



Burden of rotavirus infection in hospitalized elderly individuals prior to the introduction of rotavirus vaccination in Sweden

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ABSTRACT

Background: Rotavirus gastroenteritis (GE) in the elderly has been much less studied than in children.

Objectives: The aim of this study was to determine the morbidity and mortality for elderly hospitalized patients with rotavirus GE prior to the introduction of rotavirus vaccination in Sweden, and to investigate the epidemiology of rotavirus genotypes in these patients.

Study design: All patients 60 years or older who were hospitalized at Sahlgrenska University Hospital, Gothenburg, Sweden, and were rotavirus positive in a clinical diagnostic test from 2009 to 2016, were included.

Medical records were reviewed and rotavirus genotyping real-time PCR was performed.

Results: One hundred and fifty-nine patients were included, corresponding to an annual incidence of hospitalization due to rotavirus GE of 16/100 000 inhabitants aged 60 years or older. G2P[4] was the most common genotype, followed by G1P[8] and G4P[8]. The majority of patients had community-onset of symptoms and no or few pre-existing health disorders.

Four patients (2.5%) died within 30 days of sampling. Patients with hospital-onset rotavirus GE had a longer median length of stay following diagnosis compared with patients with community-onset of symptoms (19 vs. 5 days, $p = 0.001$) and higher 30-day mortality (8.6% (3/35) vs. < 1% (1/124), $p = 0.03$).

Conclusions: Hospitalization due to rotavirus GE among the elderly seems to mainly affect otherwise healthy individuals and is associated with low 30-day mortality.

1. Background

Rotavirus caused approximately 2 million hospitalizations and more than 400,000 deaths worldwide each year in children < 5 years of age, prior to the introduction of rotavirus vaccines [1]. The global burden of rotavirus disease in children has decreased over the last decade, with the implementation of oral rotavirus vaccines in several National Immunization Programs (NIP) [2]. In high-income countries, rotavirus infection is rarely a life-threatening condition in any age-group, but remains a frequent cause of hospitalizations and short-term morbidity [3,4]. In Sweden, where rotavirus is not yet included in the NIP, rotavirus disease in children < 5 years of age is estimated to lead to approximately 350 hospitalizations and 640 Emergency care visits, per 100 000 children per year [5]. The epidemiology in adults and the elderly, however, has been less thoroughly explored. Rotavirus appears to be a frequent cause of gastroenteritis (GE) requiring hospitalization in adults [6,7], and affects elderly and immunocompromised individuals to a larger extent [8]. Rotavirus disease in these patients has similar

clinical presentation as in other age groups, presenting with diarrhea, often accompanied by vomiting and abdominal pain [8]. Before mass vaccinations, G1P[8] was the predominant genotype in children, whereas genotype distribution in adults and elderly appears to be more diverse [8–10]. Most previous studies on the rotavirus burden in adults are based on discharge diagnosis data, which can lead to under-estimations [11,12]. Data from more robust observational cohort studies are still limited.

2. Objectives

The aim of this study was to determine the morbidity and mortality among elderly hospitalized patients with rotavirus disease across seven years, prior to the introduction of rotavirus vaccination in Sweden. Furthermore, we wanted to investigate the epidemiology of rotavirus genotypes in these patients.

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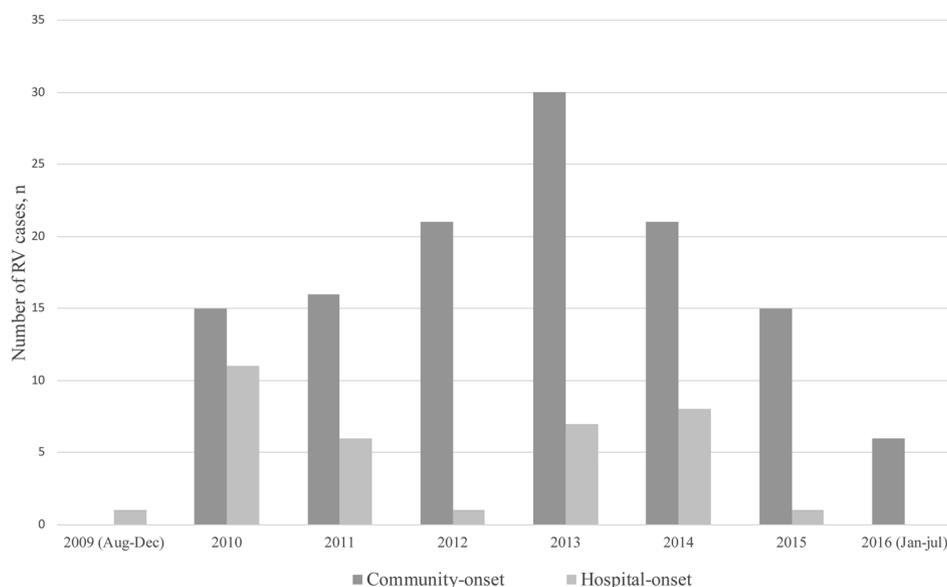


Fig. 1. Number of rotavirus cases each year in hospitalized patients 60 years or older, divided into community-onset disease and hospital-onset disease.

3. Study design

3.1. Patients and sample collection

Sahlgrenska University Hospital is a 2000 bed teaching hospital, and the only hospital serving the city of Gothenburg, Sweden with a mean population uptake of 680,000 residents, of whom approximately 140,000 were 60 years or older during the seven-year study period, August 2009 through July 2016. The database for standard of care testing of viral causes of GE at the Department of Clinical Microbiology at Sahlgrenska University Hospital, Gothenburg was reviewed retrospectively. Hospitalized patients 60 years or older with at least one positive rotavirus sample were identified and included in the study.

Standard of care testing consisted of samples from either whole stool, rectal swabs collected with flocked swabs (eSwab™ 480CE, Copan, Italy) or vomit and were obtained from all patients who were admitted with suspected viral GE and analyzed as described below. There were no changes in standard of care testing during the study period. Population hospitalization rate was calculated using population data from Statistics Sweden [13].

Medical records were reviewed for all included patients. Community-onset of symptoms was defined as GE symptoms (vomiting or diarrhea) present at admission and hospital-onset as absence of GE symptoms at admission. Comorbidities were categorized using Charlson comorbidity index, with comorbidity scores of ≥ 5 defined as severe comorbidity, as suggested in the original publication [14]. Length of stay (LOS) was calculated as the number of calendar days in the hospital, and LOS after diagnosis as the number of calendar days from rotavirus sampling to discharge.

The study was approved by the regional Ethical Review Board in Gothenburg (day-book number T522-16).

3.2. Detection of rotavirus and rotavirus genotyping by real-time PCR

Standard of care testing consisted of an in-house multiplex polymerase chain reaction (PCR) panel used for detection of five viral GE agents (adenovirus, astrovirus, norovirus, rotavirus and sapovirus), as previously described [15]. In brief, 250 μ L of feces or vomit were dissolved in 4.5 mL of saline and centrifuged for 5 min at 750 x g. For standard of care analysis, 250 μ L of the dissolved feces or vomit, alternatively rectal swab supernatant, was mixed with 2 mL of lysis buffer, and this volume was used for extraction of total nucleic acid in

an EasyMag extraction instrument (Biomerieux, Marcy l'Étoile, France). The elution volume of nucleic acid was 110 μ L where 10 μ L were used for each PCR reaction.

All included rotavirus positive samples were subsequently analyzed further to determine the genotype as previously described [16]. Rotavirus genotyping by real-time PCR was performed in 3 parallel multiplex reactions targeting rotavirus strain VP7 (G1, G2, G3, G4, G9 and G12) and VP4 (P[4], P[6] and P[8]) in a QuantStudio 6 real-time PCR instrument (Applied Biosystems, Carlsbad, CA).

3.3. Sequencing

Sequencing of rotavirus was performed in 5 cases with low cycle threshold (Ct)-values from a cluster of cases in two adjacent Oncology wards, denoted E and F, in December 2010, and on a subset of 6 samples, with low Ct-values but otherwise randomly selected, from other wards (denoted A–D) and time periods (2010–2013). The VP4 region was amplified by using a mixture of 11 primers in order to target all genotypes, as previously described [17]. Cycle sequencing was then performed using the same primers as in the PCR, followed by reading in an ABI 3130 XL sequence reader. Distance matrix and neighbor-joining analysis based on the Tamura-Nei model, including bootstrapping to 500 replicates, was performed using the MEGA7 software.

3.4. Statistical methods

The Mann-Whitney-U-test was used for univariate comparisons of continuous variables and the Chi-Square test was used for proportions. Fisher's exact test was used for small sample sizes. A p-value < 0.05 was considered significant. All calculations were made with SPSS Statistics 22 software (IBM Corp., Armonk, NY).

4. Results

During the study period, a total of 24 397 clinical samples, 14 473 from patients 60 years or older, were sent to Sahlgrenska University Hospital for analysis of viral pathogens that may cause GE. In total, 1142 of them were positive for rotavirus, and of these 508 were from in-patients. After excluding samples from children and younger adults, 159 in-house rotavirus positive patients aged 60 years or older remained and were included in the study (see Supplementary data). The

Table 1
Clinical and demographic characteristics of included patients (n = 159).

| | Community-onset n = 124 | Hospital-onset n = 35 | P-value |
|---------------------------------------|----------------------------|--------------------------|---------|
| Age (years) | 80 (72–86) ^a | 79 (74–84) ^a | 0.7 |
| Female | 74 (60) ^b | 18 (51) ^b | 0.4 |
| Charlson score ≥ 5 | 4 (3) ^b | 8 (23) ^b | 0.001 |
| Length of stay (days) | 5 (4–9) ^a | 19 (9–38) ^a | 0.001 |
| Length of stay after diagnosis (days) | 5 (3–8) ^a | 13 (3–24) ^a | 0.002 |
| Immunosuppressive therapy | 11 (9) ^b | 3 (9) ^b | 1.0 |
| 30-day mortality | 1 (0.8) ^b | 3 (8.6) ^b | 0.03 |

^a Median (Interquartile range).

^b n (%).

annual numbers of rotavirus positive samples and type of infection (community- vs. hospital-onset) in this group are presented in Fig. 1. There were no co-detections with norovirus, one co-detection with *Campylobacter* and three co-detections with *Clostridium difficile*. The highest number of rotavirus detections were made in 2013 and 2014, 37 and 29 detections, respectively. Lower number of rotavirus infections were diagnosed in 2012 and 2015, especially for hospital-onset disease. The annual number of samples sent for GE-virus testing varied, but no statistically significant variation over time was observed over the study period (data not shown).

In the Gothenburg area, the average annual rate of hospitalization with rotavirus infection was 16 per 100 000 inhabitants 60 years or older during the study period. For community-onset rotavirus infection the rate was 13 per 100 000 inhabitants 60 years or older, with the highest rate (21 per 100 000) observed in 2013. The clinical characteristics of the patients are presented in Table 1. Of 159 patients 43 (27%) had no known underlying health condition and thus a low comorbidity score. The short-term mortality was low, in total four patients (median (IQR) age 82 (67–87) years) died within 30 days of sampling. Three deaths occurred among patients with hospital-onset rotavirus disease, and the 30-day mortality rate was significantly higher in this group compared to patients with community-onset disease (Table 1). As shown in Table 1, hospital-onset disease was less frequent than community-onset. The average co-morbidity scores were similar in both groups, but a larger proportion of patients in the hospital-onset group had a Charlson score of 5 or more indicating severe comorbidity. The patients who died had a higher mean Charlson score compared to the other 155 patients (4.5 vs. 1.7 respectively ($p = 0.003$)). Patients with hospital-onset disease were diagnosed on median 7 (IQR 3–12) days into the hospitalization and had a longer LOS following diagnosis compared to patients with community-onset disease (Table 1).

The number of rotavirus infections typically increased during the winter and reached a peak in February through April each year (data not shown). Fig. 2 shows the distribution of rotavirus genotypes across the seven-year period. Overall, G2P[4] was the most common genotype, followed by G1P[8] and G4P[8] (87/159 (55%), 28/159 (18%) and 13/159 (8%) respectively). The G2P[4] infections were concentrated to the two years (2013 and 2014) when the largest total number (37/159 (23%) and 29/159 (18%) respectively) of rotavirus infections were observed (Fig. 2). The proportion of G2P[4] was also significantly higher during these two years, compared to the rest of the study period (55/66 (76%) vs. 35/93 (38%); $p = 0.001$). G2P[4] alone accounted for the total increase in rotavirus detections during these two years. In the other years, genotype distribution was more even. In 10 (6.3%) samples with high Ct-values, indicating a low viral load, the genotype could not be determined.

Of the four patients who died, three were treated in the same department (two adjacent Oncology wards denoted ward E and F) within 30 days of each other and had hospital-onset disease of identical genotype (G2P[4]). Another five patients in the same department, four of

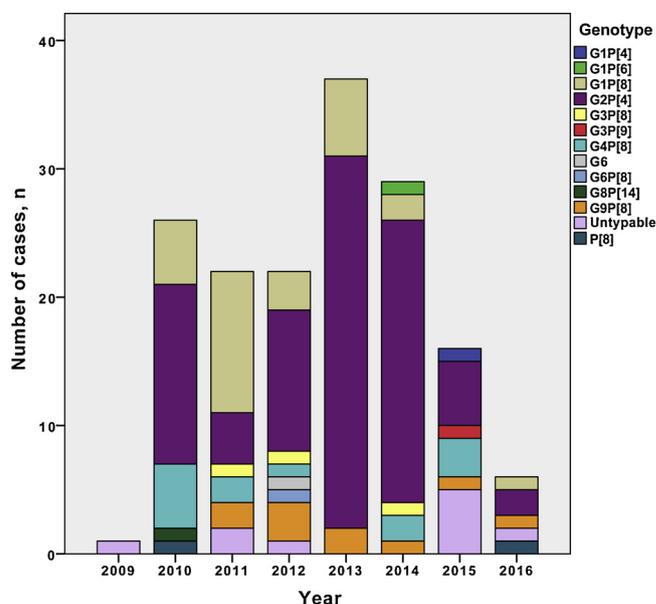


Fig. 2. Overall distribution of rotavirus genotypes in 159 patients 60 years or older hospitalized with rotavirus gastroenteritis during the study period (2009–2016). 10 samples, with high average Ct-values, were untypable.

whom had hospital-onset disease, tested positive for G2P[4] within the same month. As no other positive rotavirus samples were reported from patients in wards E or F before or after this month, this was interpreted as an unreported hospital-onset outbreak. Of 159 patients, 14 (9%) patients were on immunosuppressive therapy, four of whom were from the alleged outbreak (Table 1). Mean Charlson score was significantly higher in the patients from the two Oncology wards compared to the other patients in the study (5.1 vs 1.5 ($p = 0.001$)). To further explore the possible outbreak among hospitalized patients, we sequenced a part of the VP4 region of the rotavirus genome in a subset of five samples from the suspected outbreak. For reference, we also included sequences from three samples obtained from other wards during the same epidemic season and another three during a later epidemic season (2012/2013). As shown in Fig. 3, the sequences obtained from patients in the suspected outbreak clustered together. A similar sequence from an unrelated case, which clustered with sequences from the suspected outbreak, was also found in another part of the hospital complex (Patient 5).

5. Discussion

We found that rotavirus infection mainly affected previously healthy elderly individuals who acquired the infection in the community. Mortality was low, but the median duration of hospitalization was five days, indicating that community-onset rotavirus infection often induces severe symptoms requiring hospitalization of otherwise healthy elderly persons. Hospital-onset rotavirus infections were typically seen in individuals with multiple comorbidities and rotavirus disease may have contributed to short-term mortality in some cases. Our findings are in line with a case-control study from Denmark, where only 15% of adults with rotavirus GE had underlying medical conditions [18].

Our results suggest that the epidemiology of rotavirus infection in the elderly may differ from that of other types of viral gastroenteritis [19]. A study surveying mortality in the elderly in relation to enteric infections did not show any additional mortality due to rotavirus infection, but a significant increase in deaths among elderly patients with norovirus infection [20]. It is believed that elderly individuals acquire rotavirus infections in the community, from close contact with small children or their household contacts [18,21,22]. This hypothesis is supported by the finding that widespread implementation of pediatric

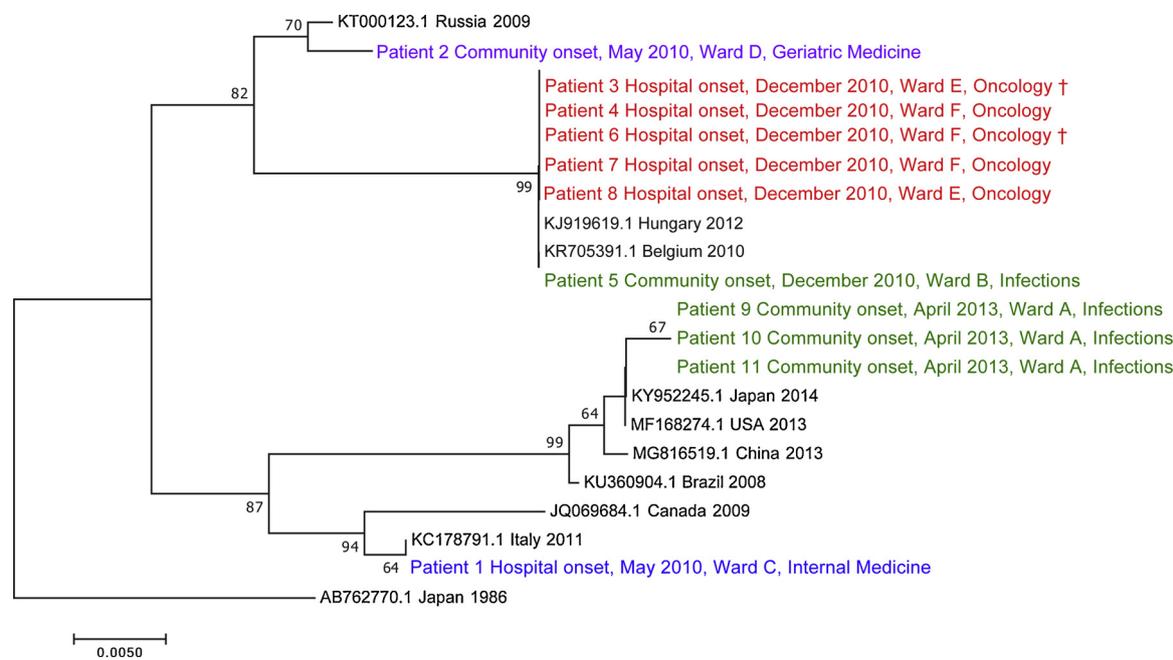


Fig. 3. Neighbor-joining phylogenetic tree of partial sequences of the VP4 gene of rotavirus (419 nucleotides). Bootstrap values are shown at the nodes. Five samples from a cluster of putative nosocomial cases in wards E and F were sequenced, along with 6 samples from other wards and time periods. †, patients who died.

rotavirus vaccination has resulted in a marked decrease in rotavirus incidence also among adults in the US [23].

Our findings are in line with two studies from the USA and Canada, which estimated the overall burden of rotavirus in terms of hospitalizations [3,11]. In two additional studies from the Netherlands and Australia, the estimated hospitalization rates were significantly lower [7,12]. The Dutch study included few elderly patients with rotavirus infection, however, and the Australian study included rotavirus admissions coded according to discharge diagnosis in medical records, which may underestimate the rate of hospitalization due to viral GE. If the admission rates in the Australian study are adjusted with the correction model for GE discharge diagnoses presented by Lopman et al [11], the adjusted estimate comes close to our findings.

Although rotavirus infection is generally not associated with mortality in the elderly, it is likely that it could contribute to death if transmitted to hospitalized individuals with multiple comorbidities [4]. A larger sample size would be necessary for such an investigation, as mortality in our study in general was low. The median LOS after sampling was more than one week longer among patients with hospital-onset compared with community-onset rotavirus infection. Similar prolongation of LOS has been observed in hospital-onset norovirus infections [24]. In the comparison of LOS between hospital-onset and community-onset rotavirus GE the groups differed regarding underlying disease and comorbidities. These factors likely contributed to the difference.

The distribution of rotavirus genotypes varied over the study period. In 2012/13 and 2013/14 an increase of genotype G2P[4], and a decline in G1P[8] was noted. This pattern has also been described among Swedish children, and persons of all ages in other countries [16,25,26]. In the two latter studies, the decrease of infections with G1P[8] followed the introduction of nationwide rotavirus vaccination. However, these observations are probably due to natural fluctuation of circulating genotypes. The two-year peak of G2P[4], followed by much lower levels, is in line with recent observations of variations in genotype distribution in other areas [27,28].

We identified eight in-patients, who we believe represent a previously unrecognized nosocomial outbreak. Reports about healthcare associated outbreaks of rotavirus in the elderly are rare, and the magnitude of this problem is largely unknown. Hospital-acquired rotavirus

infection in children seems to be more common in wards that seldom treat patients with gastroenteritis [29] and in outbreaks of hospital-acquired GE in neonatal intensive care units, rotavirus has been reported to be the most common agent found [30].

This study has several limitations. All the patients in our study were hospitalized, and therefore general conclusions about rotavirus in the elderly in the society cannot be drawn. However, since there are no other hospitals serving the geographical area, we believe that the hospitalization rate due to rotavirus infection we found is a good estimate. The retrospective design is associated with risk of bias and of missing relevant clinical or epidemiological data that cannot be corrected for. Samples were taken as standard of care testing and changes in the frequency of testing might have occurred. We did not identify any such trends, however, in standard of care testing during the study period. Due to unreliable data regarding the exact time point of infection, we chose to define the subgroups based on the onset of symptoms, as either community-onset or hospital-onset.

In conclusion, we showed that hospitalization due to rotavirus GE among the elderly seems to mainly affect otherwise healthy individuals and is associated with low 30-day mortality.

Declaration of Competing Interest

None of the authors have any conflicts of interest to declare.

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Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

CRediT authorship contribution statement

Thomas Beck-Friis: Conceptualization, Validation, Formal

analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Maria Andersson:** Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing. **Lars Gustavsson:** Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Magnus Lindh:** Conceptualization, Methodology, Software, Validation, Resources, Writing - review & editing, Visualization, Funding acquisition. **Johan Westin:** Conceptualization, Formal analysis, Writing - review & editing, Supervision, Funding acquisition. **Lars-Magnus Andersson:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jcv.2019.07.005>.

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