



Gunta Laizāne

**Clinical Peculiarities of Rota Viral  
Infection, Molecular Epidemiology  
and Health-Related Quality of Life  
for Hospitalised Children in  
Children's University Hospital and  
Their Family Members**

Summary of the Doctoral thesis  
for obtaining a doctoral degree (*Ph.D.*)

Sector – Clinical Medicine  
Sub-sector – Paediatrics

Rīga, 2020



Gunta Laizāne

ORCID 0000-0002-1866-9517

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Supervisor of the Doctoral Thesis :

*Dr. habil. med.*, Professor **Dace Gardovska**,  
Rīga Stradiņš University, Latvia

Official Reviewers:

*Dr. habil. med.*, Professor **Ludmila Viksna**,  
Rīga Stradiņš University, Latvia

*Dr. med.*, Professor **Ilva Daugule**,  
University of Latvia, Faculty of Medicine, Department of Paediatrics

*MD, PhD*, Professor **Timo Vesikari**,  
Director of Vaccine Research Centre at the Tampere University, Finland

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## Abstract

Despite available specific prophylaxis, Rotavirus is still one of the leading causes of severe diarrhoea in young children and infants worldwide, representing a heavy public health burden. Limited data regarding the impact of rotavirus gastroenteritis and the quality of life of affected children and their families is available.

The aim of the study was to estimate clinical peculiarities, molecular epidemiology and the impact of rotavirus infection on health-related quality of life (HRQL), to assess the social and emotional impact on the families of affected children, hospitalised in Children's Clinical University Hospital in Riga.

The study was designed as a quantitative cross-sectional study consisting of two sections, where in the first section clinical, socio-epidemiological characteristics and molecular epidemiology of Rota viral infection were investigated, whereas in the second section emotional, social and economic burden of the patient family, as well as the quality of the child's life and the factors associated with it were analysed. Descriptive statistics were used for data analysis. Results were considered to be statistically significant if  $p < 0.05$ . Data processing was performed using IBM SPSS Statistics (Statistical Package for the Social Science, Version 22.0).

The study enrolled 527 Rota positive (with further PCR detection) cases (0–18 years of age) hospitalised between April 2013 and December 2015 and their caregivers respectively. P and G genotypes were detected in 462 samples, and paired with clinical and socio-epidemiological data for further interpretation. For the second section, data of all cases ( $n = 527$ ) were used for further interpretation.

92.9 % ( $n = 429$ ) of the patients were less than 5 years old. Clinical symptoms were categorised as severe, according to the Vesikari score, in 87.0 % patients ( $n = 402$ ) ( $p < 0.001$ ), and on average most of the children were

hospitalised 2.5 days after the onset of symptoms. In single-type infections, the predominant G/P combinations were G4P[8], 60.2 % (n = 278), G9P[8], 12.1 % (n = 56), G2P[4] 10.6 % (n = 49), G1P[8] 6.5 % (n = 30), G3P[8] 4.1 % (n = 19), G8P[8] 3.5 % (n = 16), G2P[8] 2.6 % (n = 12), G1P[4] 0.2 % (n = 1) and G4P[4] 0.2 % (n = 1).

Statistically significant correlations were found among certain genotypes and severity of vomiting as a separate symptom. Infections with genotypes G4P[8], G8P[8] and G9P[8] had significantly more vomiting episodes than G1P[8] ( $p < 0.05$ ).

A significant correlation was found among stress/anxiety and irritability, tearfulness of the child ( $p < 0.001$ ) and of fever level ( $p = 0.02$ ). Analysis of social burden showed statistically significant associations with different sociodemographic factors – older age of a child ( $p < 0.001$ ), older age of a mother ( $p < 0.001$ ) and higher education level of the mother ( $p < 0.001$ ) corresponded to higher proportions of caregivers reporting a need to introduce changes in their daily routine.

To estimate economic burden of Rota virus infection, lost working days and additional financial expenditures were analysed. 55.3 % (n = 289) of care givers had to take days off work and 75.2 % (n = 380) reported additional expenditures, but no statistically significant correlations were found in association with the analysed factors.

A better understanding on how an acute episode of Rota virus infection can affect the child and the child's family could help to minimise parental fears and advise the parents on characteristics of rotavirus infection as well as optimal care of an affected child.

Current study results proved the total burden of Rota viral infection, and emphasises the routine immunisation to be used as evidence based prophylaxis of the disease in Latvia.

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## Abbreviations

AGE	Acute gastroenteritis
AV	Antibodies
CCUH	Children's Clinical University Hospital
CDPC	Centre for Disease Prevention and Control
CI	Confidence interval
ELISA	Enzyme-linked immunosorbent assay
FDR	False discovery rate
GP	General Practitioner
HRQL	Health-related quality of life
I/V	Intravenous access, injection
mRNA	messenger RNA
NIP	National Immunisation Programme
OR	Oral rehydration
PCR	Polymerase chain reaction
RV	Rotavirus
RV5	Pentavalent Rotavirus vaccine
RVI	Monovalent Rotavirus vaccine
ssRNA	positive sense single-stranded RNA
VP	Structural viral proteins
WHO	World Health Organisation

# Introduction

## Background

Acute gastroenteritis in paediatric patients remains a globally relevant problem due to its high morbidity and mortality. In developed countries, mortality rates are relatively low. However, one considers acute gastroenteritis to be the most common cause of hospitalisation after pneumonia and represent a significant socio-economic burden on health care in general (Florez et al., 2020; Hartmann et al., 2019; ECDC, *Expert Opinion on Rotavirus Vaccination in 2017*; Walker et al., 2013).

Until 1973, when Rotavirus was discovered, the diagnosis of gastroenteritis of unspecified aetiology had been dominating (Bishop et al., 2009; Bishop et al., 1973; Davidson et al., 1975; Flewett et al., 1975). Since the thirties of the last century, scientists have begun to differentiate between the aetiology of viral and bacterial acute gastroenteritis, but they are unable to identify all the agents. At the time some of the enteropathogenic bacteria responsible for “Cholera infantum”, which has a high mortality rate in Europe, were recognisable (Bishop et al., 2009). Depending on symptoms and seasonality, one speaks about “summer diarrhoea” and “winter vomiting disease” (Bishop et al., 1973).

The leading aetiology of gastroenteritis in developed countries is viral nowadays (Hartmann et al., 2019; Randy et al., 2018). Studies on the role of Rotaviruses in the common paediatric gastroenteritis group began after its discovery in 1973. Research is being launched by American scientists with the parallel development of specific prevention – vaccine against Rota viral infection (Plotkin et al., 2008). Finland was one of the European countries actively involved in the study of the viral aetiology of acute gastroenteritis. The first vaccine, Rotashield®, was developed in the early 1990s to protect children from

severe rotavirus infection, but its use for prevention was soon discontinued due to the increased risk of complications (ACIP, 1999). New safe vaccines are available since 2006 such as pentavalent Rotateq® and monovalent Rotarix® vaccines, which reduce morbidity and mortality of rotavirus infection significantly, in the countries where vaccination is included in the National Immunisation Programme, and reaches sufficient coverage (Richardson et al., 2010). Vaccination results in protection against severe, moderate forms and complications of the disease (Burke et al., 2020; ECDC, *Expert opinion on rotavirus vaccination in infancy*, 2017; Velazquez et al., 1996; Franko et al., 2006).

The dominant genotypes of Rotaviruses in Europe are G1-G4 and G9, and used vaccines protect children from moderate to severe forms of disease and possible complications by production of cross-immunity (Burke et al., 2020; Van Damme et al., 2007; WHO, 2018).

Despite possible prevention, Rotavirus as an etiological agent remains relevant in the common gastroenteritis group by causing clinically severe infection in unvaccinated children and maintaining a high risk of hospitalisation (Florez et al., 2020; Zavadska et al., 2016; WHO, 2018; ECDC, *Expert opinion on rotavirus vaccination in infancy*, 2017).

The impact of acute illness on the quality of life of patient caregivers is poorly studied in general. Literature provides data on the statistically significant socio-economic and emotional burden in acute Rotavirus infection (Mast et al., 2009; Giaquinto et al., 2007).

In Latvia, routine immunisation of infants against Rotaviral infection was implemented in January 2015. The number of proven cases of Rotavirus reached 150.5 cases per 100,000 population in 2015, while the number has decreased to 63.1 cases per 100,000 population in 2018 (CDPC 2019). In 2017, 21,465 children under 1 year of age had to be immunised against Rotavirus in Latvia. There are 18,521 children who received the full vaccination course. There

were 1,224 written refusals of vaccination received in 2017, and the reasons for not vaccinating 1,372 children are unknown (CDPC 2019). Following the statistics of the CDPC, there is a clear need for local evidence on the safety and efficacy of Rotavirus vaccination to be used for raising public awareness.

From 2015 to 2018, the Children's Clinical University Hospital (C has treated 2,459 patients with the diagnosis of Rotavirus (ICD code A08.0). The average hospital stay per patient is 3.54 days. In total, this group of patients spent 8,721 bed-days in the hospital during this period. The cost of one bed-day in CCUH constitutes 286.27 EUR, which means that the average cost per clinical case of Rotaviral infection is 1,013 EUR (CCUH Department of Economics and Finance, 2019).

To date, no studies have been conducted in Latvia on the clinical peculiarities and the molecular epidemiology of Rotaviral infection, and the impact on the health-related quality of life of a family. Consequently, the topic of research is considered as relevant.

## **Aim of the study**

The aim of the study was to investigate the clinical peculiarities of Rotaviral infection, molecular epidemiology, and health-related quality of life for hospitalised children in Children's Clinical University Hospital and their family members.

## **Study objectives**

1. To determine Rotavirus genotypes in hospitalised children in CCUH.
2. To analyse potential correlation between clinical signs and socioepidemiological peculiarities of Rotaviral infection with specific circulating genotypes of the virus.

3. To analyse the compliance of the isolated genotypes for continued safe prevention with the Rotavirus vaccines available in Latvia.
4. To identify the emotional, social, and economic burden caused by Rotaviral infection and the factors related to it in the families of the patients.

### **Questions raised for the study**

1. Is there a correlation between the molecular characterisation of Rotaviruses and the severity of the disease?
2. Does molecular epidemiology in Latvia differ from other countries?
3. Is there a possibility of local Rotavirus genotypes?
4. Has the Rotaviral infection caused a psycho-emotional and socio-economic burden on caregivers of the children admitted to CCUH and their family members in general, and what factors are involved?

### **Scientific novelty of the Thesis**

1. The study on the molecular epidemiology of Rotaviruses and health-related quality of life of the family is carried out in Latvia for the first time. Also, the health-related quality of life in acute illness in the paediatric population was researched for the first time.
2. Circulating Rotavirus genotypes are identified, and their relevance to immunity development about Rotavirus vaccines used in Latvia is determined.
3. There is statistically reliable evidence of the emotional and social burden caused by Rotaviral infection.

# **1. Materials and methods**

## **1.1 Structure of the research**

Research “Clinical Peculiarities of Rota Viral Infection, Molecular Epidemiology, and Health-Related Quality of Life for Hospitalised Children in Children’s University Hospital and Their Family Members” was launched in 2013 and consists of two chapters, namely, Part I and Part II.

Part I studied the clinical and socio-epidemiological peculiarities of Rotavirus and included molecular biological analysis of biological material, dsRNA isolation and sequencing by VP4 and VP7 sequences.

Part II is devoted to the analysis of the emotional, social, and economic burden and health-related quality of life, and the factors correlated to that for patients and their parents enrolled in the study during an episode of the disease.

## **1.2 Literature review**

The author carried out a selection of the literature based on the principles of systematic literature review to identify, select, and analyse scientific literature of evidence-based medicine on the researched subject.

For the literature review, websites of the international association of experts (EuroRotaNet, European Society for Paediatric Infectious Diseases, etc.), as well as national guidelines of other countries, data of National Health Statistics Centres, data of various health organisations (National Institute for Health and Clinical Excellence, The NHS Centre for Reviews and Dissemination, National Institute of General Medical Sciences (NIGMS), WHO) and medical databases referable to the issue under research were used:

- PubMed,
- ScienceDirect,

- The Cochrane Library,
- The Centre for Evidence-Based Medicine ([www.cebm.net](http://www.cebm.net)),
- SCOPUS.

One defined selection restrictions regarding publications: only units with summaries and there was no time limit for the publications sought. Duplicate publications were excluded.

The following keywords were used: Rotavirus, Rotavirus gastroenteritis, clinical characterisation, acute, molecular epidemiology, genotyping, viral immunity, health-related quality of life, childhood, impact, and family.

### **1.3 Study design**

Research “Clinical Peculiarities of Rota Viral Infection, Molecular Epidemiology, and Health-Related Quality of Life for Hospitalised Children in Children’s University Hospital and Their Family Members” is a quantitative cross-sectional study, carried out at State-owned Limited Liability Company “Children’s Clinical University Hospital” (CCUH), Department of Paediatrics of Rīga Stradiņš University, Laboratory of Molecular Biology of RSU Institute of Oncology, and Department of Public Health and Epidemiology from March 2013 to December 2015. The study was approved by the Ethics Committee of Rīga Stradiņš University.

### **1.4 Inclusion and Exclusion Criteria**

**Part I** – The study included 527 patients aged 0–18 years who were hospitalised to CCUH with a diagnosis of Rotaviral acute gastroenteritis. All patients or their legal caregivers included in the study signed a consent form for participation in the study. Samples of the biological material of 497 patients with

dsRNA isolated were validated for molecular analysis that served as inclusion criterion.

**Part II** – The study included 527 caregivers of patients who signed a consent form to participate in the study.

The exclusion criterion was either lack of written consent for Part I and Part II.

## **1.5 Materials and Methods**

The target population of the study was hospitalised children (aged up to 17 years incl.) with confirmed Rotaviral infection. According to CCUH statistics, the target population consisted of 3,034 children between January 2013 and December 2015. By choosing traditional (95 %) credibility rate and margin of error (5 %) and assuming a 50 % probability distribution of the trait in the population (Since the distribution of different traits is studied instead of one specific and this type of study is carried out for the first time in the country, the expected prevalence (distribution) is traditionally selected at this 50/50 level), one has calculated that the minimum sample size required for the cross-sectional randomised study is 327 children.

### **Part I**

Between 2013 and 2015, there were 527 children with AGE enrolled in the study, which approached Emergency Department of the Children's Clinical University Hospital (CCUH) and were hospitalised with the diagnosis of acute Rotaviral gastroenteritis (ICD Code A08.0) sequentially. The aetiology of Rotaviruses in faeces has been detected by rapid test RIDA QUICK Rotavirus based on immuno-chromatographic method.

The author drafted a questionnaire for this part of the study. After enrolment into the study, each patient was assigned an anonymous identification number.

The researcher filled out the questionnaire for all patients enrolled in the study on the first day of the study. This information included:

- Patient demographics,
- History of the disease,
- Epidemiological history,
- Social history,
- Laboratory test results (Leukocytes, erythrocytes, platelets, haematocrit, haemoglobin, and CRO),
- Assessment of the severity of dehydration as per the WHO scale,
- Determining the severity of the disease according to the Vesikari scale.

A 20-point Vesikari scale has been used to determine the overall severity of the disease, which takes into account the following features:

- Maximum number of stools and vomiting per 24 hours,
- Duration of diarrhoea and vomiting in days,
- Axillary temperature,
- Degree of dehydration,
- Therapy.

Interpretation of the scale corresponds to > 11 points for severe disease and 7–10 points for moderate disease (Ruska et al., 1990).

Biological material – faeces were collected from all participants of Part I of the study and frozen (– 200 °C). The frozen biological material was taken to the Molecular Biology Laboratory of the RSU Institute of Oncology for further analysis. The total number of samples was  $n = 527$ .

## **RNA isolation from biological material**

RNA was isolated in 497 samples (n = 497). Rotaviral RNA from faeces was isolated by using QIAamp Viral, an RNA mini reagent kit (Qiagen, Germany), based on a protocol developed by the manufacturer. One designed the protocol to minimise contamination between different samples.

Approximately 0.5 ml to 1 ml of faeces was diluted in saline in the proportion of 1:10. After thorough shaking, one centrifuged the samples for 20 min at 1000 rpm at 4 °C. RNA was isolated from 140 µl of faecal suspension. Initially, the sample was lysed in denaturing buffer to inactivate RNase and ensure isolation of intact viral RNA. Messenger RNA was added to the samples, and the resulting mixture was applied to the QIAamp Mini Silicone Membrane. The salt concentration and pH of the lysate ensure that proteins and other potentially contaminating impurities that can inhibit enzymatic reactions do not settle on the membrane. The membrane-bound RNA was washed by using two different buffers developed by the manufacturer, which, without affecting the binding of the RNA to the membrane, improved the purity of the RNA significantly by removing residual impurities. The resulting RNA in concentrated form was eluted in a buffer developed by the manufacturer and stored at – 20 °C.

## **Identification of VP7 (G) and VP4 (P) genotypes**

G and P genotyping was performed by using reverse transcription, that is, polymerase chain reaction (RT-PCR) and Sanger sequencing. The GoTaq 1-Step-RT-qPCR System Reagent Kit (Promega, USA) was used for RT-PCR based on the protocol developed by the manufacturer and with the reagents of the manufacturer (Promega, USA). This kit enables cDNA synthesis and amplification of target regions in a single reaction.

The amplification resulted in a 663bp long product for the VP4 genotype and an 880bp long product for the VP7 genotype (Table 1.1, Table 1.2, Table 1.3, and Table 1.4).

Table 1.1

**Primers Used in the study (Aly et al., 2015)**

Sequence of primers	Amplifiable genotype	Reference
5`-TAT GCT CCA GTN AAT TGG-3` F	VP4	Aly M et al., 2015
5`-ATT GCA TTT CTT TCC ATA ATG-3` R		
5`-ATG TAT GGT ATT GAA TAT ACC AC-3` F	VP7	
5`-ATG TAT GGT ATT GAA TAT ACC AC-3` R		

Table 1.2

**Reaction Mixture for Single-Step RT-PCR and Target Region Amplification**

Reagent	Quantity for 1 reaction	Final concentration
GoTaq® qPCR Master Mix, 2X	25 µl	1x
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	1 µl	1x
Primer F	5 µl	50 nM
Reagent	Quantity for 1 reaction	Final concentration
Primer R	5 µl	50 nM
RNA	5 µl	5 ng/ µl
RNase free water	9 µl	-
Total reaction volume	50 µl	-

Table 1.3

**Programme Used for Reverse Transcription and VP4 Amplification (Aly et al., 2015)**

	Number of cycles	Temperature/ time
Reverse transcription	1	50 °C/ 30 min
Inactivation of reverse transcriptase	1	95 °C/ 15 min
Denaturation	35	94 °C/ 1 min
Hybridisation		50 °C/ 1 min
Polymerisation		72 °C/ 1 min
End of amplification	1	72 °C/ 10 min

Table 1.4

**Programme Used for Reverse Transcription and VP7 Amplification**  
(Aly et al., 2015)

	Number of cycles	Temperature/ time
Reverse transcription	1	50 °C/ 305 min
Inactivation of reverse transcriptase	1	95 °C/ 15 min
Denaturation	40	94 °C/ 1 min
Hybridisation		Gradients 45 °C → 65 °C/ 45 sec
Polymerisation		72 °C/ 1 min
End of amplification	1	72 °C/ 10 min

To remove excess salts and nucleotides, PCR (Polymerase Chain Reaction) product purification was performed by using the MinElute 96UF PCR Purification Reagent Kit (Qiagen, Germany) based on the manufacturer's protocol and reagents developed by the manufacturer. After purification of the samples, one measured their concentration using spectrophotometer NanoDrop ND-1000 (Thermo Fisher Scientific, USA). This concentration should be approximately 5 ng /  $\mu$ l after purification. One sequencing reaction required 7.5 ng of PCR product. If this concentration was higher than required, the purified PCR product was diluted to the required concentration, while in the event of reduced concentration, a larger volume of PCR product was added to the sequencing reaction by reducing the amount of water in the reaction.

The sequencing reaction was performed by using the ABI PRISM BigDye terminator cycle reagent kit (Applied Biosystems, USA). The next step was a sequencing reaction, which resulted in fluorescent-labelled products differing in length by one nucleotide. The resulting fragments were purified by precipitating them with ethanol and sodium acetate. Then the samples were denatured by using formamide as a denaturing agent and incubated at 95 °C/ 5min. The prepared samples were analysed in a genetic analyser, Applied Biosystems (ABI) Prism 3130 (Life Technologies, USA), according to the principle of capillary

electrophoresis, where fluorescent colour emission was read as they were moving along the detection chamber (Table 1.5, Table 1.6).

Table 1.5

**Reaction mixture for sequenation**

Reagent	Quantity for 1 reaction	Final concentration
Sequencing buffer 5X	2 µl	2.5x
BigDye Terminator Mix	0.3 µl	-
Primer (F or R)	1 µl	10 nM
Purified PCR product	4 µl	-
RNase free water	2.7 µl	-
Total reaction volume	10 µl	-

Table 1.6

**Programme used for sequenation**

	Number of cycles	Temperature/ time
Start of amplification	1	96 °C/ 1 min
Denaturation	40	96 °C/ 10 sec
Hybridisation		50 °C/ 20 sec
Polymerisation		60 °C/ 4 min

All isolate sequences were analysed against the VP4 and VP7 international rotavirus sequences available in the database of National Centre for Biotechnology Information (NCBI) by using the Basic Local Alignment Tool (BLASTn-2).

**Part II**

The care givers, whose children were enrolled in the study, were invited to participate in the part of the study on the quality of life of a child and a family associated with the given RV AGE episode. The author received 527 signed

consent forms. One interviewed the parents sequentially and recorded their responses in a separate questionnaire drafted for this part of the study.

The questionnaire was developed based on concepts and research methods used in similar studies and covered the following domains of the impact of paediatric rotavirus on the family: (Javier Diez et al., 2012; Van der Wielen et al., 2010; Mast et al., 2009; Huppertz et al., 2008).

1. Parental emotional well-being and feelings (distress; helplessness; mental exhaustion; worry; anxiety for the child; fear of being infected; feelings of guilt).
2. Social burden of disease (or the disease impact on parents' daily activities (work schedule, training plans (syllabus), leisure activities, household activities).
3. Economic burden of the disease (work days off the work due to child disease and additional financial expenditures).
4. Parental opinion about the child's physical symptoms (diarrhoea, vomiting, fever, abdominal pain, dehydration, loss of appetite) and behavioural changes (apathy, sleeping disorders, irritability, anxiety)
5. Parental opinion on Rotavirus vaccination (awareness of vaccine existence (yes/no); use of vaccine (yes/no; if answered "no", the parents were asked to define reasons for refusal).

## 1.6 Statistical data processing methods

Descriptive statistical methods were used for **Part I**, and the Spearman rank correlation coefficient  $|\rho|$  was calculated. The results obtained were interpreted as follows:  $|\rho| \leq 0.25$  – weak correlation,  $0.25 < |\rho| < 0.75$  – average correlation,  $|\rho| \geq 0.75$  – strong correlation. Results were considered statistically significant if  $p < 0.05$ . Study results using multiple statistical tests were adjusted using the Benjamini-Hochberg procedure (Chosen FDR-adjusted  $q = 0.05$ ). The

author carried out data analysis in SPSS (Statistical Package for the Social Science, Version 22.0).

This part analysed the data from 462 study patients (Samples of biological material, where one managed to determine genotypes). Appropriate data from the first part was used to analyse the correlation of age with particular genotypes, seasonality, and prevalence of particular genotypes by year, etc.

Part I and II also use descriptive statistical methods such as mean values for parametric variables and proportions for non-parametric variables.

To evaluate the statistical significance of the differences of proportions of severe/very severe cases between subgroups, a Chi-square test or Fisher's exact test were used. Results were considered statistically significant if  $p < 0.05$ . Data analysis was carried out in SPSS (Statistical Package for the Social Science, Version 22.0).

## **2. Results**

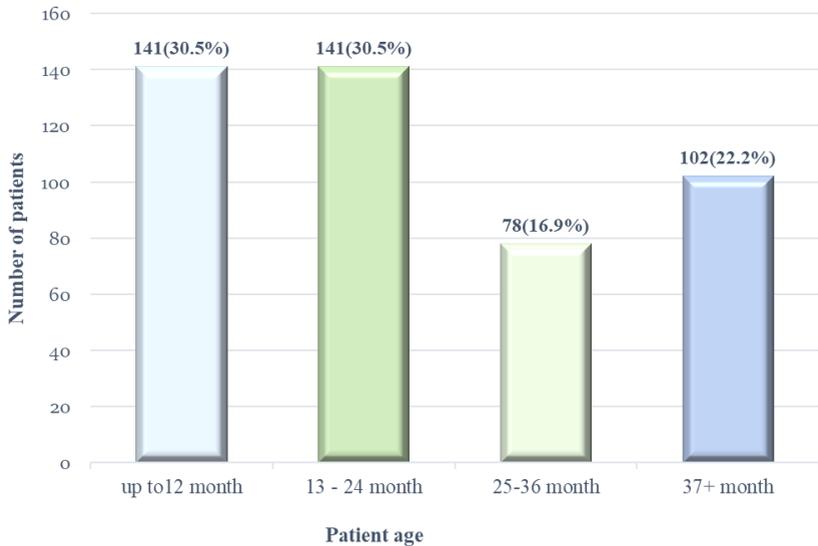
The analysis of the demographic information and clinical data of the study patients included 527 paediatric patients who were hospitalised at CCUH between March 2013 and December 2015 and in whose faeces Rotavirus was identified in the CCUH Lab of Microbiology. Patients aged between 0 and 18 years were included in the study. Then the data obtained were analysed in two steps by interlinking the parts of the study. Calculations may be inconsistent in absolute numbers because the information has been missing when filling out the questionnaires. When processing data, the software reports it as missing.

### **2.1 Results I**

The study group, where demographic, clinical and socio-epidemiological data were analysed related to the molecular epidemiology of Rotaviruses, included data from 462 patients.

The mean age in this patient group was 26.1 months or 2.2 years. The youngest patient was 1 month old, while the oldest patient was 17.4 years old. So, 92.9 % (n = 429) of patients were under 5 years of age. In its turn, one should emphasise that RV AGE was affecting children under 2 years of age more often by 61.0 % (n = 282).

For further interpretation of the results, the author should outline the more detailed patient distribution by age (Figure 2.1).



**Figure 2.1 Patient distribution by age**

Distribution by gender was even, with 49.4 % (n = 228) girls and 50.6 % (n = 234) boys, respectively.

No particular seasonality in the prevalence of RV infection was observed. RV infection occurs throughout the year, with a relative increase in the number of patients during the winter months. The incidence was 27.7 % (n = 128) in spring, 16.8 % (n = 78) in summer, 17.7 % (n = 82) in autumn, and 37.7 % (n = 174) in winter. The peak of incidence was observed in January at 20.3 % (n = 94), while the lowest incidence was recorded in December at 2.4 % (n = 11) (Figure 2.2).

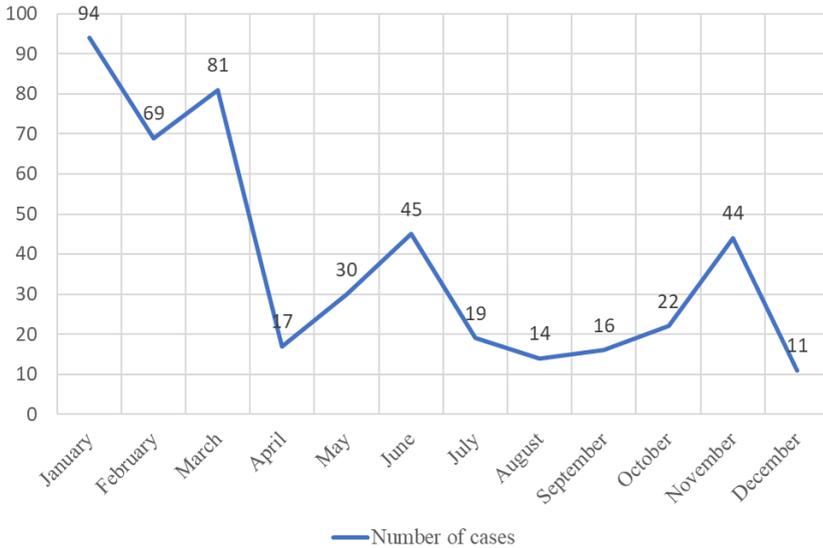


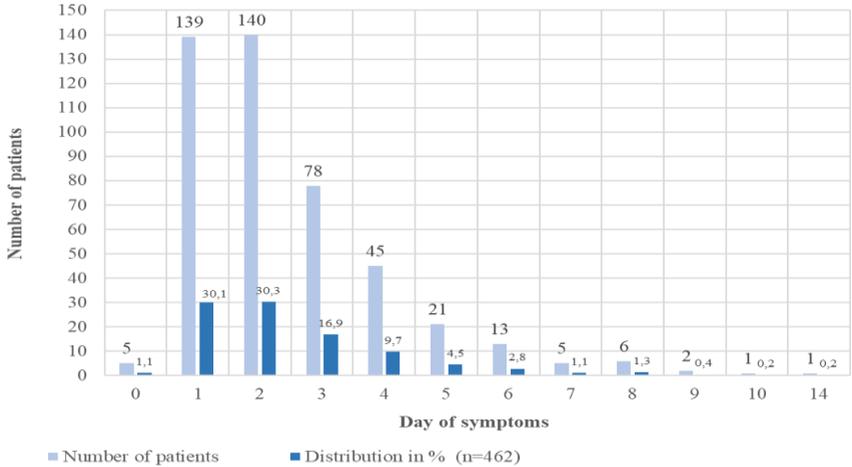
Figure 2.2 **Rotavirus seasonality**

Majority of patients, that is, 90.5 % (n = 418) were hospitalised from home. The remaining 6.7 % (n = 31) were the patients transferred from other CCUH departments, 1.3 % (n = 6) were the patients transferred from regional inpatient units, and 1.5 % (n = 7) were the children transferred from the childcare institutions. Nosocomial Rotaviral infection has been proven in those patients. The total incidence of nosocomial Rotaviruses constitutes 9.5 % (n = 44).

Even distribution of results was observed among patients who had visited a General Practitioner (GP) before a hospital and those who had not sought help of GP, i.e., 49.7 % (n = 223) and 50.3 % (n = 226), respectively. Rotaviral infection affected children attending kindergarten in 37.1 % (n = 166) of cases and 62.9 % (n = 282) children not attending kindergarten.

One should outline that children were hospitalised on 2.5 days from onset of the disease at an average that indicates severe disease and all patients received

intravenous fluid replacement therapy on their first day of hospitalisation (Figure 2.3).



**Figure 2.3 Patient distribution by the day of hospitalisation**

After evaluating severity of the disease among patients according the Vesikari scale in this group, it was established that 92.6 % (n = 402) of the patients had severe disease and only 7.4 % (n = 32) of the patients had the moderate disease. No mild cases had been reported in any patient. During the study period, only 2.2 % (n = 10) of the patients in the study had received Rotavirus vaccination.

Genotyping data demonstrates the prevalence of P and G genotype combinations in the study population clearly (Figure 2.4).

### Detected genotypes n=462

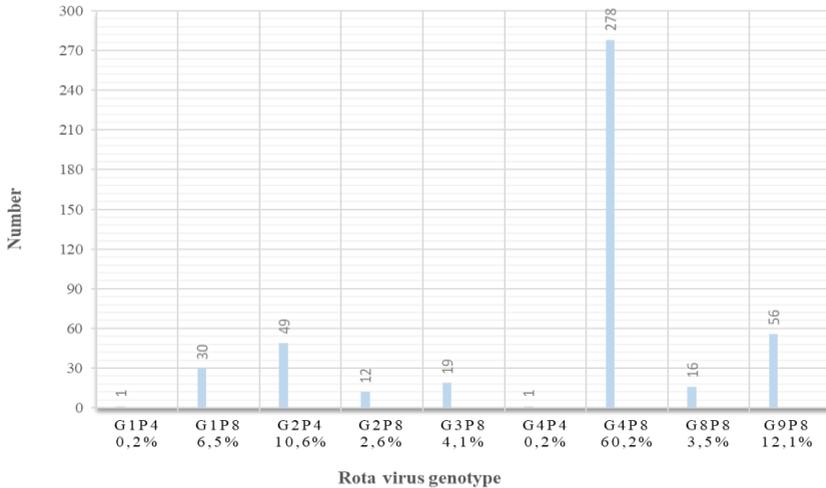
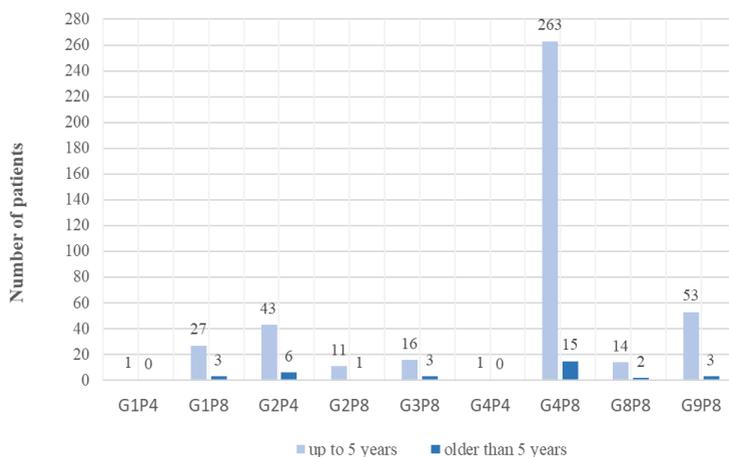


Figure 2.4 **Detected Rotavirus genotypes in the study population**

There were 497 samples analysed for VP7 and VP4 sequences, of which only the G genotype was isolated in 3.0 % (n = 15) and only the P genotype in 4 % (n = 20). The G and P combinations were detected in 462 samples. Samples, where both genotypes were isolated, were used for further data analysis and interpretation of the results. The following Rotavirus genotype combinations were detected in the analysed samples: G1P[8], G2P[4], G2P[8], G3P[8], G4P[8], G9P[8], G8P[8], G1P[4], and G4P[4].

The predominant combination of P and G genotypes in the study population was G4P [8] in 60.2 % (n = 278) of cases followed by 12.1 % (n = 56) of G9P[8], 10.6 % (n = 49) of G2P[4], 6.5 % (n = 30) of G1P[8], 4.1 % (n = 19) of G3P[8], 3.5 % (n = 16) of G8P[8], and 2.6 % (n = 12) of G2P[8]. One can consider G1P[4] the rarest genotype in this study found only in 0.2 % (n = 1) of cases, as well as G4P[4] that was detected only in one sample and accounting for 0.2 % (n = 1) of the total number of respondents.

Analysing the results leads to a slight trend for genotype differences in children younger than 5 years and older than 5 years of age, although no statistically significant difference was found ( $p = 0.27$ ). Up to the age of 5 years, G4P[8] prevails at 61.0 % ( $n = 263$ ), followed by G9P[8] at 12.4 % ( $n = 53$ ), and G2P[4] at 10.0 % ( $n = 43$ ). In children older than 5 years, the most common genotype is G4P[8] in 45.0 % ( $n = 15$ ) of cases, while the prevalence of other genotypes is different in this age group, followed by G2P[4] in 18.0 % ( $n = 6$ ) of cases and equally common G9P[8], G3P[8], and G1P[8] in 9.1 % ( $n = 3$ ) of cases respectively (Figure 2.5).



**Figure 2.5 Distribution of Rotavirus genotypes in children younger than 5 years and older than 5 years of age**

A more detailed analysis of the correlation between age groups and a particular genotype did not reveal a statistically significant correlation ( $p = 0.89$ ), although, interestingly, the only case of the disease caused by G1P[4] was in the age group up to 12 months and the only case caused by G4P[4] in the age group 13–24 months.

G4P[8] genotype prevails in all age groups by constituting 63.1 % (n = 89) in the age group up to 12 months, 56.0 % (n = 179) in the age group 13–24 months, 61.5 % (n = 48) in the age group 25–36 months, and 60.8 % (n = 62) in paediatric patients aged  $\geq 37$  months.

However, after processing the data of the study, statistically significant correlations were found with the circulation of different genotypes among the study years.

Stable statistically significant trends are observed for G4P[8], G8P[8], G9P[8] genotypes. There is a clear statistically significant correlation between the growing incidence of G4P[8], G8P[8] genotype caused disease from 2013 to 2015 ( $p < 0.001$ ). In its turn, the number of cases caused by G9P[8] genotype has statistically significant decrease ( $p < 0.001$ ) between 2013 and 2015. Similarly, statistically significant correlations were observed between different genotypes of different years (G2P[4], G3P[8]), but the finding has no value considering the fluctuations of the obtained results within one genotype from year to year.

Some statistically significant correlations were found between circulating genotypes and clinical symptoms. For example, a statistically significant positive correlation was found for the G8P[8] genotype with symptoms of dehydration (more intense signs of dehydration were observed in children with the disease caused by this genotype) and fever (higher temperature was recorded than in the event of other genotypes) ( $\rho = 0.167$ ;  $\rho = 0.142$ ;  $p < 0.05$ ). In its turn, G3P[8] was statistically positively correlated with the duration of diarrhoea in days (duration of diarrhoea in days is longer than in other genotypes) ( $\rho = 0.150$ ;  $p < 0.001$ ).

The finding of a statistically significant correlation between the number of vomiting episodes per 24 hours and individual genotypes should be stressed (Table 2.1).

Table 2.1

**Virus genotype coherence with vomiting episodes**

			Vomiting episodes per 24 hours				Total
			0	1	2-4	5+	
<b>Genotype</b>	G1P[4]	Count	0	1	0	0	1
		Genotype (%)	0.0	100.0	0.0	0.0	100.0
	G1P[8]	Count	0	14	11	4	29
		Genotype (%)	0.0	48.3	37.9	13.8	100.0
	G2P[4]	Count	0	12	20	15	47
		Genotype (%)	0.0	25.5	42.6	31.9	100.0
	G2P[8]	Count	0	4	6	2	12
		Genotype (%)	0.0	33.3	50.0	16.7	100.0
	G3P[8]	Count	0	1	8	7	16
		Genotype (%)	0.0	6.2	50.0	43.8	100.0
	G4P[4]	Count	0	1	0	0	1
		Genotype (%)	0.0	100.0	0.0	0.0	100.0
	G4P[8]	Count	2	46	121	84	253
		Genotype (%)	0.8	18.2	47.8	33.2	100.0
	G8P[8]	Count	0	2	4	10	16
		Genotype (%)	0.0	12.5	25.0	62.5	100.0
	G9P[8]	Count	0	8	33	15	56
		Genotype (%)	0.0	14.3	58.9	26.8	100.0
<b>Total</b>		Count	2	89	203	137	431
		Genotype (%)	0.5	20.6	47.1	31.8	100.0

According to data summarized in Table 2.1, genotype combinations were analyzed, to determine genotype pairs with statistically significant difference according to amount of vomiting episodes (Table 2.2).

Table 2.2

**Determination of genotype pairs according to amount of vomiting episodes**

	G1P [4]	G1P [8]	G2P [4]	G2P [8]	G3P [8]	G4P [4]	G4P [8]	G8P [8]	G9P [8]
<b>G1P[4]</b>		p = 1.00	p = 0.27	p = 0.54	p = 0.12	–	p = 0.19	p = 0.18	p = 0.16
<b>p value correction</b>		p = 1.00	p = 0.45	p = 0.63	p = 0.37	–	p = 0.37	p = 0.37	p = 0.37

Table 2.2 continued

	G1P [4]	G1P [8]	G2P [4]	G2P [8]	G3P [8]	G4P [4]	G4P [8]	G8P [8]	G9P [8]
G1P[8]			p = 0.08	p = 0.65	<b>p = 0.01</b>	p = 1.00	<b>p = 0.004</b>	<b>p = 0.002</b>	<b>p = 0.003</b>
<i>p</i> value correction			p = 0.37	p = 0.70	<b>p = 0.09</b>	p = 1.00	<b>p = 0.047</b>	<b>p = 0.047</b>	<b>p = 0.047</b>
G2P[4]				p = 0.65	p = 0.25	p = 0.27	p = 0.66	p = 0.10	p = 0.20
<i>p</i> value correction				p = 0.70	p = 0.43	p = 0.43	p = 0.70	p = 0.37	p = 0.37
G2P[8]					p = 0.14	p = 0.54	p = 0.36	p = 0.06	p = 0.28
<i>p</i> value correction					p = 0.37	p = 0.63	p = 0.53	p = 0.35	p = 0.43
G3P[8]						p = 0.12	p = 0.54	p = 0.43	p = 0.48
<i>p</i> value correction						p = 0.37	p = 0.63	p = 0.61	p = 0.63
G4P[4]							p = 0.19	p = 0.18	p = 0.16
<i>p</i> value correction							p = 0.37	p = 0.37	p = 0.37
G4P[8]								p = 0.13	p = 0.54
<i>p</i> value correction								p = 0.37	p = 0.63
G8P[8]									<b>p = 0.03</b>
<i>p</i> value correction									<b>p = 0.21</b>
G9P[8]									

The results obtained show that G1P[8] genotype has statistically significant differences in the number of vomiting episodes compared to G3P[8], G4P[8], G8P[8], and G9P[8] genotypes. Statistical reliability remained after correction of *p*-value for most of the results. Sequential analysis of which groups of vomiting were distinct among the pairs of particular genotypes was carried out.

- In patients with proven G4P[8] genotype, the incidence of vomiting “2 to 4 times” and “5 and more times” are 3.4 (OR 3.4 (95 % CI 1.4–7.9); *p* = 0.001) and 6.4 (OR 6.4 (95 % CI 2.0–20.6); *p* = 0.001) times more common than for G1P[8] genotype respectively compared to the number

of vomiting “once”. In particular, one can conclude that a higher incidence of vomiting is characteristic for patients with G3P[8] infection than for patients with G1P[8] infection.

- In patients with infection caused by G8P[8], the incidence of vomiting “5 times or more” is 17.5 (OR 17.5 (95 % CI 2.7–114.8);  $p = 0.001$ ) times more common than the one caused by G1P[8] compared to vomiting “once” and 6.9 (OR 6.9 (95 % CI 1.3–35.1);  $p = 0.02$ ) times more common than vomiting “2 to 4 times”. It means that patients with G8P[8] genotype-associated infection have a particularly high incidence of vomiting, whereas patients with G1P[8] genotype associated infection have a less pronounced symptom of vomiting.
- Similarly, for the G9P[8] genotype-associated disease, the incidence of vomiting in the group “2 to 4 times” and “5 and more times” was 5.3 (OR 5.3 (95 % CI 1.7–15.8);  $p = 0.002$ ) and 6.6 (OR 6.6 (95 % CI 1.6–26.7);  $p = 0.006$ ) more common than for the G1P[8] genotype compared to the number of vomiting “once”. It means that a higher incidence of vomiting is characteristic for the patients with the G9P[8] caused infection than for the patients with the G1P[8] genotype associated infection.

No other statistically significant correlations were obtained by correlating the data on Rotavirus genotypes and clinical manifestations.

## **2.2 Results II**

This part summarises the results about all the patients enrolled in the study ( $n = 527$ ) and analyses demographic, socio-epidemiological data, and data on the emotional, social, and economic burden, health-related quality of life of the patient and the parents, and the factors associated to it.

## **2.2.1 Demographic characteristics of study subjects and their parents**

The average age of the patients was 26.1 months, and the distribution by gender of the study population was similar, with 51.0 % (n = 269) of boys and 49.0 % (n = 258) of girls. Most parents are in the age group of 25 to 34 years. This age group includes 63.8 % (n = 335) of mothers and 55.1 % (n = 281) of fathers. There was a marked difference in the level of education among the parents, with 58 % (n = 304) of mothers having higher education and 36.2 % (n = 189) of mothers having secondary/vocational education. Majority of fathers had secondary vocational and higher education, 48.7 % (n = 245) and 44.1 % (n = 222), respectively. The place of residence of the study population indicates that 87.2 % (n = 449) of study participants live in urban areas and 12.8 % (n = 66) live in rural areas. Social status of the family was defined following Cabinet Regulation No 229, stipulating the status of low-income family where the income did not exceed 128.09 EUR per family member per month (Cabinet Regulation No 229, 2014). Most of the families included in the study were socially insured (94.6 %; n = 491), while 5.4 % (n = 28) had the status of low-income family.

## **2.2.2 Objective and subjective assessment of the health of a child**

The Vesikari scale (Vesikari, 2011) was used to determine severity of the patient's disease. It is essential that 93.0 % (n = 493) of children had severe disease, and only 7.0 % (n = 35) of children had moderate disease. No mild disease condition was identified in any patient.

In the results where parents assessed the severity of the symptoms of their child subjectively, some trends emerged, that is, 53.3 % (n = 280) of the parents rated the diarrhoea symptom of their child as very severe, followed by insufficient hydration, what 49.6 % (n = 259) of the parents noted as very severe.

Lack of appetite was rated as very severe by 41.5 % (n = 215) of the parents. Vomiting, fever, and abdominal pain were rated as a severe symptom by approximately 30 % of the parents for each symptom separately.

### **2.2.3 Assessing the impact of the emotional, social, and economic burden associated with the disease on the quality of life of the family**

When analysing the results of this part, compassion stood out as the most pronounced emotional factor, which the parents marked as very severe in 76.4 % (n = 402) of cases, followed by agitation in 59.6 % (n = 311) of cases, and stress/anxiety in 37.8 % (n = 199) of cases. Changes in daily plans (work, study, leisure, and household-related plans) were chosen as the social burden criterion, and 79 % (n = 413) of the parents noted that they had to make changes in this regard.

The economic burden of the disease was assessed concerning absenteeism of parents, where only 33.1 % (n = 173) of them noted that they had not been absent due to the child's disease. Other respondents had to miss work for 1–5 days. Here, the percentage breakdown is similar when analysing the number of days. Extra costs associated with the disease episode was another factor in assessing the economic burden, and 75.2 % (n = 380) of the families had additional costs associated with paying for medication, hygiene products, cleaning products, or babysitting services.

### **2.2.4 Relationship of factors to the impact of the disease on the quality of life of the family**

#### ***Emotional burden***

To measure the impact of the emotional burden associated with the disease, the author selected three indicators that were reported most frequently

after compiling the results, namely: stress/anxiety, worry and compassion. For better data interpretation, the categories “severe” and “very severe” were combined, and the categories “mild” and “not at all” were combined.

The first of those indicators to be analysed was “compassion” resulting in the fact that none of socio-demographic, objective and subjective factors of child’s health appeared to be significantly associated with the emotional burden of RV infection. Among those indicators, only the level of education of a father and compassion have a statistically significant correlation. Namely, the higher the level of education of the father, the more often the level of “high” or “very high” compassion is marked ( $p = 0.01$ ). By adjusting the p-value, the statistical reliability of the result is lost.

A statistically significant correlation was found between stress/anxiety and fever. Namely, the more pronounced the fever was, the higher the level of stress/anxiety, the caregivers of the child felt ( $p = 0.02$ ). Similarly, a statistically significant correlation was found between stress/anxiety and the child’s degree of irritability and tearfulness, i.e., the more annoyed or severely tearful a child was, the more often caregivers noted high or very high levels of stress/anxiety ( $p < 0.001$ ).

Worry was analysed as the last indicator in this group, and as before, a statistically significant correlation was found between worry and tearfulness of the child. The more pronounced this feature was in the child, the proportionally more worried the caregivers of the child were ( $p < 0.007$ ). High levels of child anxiety/irritability also caused greater anxiety in the parents ( $p < 0.002$ ).

None of the emotional burden indicators showed a statistically significant correlation between the objective variables of the child’s health condition and the majority of the subjective variables of the health condition.

## ***Social burden***

To assess the social burden, the caregivers expressed their views on the changes in their daily routine associated with the illness episode (work, study, leisure, and household-related plans).

Interestingly, no statistical correlation was found between the need for the child's caregiver to change the daily routine and the objective indicators of the child's health condition. However, one should indicate a statistically significant correlation between social burden and various socio-demographic factors. For example, the older the child ( $p < 0.001$ ), the older the mother ( $p < 0.001$ ), and the higher the mother's level of education ( $p < 0.001$ ), the proportionally larger number of caregivers have had to change their daily routine (sporting, educational, cultural events/activities). Statistical reliability remained after correction of the p-values. A statistically significant correlation was found with the age of the father ( $p = 0.03$ ), reliability is lost when correcting the p-value.

Only fever and insufficient hydration correlated statistically significantly with the social burden of RV infection out of all the subjective indicators of the child's health conditions analysed. Correspondingly, if the child had a more explicit fever ( $p = 0.02$ ) or more pronounced dehydration ( $p = 0.04$ ), the caregivers had to change their daily routine more frequently.

Statistical reliability is lost here as well when correcting the p-value.

## ***Economic burden***

The results of the factors affecting the economic burden of RV infection are described below, where parents noted absenteeism in days, financial loss related to absenteeism, and other additional expenses.

Interesting results were obtained here as well because none of the objective indicators of the child's health conditions has influenced the workflow of the parents. Only two of the socio-demographic factors, i.e., the older the child

( $p = 0.01$ ) and the higher education of the mother ( $p = 0.02$ ), correlated statistically significantly with the need of caregivers to be absent from work for at least 1 day. Out of all the subjective indicators of the child's health condition analysed in this study, only dehydration ( $p = 0.02$ ) and gluteal inflammation ( $p = 0.03$ ) correlated statistically significantly with the economic burden associated with RV infection, namely, missed working days. One must note that adjusting the p-value resulted in lost statistical reliability for all associated factors.

It is important to mark that 75.2 % ( $n = 380$ ) of the parents have mentioned additional expenses related to their child's disease. Parents were asked to answer what type of expense was associated with the disease episode by choosing one or more options (purchase of medication, patient co-payment, costs of the parents for staying at the hospital, purchase of cleaning agents and disinfectants, purchase of personal hygiene items, babysitting services, and travel expenses).

### 3. Discussion

Acute diarrhoea is considered one of the most common causes of morbidity and mortality in children under 5 years of age (Florez et al., 2020; Hosny et al., 2019). Viral aetiology is leading in the gastroenteritis group in both developing and developed countries (Poelaert et al., 2018).

Group A Rotaviruses can cause severe gastroenteritis and are characterised by the ability to induce manifestations of extraintestinal infection not only in immunosuppressed individuals but also in immunocompetent individuals (Hosny et al., 2019). Despite the positive effect of specific prevention on morbidity and mortality rates, research of Rotaviruses is ongoing for both epidemiological and molecular biological purposes (ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017).

In Latvia, infant vaccination against Rotavirus was introduced in the NIP in January 2015. This study was initiated in the pre-vaccination period and continued for the first year after the introduction of vaccination in the NIP. According to CDPC data, the incidence of Rotaviral infection has been decreasing in Latvia in recent years. While the number of reported cases reached 150.5 cases per 100,000 population in 2015, it was 79.3 cases in 2017, and 63.1 cases per 100,000 population in 2018.

It should also be noted that no deaths due to Rotaviral infection have been detected in Latvia between 2013 and 2018 (CDPC, 2019).

If global and local statistics demonstrate the efficiency of vaccination in reducing mortality and morbidity clearly, the question arises as to why so much research is continuing in this area. Despite the research work already done, there is still a lack of clarity about the mechanisms of pathogenesis, replication, and immune response in Rotaviral infection. In each of the literature sources used in this research, one can find a phrase about the need for further research to identify,

clarify, understand, which indicates the need for further research in this area explicitly.

In Latvia, the movement of anti-vaccine advocates has been active in recent years by provoking discussions in social media such as radio, television, and internet portals, where no evidence-based information is provided. This places an additional burden on healthcare staff in convincing parents of the need for vaccination, as well as an unreasonable emotional burden on the parents who must decide on immunisation of their child.

The economic burden of Rotaviral infection can be assessed both from a family perspective and from a national perspective. One should emphasise the care of the emotional, social, and economic well-being of the family primarily, hence the health-related quality of life in general. However budget allocated to healthcare in Latvia, should be spent for the disease groups, where prevention is not possible.

To date, no studies have been conducted on clinical peculiarities, molecular epidemiology of Rotaviral infection and their impact on the quality of life of the child and family members in Latvia.

The demographic data collected in this study show that there is no significant difference between the genders in the case of Rotaviral infection because boys and girls have fallen ill equally often. The average age of children coincides with the average age of children indicated in other studies, namely, most patients in the age group up to 5 years with a peak in the age group below 2 years. A prospective multicentre research conducted in various European countries (Belgium, France, Germany, Italy, Spain, Sweden, and United Kingdom) found similar results for the age of children, that is, the highest number of patients were up to 5 years old with a peak in the age group from 6 to 23 months (Burnett et al., 2020; Van Damme et al., 2007; Randy et al., 2018; Shane et al., 2018; D'Souza et al., 2008). Globally, one faces the highest mortality rates

in this age group, so scientific efforts for full protection focus on this age group in particular (Burnett et al., 2020).

Given the immunisation status of the study population, that is, only 2.2 % (n = 10) of the children in the study group were vaccinated, one can possibly explain a predominantly severe general condition, defined according to the Vesikari severity scale in 87.0 % (n = 402) of cases. However, one must indicate that this study cannot be generalised, as only hospitalised patients were included in the study, which is considered as one of the limiting factors of the study. In general, it has been reported in the literature that unvaccinated individuals, with the first and second episodes of Rotaviral infection, are more likely to develop severe disease, as cross-type immunity develops naturally during the second episode to protect against serious forms and complications of the disease (Vesikari et al., 2008; Vesikari, 2012; ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017). A similar study assessing the severity of RV disease in hospitalised children was conducted in Estonia in 2012 (Soeorg et al., 2012). Comparatively, patients in the Estonian study had predominantly moderate disease in 76.9 % of cases and mild disease in 14.7 % of cases. In its turn, only 7.6 % of patients had severe disease. The severity of the disease was assessed, thereby using the Clark's Severity Grading Scale, which differs from the study carried out in Latvia. The use of different scales, as well as the human factor (clinical experience, accuracy, time taken to evaluate the general condition of the patient), may be responsible for different results partially.

Patients in the Doctoral research study were hospitalised on 2.5 days of disease at an average, which coincides with the peak period of symptoms reported in the literature (Shane et al., 2017). The author remarks that Rotaviral infection is not a strictly seasonal disease according to the results of this study. The circulation of the virus is observed throughout the year with a trend to increase during winter months. A similar seasonal circulation of the virus is also observed in other climatically similar countries in the world (Patel et al., 2013).

If interpretation of the severity of the disease cannot be attributed to the overall nationwide population of RV infection, as only hospitalised patients participated in the study, one can discuss the most commonly circulating genotypes in Latvia when interpreting the results of molecular epidemiology, because no other studies have been conducted.

Molecular epidemiology data for RV patients hospitalised to CCUH are not significantly different compared to other European countries. According to EuroRotaNet data, the six dominant genotypes of serogroup A G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] cause 90 % of Rotavirus cases in the European Union/European Economic Area and globally (EuroRotaNet, 2018). One has proven that G1P[8] has been a predominant genotype before introduction of vaccination (G1P[8] is included in the RotaRix monovalent vaccine), but all six genotypes mentioned above have been found in all countries studied (EuroRotaNet, 2018). Genotypes were detected by molecular biological methods in the children hospitalised in CCUH between 2013 and 2015, with 87 % (n = 402) causing severe and 6.9 % (n = 32) causing moderate RV infections. In total, nine combinations of the P and G genotypes were identified: G1P[8], G2P[4], G2P[8], G3P[8], G4P[8], G9P[8], G8P[8], G1P[4], and G4P[4], of which G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] coincide with European and World Registry data. One must remark that the G4P[8] genotype prevailed in this study, which caused the disease in 60.2 % (n = 278) of cases. The study did not record any G12P[8] associated case that was discovered in all countries under surveillance for molecular epidemiology of RV (ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017). Perhaps, an explanation may be found with non-genotyped samples in the event of this study, which has reached 7.0 % (n = 35) and is not considered an incomplete result. Evidence-based literature reports varying percentages of non-genotyped or partially genotyped cases. For example, in a multicentre study involving five European countries, genotype all P types were possible, but one study carried out in Italy failed to genotype 38.2

% of cases (Forster et al., 2012; Ansaldi et al., 2007). Other Central and East European countries report between 0.6 % and 13.7 % of cases without determination of genotype (Ogilvie et al., 2011). They mention genetic mutations, reduced efficiency of reverse PCR due to mismatched primer sequences, and practical laboratory performance as the reasons able of influencing the process (Kühne et al., 2008; Ogilvie et al., 2011).

It should be noted that in other European countries, where they carried out similar studies, they have detected the genotypes that are proven in our study, but with different prevalence. For instance, G2P[4] prevailed in the study conducted in Estonia before the introduction of vaccination, followed by G4P[8], whereas G1P[8] genotype followed in the United Kingdom, Spain, Sweden, and Slovenia (EuroRotaNet 2018; Soeorg et al., 2012).

The discovery of “non-dominant” genotypes in Europe and worldwide, such as in the current study G2P[8], G8P[8], G1P[4], and G4P[4] can be possibly explained by the development of new genotypes in co-infection with two different RV genotypes, as well as through the transmission of infections from animals, or through the interaction of RV genotypes pathogenic to animals and humans (ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017). One has observed similar situations in the studies of other countries, where the dominant serotype has changed from year to year, which might not be found among the more common serotypes circulating in Europe or worldwide (Steyer et al., 2014; Almalki, 2018). However, those changes do not affect the efficacy of immunisation boosted by Rotavirus vaccines (Dornbusch et al., 2020; ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017).

It is also essential to mention that prevalence of specific genotypes has been observed in the seasonal prevalence of RV genotypes that vary from year to year, and no difference has been found between countries, where immunisation is introduced or is not introduced (ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017).

Clinical trials in Europe and the United States of America have demonstrated that the immunity conferred by the commonly used vaccines RotaRix (RV1) and RotaTeq (RV5) is efficient against genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] in the case of RotaRix and to G12P[8] in addition in the case of RotaTeq (EMA, 2018).

The author would like to emphasise that worldwide studies have proven the development of cross-sectional type immunity also against other genotypes that are rare in Europe. In the studies conducted in Malawi, South Africa, and Ghana researching both vaccines mentioned above, vaccine efficacy ranged from 49.0 % to 76.9 %, which is considered as a proof of a cross-type protection hypothesis. One stresses that those geographical regions have the most diverse genotype variability and the G1P[8] genotype is found only in 13.0 % of cases (Armah et al., 2010; Madhi et al., 2010). Although the data obtained in those studies demonstrate the development of cross-type immunity, the level of vaccine efficacy is considered incomplete (Clark et al., 2019). Latest studies demonstrate more and more that vaccination efficacy is not solely dependent on circulating genotypes but on the presence of contiguous factors as well like the presence of morphological blood cell antigens and secretory IgA activity (Clark et al., 2019; EuroRotaNet, 2018, ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017). It is important to outline that despite advances in Rotavirus prevention, an open question remains about the development of full-fledged protection in regions of the world, where vaccine efficacy does not achieve the desired results due to the reasons that have not been elucidated in full yet. Besides, with the reducing global burden caused by Rotaviruses thanks to the opportunity of immunisation, the burden caused by Noro viral infections is taking its place. For this reason, global vaccine experts are working on a new vaccine model that will be a Noro-Rota virus combined parenteral vaccine (Glassab et al., 2018).

The sudden onset of the disease and the rapid cascade of symptoms typical of RV infection can potentially affect the daily routine of the family and subsequently lead to unexpected daily changes by affecting the social and emotional well-being of the family directly. A family with a child is considered a whole, and any subjective or objective disease-related dysfunction affects the quality of life in the family (Spieth et al., 1996; Van der Wielen et al., 2012). Evidence-based literature describes relatively little studies on the impact of acute diseases, including Rota viral infection on the quality of life of the family (Van der Wielen et al., 2012; Diez et al., 2012; Mast et al., 2009). Health-related quality of life has been studied with chronic diseases more frequently (Goldbeck et al., 2005; Grootenhuis et al., 2007; Matza et al., 2004; Bullinger et al., 2003). The results summarised in this study demonstrate that acute disease can have an adverse effect on the family and cause a social, emotional, and economic burden although some of the results lost statistical reliability in the result of correcting the p-value. When describing the study group in general, where one analysed the emotional burden, one can say that the parents of children have felt unhappy by feeling high levels of stress and anxiety, as well as physically exhausted and helpless. Similar results can be found in other studies on the impact of the Rotaviral infection on the family where parents experience the same emotions (Van der Wielen et al., 2012; Diez et al., 2012; Vesikari, 2011; Huppertz et al., 2008). In addition to the emotional burden, the care givers of the patients in this study have also faced the need to change their daily routine similar to the study on the social burden of RV infection in the United States (Mast et al., 2009).

The study provides evidence of the economic burden caused by Rotaviral infection as 55.3 % of the parents had to be absent from work and 75.2 % of the parents surveyed had to spend extra money on the disease of their child similar to the studies carried out in other countries (O'Brien et al., 2015; Mast et al., 2009). The older the child, the proportionally higher number of parents have been absent from work for one day at least. This may be due to the parental benefit in

Latvia up to 1 year of child's age. Many parents whose children have reached the age of one year are employees, and they had to take a sick-leave certificate. In most cases, absenteeism of at least 1 day was required for mothers with higher education, which possibly is associated with job specificities, sense of duty and responsibility, and potentially better social security.

Although detailed factor analysis regarding the economic burden does not show statistically significant correlations, the overall economic burden of Rota viral infection is undeniable after adjusting the p-values. Given the lack of research in Latvia on health-related quality of life of the family in the paediatric population until now, the need for further research in this area is evident.

One still considers that further research is required to identify the factors and their interconnections that would enable a comprehensive view of the impact of acute gastroenteritis on health-related quality of life (Johnston et al., 2013).

In this study, stress/anxiety, worry, and compassion were the most common emotions that the parents experienced during the acute illness of their child. Based on the subjective assessment of the general condition of the child, a correlation emerged that the higher fever, the more pronounced tearfulness and irritability the child, the higher the level of stress the parents had. Emotional reactions are socially determined to some extent (Von Scheve, 2014). Parental reaction to the symptoms of the child's illness and the following emotions can be viewed and interpreted from a cultural perspective. Perhaps, fever/high temperature in a child might be perceived as a common feature of phobia both among medical staff and parents in Latvia. Fever is often perceived as a risk associated with a potentially life-threatening condition and makes one addressing an emergency department of a hospital immediately, or is one of the most common causes of calling an ambulance. In 2018, 65,435 patients attended the CCUH Emergency department, of whom more than 43,000 patients had acute symptoms not related to trauma or surgical pathology. An acute disease accompanied by fever/high body temperature was among the most common

reasons. Only 9,000 patients out of the total patient population required for hospitalisation (CCUH, 2018).

Similarly, there is clear evidence from a retrospective study conducted in the CCUH, which analysed outpatient or inpatient case records of 1,921 fever patients. The results of that study demonstrate clearly that the lack of understanding of the symptoms of the disease as well as the inability to receive outpatient assistance place a heavy burden on the CCUH Emergency and Observation Department and the hospital in general (Balode et al., 2017). The summary of the results shows that the predominant cause of fever was related to viral aetiology and did not endanger the life of the child. A multifactorial lack of understanding of the causes of fever might make parents react with excessive levels of stress. One should also outline that individual and collective public views influence the assumption of how a healthy child should “look like” and “behave” (Barr, 2008), that is, tearfulness and irritability are not associated with the perception of a healthy child, and this is probably why those symptoms have caused higher levels of stress/anxiety and worry in the parents additionally.

One has analysed the multifactorial impact on the quality of life of the family associated with Rotaviral infection in an ethnographic study conducted in Taiwan and Vietnam in 2013, as well as in other multicentre studies comparing the parental emotions among the parents in Spain, Belgium, Italy, and other countries (O’Brien et al., 2015, Van der Wielen et al., 2012).

Taking into account the proof of the present, high emotional burden, one needs to raise awareness of the parents to promote successful understanding of the symptoms and the needs of the child during acute disease, which would reduce misunderstandings and individual emotional reactions (Betz et al., 2008). Successfully implemented a multidisciplinary approach to treatment of the patient and multidisciplinary communication with the parents are considered the cornerstone of social family support aimed at reducing the emotional burden during an acute disease (Levetown, 2008). The social burden is a vital component

of health-related quality of life. This study demonstrates that higher stress level of the mother and higher education of the mother have a statistically significant correlation with the need to change daily routine unexpectedly due to the child's disease. Perhaps one can explain that parents have employment commitments, more social responsibilities, and activities in the age group of 35–44 years old. It also correlates with the age of the child. Main reasons for changing daily routine include symptoms such as fever and dehydration. Here, it might be related to common cultural traits, the level of education of parents, and a common understanding of public health. As part of public health promotion, parents have access to vast information on tactics for dealing with a variety of health-related situations. For example, there are guidelines developed for parents and medical staff in the form of a step algorithm to prevent dehydration and fluid replacement (Zavadska et al., 2016).

This study had limiting factors that prevented attribution of the results to all RV AGE cases in childhood; since only hospitalised children with severe disease were included in the study, results did not apply to outpatient, milder cases of RV AGE.

Looking back at the results of the study, several strategically important findings stand out that enable highlighting the essential role of Rotaviral infection in the overall context of paediatric healthcare irrespective of the fact that only hospitalised patients have participated in the study. In general, the concern for the emotional, social, and economic well-being of the family must be stressed, hence, the health-related quality of life, but spending the public funds allocated to healthcare for the disease groups, where prevention is possible, is considered unjustified. The Rotavirus vaccine is included in the NIP in Latvia since January 2016. It is understood that all infants in the age group of 2 to 6 months can receive a state-funded vaccination against Rotaviral infection. Apart from the proof in the study on the multifaceted burden associated with Rotaviral infection, other equally important factors should be taken for granted to facilitate

the development of broader vaccination coverage in the country. One finds significant to highlight the effect of collective immunity, which protects the part of population that cannot be vaccinated (immunosuppressed patients, neonates, and infants under 2 months of age) (Dornbusch et al., 2020; EuroRotaNet, 2018). The role of vaccination in limiting the spread of nosocomial RV infection is also important. In this study, 9.5 % (n = 44) of RVs were reported as inpatient infections. According to the EuroRotaNet summary, Rotaviral infection as a hospital infection occurs in 25–30 % of cases in the European hospitals during the “peak season” of the virus. It affects patients with chronic secondary conditions more often, which worsens the general condition of the patient, prolongs the time spent in hospital, and increases the total cost of hospitalisation (Badur et al., 2019; ECDC, *Expert Opinion on Rotavirus Vaccination in Infancy*, 2017). As a result, healthcare personnel providing infant vaccination and the public at large must undertake the responsibility for partially unjustified spending of the funds allocated to healthcare and the overall outcome of healthcare.

Given the cost of inpatient treatment of one Rotavirus clinical case, which averages 1,013 euros, vaccination against Rotaviral infection is considered efficient prevention.

When emphasising the family-oriented model of healthcare, a clear need to work even more intensively emerged (using the results of evidence-based research) by cooperating among competent healthcare professionals and public media in raising awareness of the families on the possible prevention against Rotaviral infection. The results of this study shall be used to inform the public, by notifying the Latvian public on the total burden associated with the Rotaviral infection on the health sector.

## Conclusions

1. RV infection is more common in children under five years of age, with an emphasis on the age group below two years. There were 92.9 % (n = 429) of patients under 5 years of age, with 61.0 % (n = 282) of patients up to 2 years of age, that indicates a high risk of developing the severe disease in this patient population.
2. Proven correlation between the severity of disease and the need for hospitalisation. Severe disease was reported in 87 % (n = 402) of patients (p < 0.001) coinciding with the studies in other countries carried out before the introduction of vaccination by using the Vesikari severity grading scale.
3. There are no statistically significant differences in the correlation between the visit to a General Practitioner and the need for hospitalisation. 48.3 % (n = 223) of the patients visited the GP before hospitalisation and 48.9 % (n = 226) of the patients did not seek the help of a GP, which indicates the peculiarities of Rotaviral infection and trend of severe disease manifestation in non-vaccinated children.
4. No local circulating RV genotypes were found in the study, and no significant differences were found in the molecular epidemiology of Rotaviruses in the CCUH inpatient population compared to other European countries with similar studies. Namely, isolated combinations of Rotavirus genotypes, that is, G1P[8], G2P[4], G2P[8], G3P[8], G4P[8], G9P[8], G8P[8], G1P[4], G4P[4] with overwhelmingly dominant G4P[8] genotype – are similar to other European genotype combinations prior to the introduction of the Rotavirus vaccine in the National Immunisation Programme.
5. A statistically significant correlation was found between a particular genotype and the frequency of vomiting symptoms. Genotype G1P[8] has statistically significant differences in the number of vomiting episodes compared to genotypes G4P[8], G8P[8], and G9P[8]. Similarly, a statistically

significant positive correlation was found between the G8P[8] genotype and signs of dehydration and febrility ( $p < 0.05$ ), and G3P[8] was statistically positively correlated with the duration of diarrhoea in days ( $p < 0.001$ ).

6. During the study, no statistically reliable evidence was found for the association of Rotavirus molecular biology with the severity of the disease. Severe disease progression was observed in 87.0 % ( $n = 402$ ) of the patients ( $p < 0.001$ ), and the results showed no statistically significant correlations with specific genotypes.
7. Having summarised data from the study, statistically significant results were obtained for the correlation of Rotaviral infection with the emotional and social burden ( $p < 0.05$ ).
  - Compassion was most pronounced among the emotional factors, which parents marked as very severe 76.4 % ( $n = 402$ ) of cases, followed by agitation in 59.6 % ( $n = 311$ ) of cases, and stress/anxiety in 37.8 % ( $n = 199$ ) of cases. A statistically significant correlation was found between the level of stress/anxiety and the child's level of irritability and tearfulness ( $p < 0.001$ ).
  - The social burden indicator, i.e., the need for changes in daily routine (work, study, leisure, and household) was noted by 79 % ( $n = 413$ ) of caregivers of the child. There was a statistically significant correlation proved between the age of the child, age of the mother, and educational attainment of the mother ( $p < 0.001$ ).
  - Absenteeism was chosen as an indicator for assessing the economic burden, and no statistically reliable results were obtained that would be correlated to the economic burden associated with RV infection, namely, the absenteeism in days.
8. All the genotypes isolated in the result of the study that caused the disease correspond to the prevention possibilities with the Rotarix and RotaTeq vaccines available in Latvia. The overall results of the study demonstrate

clearly that vaccination with the vaccines used in Europe is an evidence-based method of prevention.

## **Practical recommendations**

1. Given the overall burden of Rotaviral infection, the results of the study demonstrate that vaccination against Rotaviral infection is recommendable.
2. Multidisciplinary communication with parents about the disease and the sequence of symptom development is likely to reduce the psycho-emotional burden, thereby reducing the negative impact on health-related quality of life.

## Publications and reports of the author

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