VILNIUS UNIVERSITY

INGA IVAŠKEVIČIENĖ

PARALELLS OF MOLECULAR EPIDEMIOLOGY AND CLINICAL FEATURES OF ROTAVIRUS INFECTION IN CHILDREN

Summary of doctoral dissertation
Biomedical sciences, medicine (06B)

Vilnius, 2014

The dissertation has been prepared at Vilnius University in 2008 – 2014

Scientific superviser – Prof. Habil. Dr. Vytautas Usonis (Vilnius University, Biomedical Sciences, Medicine – 06B).

The Doctoral Dissertation will be defended at the Medical Research Council of Vilnius University:

Chairman – Prof. Dr. Janina Tutkuvienė (Vilnius University, Biomedical Sciences, Medicine – 06B).

Members:
Prof. Dr. Ron Dagan (Ben-Gurion University of the Negev, Israel, Biomedical Sciences, Medicine – 06B)
Dr. Audronė Eidukaitė (State Research Institute Centre for Innovative Medicine, Biomedical Sciences, Medicine – 06B)
Prof. Dr. Regina Ėmužytė (Vilnius University, Biomedical Sciences, Medicine – 06B)
Prof. Habil. Dr. Rūta Dubakienė (Vilnius University, Biomedical Sciences, Medicine – 06B)

Opponents:
Prof. Dr. Timo Vesikari (University of Tampere, Finland, Biomedical Sciences, Medicine – 06B)
Prof. Dr. Augustina Jankauskienė (Vilnius University, Biomedical Sciences, Medicine – 06B)

The dissertation will be defended at the open session of the Medical Research Council of Vilnius University at 2:00 p.m. on 27th of October, 2014 in the Auditorium of Children's Hospital, Affiliate of Vilnius University Hospital Santariškių Klinikos.

Address: Santariškių 7, LT-08406 Vilnius, Lithuania.

The summary of this doctoral dissertation was mailed out on the 26th of September, 2014.

This dissertation is available in the Library of Vilnius University.

VILNIAUS UNIVERSITETAS

INGA IVAŠKEVIČIENĖ

VAIKŲ ROTAVIRUSINĖS INFEKCIJOS MOLEKULINĖS EPIDEMIOLOGIJOS IR KLINIKOS PARALELĖS

Daktaro disertacija
Biomedicinos mokslai, medicina (06 B)

Vilnius, 2014

Disertacija rengta 2008 – 2014 metais Vilniaus universiteto Medicinos fakultete.

Mokslinis vadovas – prof. habil. dr. Vytautas Usonis (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B)

Disertacija ginama Vilniaus universiteto Medicinos mokslo krypties taryboje:

Pirmininkė:
prof. dr. Janina Tutkuvienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B)

Nariai:
prof. dr. Ron Dagan (Negevo Ben-Gurion universitetas, Izraelis, biomedicinos mokslai, medicina – 06B)
dr. Audronė Eidukaitė (Valstybinis mokslinių tyrimų institutas, Inovatyvios medicinos centras, biomedicinos mokslai, medicina – 06B)
prof. dr. Regina Ėmužytė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B)
prof. habil. dr. Rūta Dubakienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B)

Oponentai:
prof. dr. Timo Vesikari (Tamperės universitetas, Suomija, biomedicinos mokslai, medicina – 06B)
prof. dr. Augustina Jankauskienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B)

Disertacija bus ginama viešame Medicinos mokslo krypties tarybos posėdyje 2014 m. spalio mėn. 27 d. 14 val. Vaikų ligoninės Všį Vilniaus universiteto ligoninės Santariškių klinikų filialo didžiojoje auditorijoje.

Adresas: Santariškių 7, LT-08406 Vilnius, Lietuva

Disertacijos santrauka išsiuntinėta 2014 m. rugsėjo 26 d.

Disertaciją galima peržiūrėti Vilniaus universiteto bibliotekoje.

CONTENTS

ABBREVIATIONS 6
1. INTRODUCTION 7
   1.1. Background 7
   1.2. Scientific novelty 8
   1.3. European Rotavirus Network - EuroRotaNet 8
2. AIM AND OBJECTIVES OF THE STUDY 11
3. MATERIALS AND METHODS 12
   3.1. Clinical and demographical data of children hospitalised due to RVI 12
   3.2. Collection of faecal samples and rotavirus genotyping 13
   3.3. Genotyping data of Lithuania versus other EuroRotaNet participants 14
   3.4. Evaluation of potential RV vaccine effectiveness 15
   3.5. Statistical methods 15
4. RESULTS 16
   4.1. Common characteristics of study subjects 16
   4.2. Genotype distribution 16
      4.2.1. Single rotavirus strains 17
         4.2.1.1. Common rotavirus genotypes 17
         4.2.1.2. Other rotavirus genotypes 17
      4.2.2. Mixed rotavirus infections 17
      4.2.3. Partially typed rotavirus strains 17
   4.3. Comparison of Lithuanian and EuroRotaNet rotavirus genotyping data 24
      4.3.1. Single rotavirus strains 24
         4.3.1.1. Common rotavirus genotypes 24
         4.3.1.2. Other rotavirus genotypes 24
      4.3.2. Mixed rotavirus strains 25
      4.3.3. Partially typed rotavirus strains 25
   4.4. Evaluation of potential rotavirus vaccine effectiveness 26
   4.5. Evaluation of the clinical severity of the rotavirus disease 26
5. DISSCUSION 28
6. CONCLUSIONS 31
7. SUMMARY IN LITHUANIAN 32
8. REFERENCES 38
9. PUBLICATIONS AND SCIENTIFIC CONFERENCE REPORTS 43
10. BIOGRAPHY 46
11. ACKNOWLEDGEMENTS 50

Abbreviations

RV - rotavirus

RVA – group A rotavirus

RVI - rotavirus infection (the terminology of "rotavirus infection" in this study is used to define clinically pronounced disease)

RV1 – single-strain human rotavirus vaccine (Rotarix™)

RV5 – vaccine containing of five bovine-human reassortant rotaviruses (RotaTeq™)

ERN - European Rotavirus Network (EuroRotaNet)

UD – undetermined

SD - standard deviation

ICU – intensive care unit

1. INTRODUCTION

1.1. Background

Rotavirus is the most common etiological agent of childhood diarrhoea. It is estimated that almost every child is infected by rotavirus by the age of 5 years [1]. Rotavirus is estimated to cause a huge worldwide health and economic burden [2]. Although RV mortality rates are very low in developed versus developing countries, the burden of RV-associated disease is considerable in terms of pressure on healthcare services, financial impact and the impact on quality of life (both of affected children and their parents) [3-8].

Due to the absence of good reliable epidemiological studies the health burden of RVI in Lithuania is uncertain. Despite this absence the problem remains relevant as the morbidity rates associated with RVI are high (the prevalence rate of RVI in 2013 was 127,1 cases/100.000) [9].

The range of RVA strains circulating in the human population represents a huge diversity due to rotavirus potential to generate many different combinations of G and P proteins. It is understood that theoretically 800 different rotavirus genotypes may occur [10]. However, the number of different rotavirus strains is less in real life than in theory. In many parts of the world at least 27 different G and 37 P types have been identified so far and at least 12 G and 15 P types infect humans [11-13]. While a great diversity of rotaviruses is observed worldwide, five of the most common human genotypes (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) predominate in Europe and other industrialised countries [10, 14, 15]. In addition, G12P[8] genotype strains have been found to circulate in most parts of the world since the start of the new millennium, and this emerging G12P[8] might become the sixth major human RV genotype [12].

Epidemiological data of circulating RV strains are available from many countries of the world, whereas Lithuanian data until this study were undiscovered.

After recognition of the great diversity of rotavirus genotypes, clinical severity due to particular rotavirus strain generates huge interest for many scientists of the world. There are controversial data in scientific literature regarding the relationship between rotavirus disease severity and rotavirus genotypes; therefore the opportunity to use our genotyping data and to investigate the relationships with the clinical severity of the disease seemed provide a challenge.

Control of rotavirus infection is extremely important in order to reduce burden of the disease. Nonspecific prophylactic measures are very important, but it is demonstrated to have limited benefit for the prevention of rotavirus infection. New possibilities appeared after the introduction of two rotavirus vaccines (RV1 and RV5), which have demonstrated great efficacy results in preventing rotavirus disease caused by the five human rotavirus genotypes [16-24]. Due to the absence of rotavirus molecular epidemiology data in Lithuania it wasn't known if rotavirus vaccines registered in our country, as well as in many other European countries, will be effective to Lithuanian children. Epidemiological studies of circulating rotavirus genotypes in the neighbouring countries are also lacking.

The first attempt to get Lithuanian rotavirus molecular epidemiology data was started in 2005 when the first faecal samples of children sick with RVI were collected. Unfortunately, efforts to find laboratory partners, who could be able to genotype rotaviruses in Lithuania, were unsuccessful. The study was suspended. After a hard search of laboratory partners, the study was continued in 2007 as a part of the international project European Rotavirus Network –EuroRotaNet (refer to section 1.3). After joining the EuroRotaNet the first collection of faecal samples gathered in 2005/2006 was genotyped according to the united project protocol and has contributed to more comprehensive Lithuanian data.

1.2. Scientific novelty

Literature search in PubMed (US National Library of Medicine, national Institutes for Health) alone provides nearly 12,000 publications on different aspects of RVI, however there are only two articles published from Lithuania, analysing clinical peculiarities and treatment of RVI.

The absence of Lithuanian molecular rotavirus epidemiology data as well as unsolved discussion about relationship between rotavirus genotypes and clinical severity of the disease, necessitate the choice of present topic. This study is the first to provide representative data of circulating rotavirus genotypes in Lithuania.

Partnership with EuroRotaNet enabled not only the first data of rotavirus strains diversity in Lithuania, but also contributed to more comprehensive data of the circulating rotavirus strains in Europe. Lithuania was the only one out of all neighbouring countries which took part in this relevant project.

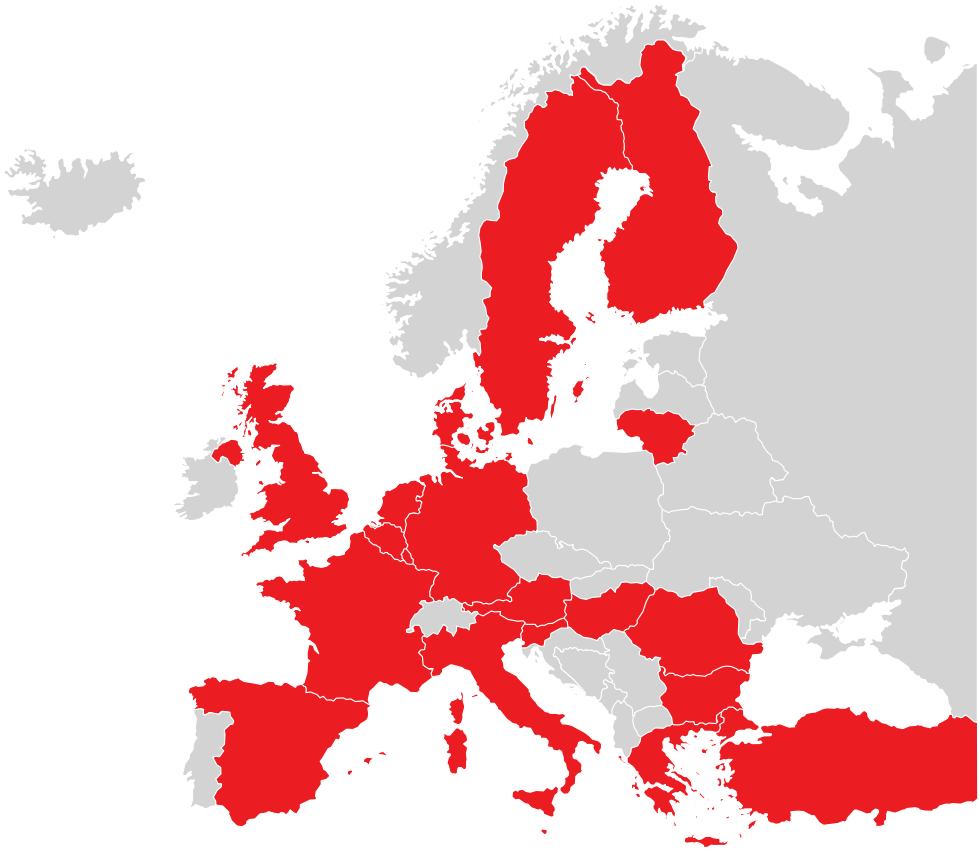
1.3. European Rotavirus Network - EuroRotaNet

The European Rotavirus Network (EuroRotaNet), a laboratory network, was established in 2007 in order to determine the diversity of co-circulating

rotavirus strains in Europe over consecutive rotavirus seasons [25]. The main goals of the network are (1) to develop methods and algorithms for effective rotavirus typing (G and P), (2) to describe in detail the molecular epidemiology of rotavirus infections in Europe, during consecutive rotavirus seasons, through genotyping of rotavirus-positive samples collected throughout each country; and (3) to monitor the emergence and spread of common and novel rotavirus strains within Europe. It is expected that comprehensive data on circulating rotavirus strains will help to evaluate the effectiveness of a rotavirus vaccines in the general population, through monitoring the reduction in disease associated with common rotavirus types, as well as to recognise the possible emergence of genotypes other than those included in the vaccine.

The project was led by Dr. Miren Iturriza-Gómara and Prof. Jim Gray, Health Protection Agency, London, UK. In 2007, the study area consisted initially of 11 European countries, including Denmark, Finland, France, Germany, Hungary, Italy, The Netherlands, Slovenia, Spain, Sweden, and the United Kingdom. An additional 3 European countries, Belgium, Bulgaria, and Lithuania, joined in 2008, and Greece and Romania joined in 2009 (Figure 1). Currently EuroRotaNet provides rotavirus strains surveillance in 18 European countries, encompassing approximately half the total European population.

Figure 1. EuroRotaNet participating countries (shown in red)



All collaborating countries provided rotavirus genotyping data during several RV seasons and uploaded it to the EuroRotaNet database. The sample size for each country was calculated according to the population size and expected cases of rotavirus disease per year. Strains circulating with a prevalence of less than 1% could have been identified with this sample size. This allowed comprehensive data on rotavirus diversity to be collected, not only for Europe as a whole, but also for each individual collaborating country. All faecal samples were analysed according to the standardised EuroRotaNet rotavirus genotyping algorithm, therefore the results of each participating country could be compared to each other.

Activities of EuroRotaNet started in 2007 and continue up to the present day. Currently the data from seven RV seasons have been collected. Project results are represented in two scientific publications [15, 26].

2. AIM AND OBJECTIVES OF THE STUDY

The aim of the study was to determine the diversity of circulating rotavirus genotypes and clinical features of rotavirus disease, caused by isolated genotypes.

Objectives:

1. To evaluate the diversity of circulating rotavirus genotypes, during different rotavirus seasons, by investigating faecal samples of children, hospitalised due to rotavirus disease.

2. To compare Lithuanian and EuroRotaNet rotavirus genotyping data.

3. To evaluate the potential rotavirus vaccine effectiveness in Lithuanian children.

4. To evaluate the relationship between isolated rotavirus genotypes and the clinical severity of the rotavirus disease.

3. MATERIALS AND METHODS

The present study was conducted at Vilnius University Children's Hospital (since 2011 - Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos) in collaboration with the Centre of Laboratory Diagnostics of Vilnius University Hospital Santariskiu Klinikos and Health Protection Agency, London, UK. The study covered seven RV seasons, since 2005 until 2013. Rotavirus seasonal year is defined as a period of time from September to August next year and as such does not correspond to the calendar year.

Clinical and demographical data of children hospitalised due to RVI were collected during the study. Faecal samples from these children were stored for rotavirus genotyping. Rotavirus genotyping data of present study were compared to EuroRotaNet genotyping data. Potential RV vaccine effectiveness in Lithuanian children was evaluated.

The study was approved by the Vilnius Regional Biomedical Ethics Committee, registry Number 158200-05-183-056LP20 and Number 158200-183-PP1-4 after extension of the study.

3.1. Clinical and demographical data of children hospitalised due to RVI

1. Limited clinical and demographical data including age, sex, pathological syndrome (vomiting, diarrhoea or vomiting and diarrhoea) and geographical location was obtained for all patients included in the study.

2. In order to evaluate the severity of the rotavirus infection episode, all clinical data to fit the requirements of the Vesikari scale (as shown in Table 1) were obtained retrospectively from patients' case histories. To determine the differences in disease severity between the 5 common human rotavirus genotypes (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]) a minimum of 30 different cases for each genotype were analysed. These cases were randomly selected. According to the Vesikari 20-point scale, an episode of gastroenteritis with a score ≥11 was considered to be severe. An internationally agreed criterion for the definition of acute diarrhoea was used: "an increase in the child's stool frequency with the passing of at least 3 watery stools in 24 hours or looser stools than usual". Cessation of diarrhoea was defined as the time when the first firm stool was passed. Children, who appeared to be sick with another acute disorder or presenting a chronic disease, or if a case of nosocomial rotavirus infection were suspected, were excluded from further analysis, as it could misrepresent severity of the disease.

Table 1. The Vesikari severity scoring scale for the evaluation of gastroenteritis in children [27]

Symptom or sign	Points		
	1	2	3
Duration of diarrhoea (days)	1-4	5	≥6
Max number of diarrhoea stools/24h	1-3	4-5	≥6
Duration of vomiting (days)	1	2	≥3
Max number of vomiting episodes/24h	1	2-4	≥5
Temperature (°C)	37.1-38.4	38.5-38.9	≥39.0
Dehydration	-	Mild (1-5%)	Moderate to severe (≥6%)
Treatment	Rehydration	Hospitalisation	-

3.2. Collection of faecal samples and rotavirus genotyping

All faecal samples, included in the study, were gathered at Laboratory of Microbiology at Vilnius University Children's Hospital. Faecal samples routinely tested for enteric viruses and positive for group A rotavirus antigen (Rida® Quick Rotavirus/Adenovirus Combi (N1002)) were collected for genotyping. The sample size for rotavirus genotyping was defined by the EuroRotaNet study protocol [28]. According to Lithuanian population size and expected cases of rotavirus disease per year, 470 stool samples each RV season were required in order to identify strains circulating with a prevalence of less than 1% (Table 2). Not all faecal samples submitted at Laboratory of Microbiology at Vilnius University Children's Hospital microbiology laboratory and positive for group A rotavirus antigen were collected for genotyping, but only those, to fit the requirements of the sample size. Faecal samples, included in the study, were collected in proportion during every month of RV season.

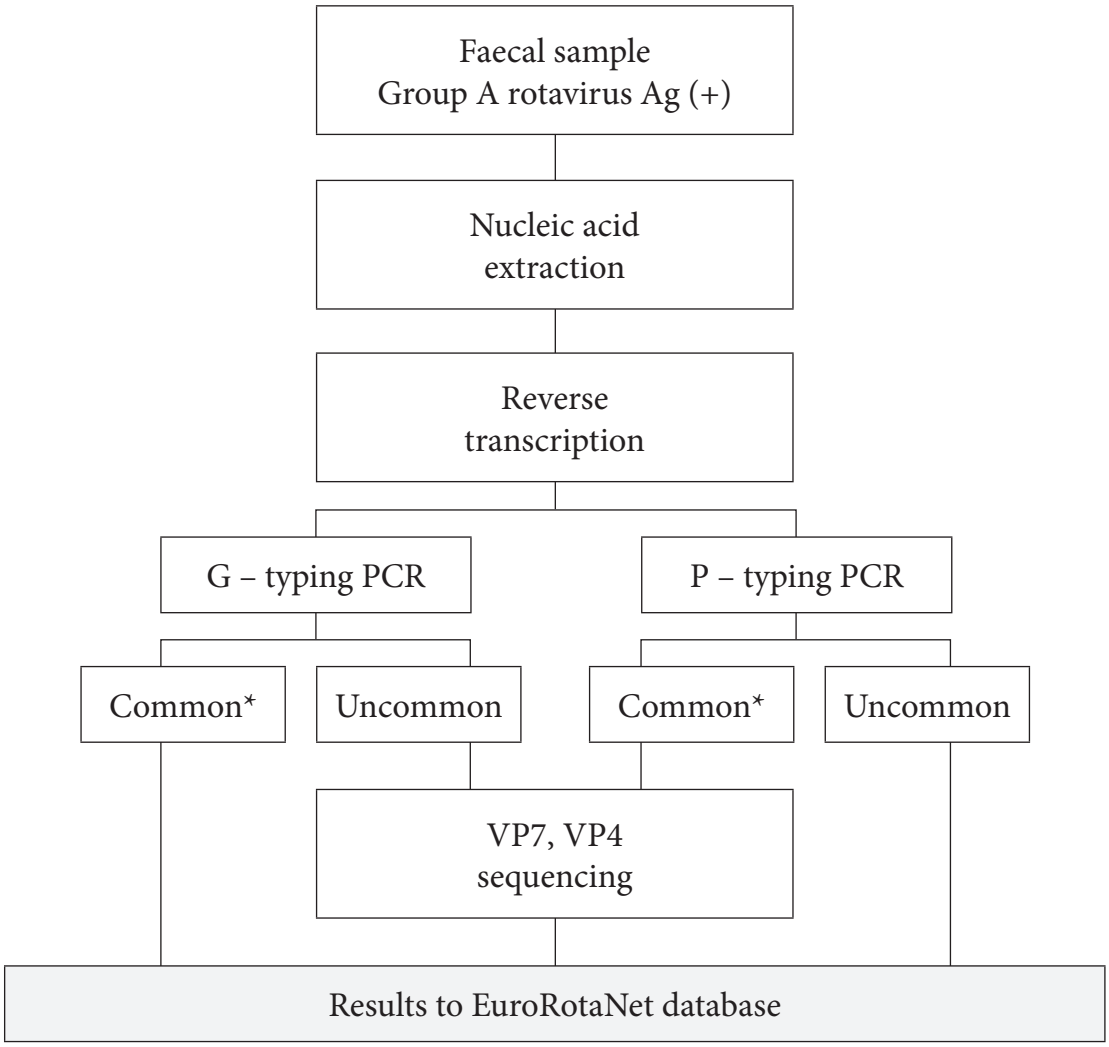
Table 2. Population size, expected cases of rotavirus disease per annum and the study sample size as a target

Country	Population (th)	Expected cases/year of rotavirus disease	Sample size/year
Lithuania	3400	34000	470

Faecal samples collected for genotyping were stored at -70°C. Rotavirus strains were characterised through genotyping using standardised EuroRotaNet approved methods to identify their G and P types [15, 29]. Testing algorithm is described in Figure 2.

All rotavirus samples were genotyped at the Enteric Virus Unit of Health Protection Agency, London, except for 349 samples that were genotyped at Centre of Laboratory Diagnostics of Vilnius University Hospital Santariskiu Klinikos.

Figure 2. EuroRotaNet rotavirus genotyping algorithm



*According to EuroRotaNet protocol common strains are considered as those circulating with a prevalence of > 1%.

3.3. Genotyping data of Lithuania versus other EuroRotaNet participants

To compare Lithuanian and EuroRotaNet genotyping data, two key publications representing ERN results were used [15, 26], as well as the 5th ERN report, which contains unpublished data for two additional RV seasons (2009/2010 and 2010/2011). EuroRotaNet data analysis of two last RV seasons (2011/2012 and 2012/2013) are still in progress, therefore these data were not included in the further comparison.

3.4. Evaluation of potential RV vaccine effectiveness

To evaluate potential RV vaccine effectiveness in Lithuanian children the European Public Assessment Report (EPAR) for RV1 and RV5 were used (published by the European Medicines Agency) [30, 31]. According to these official documents RV vaccines induce immunity to certain rotavirus genotypes. These genotypes were compared to the most common genotypes circulating in Lithuania.

3.5. Evaluation of potential RV vaccine effectiveness

Descriptive statistics, including mean (M), standard deviation (SD), median (Md), minimum (min) and maximum (max) values, were used to summarize investigative data. One-way ANOVA and the *post hoc* criterion were used for multiple comparisons. If data showed normal distribution, parametric statistics were used, if not, nonparametric statistics were used. Chi-squared test was used for the comparison of proportions. Statistical analyses were performed using MS Excel and SPSS 17.0 software for Windows. Significance was accepted with a P value < 0.05. The power of experiment was set at 0.8 and better.

4. RESULTS

In total, clinical data and faecal samples were collected from 3440 patients during seven RV seasons. This number represents 104.6% of the target reached. The exact sample number gathered during different RV seasons is shown in table 3.

Figure 3. Number of genotyped samples collected during different RV seasons

RV season	Number of genotyped samples	Percent of target reached
2005/2006	473	100.6
2007/2008	557	118.5
2008/2009	470	100
2009/2010	474	100.9
2010/2011	480	102.1
2011/2012	492	104.7
2012/2013	494	105.1
Total	3440	104.6

4.1. Common characteristics of study subjects

Out of 3440 patients, included in the study, 52% were males and 48% were females. The vast majority of all children (89.1%) were under 5 years of age. The leading pathological syndrome was rotaviral gastroenteritis (85.0%), enteritis and gastritis were found in 8.4% and 6.6% respectively. No significant differences in the distribution of rotavirus strains were found between the pathological syndromes ($p>0.05$) and the age of the patients ($p=0.62$). Of the patients included in the study 90% were from Vilnius district and 10% were from other regions of Lithuania.

4.2. Genotype distribution

Of the total 3440 samples characterised, 96%, contained a single rotavirus strain. Strain mixtures were reported in 3.3% of the samples and 0.7% of the strains were only partially characterised. A total of 68 different combinations of G and P types were found between 2005 and 2013. The following G and P types were included in either single or multiple rotavirus infections G1, G2, G3, G4, G5, G6, G8, G9, G10, G12 and P[3], P[4], P[6], P[8], P[9], P[14.

4.2.1. Single rotavirus strains

4.2.1.1. Common rotavirus genotypes

The five most common rotavirus genotypes causing disease in children hospitalised due to RVI were G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]. Their prevalence varied during different RV seasons from 85.5% to 98.8% (Table 4).

G1P[8] rotavirus genotype wasn't predominant in all seven seasons and accounted from 5.2% to 61% of the strains depending on the season (Table 4). The prevalence of G4P[8] genotype was extremely high in 2007/2008, it reached 81.3%, but declined year on year. The prevalence of G3P[8] was high in 2005/2006 and 2009/2010 (51.7% and 55.6% respectively), whereas in 2007/2009 and 2012/2013 was relatively low (1.5% - 3.8%).

4.2.1.2. Other rotavirus genotypes

Excepting the five the most common RV strains, the other RV genotypes accounted only a small part of all genotyped samples (from 0.8% to 5.8% depending on the season). Data displayed in Table 4.

The prevalence of G12P[8] increased significantly during two last RV seasons ($\chi 2 = 35.56$; $p \leq 0.001$). G12P[8] is emerging rotavirus genotype in Lithuania. The prevalence of G12P[9] also increased significantly ($\chi 2 = 19.25$; $p = 0.004$), but only in 2012/2013.

4.2.2. Mixed rotavirus infections

The highest number of mixed infections were registered in 2005/2006, at 11.8%, meanwhile the lowest number (only one case) was detected in 2008/2009 (0.2%). All mixed RV infections could be classified as single G type with two or more P types, multiple G types with a single P type or multiple G and P types. Mixed rotavirus strains are shown in Table 5.

4.2.3. Partially typed rotavirus strains

A total of 23 strains were partially typed, in 14 only the P-type was obtained, and in 9 only the G type (Table 6).

Table 4. Distribution of rotavirus genotypes found in infections with a single virus strain in seven rotavirus seasons between 2005 and 2013

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Common RV genotypes:	404	85.8	521	93.5	431	91.8	434	91.6	474	98.8	473	96.2	453	91.8
G1P[8]	24	5,2	31	5,6	250	53,2	76	16,0	255	53,1	300	61,0	227	46,0
G2P[4]	15	3,2	13	2,3	67	14,3	52	11,0	20	4,2	11	2,2	110	22,3
G3P[8]	244	51,7	9	1,6	7	1,5	263	55,6	122	25,5	95	19,4	19	3,8
G4P[8]	37	7,8	453	81,3	106	22,6	41	8,6	76	15,8	34	6,9	12	2,4
G9P[8]	84	17,9	15	2,7	1	0,2	2	0,4	1	0,2	33	6,7	85	17,3
Other RV genotypes:	11	2	14	2,5	15	3,1	24	5	4	0,8	14	2,8	29	5,8
G12P[8]	0	0	3	0,5	4	0,9	0	0	1	0,2	8	1,6	14	2,8
G12P[9]	0	0	4	0,6	2	0,4	1	0,2	2	0,4	1	0,2	9	1,8
G1P[4]	1	0,2	0	0	1	0,2	0	0	0	0	5	1,0	5	1,0
G2P[8]	1	0,2	1	0,2	3	0,6	0	0	0	0	0	0	0	0
G3P[4]	3	0,7	0	0	0	0	0	0	0	0	0	0	0	0
G4P[4]	2	0,4	0	0	2	0,4	0	0	1	0,2	0	0	0	0
G9P[4]	0	0	0	0	0	0	3	0,7	0	0	0	0	0	0
G3P[3]	1	0,2	0	0	0	0	0	0	0	0	0	0	0	0
G3P[6]	0	0	0	0	0	0	1	0,2	0	0	0	0	0	0

Table 4. (continuation)

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
G3P[9]	0	0	0	0	0	0	3	0,7	0	0	0	0	0	0
G5P[6]	0	0	0	0	0	0	1	0,2	0	0	0	0	0	0
G6P[9]	0	0	2	0,4	0	0	10	2,1	0	0	0	0	0	0
G8P[14]	0	0	0	0	0	0	1	0,2	0	0	0	0	0	0
G9P[9]	1	0,2	1	0,2	0	0	0	0	0	0	0	0	1	0,2
G4P[6]	0	0	0	0	0	0	2	0,4	0	0	0	0	0	0
G8P[8]	0	0	1	0,2	0	0	0	0	0	0	0	0	0	0
G8P[4]	0	0	2	0,4	0	0	1	0,2	0	0	0	0	0	0
G10P[6]	0	0	0	0	0	0	1	0,2	0	0	0	0	0	0
G12P[4]	2	0,4	0	0	0	0	0	0	0	0	0	0	0	0
G12P[6]	0	0	0	0	3	0,6	0	0	0	0	0	0	0	0
Total	415	87,8	535	96	446	94,9	458	96,6	478	99,6	487	99	482	97,6

Table 5. Distribution of mixed rotavirus strains in seven rotavirus seasons between 2005 and 2013

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Mixed infections (total)	56	11.8	11	2.0	18	3.8	14	3.0	1	0.2	4	0.8	12	2.4
Single G type with multiple P types														
G1P[4]+P[8]	2	0,4	0	0	0	0	0	0	0	0	0	0	0	0
G2P[4]+P[8]	3	0,6	0	0	0	0	0	0	0	0	0	0	0	0
G3P[4]+P[8]	5	1,1	0	0	0	0	0	0	0	0	0	0	0	0
G4P[4]+P[8]	2	0,4	0	0	0	0	0	0	0	0	0	0	0	0
G9P[4]+P[8]	4	0,8	0	0	0	0	0	0	0	0	0	0	0	0
G12P[4]+P[8]	0	0	0	0	1	0,2	0	0	0	0	0	0	0	0
G12P[8]+P[9]	0	0	0	0	1	0,2	0	0	0	0	0	0	0	0
Single P type with multiple G types														
G1+G2P[4]	0	0	0	0	0	0	1	0.2	0	0	0	0	2	0.4
G1+G3P[8]	0	0	0	0	1	0.2	4	0.8	0	0	0	0	2	0.4
G1+G4P[8]	0	0	5	0.8	5	1.1	1	0.2	0	0	3	0.6	1	0.2
G1+G4+G9P[8]	0	0	1	0.2	0	0	0	0	0	0	0	0	0	0
G1+G8P[4]	0	0	0	0	0	0	1	0.2	0	0	0	0	0	0
G1+G9P[8]	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
G1+G12P[8]	0	0	0	0	0	0	1	0.2	0	0	0	0	1	0.2
G2+G3P[4]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. (continuation)

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Single P type with multiple G types														
G2+G3P[8]	0	0	0	0	0	0	1	0.2	0	0	0	0	0	0
G2+G10P[4]	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
G3+G4P[3]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
G3+G4P[8]	6	1.3	0	0	0	0	2	0.5	0	0	1	0.2	0	0
G3+G8P[8]	0	0	0	0	6	1.3	0	0	0	0	0	0	0	0
G3+G9P[4]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
G3+G9P[8]	17	3.6	0	0	0	0	0	0	0	0	0	0	0	0
G3+G9+G12P[8]	1	0.2	2	0.4	0	0	0	0	0	0	0	0	0	0
G4+G9P[8]	1	0.2	2	0.4	0	0	0	0	0	0	0	0	0	0
G4+G10P[8]	0	0	0	0	1	0.2	0	0	0	0	0	0	0	0
G9+G12P[9]	0	0	0	0	0	0	0	0	1	0.2	0	0	0	0
G10+G12P[6]	0	0	0	0	1	0.2	0	0	0	0	0	0	0	0
G12+G10P[9]	0	0	0	0	0	0	1	0.2	0	0	0	0	0	0

Table 5. (continuation)

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Multiple G and P types														
G1+G2P[4]+P[8]	1	0.2	0	0	1	0.2	0	0	0	0	0	0	3	0.6
G1+G2+G4P[4]+P[8]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
G1+G4P[4]+P[8]	3	0.6	0	0	0	0	0	0	0	0	0	0	0	0
G1+G9P[4]+P[8]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
G2+G4P[4]+P[8]	0	0	0	0	1	0.2	0	0	0	0	0	0	0	0
G2+G3P[4]+P[8]	1	0.2	0	0	0	0	2	0.5	0	0	0	0	0	0
G2+G9P[4]P[8]	0	0	0	0	0	0	0	0	0	0	0	0	1	0.2
G3+G9P[4]+P[8]	3	0.6	0	0	0	0	0	0	0	0	0	0	0	0
G3+G4+G9P[4]+P[8]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0
G3+G4+G9P[8]+P[9]	0	0	1	0.2	0	0	0	0	0	0	0	0	0	0
G4+G9P[4]+P[8]	1	0.2	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Distribution of partially typed rotavirus strains in seven rotavirus seasons between 2005 and 2013

Genotype	Season													
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011		2011/2012		2012/2013	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Partially typed RV strains:	2	0,4	11	2,0	6	1,3	2	0,4	1	0,2	1	0,2	0	0
G4P-UD	1	0,2	5	0,9	1	0,2	1	0,2	0	0	0	0	0	0
G8P-UD	0	0	1	0,2	0	0	0	0	0	0	0	0	0	0
G12P-UD	1	0,2	0	0	1	0,2	1	0,2	1	0,2	1	0,2	0	0
G-UDP[8]	0	0	5	0,9	4	0,9	0	0	0	0	0	0	0	0

4.3. Comparison of Lithuanian and EuroRotaNet rotavirus genotyping data

4.3.1. Single rotavirus strains

4.3.1.1. Common rotavirus genotypes

In Lithuania, as well as in other EuroRotaNet participating countries, the five most common human rotavirus genotypes associated with RVI in children were found to be the most prevalent (Table 7). Their prevalence varied from season to season. The main difference in LTU vs ERN data was that in Lithuania G1P[8] wasn't predominant in all five seasons as it was in ERN.

Despite that G1P[8] is the most common genotype in terms of a total ERN data, only in six ERN countries (UK, The Netherlands, Sweden, Denmark, Italy and France) it was predominant in all five seasons. In Spain in 2005/2006 G1P[8] accounted 10% of all RV strains, the same prevalence of G1P[8] was in Greece in 2008/2009, in Hungary and Bulgaria – in 2009/2010, in Austria and Slovenia – in 2010/2011.

The next peculiarity of Lithuania was high prevalence of G3P[8] in 2005/2006 and 2009/2010 (51.7% and 55.6% respectively). In the vast majority of ERN countries the prevalence of G3P[8] reached from 5% to 10%. The highest prevalence of G3P[8] except of Lithuania was found in Belgium in 2010/2011 (32%) and in the Netherlands in 2009/2010 (30%) only.

First of all increasing prevalence of G4P[8] was registered in Lithuania in 2007/2008. This genotype accounted 81% of all LTU strains in that particular season. This was the highest prevalence of G4P[8] ever registered in ERN countries. Later on, in 2008/2009 an increasing prevalence of G4P[8] was registered in Germany, Bulgaria, Greece, in 2009/2010 – in Finland, Hungary, Slovenia. In all these countries G4P[8] accounted from 50% to 62%.

According to ERN data, the prevalence of G9P[8] was the highest in 2005/2006, it reached 37.2%. Among ERN countries the highest prevalence of G9P[8] was registered in Spain in 2006/2007, it reached 50% of all strains. In Lithuania the prevalence of G9P[8] was low in all seasons, except the first one, when G9P[8] accounted about 18%.

4.3.1.2. Other rotavirus genotypes

Excepting the five most common strains, prevalence of other RV genotypes was similar in Lithuania and in ERN. The diversity of RV strains, circulating in ERN countries was great, but the most important were G12 strains,

as they are defined as emerging RV strains in Europe. According to ERN data, G12P[8] strains were found circulating in several participating countries since 2005/2006, but the prevalence of infections with these strains was relatively low. In 2008/2009 the prevalence of G12 strains increased significantly (G12 $\chi 2$ = 22.83, P<0.0001) and kept increasing in further RV seasons. In 2010/2011 the prevalence of G12P[8] in Spain reached 20%, in the Netherlands – 11%, in the UK – 5% and in Slovenia – 2.7%. In Lithuania first G12P[8] genotypes were found in 2007/2008, but increased significantly only in 2011/2012.

4.3.2. Mixed rotavirus strains

4.3.3. Partially typed rotavirus strains

In all five RV seasons the number of partially typed strains were lower in Lithuania as compared to ERN. In Lithuania the failure to identify G-types were associated with P[8] and all P-types were associated with G4, G8 and G12. According ERN data the failure to identify G-types were associated with P[4], P[6], P[8], P[9], P[10], P[11] and P[14], and all P-types were associated with G1, G2, G3, G4, G8, G9, G10 and G12. The vast majority of partially typed strains were associated with G or P types seen in fully genotyped strains. Although inability to determine G-type in association with P[6], P[10], P[11], P[14] or P-type in association with G8 and G10 could have been associated with the presence of unusual strains, such cases were very rare in all participating countries.

Table 7. Distribution of rotavirus genotypes according to Lithuanian (LTU) and EuroRotaNet (ERN) data in five rotavirus seasons between 2005 and 2011

RV strains	Percent of strains per season									
	2005/2006		2007/2008		2008/2009		2009/2010		2010/2011	
	LTU	ERN	LTU	ERN	LTU	ERN	LTU	ERN	LTU	ERN
Common RV genotypes:	85.8	91.1	93.5	90.8	91.8	87.7	91.6	90.3	98.8	90.2
G1P[8]	5.2	43.3	5.6	52.9	53.2	45.8	16.0	42	53.1	52.2
G2P[4]	3.2	2.4	2.3	8.7	14.3	8.8	11.0	14.3	4.2	11.4
G3P[8]	51.7	3.8	1.6	3.8	1.5	5.6	55.6	7.1	25.5	7
G4P[8]	7.8	4.2	81.3	14.9	22.6	19.5	8.6	16	15.8	12.2
G9P[8]	17.9	37.3	2.7	10.5	0.2	8	0.4	10.9	0.2	7.4
Other RV genotypes	2	3.1	2.5	2.5	3.1	4.3	5	3.7	0.8	4.9
Partially typed strains	0.4	4.6	2.0	2.9	1.3	3.8	0.4	1.8	0.2	2.2
Mixed RV strains	11.8	1.2	2.0	3.8	3.8	4.2	3.0	4.2	0.2	2.7
Total	100	100	100	100	100	100	100	100	100	100

4.4. Evaluation of potential rotavirus vaccine effectiveness

Since 2006 there are two officially registered rotavirus vaccines (RV1 and RV5) in Lithuania. According to the European Public Assessment Reports both vaccines provide protection against disease, caused by most common RV genotypes: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]. Exactly the same genotypes were found to be the most frequently isolated in Lithuanian children sick with RVI, therefore both rotavirus vaccines are potentially effective for Lithuanian children.

4.5. Evaluation of the clinical severity of the rotavirus disease

A total of 251 rotavirus infection episodes were evaluated according to the Vesikari 20-point clinical severity scoring scale. Exact numbers of analysed cases for each different RV genotype are provided in Table 8.

There were no statistically significant differences in clinical severity among different rotavirus genotypes ($p = 0.086$). As this study was conducted in a hospital and the severity score of rotavirus infection episode was only calculated for hospitalised patients, it should be taken in to account that very mild cases may not be adequately reflected in this study. The low numbers of mild (<11 points) rotavirus infection cases obtained support this assumption (Table 8).

Of all patients, included in the clinical evaluation, 53% were males and 47% were females. The mean age of patients was 21 months (the youngest child was 2 months of age and the oldest was 6 years). Statistical analysis of data revealed that the age and gender distribution were similar in all groups according to different rotavirus genotypes ($p=0.174$ and $p=0.872$ respectively).

Table 8. Descriptive statistics of clinical scoring of rotavirus infection episodes, caused by the different rotavirus genotypes

RV genotype	Total number of cases N=251	Vesikari severity score					
		Mild cases (<11 points) N=18	Severe cases (≥11points) N=233	Max score	Min score	Median	Mean of the score (±SD)
G1P[8]	40	3	37	19	9	13.0	13.95 (±2.33)
G2P[4]	40	2	38	18	8	14.0	13.70 (±2.17)
G3P[8]	64	8	56	18	7	14.0	13.71 (±2.86)
G4P[8]	67	4	63	17	9	14.0	13.56 (±1.97)
G9P[8]	40	1	39	18	7	15.0	14.72 (±2.32)

Summarizing clinical data of all 251 patients the following results were derived: of all patients 78.9% had fever ≥38°C (the highest registered temperature was 40.3°C), in 14.7% of patients the temperature ranged from 37°C to 38°C and 6.4% of patients had no fever; the mean duration of diarrhoea was 2.8 days (max – 8, min – 1), the mean number of diarrhoea stools/24h was 5.8 (max – 18, min – 2); the mean duration of vomiting was 2 days (max – 4, min – 1), the mean number of vomiting episodes/24h was 5.4 (max – 20, min - 1). Oral rehydration only was used for 12% of patients and for 88% of patients intravenous rehydration was required. The mean duration of intravenous rehydration was 1.7 days (max – 5, min – 1). Of all patients 10.9% were treated at the intensive care unit, the mean duration at the ICU was 2.4 days (max – 5, min -1). The mean duration of the hospitalisation due to RVI was 3 days (max – 10, min -1). Summarising all these clinical data 92.8% of patients had severe RVI (according to Vesikari scale).

5. DISSCUSION

Data from the molecular rotavirus epidemiology in Lithuania revealed nothing unexpected. No novel rotavirus genotypes were identified in Lithuania throughout the study period. Although diversity of different rotavirus strains in Lithuania was great, the five most common rotavirus genotypes (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]) were the most prevalent. Data, showing the high prevalence of five most common rotavirus genotypes in Lithuania are compatible with reports from other countries of Europe or other countries of temperate climate such as United States, Canada, Australia or New Zealand [14, 15, 32, 33].

The distribution of different rotavirus genotypes in Lithuania varied among seasons. This is called natural fluctuation of rotavirus strains diversity. A similar tendency of fluctuation in the interseasonal diversity of rotavirus strains is well known in other European countries and was described in several papers [10, 15, 26, 33].

Excepting the five most common genotypes, other rotavirus strains covered only small proportion of all analysed rotavirus genotypes. The most important of these were G12 strains, as their prevalence increased significantly not only in Lithuania but in other ERN countries as well. Emerging strains such as G12 have been identified so early only due to the ability of ERN participants to identify strains circulating with a prevalence of 1%. Currently, it is important whether G12P[8] will become the sixth major human RV genotype.

Going back into the history of molecular rotavirus epidemiology it was known that before 1995 four rotavirus genotypes (G1P[8], G2P[4], G3P[8], G4P[8]) were responsible for about 90-95% of all hospitalised RV cases in industralised countries [34]. Later G9 strains have emerged and became predominant in different parts of the world. Currently G9P[8] is considered to be the fifth major human RV genotype [32, 35]. Phylogenetic analyses have revealed that a single sublineage of G9 RV strains was responsible for this worldwide spread [12]. Although a number of genetically different variants of G12 RVs have been isolated from humans, but comparable with G9, a single G12 lineage (Lineage III for G12) have been able to spread and cause disease across the globe [12, 36].

Data of complete RVA genome sequence comparisons suggests that only two major genotype constellations of the non-G, non-P genes; I1-R1-C1-M1-A1-N1-T1-E1-H1 (Wa-like) and I2-R2-C2-M2-A2-N2-T2-E2-H2, (DS-1-like),

have been successful in sustaining infection of humans worldwide over longer periods of time [37-40]. RVAs of other genotype constellations such as AU-1 like or other were able to spread to a limited extent [40].

In summary, these data suggests that G12P[8] genotype, as G9P[8] in those days, may became a sixth major human RV genotype worldwide.

Rotavirus vaccination coverage in Lithuania is not very well documented. WHO is the only one official source which refers that rotavirus vaccination coverage in Lithuania in 2013 was about 10% [41]. As rotavirus vaccination costs are not covered by the government, it is not expected that vaccination coverage throughout the study period was higher than 10%. It is supposed, that rotavirus vaccination did not influenced distribution of circulating rotavirus genotypes, as vaccination coverage was very low. Furthermore, the faecal samples of the first season (2005/2006) were collected before introduction of rotavirus vaccination in Lithuania.

None of rotavirus vaccine efficacy or effectiveness studies were performed in Lithuania and the present study does not pretend to be equal to it. However, after determination of most common rotavirus strains, it is expected that two registered rotavirus vaccines are effective in Lithuania, as it covers all the most popular genotypes circulating in the country. Natural interseasonal fluctuation of rotavirus strains is thought to be of limited importance regarding effectiveness of rotavirus vaccines, as vaccines provide protection against all five common rotavirus strains, no matter how often they do occur in particular season.

The retrospective analysis of patients' case histories as well as the study performance in the hospital setting is assumed as a limitation of the study. As this study was conducted in the hospital and severity score of rotavirus infection episode was calculated only for hospitalised patients, mild cases were not reflected and the certain prevalence of these cases was not known. However, according to REVEAL study data which indicates that regardless of where the child was treated (hospitalised or not), the incidence of genotypes was the same [42]. Meanwhile, severe cases or rotavirus disease are very important due to their clinical outcome as well as epidemiologically, whereas mild cases are mostly important only for epidemiological studies. Nevertheless, there were no differences found regarding severity of the disease comparing it with common rotavirus genotypes.

According to the controversial literature data the relationship between genotype and clinical outcomes seems to be complex and varies among different parts of the world [43-49]. Differences in disease severity can be caused by

variation in virulence between different rotavirus strains or the introduction of new strains into a community [33]. Other factors, such as age as well as immune status of the child or accessibility of medical services are very important and may cause different disease severity, but it was not researched in any of the study.

6. CONCLUSIONS

1. The most common rotavirus genotypes, isolated in children hospitalised due to RVI, were G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]; their distribution varied among different seasons. Excepting the five common rotavirus stains, other rotavirus genotypes made only small number out of all identified strains. G12P[8] is emerging rotavirus genotype in Lithuania.

2. In Lithuania, as well as in other EuroRotaNet participating countries, the five most common rotavirus genotypes associated with RVI in children were found to be the most prevalent.In contrast to other EuroRotaNet countries the incidence of G3P[8] and G4P[8] in Lithuania was very high. G12P[8] rotavirus genotype is emerging not only in Lithuania, but also in other EuroRotaNet countries.

3. Rotavirus vaccines are potentially effective to Lithuanian children, as vaccines induce immunity to those rotavirus genotypes, which were the most commonly isolated in our country.

4. There was no relationship found between the most commonly isolated rotavirus genotypes and the clinical severity of the disease.

7. SUMMARY IN LITHUANIAN

Įvadas

Rotavirusinė infekcija (RVI) yra viena dažniausių vaikų viduriavimo priežasčių visame pasaulyje. Dėl didelio sergamumo ir dažnų hospitalizacijų RVI yra aktuali pediatrinė problema ir Lietuvoje. Nuo 2006 metų mūsų šalyje yra įregistruotos rotavirusų vakcinos, kurios apsaugo vaikus nuo RVI, sukeltos 5 pagrindinių rotavirusų (RV) genotipų. Iki pradedant mūsų tyrimą, Lietuvoje nebuvo atlikta jokių RV genotipų įvairovę nagrinėjančių darbų, nebuvo žinoma, ar esamos vakcinos bus efektyvios ir Lietuvos vaikams. Mokslinėje literatūroje iki šiol nėra bendros nuomonės, ar RV genotipai daro įtaką RVI eigos sunkumui. Šiuo aspektu RVI Lietuvoje nebuvo nagrinėta.

Mokslinėje literatūroje publikacijų, nagrinėjančių įvairius RVI aspektus, yra be galo daug, tačiau iš Lietuvos - tik dvi. Didelė straipsnių iš viso pasaulio gausa ir publikacijų iš Lietuvos stoka dar kartą pabrėžia disertacijos temos aktualumą. Apibendrinant literatūros duomenis, akivaizdu, kad rotavirusas – dažniausias mažamečių vaikų gastroenterito sukėlėjas visame pasaulyje. Cirkuliuojančių rotavirusų genotipų įvairovė yra labai didelė, tačiau industrinėse šalyse dominuoja penki pagrindiniai RV genotipai: G1P[8], G2P[4],G3P[8], G4P[8], G9P[8.]. Pietų Amerikoje, Afrikoje, Azijoje dominuojančių RV genotipų įvairovė daug didesnė, šiose šalyse dažnai aptinkamos mišrios RVI bei nemažai netipuojamų RV. Remiantis mokslinės literatūros duomenimis, manoma, kad G12 – naujai plintantis RV tipas pasaulyje. Darbai, nagrinėjantys RVI sunkumo priklausomybę nuo RV genotipo, prieštaringi.

Šis darbas yra sudėtinė tarptautinio projekto *EuroRotaNet* (ERN) dalis. Pagrindinis šio projekto tikslas - surinkti kuo išsamesnius duomenis apie cirkuliuojančius RV genotipus Europoje. *EuroRotaNet* veikloje dalyvauja 18 Europos šalių. Per kelerius metus kiekviena iš jų įsipareigojo pateikti reprezentatyvios imties RV genotipavimo duomenis. Visos projekte dalyvaujančios šalys taikė vienodą darbo metodiką. Gauti rezultatai apibendrinami ir publikuojami. Lietuva – vienintelė iš kaimyninių ir Baltijos šalių, dalyvaujanti šiame projekte.

Darbo tikslas

Nustatyti cirkuliuojančių rotavirusų genotipų įvairovę bei šių genotipų sukeltos infekcijos klinikinius ypatumus.

Darbo uždaviniai

1. Įvertinti cirkuliuojančių rotavirusų genotipų įvairovę skirtingų sezonų metu, tiriant vaikų, hospitalizuotų dėl rotavirusinės infekcijos, išmatų bandinius.
2. Palyginti Lietuvos rotavirusų genotipavimo rezultatus su *EuroRotaNet* projekte dalyvaujančių šalių rezultatais.
3. Įvertinti potencialų dviejų rotaviruso vakcinų efektyvumą Lietuvos vaikams.
4. Įvertinti rotavirusinės infekcijos sunkumo priklausomybę nuo ligą sukeliančio rotaviruso genotipo.

Darbo metodika

Mokslinis darbas atliktas Vilniaus universiteto Medicinos fakulteto Vaikų ligų klinikoje ir Vaikų ligoninėje VšĮ Vilniaus universiteto ligoninės Santariškių klinikų filiale, bendradarbiaujant su Jungtinės Karalystės Sveikatos apsaugos agentūra Londone bei VšĮ Vilniaus universiteto ligoninės Santariškių klinikų Laboratorinės diagnostikos centru. Tyrimas truko 7 RVI sezonus (2005-2013 m., išskyrus 2006-2007 m.). Darbui gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas Nr. 158200-05-183-056LP20 bei leidimo papildymas Nr. 158200-183-PP1-4.

Tyrimo medžiaga

1. Klinikiniai bei demografiniai duomenys apie vaikus, sirgusius RVI:

a) minimalūs visų pacientų duomenys; b) išsamūs klinikiniai duomenys, rinkti pagal Vesikari skalę, siekiant išsiaiškinti, ar yra priklausomybė tarp ligos sunkumo ir RV genotipo.

2. Į tyrimą įtrauktų vaikų išmatų bandiniai.

3. *EuroRotaNet* projekte dalyvaujančių šalių ir Lietuvos RV genotipavimo duomenys.

Tyrimo metodai:

Išmatų bandinių tyrimai: visi išmatų bandiniai rinkti Vaikų ligoninės VšĮ Vilniaus universiteto ligoninės Santariškių klinikų filialo Mikrobiologijos laboratorijoje. Į tyrimą įtraukti tik RV Ag turintys bandiniai (RV Ag patvirtintas imunochromatografiniu testu). Pagal ERN protokolą Lietuvai numatyta surinkti 470 RV bandinių per RVI sezoną. Visi RV bandiniai buvo genotipuojami pagal bendrą ERN genotipavimo algoritmą.

EuroRotaNet ir Lietuvos rotavirusų genotipavimo rezultatų palyginimas: šiam uždaviniui įgyvendinti buvo naudoti 5-ųjų ERN veiklos metų ataskaitos ir dviejų, EuroRotaNet rezultatus apibendrinančių, publikacijų duomenys.

Potencialaus RV efektyvumo vertinimas: vadovaujantis oficialiomis vakcinų preparatų charakteristikomis [30, 31], buvo vertinama, nuo kokių RV genotipų apsaugą užtikrina abi rotaviruso vakcinos, ir šie genotipai lyginami su Lietuvoje cirkuliuojančių RV genotipų duomenimis.

Statistinė duomenų analizė atlikta, naudojant „MS Excel“ ir „SPSS 17.0“ programas.

Rezultatai

Per septynis RVI sezonus iš viso gauti 3440 rotavirusų bandinių genotipavimo rezultatai, tai sudaro 104,6 proc. užsibrėžto tikslo, todėl daroma išvada, kad nustatyta tyrimo imtis buvo pasiekta, o gauti duomenys reprezentatyvūs.

Bendroji tiriamųjų charakteristika:

Iš visų vaikų, kurie buvo įtraukti į tyrimą, 52 proc. buvo berniukai ir 48 proc. - mergaitės. Vaikai buvo įvairaus amžiaus, tačiau didžioji dauguma (89,1 proc.) - vaikai iki penkerių metų. Statistiškai patikimo skirtumo tarp pacientų amžiaus ir skirtingų rotavirusų genotipų nebuvo ($p = 0,62$). Dažniausiai (85 proc. atvejų) hospitalizuotiems vaikams buvo diagnozuotas rotavirusinis gastroenteritas, žymiai rečiau pasitaikė rotavirusinis enteritas (8,4 proc. atvejų) ar gastritas (6,6 proc. atvejų). Statistiškai patikimo skirtumo tarp rotavirusų genotipų ir vyraujančių patologinių sindromų (gastroenterito, gastrito, enterito) nebuvo rasta ($p>0,05$). Visų tyrime dalyvavusių pacientų išmatų bandiniai buvo renkami viename centre, tačiau pacientai, sirgę RVI ir gydyti Vaikų ligoninėje, buvo atvykę iš 32 skirtingų Lietuvos vietovių. Didžioji dalis pacientų buvo Vilniaus miesto (78 proc.) ar Vilniaus apskrities gyventojai (12 proc.). Pacientai, atvykę iš kitų miestų, sudarė 10 proc.

Rotavirusų genotipų įvairovė

Iš visų 3440 genotipuotų bandinių vieno RV genotipo infekcijos (angl. - *single rotavirus strain*) sudarė 96 proc. Mišrių RV genotipų infekcijos, t.y. kai vieną pacientą infekuoja keli skirtingi RV genotipai, sudarė 3,3 proc., o iš dalies genotipuoti virusai - 0,7 proc. atvejų.

Per septynis RVI sezonus iš viso rastos 68 skirtingos G- ir P- tipų kombinacijos, kurias sudarė pavienės ar mišrios infekcijos G1, G2, G3, G4, G5, G6, G8, G9, G10, G12 ir P[3], P[4], P[6], P[8], P[9], P[14] genotipais.

Patys dažniausi RV genotipai, sukėlę infekcijas dėl RVI hospitalizuotiems vaikams, buvo šie: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]. Jų dažnis skirtingais sezonais sudarė nuo 85,5 iki 98,8 proc. visų RV genotipų. Šių 5 pagrindinių RV genotipų pasiskirstymas per skirtingus sezonus labai skyrėsi. Per visą tyrimo laikotarpį, G1P[8] genotipas pasitaikė dažniausiai. Nors jo dažnis pirmus du RVI sezonus yra mažas ir siekia iki 5,6 proc., tačiau 2008-2009 bei 2010-2012 metais jis viršija 50 proc. G3P[8] genotipas buvo pats dažniausias 2005-2006 m. ir 2009-2010 m., kuomet jis sudarė 51,6 proc. ir 55,5 proc. visų RV genotipų. G4P[8] genotipo dažnis iš visų kitų ryškiai išsiskiria 2007-2008 metais, jis siekia 81,3 proc. Per visus septynis RVI sezonus pats didžiausias G2P[4] genotipo dažnis buvo 2012-2013 m., jis sudarė 22,3 proc. visų genotipų. 2005-2008 bei 2010-2012 metais G2P[4] dažnis neperkopė 3,2 proc. ribos. G9P[8] genotipas per septynis RVI sezonus pasitaikė rečiausiai. Tik 2005-2006 bei 2012-2013 m. šio genotipo dažnis buvo apie 17-18 proc., kitais metais jis buvo mažas ir svyravo nuo 0,2 iki 6,8 proc.

Be penkių pagrindinių, kiti RV genotipai, sukeliantys infekcijas hospitalizuotiems vaikams, sudarė nedidelę dalį, jų dažnis skirtingų sezonų metu svyravo nuo 0,8 iki 5,8 proc.

Tyrimo rezultatai parodė, kad G12P[8] dažnio padidėjimas per du paskutiniuosius RVI sezonus yra statistiškai reikšmingas ($\chi 2 = 35,56$; $p \leq 0,001$). G12P[8] yra naujai plintantis rotaviruso genotipas Lietuvoje.

Pats didžiausias mišrių RVI dažnis buvo 2005-2006 m., jis siekė 11,8 proc. Tik vienas (0,2 proc.) mišrios RVI atvejis buvo identifikuotas 2008–2009 m., tai pats mažiausias mišrios RVI dažnis per visą tyrimo laikotarpį. Per likusius penkis sezonus mišrių RVI dažnis neviršijo 3,8 proc. ribos. Per septynis RVI sezonus iš dalies genotipuotų RV dažnis svyravo nuo 0 iki 2 proc. (M = 0,7 proc.). Iš viso rasti 23 iš dalies genotipuoti RV, devyniems iš jų nepavyko nustatyti G tipo, o likusiems – P tipo.

Lietuvos rotavirusų genotipavimo rezultatų palyginimas su kitų *EuroRotaNet* projekte dalyvaujančių šalių rezultatais

Lietuvos rotavirusų genotipavimo rezultatus, lyginant su EuroRotaNet projekto duomenis (toliau ERN duomenimis), akivaizdu, kad Lietuvoje, kaip ir kitose ERN valstybėse, dominuoja tie patys penki pagrindiniai rotavirusų genotipai. Jų dažnis skirtingais sezonais svyravo tiek Lietuvoje, tiek įvairiose ERN šalyse, didžiausi skirtumai buvo 2005-2006 m., kuomet Lietuvoje penki pagrindiniai RV genotipai sudarė 85,8 proc., o ERN duomenimis jie siekė 91,1 proc. ir 2010-2011 m., kuomet Lietuvoje jų buvo 98,8 proc. lyginant su 90,2 proc. ERN šalyse.

ERN duomenimis, G1P[8] rotavirusų genotipas buvo dominuojantis visus penkis RVI sezonus (jo dažnis svyravo nuo 42 proc. iki 52,9 proc.), o Lietuvoje G1P[8] dominavo tik 2008-2009 ir 2010-2011 metais (siekė 53,2 proc. ir 53,1 proc.)

Lietuva iš kitų ERN valstybių išsiskiria tuo, kad nei vienoje šalyje nei vieno RVI sezono metu nebuvo rasta tokio didelio G3P[8] genotipo dažnio, kuris 2005-2006 m. Lietuvoje siekė apie 52 proc., o 2009-2010 m. – apie 56 proc. Be Lietuvos, didžiausias G3P[8] dažnis užregistruotas Belgijoje 2010-2011 m., jis siekė 32 proc.

Lietuvoje, lyginant su kitomis ERN valstybėmis, anksčiausiai aptiktas G4P[8] genotipo dažnio padidėjimas. Lietuvoje 2007-2008 m. jis sudarė 81 proc. visų RV padermių ir tai yra pats didžiausias G4P[8] dažnis visose ERN šalyse per penkis RVI sezonus. Vėliau, 2008-2009 m. G4P[8] dažnio padidėjimas aptiktas ir Bulgarijoje, Vokietijoje, Graikijoje, o 2009-2010 m. Suomijoje, Vengrijoje ir Slovėnijoje, tačiau visose šiose šalyse genotipo dažnis svyravo tarp 50 - 62 proc.

Be penkių pagrindinių, kitų RV genotipų dažnis Lietuvoje ir ERN šalyse yra panašus. ERN valstybėse, kaip ir Lietuvoje, yra aptikta įvairių RV padermių, tačiau reikšmingiausi G12 turintys rotavirusai. Vadovaujantis ERN duomenimis, nustatyta, kad G12 rotaviruso dažnio padidėjimas 2008-2009, lyginant su ankstesniais RVI sezonais, buvo statistiškai reikšmingas (G12 $\chi 2 = 22,83$, $P<0,0001$). Vėliau šio RV genotipo dažnis ERN šalyse dar labiau didėjo, šiuo metu jis vadinamas naujai plintančiu RV tipu Europoje.

Potencialaus rotaviruso vakcinų efektyvumo vertinimas

Vadovaujantis oficialiomis vakcinų preparatų charakteristikomis [30, 31], yra žinoma, kad abi rotaviruso vakcinos užtikrina apsaugą nuo penkių pagrindinių RV genotipų: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]. Lietuvoje šie RV genotipai infekcijas vaikams sukelia dažniausiai (nuo 85,5 iki 98,8 proc.), todėl manoma, kad abi rotaviruso vakcinos yra potencialiai efektyvios ir Lietuvos vaikams.

Skirtingų rotavirusų genotipų sukeltos infekcijos klinikinio sunkumo vertinimas

Siekiant nustatyti, ar esama ryšio tarp 5 pagrindinių RV genotipų ir klinikinio ligos sunkumo, iš viso buvo išnagrinėtos atsitiktinės atrankos principu atrinktos 251 paciento, hospitalizuoto dėl RVI, ligos istorijos. Skirtingų RV genotipų sukeltos infekcijos sunkumo vertinimo rezultatai apskaičiuoti pagal Vesikari skalę. Statistiškai patikimo skirtumo tarp RV genotipų ir ligos sunkumo, išreikšto Vesikari balais, nebuvo ($p = 0,086$).

Pacientų, kurių ligos istorijos buvo analizuojamos, grupę sudarė 53 proc. berniukų ir 47 proc. mergaičių. Vaikų amžiaus vidurkis buvo 21 mėnuo. Statistinė duomenų analizė patvirtino, kad skirtingų RV genotipų grupėse vaikų amžiaus ir lyčių pasiskirstymas buvo panašus ($p = 0,174$ ir $p = 0,872$, atitinkamai). Apibendrinus visų 251 paciento klinikinius RVI duomenis, gauti šie rezultatai: iš visų tirtųjų 78,9 proc. kūno temperatūra buvo 38°C ir daugiau, 6,4 proc. iš viso nekarščiavo; vidutiniškai viduriavimas truko 2,8 paras, vidutinis viduriavimų skaičius per parą - 5,8; vėmimo trukmė vidutiniškai buvo 2 paros, o vidutinis vėmimų skaičius per parą buvo 5,4. Geriamoji rehidratacinė terapija buvo taikyta 12 proc. pacientų, o intraveninė – 88 proc. Vidutiniškai intraveninė rehidratacinė terapija truko 1,7 paros. Gydymo vaikų intensyvios terapijos skyriuje prireikė 10,8 proc. pacientų, vidutinė jų gydymo VRITS trukmė buvo 2,4 paros. Vidutinė hospitalizacijos trukmė dėl RVI buvo 3 paros. Apibendrinus visų šių pacientų klinikinius duomenis, nepriklausomai nuo RV genotipo, 92,8 proc. pacientų, vertinant pagal Vesikari skalę, buvo nustatyta sunki RVI.

Išvados

1. Dėl rotavirusinės infekcijos hospitalizuotiems vaikams ligą dažniausiai sukėlė penki pagrindiniai rotavirusų genotipai: (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]), o kiti genotipai sudarė tik nedidelę visų identifikuotų rotavirusų padermių dalį. Per skirtingus RVI sezonus rotavirusų genotipų pasiskirstymas skyrėsi. G12P[8] yra naujai plintantis rotaviruso genotipas Lietuvoje.

2. Lietuvoje, kaip ir kitose *EuroRotaNet* projekte dalyvaujančiose Europos šalyse, dažniausiai aptikti penki pagrindiniai, infekcijas vaikams sukeliantys, rotavirusų genotipai, tačiau Lietuva iš kitų *EuroRotaNet* dalyvių išsiskyrė dideliu G3P[8] ir G4P[8] genotipų dažniu. G12P[8] rotaviruso genotipas plinta ne tik Lietuvoje, bet ir kitose *EuroRotaNet* šalyse.

3. Mūsų šalyje dažniausiai aptikti tie rotavirusų genotipai, nuo kurių sukeltos ligos apsaugo rotaviruso vakcinos, todėl Lietuvos vaikams šios vakcinos yra potencialiai efektyvios.

4. Rotavirusinės infekcijos sunkumas nepriklausė nuo ligą sukeliančio rotaviruso genotipo.

8. REFERENCES

1. Parashar UD, Bresee JS, Gentsh JR, et al. Rotavirus. *Emerg Infect Dis.* 1998;4 (4):561-70.

2. Das JK, Salam RA, and Bhutta ZA. Global burden of childhood diarrhea and interventions. *Curr Opin Infect Dis.* 2014.

3. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012;12 (2):136-41.

4. Soriano-Gabarró M, Mrukowicz J, Vesikari T, et al. Burden of Rotavirus Disease in European Union Countries. *Pediatr Infect Dis J.* 2006;25 Suppl 1:7-11.

5. Mast TC, Walter EB, Bulotsky M, et al. Burden of childhood rotavirus disease on health systems in the United States. *Pediatr Infect Dis J.* 2010;29 (2):e19-25.

6. Van der Wielen M, Giaquinto C, Gothefors L, et al. Impact of community-acquired paediatric rotavirus gastroenteritis on family life: data from the REVEAL study. *BMC family practice.* 2010;11:22.

7. Van Damme P, Giaquinto C, Huet F, et al. Multicenter prospective study of the burden of rotavirus acute gastroenteritis in Europe, 2004-2005: the REVEAL study. *J Infect Dis.* 2007;195 Suppl 1:4-16.

8. Diez Domingo J, Patrzalek M, Cantarutti L, et al. The impact of childhood acute rotavirus gastroenteritis on the parents‘ quality of life: prospective observational study in European primary care medical practices. *BMC Pediatr.* 2012;12:58.

9. Belskij A, Čaplinskienė I, Davidavičienė E. ir kt. Sergamumo užkrečiamosiomis ligomis Lietuvoje 2012 m. apžvalga *Užkrečiamųjų ligų ir AIDS centras.* 2013.

10. Usonis V, Ivaskeviciene I, Desselberger U, et al. The unpredictable diversity of co-circulating rotavirus types in Europe and the possible impact of universal mass vaccination programmes on rotavirus genotype incidence. *Vaccine.* 2012;30 (31):4596-605.

11. Matthijnssens J, Ciarlet M, McDonald SM, et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol.* 2011.

12. Matthijnssens J, Heylen E, Zeller M, et al. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. *Molecular biology and evolution.* 2010;27 (10):2431-6.

13. Trojnar E, Sachsenroder J, Twardziok S, et al. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. *J Gen Virol.* 2013;94 (Pt 1):136-42.

14. Banyai K, Laszlo B, Duque J, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. *Vaccine.* 2012;30 Suppl 1:A122-30.

15. Iturizza-Gomara M, Dallman T, Banyai B, et al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. *Epidemiol Infect.* 2010:1-15.

16. Ruiz-Palacios G, M., Perez-Schael I, Velazquez FR, et al. Safety and Efficacy of an Attenuated Vaccine against Severe Rotavirus Gastroenteritis. *N Engl J Med.* 2006;354 (1):11-22.

17. Linhares AC, Velazquez FR, Perez-Schael I, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet.* 2008;371 (9619):1181-9.

18. Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet.* 2007;370 (9601):1757-63.

19. Phua KB, Lim FS, Lau YL, et al. Safety and efficacy of human rotavirus vaccine during the first 2 years of life in Asian infants: randomised, double-blind, controlled study. *Vaccine.* 2009;27 (43):5936-41.

20. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med.*362 (4):289-98.

21. Vesikari T, Matson D, O., Dennehy P, et al. Safety and Efficacy of a Pentavalent Human-Bovine (WC3) Reassortant Rotavirus Vaccine. *N Engl J Med.* 2006;354 (1):23-33.

22. Vesikari T, Itzler R, Karvonen A, et al. RotaTeq, a pentavalent rotavirus vaccine: Efficacy and safety among infants in Europe. *Vaccine.* 2009;28 (2):345-51.

23. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376 (9741):606-14.

24. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2010;376 (9741):615-23.

25. European Rotavirus Surveillance Network. www.eurorota.net. Accessed 2014 March 23.

26. Iturriza-Gomara M, Dallman T, Banyai K, et al. Rotavirus surveillance in Europe, 2005-2008: web-enabled reporting and real-time analysis of genotyping and epidemiological data. *J Infect Dis.* 2009;200 Suppl 1:S215-21.

27. Ruuska T, and Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis.* 1990;22 (3):259-67.

28. Iturizza-Gomara M, and Gray J. EuroRotaNet project: collection of samples within the European rotavirus network. http://www.eurorota.net/project.php. Accessed March 20, 2014.

29. Gray J, and Iturizza Gomara M. EuroRotaNet project : Rotavirus Detection and Typing. http://www.eurorota.net/docs.php. Accessed 20 March 2014.

30. European Medicines Agency. Rotarix: EPAR - Product Information. http://www.ema.europa.eu/. Updated 14 May 2014. Accessed August 15, 2014.

31. European Medicines Agency. Rotateq: EPAR - Product Information. http://www.ema.europa.eu/. Updated 03 July 2014. Accessed August 15, 2014.

32. Santos N, and Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *RevMedVirol.* 2005;15 (1):25-56.

33. Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, et al. Rotavirus Types in Europe and Their Significance for Vaccination. *Pediatr Infect Dis J.* 2006;25 Suppl 1:30-41.

34. Gentsch JR, Woods PA, Ramachandran M, et al. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. *J Infect Dis.* 1996;174 Suppl 1:S30-6.

35. Ramachandran M, Kirkwood CD, Unicomb L, et al. Molecular characterization of serotype G9 rotavirus strains from a global collection. *Virology.* 2000;278 (2):436-44.

36. Matthijnssens J, Bilcke J, Ciarlet M, et al. Rotavirus disease and vaccination: impact on genotype diversity. *Future Microbiol.* 2009;4:1303-16.

37. Matthijnssens J, Ciarlet M, Heiman E, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *J Virol.* 2008;82 (7):3204-19.

38. McDonald SM, Matthijnssens J, McAllen JK, et al. Evolutionary dynamics of human rotaviruses: balancing reassortment with preferred genome constellations. *PLoS Pathog.* 2009;5 (10):e1000634.

39. Heiman EM, McDonald SM, Barro M, et al. Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. *J Virol.* 2008;82 (22):11106-16.

40. Matthijnssens J, and Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. *Current opinion in virology.* 2012;2 (4):426-33.

41. WHO. Immunization data and statistics. http://www.euro.who.int/en/health-topics/disease-prevention/vaccines-and-immunization/data-and-statistics.

42. Van Damme P, Giaquinto C, Maxwell M, et al. Distribution of rotavirus genotypes in Europe, 2004-2005: the REVEAL Study. *J Infect Dis.* 2007;195 Suppl 1:17-25.

43. Iturriza-Gomara M, Cubitt D, Steele D, et al. Characterisation of rotavirus G9 strains isolated in the UK between 1995 and 1998. *J Med Virol.* 2000;61 (4):510-7.

44. Linhares AC, Verstraeten T, Wolleswinkel-van den Bosch J, et al. Rotavirus serotype G9 is associated with more-severe disease in Latin America. *Clin Infect Dis.* 2006;43 (3):312-4.

45. Arista S, Vizzi E, Migliore MC, et al. High incidence of G9P[8] rotavirus infections in Italian children during the winter season 1999-2000. *Eur J Epidemiol.* 2003;18 (7):711.

46. Clark HF, Lawley DA, Schaffer A, et al. Assessment of the Epidemic Potential of a New Strain of Rotavirus Associated with the Novel G9 Serotype Which Caused an Outbreak in the United States for the First Time in the 1995-1996 Season. *J Clin Microbiol.* 2004;42 (4):1434-38.

47. Zuccotti G, Meneghin F, Dilillo D, et al. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. *BMC Infectious Diseases.* 2010;10 (218):1-8.

48. de Rougemont A, Kaplon J, Pillet S, et al. Molecular and clinical characterization of rotavirus from diarrheal infants admitted to pediatric emergency units in france. *Pediatr Infect Dis J.* 2011;30 (2):118-24.

49. Matthijnssens J, Zeller M, Heylen E, et al. Higher proportion of G2P[4] rotaviruses in vaccinated hospitalised cases compared to unvaccinated hospitalised cases, despite high vaccine effectiveness against heterotypic G2P[4] rotaviruses. *Clin Microbiol Infect.* 2014.

9. PUBLICATIONS AND SCIENTIFIC CONFERENCE REPORTS

Publications:

1. Iturriza-Gómara M, Dallman T, Bányai K, Böttiger B, Buesa J, Diedrich S, Fiore L, Johansen K, Korsun N, Kroneman A, Lappalainen M, László B, Maunula L, Mathijnssens J, Midgley S, Mladenova Z, Poljsak-Prijatelj M, Pothier P, Ruggeri FM, Sanchez-Fauquier A, Schreier E, Steyer A, **Sidaraviciute I**, Tran AN, Usonis V, Van Ranst M, Rougemont A, Gray J.

Rotavirus Surveillance in Europe 2005-2008: Web-Enabled Reporting and Real-Time Analysis of Genotyping and Epidemiological Data. Journal of Infectious Diseases. J Infect Dis 2009;200 Suppl 1:S215-21;

2. Iturriza-Gómara M, Dallman T, Bányai K, Böttiger B, Buesa J, Diedrich S, Fiore L, Johansen K, Koopmans M, Korsun N, Koukou D, Kroneman A, László B, Lappalainen M, Maunula L, Mas Marques A, Matthijnssens J, Midgley S, Mladenova Z, Nawaz S, Poljsak-Prijatelj M, Pothier P, Ruggeri F. M, Sanchez-Fauqueir A, Steyer A, **Sidaraviciute-Ivaskeviciene I**, Syriopoulou V, Tran A. N, Usonis V, Van Ranst M, De Rougemont A, Gray J.

Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. Epidemiology and Infection 2010:1-15;

3. **Ivaškevičienė I,** Mačionienė A, Kučinskienė ZA, Usonis V.

Rotavirusinė infekcija: etiopatogenezė, epidemiologija ir profilaktikos galimybės. Visuomenės sveikata 2012, 3(58): 10-18, 2012;

4. Usonis V, **Ivaskeviciene I**, Desselberger U, Rodrigo C.

The unpredictable diversity of co-circulating rotavirus types in Europe and the possible impact of universal mass vaccination programmes on rotavirus genotype incidence. Vaccine 2012, 30(31): 4596-4605.

Scientific monograph

Ivaškevičienė I, Usonis V. Rotavirusinė infekcija, knygoje Vakcinos ir skiepijimas, Homo Liber 2010:175-187

Conference reports

1. Sidaraviciute - Ivaskeviciene I.
Rotavirus infection in Lithuania: first genotyping data. 3rd European Rotavirus Biology Meeting, Loch Lomond, Scotland, September 13-16th 2009;

2. Ivaskeviciene I.
Molecular epidemiology and clinical evaluation of rotavirus infection in Lithuania. Course of Postgraduate diploma in Paediatric Infectious Diseases at the University of Oxford, United Kingdom, June 27th, 2010;

3. Ivaskeviciene I.
Distribution of Rotavirus Genotypes in Europe and Lithuania. 4th International Vilnius Course "Update on Paediatric Infections", Vilnius, Lithuania, October 7-8th 2010;

4. Ivaškevičienė I.
Rotaviruso vakcinos ir PCV. Mokslinė konferencija "Imunoprofilaktikos ruduo", Vilnius, Lietuva, spalio 12d., 2010;

5. Ivaškevičienė I.
Rotavirusinė infekcija: nuo molekulinių tyrimų iki kasdienės praktikos.
IX Lietuvos pediatrų draugijos suvažiavimas, Vilnius, Lietuva, spalio 7-8 d., 2011.

Poster presentations

1. Sidaraviciute I, Stankuniene B, Staras K, Usonis V.
Rotaviral gastorenteritis (RG) as seen by parents. 25th Annual meeting of the European Society for Paediatric Infectious Diseases, Porto, Portugal, May 2-4, 2007;

2. Stankuniene B, Sidaraviciute I, Usonis V.
Nosocomial rotavirus infection (NRVI) in the Vilnius University Children's hospital. 25th Annual meeting of the European Society for Paediatric Infectious Diseases, Porto, Portugal, May 2-4, 2007;

3. Sidaraviciute - Ivaskeviciene I, Macioniene A, Usonis V.
Rotavirus infection in Lithuania: first genotyping data. 3rd European Rotavirus Biology Meeting, Loch Lomond, Scotland, September 13-16th 2009;

4. Ivaskeviciene I, Macioniene A, Usonis V.
First rotavirus genotyping data in Lithuania. 28th Annual Meeting of the European Society for Paediatric Infectious Diseases, Nice, France, May 4-8, 2010;

5. Ivaškevičienė I, Usonis V, Nawaz S, Aladin F, Iturriza-Gómara M. Rotavirus genotyping data in Lithuania during four rotavirus seasons. 29th Annual Meeting of the European Society for Paediatric Infectious Diseases, The Hague, The Netherlands, June 7-11, 2011;

6. Iturriza-Gomara M.
On behalf of EuroRotaNet participans. EuroRotaNet: seven years of rotavirus strain surveillance in Europe. 5th European Rotavirus Biology Meeting, Valencia, Spain, 6-9 October, 2013.

10. BIOGRAPHY

Name:
Inga Ivaškevičienė (maiden name: Sidaravičiūtė)

Date of birth:
1st October 1979

Education

Sept 1997 – June 2003
Vilnius University, Faculty of Medicine, Lithuania Completion of the integral studies of Medicine Awarded the qualification of a medical doctor

Sept 2003 - June 2008
Completion of a 5 year term residency of Paediatrics, which included one year of children's pulmonology, as a subspecialty, at Vilnius University, Faculty of Medicine, Clinic of Children's diseases.
Registered as paediatrician – pulmonologist

Oct 2008 – Oct 2010
Oxford University Postgraduate Diploma in Paediatric Infectious Diseases (completion of a 2 year term studies)

Oct 2008 – Oct 2014
PhD studies at Vilnius University, Faculty of Medicine, Clinic of Children's diseases

Work experience

Nov 2010 – present
Baltic Immunoprophylaxis Association Executive secretary

Mar 2008 – present
Vilnius University Children's Hospital Department of Paediatric Infectious Diseases General Paediatrician, since 2011 Head of the Department

Sep 2009 – present
Vilnius University, Faculty of Medicine, Clinic of Children's diseases; Working as assistant

Sep 2007- Aug 2009
Vilnius University, Faculty of Medicine, Clinic of Children's diseases; Working as junior researcher

Membership

Since 2010 - member and executive secretary of Baltic Immunoprophylaxis Association (BALTIPA)

Since 2008 - member of Central European Vaccination Advisory Group (CEVAG)

Since 2007 - member of European Society for Paediatric Infectious Diseases (ESPID)

Since 2006 - member of Lithuanian Paediatric Society

Postgraduate training

2010

Residential (Rome, Italy) and online course of Paediatric HIV and AIDS (PENTA course)

Feb 2009 – Apr 2009
Nine weeks training at the University Children's Hospital Basel (UKBB), Basel, Switzerland

Nov 2008
Two weeks training at the Enteric Virus Unit, Health protection Agency, London, UK

Scientific awards

2011 - Sophie Valentina Ambroza award at Vilnius University

2011 – Junior researcher award of Lithuanian Paediatric Society

Scientific monograph

Ivaškevičienė I, Usonis V. Rotavirusinė infekcija, knygoje Vakcinos ir skiepijimas, Homo Liber 2010:175-187

Publications

1. **Sidaraviciute I.**, Stankuniene B., Audickaite I., Alisauskiene I., Usonis V. Rotavirus infection - hard to avoid, but possible to prevent. Pediatrija 2006;3:84-90.

2. **Sidaraviciute I.**, Usonis V. Prophylaxis of rotavirus infection. Pediatrija 2007;4:59-62.

3. Narkeviciute I, **Sidaraviciute I.** The prophylaxis of tick- transmitted bacterial diseases. Lietuvos bendrosios praktikos gydytojas 2007;4:268-271.

4. Suciliene E, **Sidaraviciute I**, Budgeniene D , Valiulis A. Adverse reactions of BCG vaccination. Vaikų pulmonologija ir alergologija 2008;11(1):3794-3804.

5. Iturriza-Gómara M., … **Sidaraviciute I**, et al. Rotavirus Surveillance in Europe 2005-2008: Web-Enabled Reporting and Real-Time Analysis of Genotyping and Epidemiological Data. J Infect Dis 2009;200 Suppl 1:S215-21

6. Iturizza-Gomara M., … **Sidaraviciute-Ivaskeviciene I**, at al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. Epidemiology and Infection, Published online by Cambridge University Press. 2010;139(6):895-909

7. Usonis V, … **Ivaskeviciene I**, et al. Central European Vaccination Advisory Group (CEVAG) guidance statement on recommendations for influenza vaccination in children. BMC Infectious Diseases 2010;10:1-8

8. Chlibek R, … **Ivaskeviciene I**, et al. Central European Vaccination Advisory Group (CEVAG) guidance statement on recommendations for 2009 pandemic influenza A(H1N1) vaccination. Vaccine 2010;28:3758-3766

9. Usonis V, Mickienė A, Vėlyvytė D, Nadišauskienė R, Drąsutienė G, Ambroziatis A, **Ivaškevičienė I.** Nėščiųjų gripas ir jo profilaktika (Influenza and its prevention in pregnancy) Lietuvos akušerija ir ginekologija 2010;XIII:260-271

10. Prymula R, Chlibek R, **Ivaskeviciene I**, et al. Paediatric pneumococcal disease in Central Europe. Eur J Clin Microbiol Infect Dis 2011;30(11):1311-20

11. Usonis V, ... **Ivaskeviciene I**, et al. Rubella revisited: where are we on the road to disease elimination in Central Europe? Vaccine 2011;29(49):9141-7

12. Chlibek R, ... **Ivaskeviciene I**, et al. Adult vaccination in 11 Central European countries - Calendars are not just for children.Vaccine 2012;30(9):1529-40

13. Ivaškevičius R, **Ivaškevičienė I**, Usonis V. Kokliušo ypatumai modernioje visuomenėje. Vaikų pulmonologija ir alergologija 2012; 15(1): 69-80.

14. Usonis V, **Ivaskeviciene I**, Desselberger U, Rodrigo C. The unpredictable diversity of co-circulating rotavirus types in Europe and the possible impact of universal mass vaccination programmes on rotavirus genotype incidence. Vaccine 2012;30(31):4596-605.

15. **Ivaškevičienė I**, Mačionienė A, Kučinskienė ZA, Usonis V. Rotavirusinė infekcija: etiopatogenezė, epidemiologija ir profilaktikos galimybės. Visuomenės sveikata 2012;3(58):10-18.

16. Zavadska D, ... **Ivaskeviciene I**, et al. Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). Hum Vaccin Immunother 2013;9(2):362-74.

11. ACKNOWLEDGEMENTS

I would like to express my special appreciation and thanks to my supervisor Professor Dr. Vytautas Usonis, who has been a tremendous mentor. I would also like to express my gratitude to Professor Jim Gray and Dr. Miren Iturriza-Gómara who afforded me the golden opportunity to be a part of the EuroRotaNet project. I am especially grateful to Ms. Farah Aladin and Ms. Sameena Nawaz who did all the rotavirus genotyping work at HPA, London. I would also like to thank Dr. Asta Mačionienė and Mrs. Jurgita Baubinienė for their great effort to genotype rotaviruses in Lithuania. I am thankful to Dr. Genovaitė Bernatonienė, Mrs. Daiva Dambrauskienė and Mrs. Laima Dovydėnienė for their great assistance in the Microbiology Laboratory at Vilnius University Children's Hospital. A special thanks goes to assoc. prof. Daiva Dabkevičienė for her priceless help with statistics. I am grateful to Mr. Jonas Liugaila for his assistance with producing the diagram of the rotavirus structure.

Words cannot express how grateful I am to my family for all of the sacrifices that they have made on my behalf.