirus strains from pre and post vaccine licensure period and other rotavirus reference strains from GenBank. Scottish G1P[8] rotavirus strains from pre vaccine licensure period are marked with blue circles, the post licensure samples are marked with red circles, potential vaccine derived strains are with green circles and the reference strains are marked with black circles. Trees were built with the Maximum Likelihood method (Tamura-3 parameter), and bootstrapped with 1000 repetitions; bootstrap values below 70 are not shown.

**Figure 5 (black and white print version):** Phylogenetic tree based on the partial VP7 (A) and VP4 (B) nucleotide sequences of 21 G1P[8] rotavirus strains from pre and post vaccine licensure period and other rotavirus reference strains from GenBank. Scottish G1P[8] rotavirus strains from pre vaccine licensure period are marked with clear circles, the post licensure samples are marked with black circles, potential vaccine derived strains are with black diamond and the reference strains are marked with clear diamond shapes. Trees were built with the Maximum Likelihood method (Tamura-3 parameter), and bootstrapped with 1000 repetitions; bootstrap values below 70 are not shown.

**Table 1:** Decrease in the annual incidence rates of RV infection in various age groups after the introduction of RV vaccine in Scotland.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Age groups** | **Pooled mean 2009-13 laboratory reports (Pre-vaccination)** | **Pooled mean 2014-15 laboratory reports (Post-vaccination)** | **% Difference** | **Annual Incidence rate per 100,000** | | **Rate Ratio (RR)** |
|  |  |  |  | **Pre** | **Post** |  |
| Total population (All ages) | 1481.8 | 380 | -74.40% | 27.9 | 7.1 | RR , 3.95; 95% CI, 3.53, 4.42, p<0.001 |
|  |  |  |  |  |  |  |
| <1 years | 436.2 | 154 | -64.70% | 778.1 | 274.9 | RR, 2.83, 95% CI, 2.36, 3.40, p<0.001 |
|  |  |  |  |  |  |  |
| 1-4 years | 941.6 | 231.5 | -75.40% | 400.4 | 98.4 | RR, 4.07, 95% CI, 3.52, 4.70, p<0.001 |

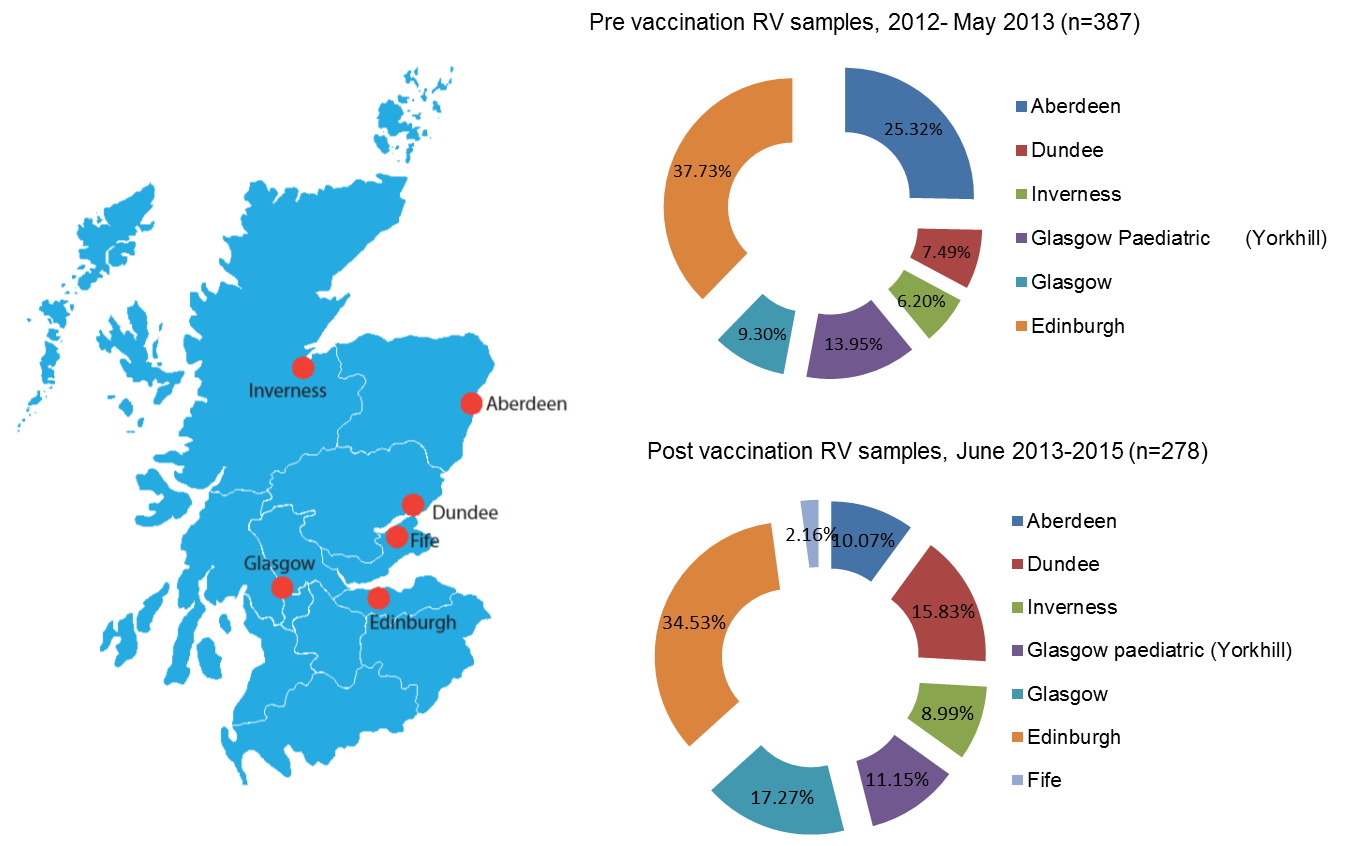
CI = Confidence Interval

**Table 2:** Overall distribution of rotavirus genotypes from Scotland pre and post introduction of the RV vaccine.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Genotype** | **Pre-vaccination** | | |  | **Post-vaccination** | | |
| **n** | **%** | **95% CI** |  | **n** | **%** | **95% CI** |
| G1P[4] | 8 | 2.07 | 1.05-4.03 |  | 4 | 1.44 | 0.56-3.64 |
| G1P[8] | 279 | 72.09 | 67.42-76.33 |  | 42 | 15.11 | 11.38-19.79 |
| G2P[4] | 28 | 7.24 | 5.05-10.26 |  | 61 | 21.94 | 17.48-27.17 |
| G3P[8] | 9 | 2.33 | 1.23-4.36 |  | 33 | 11.87 | 8.58-16.20 |
| G4P[8] | 27 | 6.98 | 4.84-9.96 |  | 14 | 5.04 | 3.02-8.27 |
| G9P[4] | 1 | 0.26 | 0.05-1.45 |  | 3 | 1.08 | 0.37-3.12 |
| G9P[8] | 13 | 3.36 | 1.97-5.66 |  | 36 | 12.95 | 9.50-17.41 |
| G10P[4] | 0 | 0.00 | - |  | 2 | 0.72 | 0.20-2.58 |
| G12P[4] | 0 | 0.00 | - |  | 4 | 1.44 | 0.56-3.64 |
| G12P[8] | 1 | 0.26 | 0.05-1.45 |  | 34 | 12.23 | 8.89-16.60 |
| Mixed | 21 | 5.43 | 3.58-8.15 |  | 45 | 16.19 | 12.32-20.97 |
| Total | 387 | 100.00 |  |  | 278 | 100.00 |  |

CI= Confidence Interval

**Figure 1**

****

**Figure 2**

**Figure 3**

**Figure 4**

PHE = Public Health England, HPS= Health Protection Scotland

**Figure 5**

Lineage I

Lineage III

Lineage VI

Lineage VII

Lineage II

Lineage IV

Lineage V

**G2**

**G1**



Lineage I

Lineage III

Lineage II

**P[4]**



**P[8]**

**A**

**B**