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MOLECULAR EPIDEMIOLOGY OF ROTAVIRUSES DURING ROTAVIRUS VACCINE INTRODUCTION IN SLOVENIA

MOLEKULARNA EPIDEMIOLOGIJA ROTAVIRUSOV V OBDOBJU UVAJANJA ROTAVIRUSNEGA CEPIVA V SLOVENIJI

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Abstract

Background *Rotaviruses are the major cause of acute watery diarrhea in children up to 5 years of age. In 2007 and 2008 an extensive rotavirus molecular epidemiology study was conducted in Slovenia in order to provide information on rotavirus molecular epidemiology in our country. This study is part of the EuroRotaNet, European rotavirus surveillance study.*

Methods *A total of 823 stool samples were collected from children with acute gastroenteritis in seven out of nine healthcare regions in Slovenia. The total RNA was extracted and first amplification of VP7 and VP4 genes was performed in RT-PCR. The RT-PCR product was used for genotyping in multiplex-nested PCR. Untypable strains were included in sequence analysis of VP7 and VP4 genes.*

Results *Genotype distribution similar to that in other European countries was observed. G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] were the most prevalent genotypes in 2007 and 2008, except of G3P[8], not detected in 2008. Relatively high rate of possible zoonotic strains was detected in 2007 (2.5%). The emerging unusual strain G10P[14] was the only potential zoonotic strain detected in 2007 and 2008.*

Conclusions *After the vaccine introduction in Slovenia in 2007, no specific changes in molecular epidemiology of rotaviruses was observed. This finding was expected since rotavirus vaccine coverage in 2007 in Slovenia was very low. Genotype G1P[8] remains the most prevalent genotype. The rotavirus strain surveillance in Slovenia should be carried on to allow monitoring the spread of some unusual rotavirus genotypes, like G10. The G10 strains should be tracked especially in vaccinated children as no data on vaccine efficiency against infection with G10 strains was presented till now.*

Key words *rotavirus; molecular epidemiology; rotavirus vaccine; genotyping; EuroRotaNet*

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Izvleček

Izhodišča *Rotavirusi so najpogostejši povzročitelji akutnega gastroenteritisa pri otrocih do petega leta starosti. Pri nevtralizaciji virusa z nevtralizacijskimi protitelesi sta najpomembnejša antigena zunanjih proteinov VP7 in VP4. Glede na njune antigenske značilnosti je uvedena dvojna tipizacija – serotip G (za VP7) in serotip P (za VP4). Vzporedno je glede na nukleotidno zaporedje genov za VP7 in VP4 uvedena genotipizacija. Tipizacija rotavirusov je v sedanjem času izjemnega pomena, saj sta se pred kratkim na trgu uveljavili dve novi rotavirusni cepivi, ki uspešno zaščitita pred okužbo z najpogostejšimi rotavirusnimi genotipi G1P[8], G2P[4], G3P[8], G4P[8] in G9P[8]. V letih 2007 in 2008 smo v Sloveniji v okviru evropskega projekta EuroRotaNet izvedli širšo raziskavo o molekularni epidemiologiji rotavirusov.*

Metode *V letu 2007 smo zbrali 400, v letu 2008 pa 423 vzorcev iztrebkov otrok do petega leta starosti, ki so zboleli za akutnim gastroenteritisom. V vzorcih smo prej z elektronsko mikroskopijo in/ali encimskoimunskimi testi dokazali rotaviruse skupine A. Iz 10-odstotne raztopine iztrebka smo osamili RNA. V prvi reakciji RT-PCR smo v ločenih reakcijah pomnoževali odseke genov za VP7 in VP4. Produkte prve reakcije smo uporabili v reakciji vgnezdene PCR s tipsko-specifičnimi začetnimi oligonukleotidi za določevanje genotipov G in P. Pomnožene DNA odseke smo ločili v gelski elektroforezi in na podlagi velikosti pomnoženih odsekov določili genotip. Neuspešno tipizirane rotavirusne seve smo vključili v analizo nukleotidnega zaporedja za gene VP7 in VP4.*

Rezultati *V letu 2007 smo zaznali premik glavnine rotavirusnih okužb v pozno pomlad (april–junij). Iz leta 2007 smo uspešno tipizirali rotaviruse v vseh 400 vzorcih. Najpogostejši genotipi v tem letu so bili G1P[8] (56 %), G4P[8] (16,5 %), G2P[4] (15,3 %), G3P[8] (4,8 %) in G9P[8] (4,0 %). V sorazmerno visokem odstotku (2,5 %) smo določili tudi manj običajne genotipe, ki so najverjetneje zoonotskega izvora: G4P[6], G9P[9], G6P[11], G6P[9] in G10P[14]. Leta 2008 smo med 400 uspešno analiziranimi vzorci zaznali povečanje pogostosti pojavljanja genotipa G1P[8] (71,9 %) in zmanjšano pogostost genotipov G4P[8], G2P[4] in G9P[8]. Genotipa G3P[8] v tem letu nismo zaznali. Povečal se je delež mešanih okužb, iz 1,0 % v letu 2007 na 3,5 % v letu 2008. Od neobičajnih genotipov smo v obeh letih določili le genotip G10P[14].*

Zaključki *Ugotovili smo, da v Sloveniji po uvedbi neobveznega cepljenja z rotavirusnim cepivom v letu 2007 ni zaznati sprememb v molekularni epidemiologiji rotavirusov. Omenjena ugotovitev ni presenetljiva, saj je bila v obdobju naše raziskave precepljenost z rotavirusnim cepivom zelo nizka. Še vedno je najpogostejši genotip G1P[8]. Glede na veliko raznolikost rotavirusnih genotipov v Sloveniji bi bilo smiselno spremljati pojavljanje genotipov še naprej. Predvsem bo pomembno spremljati nekatere manj pogoste genotipe, kot je genotip G10. Po napovedih nekaterih raziskav bi ta genotip lahko predstavljal naslednji rotavirusni genotip, ki se bo uspešno razširil po svetu. Spremljati bo potrebno pojavljanje tega genotipa predvsem pri cepljenih otrocih, saj uspešnost cepiva pred okužbo s tem genotipom še ni poznana.*

Ključne besede *rotavirus; molekularna epidemiologija; rotavirusno cepivo; genotipizacija; EuroRotaNet*

Introduction

Rotaviruses are the most common cause of acute gastroenteritis in children up to five years of age. Rotavirus infections are widespread all over the world and are responsible for more than 500.000 deaths of young children, located mostly in south and south-east Asia, Africa and some South American states. In North America and Europe the mortality rate is low, which is mostly the result of timely and efficient medical treatment.[1] As rotavirus infections could not be prevented with higher hygiene standard, incidence of rotavirus infection is high also in developed countries. Thus, it was of high interest to develop efficient vaccine against rotavirus gastroenteritis, which is also one of the common causes for children hospitalization.[2]

Rotaviruses are members of the *Reoviridae* family. They are unenveloped viruses with 70–80 nm triple-layered icosahedral capsid, enclosing the genome of 11 linear dsRNA segments.[3] Soon after the discovery of rotaviruses and after their molecular characteristics was studied, it was evident that rotavirus genetic and antigenic diversity is very high. This diversity is the result of genome reassortment and point mutations. After the co-infection of two different rotavirus strains, their genome could undergo genetic reassortment, resulting in genetic and antigenic changes of greater extension.[4] The outer layer of the viral capsid consists of two proteins carrying antigen determinants for rotavirus serotyping and crucial determinants for virus neutralization epitopes. These two proteins are

glycoprotein VP7, determining G serotype (G for glycoprotein) and hemaglutinine VP4, determining P serotype (P for protease sensitive). Following antigen determinants of these two proteins, dual typing system with serotype G and P was introduced for rotavirus classification. It was not always possible to provide monoclonal antibodies for each serotype and because of long and complicated way of serotyping, a molecular characterization based on nucleotide sequences of the VP7 and VP4 genes was introduced, determining G and P genotypes. Dual genotype classification was introduced and each rotavirus strain is characterized and described with G and P genotype, e.g. G1P[8], G10P[11], etc..[5] In 2008 a Rotavirus Classification Working Group (RCWG) was established to provide clear and uniform criteria for the classification of rotaviruses based on nucleotide sequences of all 11 genome segments. To date twenty G and twenty eight P genotypes were confirmed by the scientific committee of RCWG.[6, 7]

Currently, two rotavirus vaccines, Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck Sharp & Dohme), were released on the market and are already in use in many countries around the world. Both are live attenuated vaccines, Rotarix® is monovalent vaccine with G1P[8] human rotavirus strain and RotaTeq® is a human-bovine reassortant pentavalent vaccine, containing the most common genotypes G1–G4 and P[8].[8] During the period of vaccine introduction it is of great importance to follow rotavirus molecular epidemiology in order to define post-licensure effectiveness of these two vaccines. In clinical trials both vaccines showed to be efficient in preventing more than 90% of severe rotavirus disease requiring hospital treatment of infected children, although there was a slightly lower efficiency of protection determined against G2P[4] rotavirus strain.[9, 10] There are limited published data on rotavirus molecular epidemiology with pre- and postvaccination period. In Australia no changes in genotype distribution was observed after 12 months of vaccine use in the national immunization program.[11] In Rio de Janeiro a shift towards G2P[4] was observed one year after the nationwide rotavirus immunization program. However the increasing emergence of G2P[4] was explained rather as a global trend in seasonal emerging genotypes.[12]

Recently, an article describing rotavirus epidemiology patterns before and after the rotavirus vaccine introduction in the USA. After vaccine introduction onset and peak of rotavirus season were delayed for 15 and 8 weeks, respectively. In addition, rotavirus season was shortened to 14 weeks compared to 26 weeks in the prevaccine era.[13] The rotavirus surveillance should continue also after the introduction of rotavirus vaccine in order to detect changes in molecular characteristics of circulating strains and to detect possible »escape mutants« which could pass the immunization barrier achieved with vaccination. In Europe, EuroRotaNet (http://www.eurorota.net/), a network of laboratories working on molecular epidemiology of rotaviruses was established. EuroRotaNet is a network of 14 countries, including Slovenia. The main goal of this network is to follow changes in molecular characteristics of circulating rotavirus strains before and after the introduction of rotavirus vaccines.

In this study which is also a part of the EuroRotaNet project, molecular characterization of rotaviruses circulating in up to 5 years old children in Slovenia is presented.

Materials and methods

Stool samples of children up to 5 years of age with acute gastroenteritis were collected. Rotaviruses were detected by electron microscopy and/or enzyme-linked immunosorbent assay. In the period from January 1st 2007 to October 31st 2008 a total of 823 stool samples were collected and analyzed. Stool samples were collected at Institute of Public Health of the Republic of Slovenia (IPHRS) and six Regional Institutes of Public Health (RIPH), except RIPH Murska Sobota and Ravne na Koroškem and sent to the Institute of Microbiology and Immunology, Medical Faculty in Ljubljana (IMI) for molecular analysis. Most of the samples were sent from regions of Ljubljana and Celje, followed by Maribor, Nova Gorica, Novo mesto and Koper (Figure 1). All of the samples analyzed in this study were sent from hospitals or local pediatricians to RIPH or IMI for microbiological diagnostics of acute gastroenteritis. No extra sampling was performed.

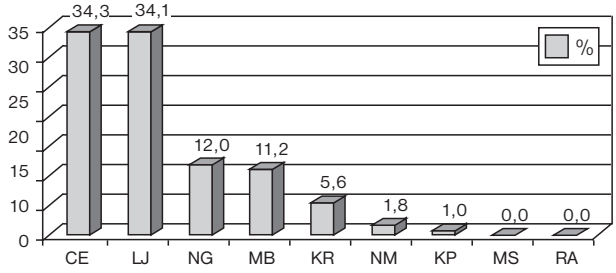

Figure 1. *Percentage distribution of investigated samples in individual healthcare regions in Slovenia. (CE – Celje, LJ–Ljubljana, NG – Nova Gorica, MB – Maribor, KR – Kranj, NM – Novo mesto, KP – Koper, MS – Murska Sobota, RA – Ravne na Koroškem).*

Sl. 1. *Delež preiskovanih vzorcev po zdravstvenih regijah. (CE – Celje, LJ–Ljubljana, NG – Nova Gorica, MB – Maribor, KR – Kranj, NM – Novo mesto, KP – Koper, MS – Murska Sobota, RA – Ravne na Koroškem).*

A 10% stool suspension was prepared in PBS (0.2 M, pH 7.4). After the clearance with centrifugation at 1600 × g for 5 minutes, 250 µl of supernatant was used for RNA isolation, using TRIzol LS reagent, following manufacturer instructions (Invitrogen). Isolated RNA was resuspended in 30 µl of sterile ddH_2O and stored at –80°C.

Isolated RNA was used in separate reactions as a template for the reverse transcription and amplification of the genome segments VP7 and VP4. For the amplification of 881 bp of VP7 gene, the primer pair VP7F/VP7R was used in RT-PCR.[14] In a separate RT-PCR reaction, a 663 bp segment of VP4 gene was amplified, using

VP4F/VP4R primer pair.[15] A commercial kit SuperScript™ One-Step RT-PCR was used for both RT-PCR reactions, following the manufacturer instructions (Invitrogen).

For the determination of genotypes G and P multiplex-nested PCR was performed, using G and P type specific primers, annealing to nucleotide sequences which are conserved within the same genotype and differ greatly comparing to other genotypes.[16] For G genotyping the VP7 RT-PCR product was used in a multiplex-nested PCR reaction mix containing type-specific primers for genotypes G1–G4, G8, G9, G10 and G12.[16, 17] For P typing the composition of reaction mix was similar to G typing, but VP4 RT-PCR product was used as a template and P type specific primers were added for P[4], P[6], P[8], P[9], P[10] and P[11].[15, 16] For G and P typing *Tfi* DNA polymerase was used, following the manufacturer instructions (Invitrogen). The G and P types were determined after the multiplex-nested reactions according to the length of the amplified products analyzed in a 1.6% agarose gel electrophoresis (running time 1 h at 90V).

Rotavirus strains which were untypable were included in a direct sequence analysis of the VP7 and VP4 amplified products. Sequences obtained after sequence analysis were blasted and compared to the sequences of reference genotype strains in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A recommended RCWG cut-off values for G and P genotype analysis were considered.[6]

Results

A total of 823 stool samples from children with rotavirus gastroenteritis were included in this study. The number of analyzed samples correlated with the dynamics of reported rotavirus cases, presented by the IPHRS (source: http://www.ivz.si) (Figure 2). In 2007 a clear shift in rotavirus epidemiology was observed. The peak of reported rotavirus cases was late in spring, between April and June (Figure 2).

The age of children, suffering from rotavirus gastroenteritis included in this study was typical for rotavirus infection. Most of children with rotavirus infection were found in the age group between 6 months and 3 years, followed by decrease numbers in older age groups (Figure 3).

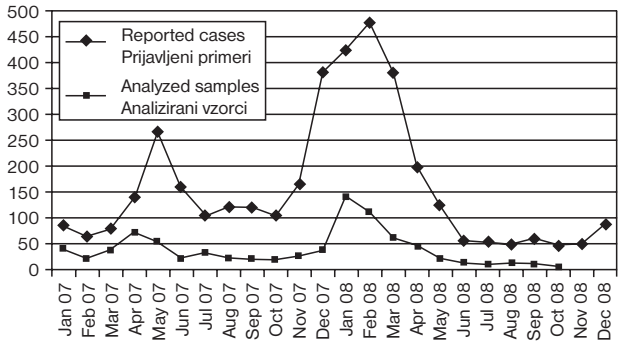

Figure 2. *The dynamics of reported rotavirus gastroenteritis cases and number of analyzed rotavirus positive stool samples in the study during 2007 and 2008.*

Sl. 2. *Gibanje števila prijavljenih primerov rotavirusnega gastroenteritisa v Sloveniji in števila analiziranih vzorcev v naši raziskavi v letih 2007 in 2008.*

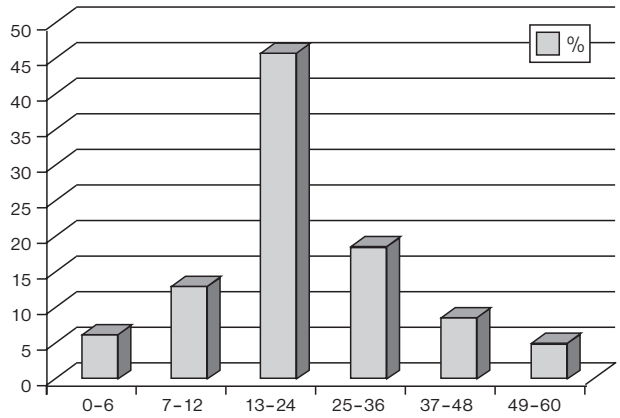

Figure 3. *Age distribution of children with rotavirus gastroenteritis included in the study.*

Sl. 3. *Starostna porazdelitev otrok z rotavirusnim gastroenteritisom, vključenih v raziskavo.*

Of 823 samples included in the study, 800 were successfully analyzed and in 23 samples rotaviruses were untypable or were only partially typed. In 2007 G1P[8] was the most prevalent genotype, detected in 56% of 400 analyzed samples. A lower prevalence was determined for genotypes G4P[8] (16.5%), G2P[4] (15.3%), G3P[8] (4.8%) and G9P[8] (4.0%) (Table 1). Rotavirus genotype G1P[8] was the most prevalent in all health care regions, except in Maribor and Novo

Table 1. *Rotavirus genotypes in Slovenia in 2007 and 2008.*

Tab. 1. *Rotavirusni genotipi v Sloveniji v letih 2007 in 2008.*

		2007		2008		Total Skupaj	
	Genotype Genotip	Number Število	% Število	Number Število	%	Number	%
Common RoV genotypes Običajni RoV genotipi	G1P[8]	224	56.0	304	71.9	528	64.2
	G2P[4]	61	15.3	20	4.7	81	9.8
	G3P[8]	19	4.8	0	0.0	19	2.3
	G4P[8]	66	16.5	44	10.4	110	13.4
	G9P[8]	16	4.0	12	2.8	28	3.4
Possible zoonotic origin Možen zoonotski izvor	G4 P[6]	1	0.3	0	0.0	1	0.1
	G9P[4]	0	0.0	1	0.2	1	0.1
	G9 P[9]	1	0.3	0	0.0	1	0.1
	G10 P[14]	5	1.3	1	0.2	6	0.7
	G6 P[9]	2	0.5	0	0.0	2	0.2
	G6 P[11]	1	0.3	0	0.0	1	0.1
	G6 P[14]	0	0.0	1	0.2	1	0.1
	G12 P[9]	0	0.0	2	0.5	2	0.2
Mixed infections Mešane okužbe	G1+G2 P[4]+P[8]	1	0.3	2	0.5	3	0.4
	G2+G4 P[4]+P[8]	0	0.0	1	0.2	1	0.1
	G1+G4 P[8]	1	0.3	6	1.4	7	0.9
	G3+G4 P[8]	1	0.3	0	0.0	1	0.1
	G9+G4 P[8]	0	0.0	3	0.7	3	0.4
	G1+G12 P[8]	0	0.0	1	0.2	1	0.1
	G1+G10 P[8]	0	0.0	1	0.2	1	0.1
	G9+G12 P[8]	1	0.3	1	0.2	2	0.2
	Untypable Netipizirani	0	0.0	23	5.4	23	2.8
	Total Skupaj	400	100.0	423	100.0	823	100.0

mesto, where G3P[8] and G2P[4] were the most often detected genotypes (data not shown). It is interesting that in 2007 relatively high percentage (2.5%) of unusual or possible zoonotic strains was detected. These strains were detected sporadically in different regions and represent rotavirus genotypes G4P[6], G9P[9], G6P[11], G6P[9] and G10P[14] (Table 1). In 2007 1.0% of mixed infections were detected, also with genotype G12, which was already detected in a previous study in Slovenia.[18]

A very similar distribution of rotavirus genotypes was detected also in 423 samples in 2008. In this year the prevalence of G1P[8] increases to 71.9%, and a decrease in G4P[8], G2P[4] and G9P[8] prevalence was observed. The genotype G3P[8] was not detected in 2008. In five samples (1.2%) unusual genotypes were detected, of which only G10P[14] was detected also in 2007. The percentage of mixed infections was higher in 2008 compared to 2007 and represents 3.5% of analyzed samples (Table 1).

Discussion

The immune protection against rotavirus infection is critical because of high antigenic diversity of rotaviruses.[19] The main goal of developed rotavirus vaccines is to elicit heterotypic immune response in order to efficiently protect against severe rotavirus infections, requiring hospitalization or medical treatment. At the moment no reports were published on general vaccine failure in protection against some rotavirus serotype/genotype. However it is of great importance for continuous surveillance of rotavirus circulating strains also after the introduction of rotavirus vaccine. Only with continuous surveillance possible »escape mutants« not covered with current vaccines could be detected soon enough to take action on vaccine modification. The real evidence of such rotavirus strains will be the simultaneously increasing incidence of rotavirus infections among vaccinated children and the increasing prevalence of the specific rotavirus strain.

In Slovenia, rotavirus vaccine Rotarix® was introduced in January 2007 and RotaTeq® was available since the end of 2008. As the cost of rotavirus vaccine is relatively high and vaccination is not obligatory, there will be probably no major effect of rotavirus vaccine introduction in Slovenia. This is also evident when comparing the population of children eligible for rotavirus vaccination and the number of vaccinated children in 2007. The official number of vaccinated children in 2007 in Slovenia according to the IPHRS data is 1080[20] and there were 19823 newborn children in 2007 (source: http://www.stat.si). Unfortunately there are no official data of rotavirus vaccinated children in 2008, but probable the percentage of vaccinated children in 2008 and in the future will be higher than in the first year of vaccination. Thus, it will be interesting to follow the incidence of rotavirus infections in up to five year old children in upcoming years.

The seasonal distribution of rotavirus infection in Slovenia is typical for the areas with climatic changes, where rotavirus infections are most common in winter months.[1] Interestingly, in 2007 there was a clear shift in rotavirus infection peak, beginning in April and lasting to the late spring, June. In 2008 there was again a typical distribution of rotavirus infection with a clear and high peak between November 2007 and April 2008 (Figure 2). The reason for this shift in 2007 is not known and should be examined in details. The dynamics of rotavirus infections probably depends on various factors which should be considered, like climatic changes in year-seasons, appearance of rotavirus infections in neighbor countries, emergence of new rotavirus strains, etc. In previous years in Slovenia similar epidemiology of rotavirus disease was observed with incidence peak between February and April, affecting children mostly between 1 and 4 years of age.[21, 22]

Rotavirus genotype distribution in Slovenia is similar to that observed in other European countries with G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] being the most prevalent genotypes.[23] However, there are also some other less frequent genotypes which are or could be possible rotavirus strains of zoonotic origin, detected also previously in Slovenia.[18, 24] Zoonotic transmitted rotavirus strains from animal to humans replicate mostly with low efficiency in human enterocites, causing mild clinical signs or asymptomatic infections and are not able to cause outbreaks of severe rotavirus disease. The zoonotic transmission became more important in case of double infections where animal and human rotavirus strains could reassort their genome segments resulting in new reassortant strains.[4, 25] The evidences of genome reassortment were clearly shown in some human rotavirus strains, originating from animals, like G12 and G9 human rotavirus strains.[26, 27] After genome reassortment with a common human genotype P[8], G9 and G12 spread efficiently in human population. For genotype G10 very similar pattern of molecular epidemiology was detected, reminding us to the history of G9 and G12 genotype stains.[28] Rotavirus G10 strains were detected in this study in combination with P[14]. It was also detected in mixed infection with G1P[8] genotype, which could be the basis for genome reassortment to G10P[8] combination. However, in Slovenia G10 was already detected in 2005/06 in combination with P[8] and P[9].[29] As G10 was already detected in combination with P[8] in humans it is not clear, why this rotavirus strain is not more prevalent. To answer this question more data on the epidemiology and molecular characteristics have to be collected in the future.

The phylogenetic analysis of human G10 rotavirus strains showed that they are closely related to bovine rotavirus strains.[28] Some genetic characteristics of G10 strains are very strongly related to G2 strains, like I2 genotype of the gene encoding for VP6 protein and E2 genotype of the NSP4 enterotoxin gene. Both characteristics are reflected in antigen determinants of VP6 protein and NSP4 enterotoxin and are at least partially important in pathogenesis and immune protection of rotavirus infections.[28] As rotavirus vaccines showed lower protection against G2P[4] rotavirus infection it is important to follow also the spread of G10 rotavirus strains in humans taking into account that both rotavirus vaccines in Europe were not tested against G10 rotavirus strains.

Genetic variability of rotaviruses is high, which is reflecting also in the antigen diversity of rotaviruses. For this reason it is crucial to follow molecular changes of rotaviruses and try to predict possible virus mutations, representing »escape mutants« of rotavirus immune protection with current vaccines. There are some theories considering the possibility of seasonal vaccine modifications after the change in molecular epidemiology of rotaviruses.[23] However, currently there are no evidences confirming the need for these measures. Comparing previous studies of rotavirus molecular epidemiology in Slovenia[18, 24, 29] with this study, there was no major change detected in rotavirus genotype distribution after the first two years using rotavirus vaccine. According to the low vaccine coverage in Slovenia it is not likely to expect changes in rotavirus disease epidemiology or in rotavirus genotype distribution.

As long as postlicensure surveillance is going on and there are no data on vaccine efficiency for new emerging serotypes and genotypes the rotavirus strain surveillance should be carried on.

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References

1. Parashar UD, Humelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9: 565–72.
2. Glass R, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, et al. Rotavirus vaccines: current prospects and future challenges. Lancet 2006; 368: 323–32.
3. Estes MK, Cohen J. Rotavirus gene structure and function. Microbiol Rev 1989; 53: 410–49.
4. Gentsch JR, Laird AR, Bielfeld B, Griffin DD, Bányai K, Ramachandran M, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J Infect dis 2005; 192: 146–59.
5. Iturriza-Gómara M, Desselberger U, Gray J. Molecular epidemiology of rotaviruses: Genetic mechanisma associated with diversity. In: Desselberger U, Gray J, eds. Viral Gastroenteritis. 1th ed. Amsterdam: Elsevier; 2003. p. 317–44.
6. Matthijnnsens J, Ciarlet M, Rahman M, Attoui H, Bányai K, Estes MK et al. Recommendations for the classification of Group A rotaviruses using all 11 genomic RNA segments. Arch Virol 2008; 153: 1621–9.
7. Solberg OD, Hasing ME, Trueba G, Eisenberg JNS. Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypable Group A rotavirus. Virology 2009; 385: 58–67.
8. Poljak M. Cepiva proti rotavirusnim okužbam. Med Razgl 2007; 46(S2): 163–74.
9, Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC et al. Safety and efficacy o fan attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006; 354: 11–22.
10. Vesicari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z et al. Safety and efficacy of a pentavalente human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006; 354: 23–33.
11. Kirkwood CD, Cannan D, Boniface K, Bishop RF, Barnes GL; Australian Rotavirus Surveillance Group. Australian Rotavirus Surveillance Program annual report, 2007/08. Commun Dis Intell 2008; 32: 425–9.
12. Gouvea VS, Dias GS, Aguiar EA, Pedro AR, Fichman ER, Chinem ES et al. Acute gastroenteritis in a pediatric hospital in Rio de Janeiro in pre- and post-rotavirus vaccination settings. Open Virol J 2009; 3: 26–30.
13. Tate JE, Panozzo CA, Payne DC, Patel MM, Cortese MM, Fowlkes AL et al. Decline and change in seasonality of US rotavirusactivity after the introduction of rotavirus vaccine. Pediatrics 2009; 124: 465–71.
14. Iturriza-Gómara M, Cubitt D, Desselberger U, Gray J. An amino acid substitution at the antigenic region A of the VP7 protein of G2 rotavirus strain associated with the failure to serotype. J Clin Microbiol 2001; 39: 3796–98.
15. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 1992; 30: 1365–73.
16. Iturriza-Gómara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. J Clin Virol 2004a; 31: 259–65.
17. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 1990; 28: 276–82.
18. Steyer A, Poljšak-Prijatelj M, Lužnik Bufon T, Marčun-Varda N, Marin J. Rotavirus genotypes in Slovenia: unexpected detection of G8P[8] and G12P[8] genotypes. J Med Virol 2007; 79: 626–32.
19. Estes M, Kapikian AZ. Rotaviruses. V: Knipe DM, Howley PM, ur. Fields Virology, 5th Edition. Philadelphia: Lippincot Williams & Wilkins, 2007: 1917–74.
20. Kraigher A, Grgič Vitek M, Metličar T, Javornik S, Pirnat N. Analiza izvajanja imunizacijskega programa v Sloveniji v letu 2007. Inštitut za varovanje zdravja Republike Slovenije Sept 2008. Dosegljivo na: http://www.ivz.si/.
21. Lužnik Bufon T, Poljšak-Prijatelj M, Steyer A, Zimšek Mijovski J, Marin J, Balog T et al. Virusni gastroenteritis. Med Razgl 2004; 43: 121–9.
22. Zakotnik B. Bolnišnične obravnave zaradi črevesnih virusnih okužb. Med Razgl 2007; 46: 89–95.
23. Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquinto C, Vesikari T. Rotavirus types in Europe and their significance for vaccination. Pediatr Infect Dis J 2006; 25: 30–41.
24. Steyer A, Poljšak-Prijatelj M, Barlič-Maganja D, Marin J. Human, porcine and bovine rotaviruses in Slovenia: evidence of interspecies transmission and genome reassortment. J Gen Virol 2008; 89: 1690–98.
25. Cook N, Bridger J, Kendall K, Iturriza Gomara M, El-Attar L, Gray J. The zoonotic potential of rotaviruses. J Infecr 2004; 48: 289–302.
26. Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, et al. Evolutionary history and global spread of the emerging G12 human rotaviruses. J Virol 2007; 81: 2382–90.
27. Iturriza-Gómara M, Cubitt D, Steele D, Green J, Brown D, Kang G, et al. Characterisation of rotavirus G9 strains isolated in the UK between 1995 and 1998. J Med Virol 2000; 61: 510–17.
28. Iturriza-Gómara M, Kang G, Mammen A, Jana AK, Abraham M, Desselberger U, Brown D and Gray J. 2004b. Characterisation of G10P[11] Rotaviruses 29. Causing Acute Gastroenteritis in Neonates and Infants in Vellore, India. J Clin Microbiol 2004b; 42: 2541–47.
29. Tcheremenskaia O, Marucci G, De Petris S, Ruggeri FM, Dovecar D, Sternak SL, et al. Molecular epidemiology of rotavirus in Central and Southeastern Europe. J Clin Microbiol 2007; 45: 2197–204.

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