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# Establishment of a national database to link epidemiological and molecular data from norovirus outbreaks in Ireland

S. KELLY[1]*, B. FOLEY[2], L. DUNFORD[3], S. COUGHLAN[3], G. TUITE[3], M. DUFFY[3], S. MITCHELL[4], B. SMYTH[5], H. O'NEILL[4], P. McKEOWN[2], W. HALL[3] AND M. LYNCH[6]

[1] *Department of Medical Microbiology, Adelaide and Meath Hospitals, incorporating the National Children's Hospital, Tallaght, Dublin, Republic of Ireland*
[2] *Health Protection Surveillance Centre, Dublin, Republic of Ireland*
[3] *National Virus Reference Laboratory, University College Dublin, Belfield, Dublin, Republic of Ireland*
[4] *Regional Virus Laboratory, Royal Victoria Hospital, Belfast, Northern Ireland*
[5] *Communicable Disease Surveillance Centre, Belfast, Northern Ireland*
[6] *Mater Misericordiae Hospital, Dublin, Republic of Ireland*

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## SUMMARY

A prospective study of norovirus outbreaks in Ireland was carried out over a 1-year period from 1 October 2004 to 30 September 2005. Epidemiological and molecular data on norovirus outbreaks in the Republic of Ireland (ROI) and Northern Ireland (NI) were collected and combined in real time in a common database. Most reported outbreaks occurred in hospitals and residential institutions and person-to-person spread was the predominant mode of transmission. The predominant circulating norovirus strain was the GII.4-2004 strain with a small number of outbreaks due to GII.2. This study represents the first time that enhanced epidemiological and virological data on norovirus outbreaks in Ireland have been described. The link established between the epidemiological and virological institutions during the course of this study has been continued and the data is being used as a source of data for the Foodborne Viruses in Europe Network (DIVINE-NET).

## INTRODUCTION

Gastroenteritis due to viral infection of the gastrointestinal tract is a common illness in humans, with high morbidity and mortality reported worldwide. Community-based studies have shown that, in developed countries, 20–25% of individuals have an episode of gastroenteritis annually [1, 2]. Acute gastroenteritis is a common illness in Ireland. A recent study of gastroenteritis in the Republic of Ireland (ROI) and Northern Ireland (NI), using a telephone questionnaire, showed that 4·5% of Irish people are affected by gastroenteritis every month [3]. However, only 2% of those affected have samples submitted for laboratory examination [3]. Noroviruses are the commonest cause of outbreaks of gastroenteritis both in the community and in health-care institutions. International studies have demonstrated that between 1% and 3% of people can expect to become infected

* Author for correspondence: Dr S. Kelly, Department of Medical Microbiology, Adelaide and Meath Hospitals, incorporating the National Children's Hospital, Tallaght, Dublin 24, Republic of Ireland.
(Email: sinead.kelly6@amnch.ie)

with norovirus each year [1, 4]. Outbreaks in health-care institutions can cause considerable disruption of services.

The increase in norovirus outbreaks in health-care institutions in the ROI in 2002 led to the publication of guidelines by the Health Protection Surveillance Centre for the diagnosis and management of outbreaks [5]. The National Virus Reference Laboratory introduced reverse transcriptase–polymerase chain reaction (RT–PCR) for diagnosis of norovirus in stool samples in 2003. The increased sensitivity of RT–PCR relative to electron microscopy which had previously been used for diagnosis has led to increasing recognition of noroviruses as the cause of outbreaks of gastroenteritis. In NI, nested reverse transcriptase–PCR (nRT–PCR), has shown high sensitivity for detection of noroviruses in faecal samples [6].

The molecular epidemiology of norovirus strains circulating in the ROI was examined for the first time in 2001 [7]. Eight samples from sporadic cases of gastroenteritis and nine isolates from separate norovirus outbreaks were examined. Of the sporadic isolates, six were shown to be genogroup II (GII) by RT–PCR, while two were genogroup I (GI). All of the outbreak isolates were GII. The majority of GII isolates were seen to cluster with Bristol/Lordsdale virus, while the two GI specimens were related most closely to the Southampton virus.

A retrospective analysis of a selection of outbreaks in 2002 from around the ROI and from various outbreak settings revealed the majority of outbreaks were attributed to genogroup II.4 strain of norovirus [8]. Eighty-eight percent of a selection of outbreaks in 2002 (16 outbreaks) sequenced as GII.4 viruses in the Bristol/Lordsdale clade. The remaining two outbreaks sequenced as Melksham-like viruses, GII.2 [8]. In 2003, norovirus strains from a selection of outbreaks were sequenced and 86% of sequenced outbreaks were GII.4. Also detected in 2003 were a Harrow strain, GII.2, a Melksham-like strain, GII.2, and BKM658, a GI.4 virus. The BKM658 strain was associated with the first documented genogroup I outbreak. This occurred in 2003 in a school setting [8]. The new variant GII.4 strain, identified as the predominant strain in epidemics across Europe from 2002 was also identified in Ireland. All GII.4 sequences from 2002 and 2003, with one exception, were found to contain the mutation from AACTTG to AATCTG at nucleotide position 4820 of the RNA polymerase region. Sequencing of selected samples from norovirus outbreaks in the ROI from November 2003 to August 2004 showed that the majority of specimens were GII.4 norovirus strains [9]. Two distinct noroviral clusters were observed – GII.4 Lordsdale-like strains and Oberhausen-like GII strains [9, 10].

The general aim of this study was to carry out a prospective study of suspected norovirus outbreaks in the ROI and in NI over a 1-year period that would combine molecular data with epidemiological data in real time to confirm that outbreaks were due to norovirus and to get a more accurate estimate of disease burden. Another objective was to describe the norovirus strains circulating in Ireland over a 1-year period and to compare these strains with those circulating in other European countries.

## MATERIALS AND METHODS

The study was carried out over a 1-year period, from 1 October 2004 to 30 September 2005. Enhanced surveillance of norovirus outbreaks was carried out during the study period. To launch the study, an outline of the methods and objectives of the study was presented to medical microbiologists and public health doctors. The study involved cross-border collaboration for collection of epidemiological and molecular data from both the ROI and NI. Methods used for collection of data in the ROI and in NI will be described separately.

### Republic of Ireland

In the ROI, outbreaks of gastroenteritis were reported to the Health Protection Surveillance Centre (HPSC). Notification of all infectious disease outbreaks, including norovirus outbreaks, became a statutory requirement in the ROI under the Amendment to the Infectious Disease Legislation in 2003 [11]. For this study an electronic database was established to combine the epidemiological and molecular data on norovirus outbreaks in the ROI. An electronic database was established between the HPSC and the National Virus Reference Laboratory in Dublin (NVRL) for the merging of epidemiological and virological data leading to an enhanced dataset. The epidemiological data collected in the database comprised: location of outbreak, numbers affected and suspected mode of transmission.

Outbreak samples of suspected viral aetiology in the ROI are referred to the NVRL which provides a

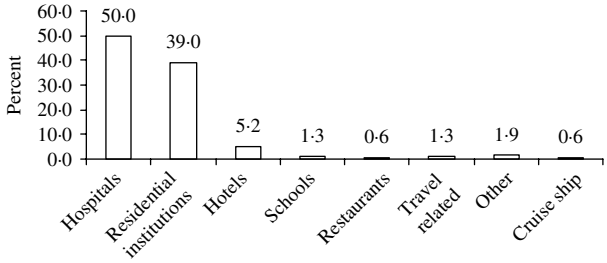


**Fig. 1.** Norovirus outbreak settings in the Republic of Ireland.

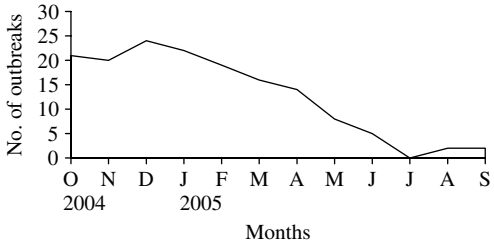


**Fig. 2.** Seasonality of norovirus outbreaks in the Republic of Ireland.

routine diagnostic service and molecular analysis on clinical samples. RT–PCR is the front-line diagnostic tool in place in NVRL for detection of noroviruses [12]. When possible, early acute stool samples (<24–48 h post-presentation) were submitted for examination. An initial batch of up to six specimens was examined from each outbreak. A further batch of 4–6 specimens was examined if fewer than two samples were positive. After two positive results from a single outbreak, no further samples were examined. RT–PCR was carried out using the NiE3 primer pair as previously described by Green *et al*. [13]. The RT–PCR fragments were further characterized by sequence analysis using the Ni and E3 primers. Phylogenetic analysis was carried out by aligning sequences in Clustal W and manually checking the alignment in MacClade software. The phylogenetic trees were constructed using Phylip 3.4/3.5 and the tree was drawn using TreeView. Reliability of the analysis was established with 1000 bootstrap replicates.

### Northern Ireland

In NI, norovirus outbreaks were reported to the Communicable Disease Surveillance Centre (CDSC-NI) in Belfast. Reporting of norovirus outbreaks is not mandatory in NI. For the duration of the study, CDSC-NI provided epidemiological data directly to the HPSC. The epidemiological data collected included the date of onset and end of the outbreak; generic location; numbers affected; suspected mode of transmission.

In NI, faecal specimens from patients with suspected norovirus infection were sent to the Regional Virus Laboratory (RVL) in Belfast. nRT–PCR was carried out as previously described [6]. The RT–PCR products were further characterized by sequence analysis using the JV12 and SM31 primers.

## RESULTS

### Outbreak characteristics

*Republic of Ireland*

Over the 1-year study period, 153 outbreaks of norovirus gastroenteritis were reported to the HPSC in the ROI. These reports included both outbreaks suspected on clinical and epidemiological grounds and outbreaks which were confirmed by detection of norovirus in stool samples by RT–PCR. Clinical recognition of outbreaks was based on the Kaplan criteria which were described in the national guidelines for management of norovirus outbreaks [5]. This represented a significant increase in the number of outbreaks relative to the previous year. Overall, there were 3053 people ill. Seventy-seven percent of all outbreaks of infectious intestinal disease reported to the HPSC were due to noroviruses.

*Settings*. Norovirus outbreaks occurred in a variety of settings but the majority of reported outbreaks occurred in hospitals and residential institutions [77 (50%) in hospitals and 60 (39%) in residential settings] (Fig. 1). Outbreaks were also reported in hotels (5·2%), schools (1·3%), restaurants (0·6%) and cruise ships (0·6%).

*Mode of transmission*. The predominant reported mode of transmission of norovirus during outbreaks was person to person [152 outbreaks (99·3%)]. One outbreak (0·7%) was foodborne.

*Number of persons affected*. The median number of people affected in all norovirus outbreaks was 13 (range 3–200).

*Seasonality*. Norovirus outbreaks occurred throughout the year but there was a marked seasonal peak in the reporting of outbreaks in the winter months (December and January) (Fig. 2). There was a decrease

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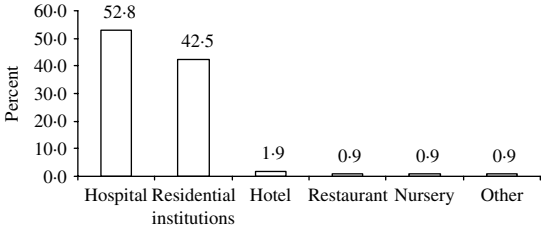


**Fig. 3.** Norovirus outbreak settings in Northern Ireland.

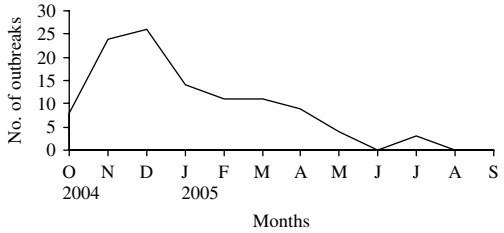


**Fig. 4.** Seasonality of norovirus outbreaks in Northern Ireland.

in the number of reported outbreaks in the summer months with no outbreaks reported in July (Fig. 2).

*Northern Ireland*

In NI, 110 norovirus outbreaks were reported to the CDSC-NI over the 1-year study period. These included outbreaks suspected on clinical and epidemiological grounds according to the Kaplan criteria (24 outbreaks) and outbreaks which were confirmed by detection of norovirus in stool samples by nRT–PCR. This represented an increase in the number of outbreaks relative to the previous year.

*Settings*. The majority of reported outbreaks occurred in hospitals and residential institutions [56 (52·8%) in hospitals and 45 (42·5%) in residential settings]. Outbreaks were also reported in hotels (1·9%), restaurants (0·9%) and nurseries (0·9%) (Fig. 3).

*Mode of transmission*. The predominant reported mode of transmission of norovirus during outbreaks was person to person [109 outbreaks (99·1%)]. One outbreak (0·9%) was foodborne.

*Number of persons affected*. The number of persons affected was available for 51 of the reported outbreaks (46%). The median number of people affected in the outbreaks for which this information was available was 16 (range 2–32).

*Seasonality*. Norovirus outbreaks occurred throughout the year but there was a marked peak in the reporting of outbreaks in the winter months (December and January) (Fig. 4). There was a decrease in the number of reported outbreaks in the summer and early autumn months with no outbreaks reported in June, August and September (Fig. 4).

### Strain characteristics

*Republic of Ireland*

Of the 153 outbreaks reported in the ROI, samples were received in the NVRL from 97 outbreaks (64%). This high rate of submission of samples was due to enhanced surveillance during the study period. Of the samples submitted, 82 outbreaks (84·5%) were positive by RT–PCR for norovirus. Nucleotide sequencing was carried out on a representative portion of the outbreaks (72 outbreaks) (87·8% of the positive samples). Overall genogroup GII.4 was the most frequently detected (70 outbreaks, 97·2%). Genogroup II.2 was detected in two outbreaks (2·8%) (Fig. 5). Genogroup GII.4 strains belonged to the GII.4-2004 strain which was first reported in Europe in The Netherlands in 2004 [14].

*Northern Ireland*

In NI, samples were received in the RVL from 73 of 110 reported outbreaks (64·5%). Norovirus was detected by nRT–PCR in 71 outbreaks (97%). Nucleotide sequencing of noroviruses from a representative portion of these outbreaks (35 outbreaks, 49·4% of the positive samples) was carried out. Genogroup GII.4-2004 norovirus strains were identified in 33 outbreaks (94·3%). Genogroup GII.2 (Melksham) was identified in two outbreaks (5·8%) (Fig. 6).

## DISCUSSION

This study represented the first time that enhanced epidemiological and virological data on norovirus outbreaks on the island of Ireland was described. Cross-border collaboration was used to collect and analyse data from both the ROI and NI. Epidemiological data on outbreaks was complemented by corresponding molecular data with identification of outbreak strains by molecular typing. Enhanced surveillance during the study period led to an increased submission of faecal samples from outbreaks for diagnosis and molecular typing. For the first time, data-sharing in real time between the epidemiological institutions (HPSC and CDSC-NI)

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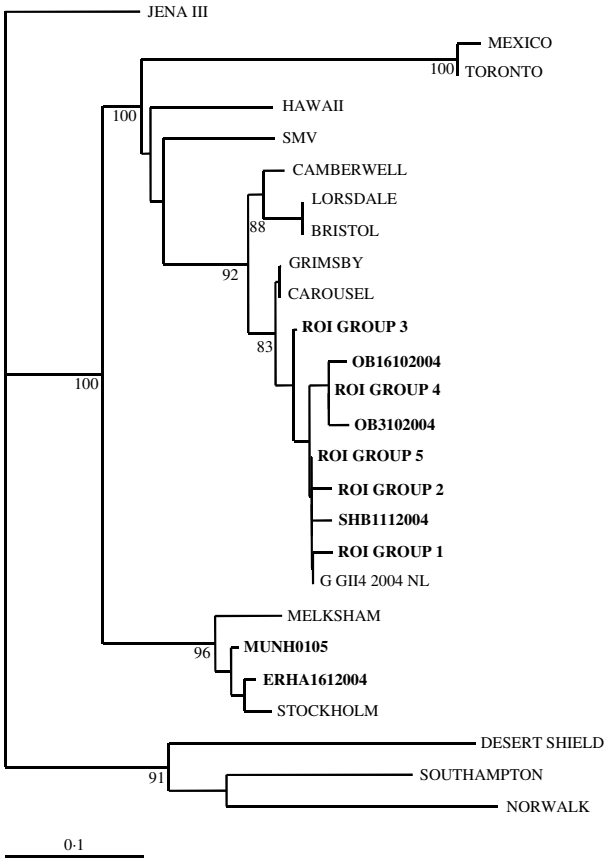


**Fig. 5.** Phylogenetic tree demonstrating genetic relationships of norovirus strains from outbreaks in the Republic of Ireland. Unrooted neighbour joining tree showing the genetic relationships of 74-bp fragments of the RNA polymerase region in open reading frame 1 of norovirus. Accession numbers for strains include: Jena/1999/UK (AJ011099), Melksham/1995/UK (X81879), Hawaii/1972/US (U07611), Snow Mountain (SMV)/1976/US (L23831), Toronto/91/CAN (U02030), Mexico/1989/MX (U22498), Camberwell (AF145896), Carousel/1998/UK (AF439539), Lordsdale/1995/UK (X86557), Desert Shield/1990/SA (U04469), Norwalk/1968/US (M87661), Southampton/1991/UK (L07418), Bristol/1993/UK (X76716), Stockholm/IV3400/2002 (AJ626603), GGII.4.2004/NL (AY900231). Grimsby/1995/UK and Harrow/2001/UK strains were received from a personal communication with HPA Colindale. The Republic of Ireland strains are identified in bold type. Bootstrap values for each node are shown if $>75\%$ after 1000 repeats. Clustal W and McClade software are utilized in sequence alignment and analysis. The phylogenetic tree was constructed using Phylip 3.4/3.5 and drawn with TreeView. Sequences that are identical are grouped together. Key for sequences: ROI group 1 refers to two outbreaks, ROI group 2 refers to three outbreaks, ROI group 3 refers to two outbreaks, ROI group 4 refers to four outbreaks and ROI group 5 refers to 56 outbreaks.

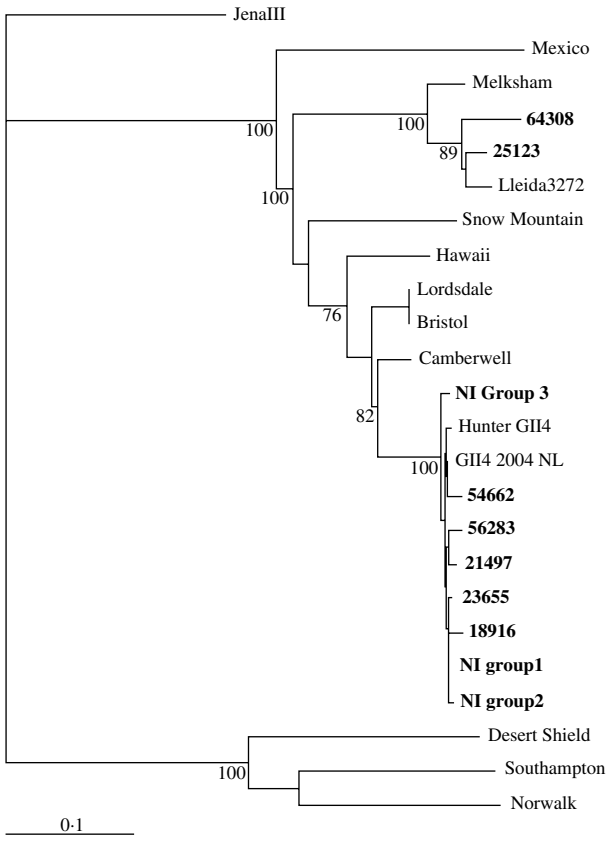


**Fig. 6.** Phylogenetic tree demonstrating genetic relationships of norovirus strains from outbreaks in Northern Ireland. Unrooted neighbour joining tree showing the genetic relationships of 259-bp fragments of the RNA polymerase region in open reading frame 1 of norovirus. Accession numbers for strains include: Jena/1999/UK (AJ011099), Melksham/1995/UK (X81879), Hawaii/1972/US (U07611), Snow Mountain (SMV)/1976/US (L23831), Mexico/1989/MX (U22498), Camberwell (AF145896), Lordsdale/1995/UK(X86557), Desert Shield/1990/SA (U04469), Norwalk/1968/US (M87661), Southampton/1991/UK (L07418), Bristol/1993/UK (X76716), Lleida327/2001 (AJ487802), GGII.4.2004/NL (AY900321), Hunter/GII.4/2004/AU (DQ078794). The Northern Ireland strains are identified in bold type. Bootstrap values for each node are shown if $>75\%$ after 1000 repeats. Clustal W and McClade software are utilized in sequence alignment and analysis. The phylogenetic tree was constructed using Phylip 3.4/3.5 and drawn with TreeView. Sequences that are identical are grouped together. Key for sequences: NI group 1 refers to seven outbreaks, NI group 2 refers to three outbreaks and NI group 3 refers to four outbreaks.

and the virology laboratories (NVRL and RVL) was established. The data-sharing link between HPSC and the NVRL in the ROI has continued after the completion of the study and the database is being used as a source of information for the DIVINE-NET network. Data-sharing links established between the RVL and the CDSC-NI have also been continued although NI is not participating in the DIVINE-NET network.

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Noroviruses were the commonest cause of reported outbreaks of infectious intestinal disease in the ROI (77%). The epidemiological data collected showed that the majority of norovirus outbreaks reported in the ROI and in NI occurred in hospitals and residential institutions (88% in the ROI and 92% in NI). A similar proportion of outbreaks occur in health-care settings in the United Kingdom and across the rest of Europe [15]. In the United Kingdom, 93% of all norovirus outbreaks reported in 2000 were reported to have occurred in residential homes and hospitals [16–18]. High levels of bed occupancy, the large size of care units, and the lack of isolation facilities in hospitals make them particularly vulnerable to norovirus outbreaks. However, surveillance systems are always subject to reporting bias and there is an inherent bias in these results due to underreporting of norovirus outbreaks in the community. A study of infectious intestinal disease in England and Wales estimated that only 1 in 300–1500 cases of norovirus gastroenteritis are reported to national surveillance [2]. Outbreaks in hospitals and institutions are more easily identified and cause more disruption than outbreaks in the community and are more likely to be reported. Outbreaks in institutions are also more likely to yield clinical specimens for identification of norovirus [19]. Over the study period, there was marked seasonal variation in the reported norovirus outbreaks with a peak in the winter months. This seasonal variation is typical of institutional outbreaks of norovirus. The seasonal peak in norovirus outbreaks in health-care institutions coincides with the respiratory infections season which increases the activity in these institutions.

The predominant presumed mode of transmission of norovirus during outbreaks in this study was person to person which is typical of outbreaks in institutions. Foodborne transmission was very rarely reported and in no instance was a particular food source identified. However, it is worth noting that person-to-person transmission is often a diagnosis of exclusion when other modes cannot be clearly identified. Biases in different surveillance systems have led to a wide variation in the estimates of the level of foodborne transmission of noroviruses in different countries. The data from this study suggested that only 0·9% of norovirus outbreaks in the island of Ireland were foodborne. This is far lower than rates reported in other countries. Estimates range from 10% in the United Kingdom [16] to 17% in The Netherlands [20] and 40% in the United States [21]. Surveillance in the United States is focused on detecting foodborne outbreaks which may account for the high estimate of the proportion of norovirus outbreaks which are foodborne. It is possible that this is also a reflection of the type of epidemiological investigation which is used to investigate outbreaks of norovirus gastroenteritis in the ROI and in NI which may be more focused on early implementation of control measures to prevent spread rather than conducting a detailed epidemiological investigation.

There was a large increase in the number of norovirus outbreaks across Europe in the 2004–2005 season [22]. This increase was analogous to the increase seen in 2002–2003 across Europe and in the United States which was associated with the emergence of a new variant (GII.4-2002). Both the ROI and NI had an increase in the number of norovirus outbreaks in 2004–2005 and it is probable that this is due to the emergence of the new variant GII.4-2004. The GII.4-2004 was first reported in Europe in The Netherlands in 2004 [22]. This strain had previously been described in Australia during the previous winter season [23] and emerged in Europe in 2004. This strain is distinct from the GII.4-2002 strain. GII.4-2004 was the predominant circulating strain in both the ROI and NI during the study period. The majority of outbreaks were due to this strain with a small number of outbreaks due to genogroup II.2. International surveillance has indicated that GII.4 is more commonly associated with outbreaks in institutional settings than other norovirus serotypes suggesting that the GII.4 norovirus genotype may have properties which facilitate transmission. Outbreaks in health-care institutions have been previously reported to show less genetic diversity than outbreaks in other settings [24] or sporadic cases [25]. The predominance of the GII.4-2004 strain in outbreaks reported in institutions in this study would appear to support this observation.

The polymerase gene, which has been shown to display diversity between different genogroups was chosen as the target for sequencing in this study [13, 26]. Short regions of the polymerase gene were used for phylogenetic analysis as the primers had to be broadly reactive in order to amplify the cDNA of a range of genogroup I and II strains. Although the sequence data generated from a small region of the RNA polymerase should be interpreted with caution, previous phylogenetic studies have revealed a strong relationship between this small amplified region and more extensive regions of the polymerase gene [27]. The use of other primer pairs, which amplify a larger

region of the polymerase gene, compromises the sensitivity of the PCR and would have led to a lower detection rate. The NiE3 primer pair RT–PCR assay detected the majority of genetic clusters in a test panel in a survey of European laboratories [28]. The current consensus is that the entire capsid gene sequence is needed to define genotypes. However, with some exceptions, norovirus strains cluster similarly when different regions of the genome are analysed [29]. While the majority of strains show good agreement between polymerase and capsid clustering [29], as more recombinants circulate, it is probable that sequencing of the polymerase region alone may not be sufficient to define genotype.

One of the aims of this study was to create an electronic database, establishing a link between the virological and epidemiological institutions which would be continued after the study period and used as a source of data for the participation of the ROI in an international surveillance network for foodborne viruses (DIVINE-NET) which was initially piloted as the Foodborne Viruses in Europe Network. The Foodborne Viruses in Europe Network was established as a 3-year European Union-funded research network to study foodborne viruses in Europe. During this project, the participant institutes have combined virological and epidemiological surveillance in order to detect national and transnational outbreaks, elucidate transmission routes, and make international comparisons of the epidemiology of viral gastroenteritis. Protocols for harmonizing the characterization of noroviruses and an outbreak questionnaire with a minimum dataset were defined. Following the success of this pilot network, the project was extended to include many more European partners. This network is known as DIVINE-NET. As a result of this study, the ROI is a participating member of the network.

The emergence of specific strains which have rapid public-health effects across geographical and political boundaries emphasizes the importance of sustained international norovirus surveillance combining molecular and epidemiological data. Observations from international surveillance in 2002 and 2004 suggest that genogroup II.4 noroviruses might have an increased propensity for epidemic spread. The increased use of molecular diagnostics and typing allows for increasing research on the disease prevalence of norovirus and the epidemiology of outbreaks. It is only by such epidemiological investigation of outbreaks that transmission routes can be determined, differences in epidemiology between strains detected and effective control measures implemented. Establishment of a system for the sharing of epidemiological and molecular data on norovirus outbreaks in Ireland is a significant step forward in developing national surveillance of viral gastroenteritis and in contributing to international surveillance of norovirus outbreaks which will lead to a greater understanding of how these viruses are transmitted and of how outbreaks can be prevented.

## CONCLUSION

The results of this study show that noroviruses are the most frequent reported cause of infectious intestinal disease on the island of Ireland causing a significant disease burden which predominantly affects healthcare institutions. Although outbreaks occurred throughout the year, there was a seasonal peak in the number of outbreaks during the winter months. Person-to-person spread was the predominant reported mode of transmission with a very low reported rate of foodborne transmission (0·9%). However, it is likely that this represents the type of epidemiological investigation of outbreaks which was carried out and foodborne transmission may be more common than this data would suggest. Over the 2004–2005 study period, there was an increase in the number of norovirus outbreaks on the island of Ireland which was associated with the emergence of a new predominant circulating strain genogroup II.4-2004. This strain was also circulating in other European countries during the same period. Enhanced surveillance during the study period led to increased submission of samples for diagnosis and molecular typing. This was the first time epidemiological and molecular data on norovirus outbreaks was collected for the island of Ireland using cross-border cooperation. It was also the first time a link to share virological and epidemiological data was established in the ROI. This data-sharing link between the NVRL and the HPSC has been continued after the duration of this study. This database is being used as a source of national surveillance data and a source of data for the Foodborne Viruses in Europe Network (DIVINE-NET), a European Surveillance Network for norovirus outbreaks.

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## DECLARATION OF INTEREST

None.

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