Rotavirus surveillance in Hungary in the pre-vaccine era

Ph.D. thesis

Krisztián Bányai

University of Pécs
Faculty of Medicine
Institute of Medical Microbiology and Immunology

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Program leader: Levente Emődy, M.D., D.Sc.
(Molecular pathogenesis of bacterial infections)

Tutor: György Szűcs, M.D., Ph.D.
(Molecular epidemiological investigation of viral gastroenteritis)

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Introduction

Based on similarities between capsid structure, genome organization, and replication strategy, rotaviruses have been classified a separate genus (*Rotavirus*) within the *Reoviridae* family. Differences in the antigenic properties, gene sequences and the genome pattern serve as the basis for further classification within the genus; serogroups (groups), subgroups, serotypes and genotypes, and electropherotypes are distinguished. The inner shell protein, VP6, is the group and subgroup-specific antigen. There are at least seven groups (designated A to G), three of which (group A, B, and C) cause disease in humans. Within group A rotaviruses, four subgroups (sg) are distinguished (sgI, sgII, sgI+II, and sg nonI-nonII). Serotype and genotype specificities are carried by the two outer capsid antigens, VP7 and VP4. The VP7 antigen determines the G (Glycoprotein) serotypes/genotypes and the VP4 antigen determines the P (Protease sensitive) serotypes/genotypes. The use of the terms serotype and genotype depends on whether antigen based detection methods (MAb-ELISA, cross-neutralization assay) or nucleic acid based detection methods (nucleotide sequencing, RT-PCR, oligonucleotide probe hybridization) are utilized. At present, 15 G types and 27 P types are known; of these, 11 G types and 12 P types have been detected in humans. Both surface antigens elicit neutralizing antibodies. This perception led to the introduction of a dual nomenclature for group A rotavirus strains (similarly to that of utilized for influenza A viruses), where the P type specificity is followed by G type specificity (e.g., P[9],G3). Rotaviruses can be classified into two major genome patterns (also called as RNA profile, electropherotype, or E-type), which are designated as ‘long’ and ‘short’ profile based on differences in the relative migration pattern of genome segments 10 and 11 in polyacrylamide gels. However, a large extent of pattern variability has been found within the two major electropherotypes.

Human rotaviruses were first described in 1973. Since then, partly due to the wide availability of rapid diagnostic tests, it has been found that these pathogens, especially group A rotaviruses, are the major cause of childhood gastroenteritis cases. An estimated 130-140 million episodes and 400-500 thousand fatal cases occur each year worldwide, most cases occur in children younger than 5 years. Although the mortality has been

estimated to continuously decrease by the 1990s, rotaviruses are still responsible for one-fourth of childhood diarrhea-associated deaths. Rotavirus infections can be efficiently treated with oral or intravenous electrolyte and fluid supplementation. However, due to the regional inequalities in medical care and to economic and societal burden, the WHO gave priority to vaccine development in the fight against rotavirus disease. One of the leading vaccine development strategies which was developed in the 1980s is based on two basic observations: (i) the majority of animal strains are naturally attenuated for humans, and (ii) serotype-specific immunity is important to prevent subsequent episodes. Multivalent animal-human reassortant vaccines have been developed, which include a mixture of human VP7, and, occasionally of VP4 type specificities (G1 to G4, and P[8]). A common feature of these vaccines is that each individual vaccine strain carries 10 gene segments from the animal parental strain (simian or bovine) and a single gene segment (encoding the VP7 and/or the VP4) is of human origin. The rhesus rotavirus based quadrivalent vaccine (rhesus-human reassortant tetravalent vaccine, RRV-TV) with the trademark name, RotaShield™, was introduced in the United States during the Autumn of 1998, but in nine months of routine use it was withdrawn from the market due to the recognition of its possible association with intussusception. Currently, a bovine-based pentavalent reassortant vaccine (RotaTeq™, Merck) and a monovalent vaccine that includes an attenuated human strain (RotaRix™, GlaxoSmithKline Biologicals) await licensure in many countries including those in the European Union.

Since the mid 1990s, in association with the possible implementation of national vaccination programs against rotaviruses, numerous countries have initiated surveys to estimate the rotavirus related disease burden and have started national rotavirus strain surveillance programs. In association with the vaccination strategies, the surveillance is focussing on determination of the sero- and genotypes of outer capsid antigens, VP7 and VP4. With the exception of a single paper, no serotyping studies were published in Hungary, resulting in significant gaps in our knowledge about the antigenic and genetic features of locally circulating human rotaviruses. The general need to gain more comprehensive and detailed data about the epidemiologic features of rotavirus infections and about circulating strains in Hungary determined the aims of this study.

Aims

Our aims were to:

- assess the relative importance of rotavirus infections in children admitted to hospital with gastroenteritis of infectious origin;
- screen a large number of rotavirus positive stool samples by using MAb-ELISA and PAGE;
- set up RT-PCR genotyping assay needed in the epidemiological surveys;
- determine the geographic and temporal variations in the distribution of VP7 serotypes;
- determine the diversity and genetic variability of the minor outer capsid antigen, VP4;
- explore the most common and less frequent allele combinations and thus identify reassortant strains by using a combination of typing methods (i.e., RNA pattern analysis, subgrouping, sero- and genotyping);
- reveal the possible phylogenetic relationships between human and animal rotaviruses;
- adapt and grow rare human strains in cell culture;
- design and utilize molecular reagents to the surveillance;
- provide information for health care policy makers about the epidemiological findings that would give supporting information when decisions are made about the possible introduction of a rotavirus vaccine in Hungary.

Materials and methods

Stool specimens: Rotavirus positive stool samples were collected from children admitted to hospitals with diarrhea from two geographical regions (Baranya County and Budapest) between 1992 and 2003. The laboratory diagnosis was performed in the Regional Laboratory of Virology (ANTSZ, Baranya County Institute of State Public Health Service, Pécs) and in the Laboratory for Diagnostic Virology ("St László" Central Hospital for Infectious Diseases, Budapest), respectively.

Detection of rotaviruses: Routine diagnostic procedures were carried out using a commercial latex-agglutination test (Rotalex, Orion Diagnostica) and an immunochromatographic test (Rota Uni-Strip, Coris BioConcept) according to the manufacturer's instruction or a home-made enzyme-linked immunosorbent-assay (ELISA). In part with the aim to confirm antigen detection results, we investigated the genome pattern analysis by polyacrylamide gel electrophoresis (PAGE). Lastly, we attempted to adapt a few rare strains to cell culture.

Molecular epidemiological investigations: PAGE was used to detect mixed infections and differences among individual strains, and to follow changes in the geographical and temporal distribution of epidemiologically major and minor strains. Subgroup determination was performed with monoclonal antibody based ELISA (MAb-ELISA). The serotype and genotype of the surface antigens were determined by serotype-specific MAb-ELISA and multiplex reverse transcription-polymerase chain reaction (RT-PCR), respectively. In certain cases, nucleotide sequencing and phylogenetic analysis was also done.

Computer analysis: Raw sequence data were edited using the GeneDoc software. Edited sequences and reference sequences downloaded from DNA data bases (GenBank/EMBL/DDJB) were aligned with ClustalX or DAMBE. Phylogenetic inference was performed with the neighbor-joining algorithm and bootstrapping for the statistical analysis using the software MEGA2 and with the maximum-likelihood using the software packages PHYLIP and TreeView.

Results

Identification of human rotaviruses in gastroenteritis cases. In general, we had only approximate data on the proportion of rotavirus positive cases. For example, at the St. László Hospital rotavirus was detected in 20-40% in stool samples of children hospitalized with gastroenteritis. [Mihály I., personal communication]. There was somewhat more reliable data available from our diagnostic practice in Baranya County, partly in association with the extensive co-operative relationship with the Infectious Ward of Baranya County Children's Hospital. Between 1996 and 2000, the proportion of rotavirus positive cases was 21% (annual

range: 15-29%) in Baranya County. By the numerical comparison of annual cases we realized that the proportion of infants and young children admitted to hospital with rotavirus infection is higher than the proportion of children admitted with infections due to enteric bacteria, and the cumulative risk for hospitalization of children by the age of 5 years is twice, thrice, and forty times higher for rotavirus than the corresponding risk associated with infections due to *Salmonella*, *Campylobacter* and *Shigella*. These data demonstrate the leading etiology of rotaviruses in childhood enteric infections.

Identification of human rotaviruses by genome pattern analysis. The electrophoretic analysis of the 11 genomic segments of rotaviruses was done to supplement antigen based diagnostic results and to perform prospective molecular epidemiological investigation. Altogether 6936 samples were analyzed between 1992 and 2003; the proportion of electropherotypeable strains was ~80%. The remaining samples were negative by this method or were not electropherotypeable due to the lack of certain genome segments. The relative proportions of the two major electropherotypes, 'long' (L) and 'short' (S), were 94% and 6%, respectively. Within both major RNA profiles we identified at least 7-10 different major patterns and an additional 7-10 minor electropherotype variants. During the 13-year study, the predominant electropherotypes were different each year in both areas (Budapest and Baranya County) and with the exception of the 1996-1997 Budapest season, the 'long' E-type strains predominated each season.

Occurrence of human rotavirus G types in Hungary. A total of 3537 samples were G typed between 1992 and 2003 by MAb-ELISA and RT-PCR. Depending on the amount of original stool samples, 15% to 82% of specimens (mean, ~51%) were subjected to G typing. Altogether, 2563 strains were examined with serotyping ELISA, of which 1971 (77%) were successfully G typed, and 1604 strains were examined with genotyping RT-PCR (including 530 samples that were not serotypeable with MAb-ELISA), of which 1556 (97%) were genotypeable. Compiling the G typing results from the ELISA and RT-PCR, we could determine the G type for 3270 strains (93%).

Among these strains, six G types (G1, G2, G3, G4, G6, and G9) were identified. When examining the relative importance of these six G types, we found that G1 strains are the most prevalent (58%). Serotype

G9 strains were found to be the second most frequent rotaviruses (16%) although their circulation in Hungary was only shown as late as 1998 and spread to a wider region only in 2000. The globally common G types, G2 (8%), G3 (1,5%), and G4 (6%) together, demonstrated a comparable frequency with the G9 strains (15.5% vs. 16%). The least frequent strains were of serotype G6 (~1%).

Remarkable seasonal and geographical variations have been seen in the strain prevalence. Among the six identified G types, G1 strains predominated in seven of eight seasons in Baranya County, and nine of 11 seasons in Budapest and the metropolitan area. The relative frequency of G1 strains varied year-by-year (range, 23% to 100%). Consistent with this finding we recognized shifts in serotype prevalence in certain seasons: in those particular seasons, serotype G2 (42%; Budapest, 1996-1997), G4 (43%; Baranya County, 1999-2000), and G9 (51%; Budapest, 2002-2003) rotaviruses emerged to become the predominant strains. An intriguing observation was that during the serotype shift in 1999-2000 in Baranya County, the number of rotavirus-associated hospitalizations has significantly increased.

Occurrence of human rotavirus P types in Hungary. The P type of circulating rotaviruses was determined with multiplex RT-PCR genotyping. A total of 1457 strains were subjected to P typing and 1413 (97%) of those strains were successfully P typed.

Although the total number of strains included in P typing was relatively high, we could not provide true prevalence data because of sampling bias in the early study period. While only 284 strains were P typed between 1992 and 2000, the total number of P typed strains in the 2000-2003 period was as high as 1173. In spite of this bias in sample selection five different, including two rare P types were identified.

These P types were P[4], P[6], P[8], P[9], and P[14], of which, with the exception of a single identified P[14] strain, all P types circulated in both regions, in each season.

Comprehensive data on the relative frequency of P types were available only for the 2000-2003 study period. In this short period we observed the dominating prevalence of genotype P[8] strains (89%); genotype P[4] (3%), P[6] (~1%), and P[9] (~2%) strains circulated at much lower frequency (in total, ~6%).

Common and rare genome patterns and antigen combinations. To explore the genetic heterogeneity of concurrently circulating strains and to understand the importance of reassortment we compiled the data from E-, G- and P typing and subgrouping, although due to the low quantity of subgroup-specific monoclonal antibodies this complete picture could be drawn only for two-hundred samples or so. The analysis of the constellation of genome pattern and different antigen specificities (VP6, VP7, and VP4) demonstrated the occurrence of the globally spread gene combinations (P[8],G1 sgII L; P[4],G2 sgI S; P[8],G3 sgII L; P[8],G4 sgII L; P[8],G9 sgII L) and several rare gene combinations, as well (P[4],G2 sgI L; P[6],G4 sgII L; P[8],G9 sgI L; P[9],G6 sgI L; P[9],G3 sgI L; P[14],G6 sgI L; P[4],G1 S).

Sequencing and phylogenetic analysis of the outer capsid genes of rare and emerging rotavirus strains. We characterized the VP7 and VP4 genes of the unusual human strain, G6, as well as the VP7 and VP4 genes of the pandemic serotype G9 human strain, and lastly the VP4 gene of P[6] rotaviruses, a relatively rare genotype in Hungary.

Molecular analysis of P[9],G6 and P[14],G6 strains. We identified three phylogenetic lineages of the VP7 gene of serotype G6 specificity. This analysis revealed close genetic relationship of these strains to representative strains of caprine, bovine, and buffalo origin. The single P untypeable G6 strain was found to be of genotype P[14], while all the remaining G6 strains carried P[9] VP4 specificity representing two distinct genetic lineages.

Molecular analysis of P[8],G9 strains. Serotype G9 rotaviruses were detected in Hungary for the first time in 1998 and soon after their first description they have emerged to become the second most frequent serotype.

Our computer analysis on the VP7 gene of G9 strains identified the globally important lineage that has also been circulating in a variety of countries throughout the world (United States, India, Australia, Malawi, Great Britain, The Netherlands, Italy, Albania, South Africa, Thailand, Japan, Belgium, Slovenia, Sweden) for the past ten years. Strains within this lineage clearly segregate from those strains that were identified in the 1980s in India, Japan, and the United States. The presumed ancestor of these strains is porcine of origin.

Molecular analysis of P[6],G4 strains. We identified two phylogenetic lineages among the uncommon Hungarian VP4 specificity, P[6], none of which was known from earlier studies. Later, in a cooperation with our Italian colleagues, we analyzed a number of porcine P[6] strains. This study revealed that at least one of our human P[6] lineage may have originated from swine rotaviruses.

In conclusion, in agreement with others' observations our phylogenetic analyses demonstrate that serotype G6, G9 and genotype P[6] strains have transmitted from their respective hosts to humans on several, independent occasions.

Summary; presentation of novel findings

Since the mid 1990s, the aim to alleviate the burden of rotavirus-related infections prompted the initiation of national surveillance program in numerous countries in order to understand the true significance of rotavirus disease and to explore the genetic and antigenic diversity of concurrently circulating rotavirus strains.

Determination of the social and economic losses resulting from rotavirus infection provides crucial information for health care policies to understand the possible local benefits of a national immunization program (whether decrease in fatal cases and/or in costs associated with the treatment can be expected). In our country, a single multi-center survey has been conducted in this topic, and its remarkable conclusions were published in 1999 in the Supplement of Acta Paediatrica. Those results demonstrated that the incidence of hospitalization due to rotavirus is among the highest across Europe and the estimated direct costs of treatment are ~5 Million US$ each year.

The perception that serotype-specific (homotypic) immunity is important in the protection against (re-)infections with rotavirus resulted in the idea of constructing polyvalent vaccines and a decade later, when the introduction of the first rotavirus vaccine, RotaShield^{TM}, was at threshold, has prompted to collect more comprehensive data on serotypes and genotypes of rotaviruses circulating in the community. The original aim of the present study was to participate in these international efforts.

These surveillance studies focus on the sero- and genotyping of the outer capsid antigens. However, in order to follow the epidemic spread of individual rotavirus strains, to detect infections with multiple strains (i.e., mixed infections), and to identify unusual and novel strains, it might be necessary to include other typing methods (e.g., subgrouping, electropherotyping, and nucleic acid sequencing) in the diagnostic arsenal. Both serotype-specific MAb-ELISA and multiplex genotyping RT-PCR are widely used methods in the analysis of antigen specificities. In our survey, we utilized both techniques for G typing but only RT-PCR based genotying was used for P typing as reliable, type-specific MAbs to determine the VP4 specificity are currently not available. In certain instances nucleic acid sequencing and phylogenetic analysis were used to confirm the typing results.

In the view of our aims, the following conclusions and achievements were made:

- Among children admitted to hospital with infectious intestinal disease, the proportion of rotavirus-positive cases is higher than proportion of cases associated with infection with enteric bacteria (*Salmonella*, *Shigella* és *Campylobacter*).
- By the routine use of PAGE we were able to perform basic molecular epidemiologic investigation on the majority of strains (~80%), this method was used as a screening technique to distinguish common and rare strains and was proven to be useful to select individual strains for further characterization.
- Serotyping MAb-ELISA allowed us to determine the G type for a large number of strains.
- We set up multiplex RT-PCR based genotyping assays in the epidemiological study of rotaviruses. (This was a great breakthrough not only in the enhancement of sensitivity but also in the increase of specificity.) A number of strains untypeable by serotyping MAb-ELISA was typeable by RT-PCR.
- The combination of MAb-ELISA and RT-PCR results helped us observe the trends in the epidemiology of rotavirus strains. We observed substantial differences in both temporal and geographical distribution of serotypes.
- We identified the globally important, pandemic serotype G9 strains, which have been in circulation in Hungary since their first detection. Our sequence- and phylogenetic analysis demonstrated

that the G9 strains having circulated in Hungary between 1998 and 2001 belong to the globally emerging predominant phylogenetic lineage and are easily separated from strains that were identified in some exotic areas as well as from strains that were identified in the 1980s.

- The genotyping RT-PCR assay and the molecular characterization revealed the circulation of five genotypes of the minor outer capsid antigen, VP4. However, unlike for the G type specificities, we were unable to fully explore the geographical and temporal distribution of the identified P types.
- Compiling the RNA profile, subgroup, and sero/-genotype specificities we identified the epidemiologically major and minor strains including the presumably reassorted rare rotaviruses. In the study period (between 1992 and 2003), partly due to the introduction of molecular techniques we identified several antigen combinations, which were not detected earlier (e.g., P[9],G3; P[9],G6; P[14],G6; P[6],G4; P[4],G1).
- The outer capsid antigens of selected unusual strains (P[14],G6; P[9],G6 and P[6],G4) were characterized by sequencing and phylogenetic analysis. Some of these strains demonstrated genetic relatedness with certain animal strains, mainly of porcine and bovine origin, suggesting that they were introduced in the human population via interspecies transmission. A few of these unusual strains have not yet been identified in humans in other parts of the world, although their possible animal ancestors were detected.
- At present, it seems to be a unique phenomenon that serotype G6 strains are endemic in the Hungarian population, even though these are only minor members of the circulating rotaviruses (with a relative prevalence of ~1%); these strains were detected for the first time in 1995 and since then they have been circulating as far as the last studied season, 2002-2003. The molecular characterization of these G6 strains revealed that they may have been introduced to humans on several, independent interspecies transmission events.
- Some of the rare Hungarian rotavirus strains have been adapted to cell culture and these strains are now in the rotavirus strain collection at the NIH.
- It is well known from the literature that as much as 5-40% of strains subjected to antigen typing remain untypeable. Our findings

correlate with these data. Therefore, one can assume that, at least a small proportion of these samples might be serotype G6. To try to help explore the epidemiological importance of these strains in humans, we designed and utilized type-specific oligonucleotide primers for RT-PCR based genotyping assays. The primers were proven to be useful in the detection of a variety of human G6 strains having isolated in Hungary and other parts of the world.

- In order to help health policy makers in their future decision about the possible introduction of rotavirus vaccines in Hungary we presented the results of our survey in several national and international conferences, and in a variety of Hungarian and international scientific journals.

List of publications

Publications served as basis for the thesis

- ***Articles:***

1. **Bányai K,** Gentsch JR, Glass RI, Szűcs G. 2003. Detection of human rotavirus serotype G6 in Hungary. Epidemiology and Infection 130:107-112 (IF: 1.509)
2. **Bányai K**, Gentsch JR, Griffin DD, Holmes JL, Glass RI, Szűcs G. 2003. Genetic variability among serotype G6 human rotaviruses: identification of a novel lineage isolated in Hungary. Journal of Medical Virology 71:124-134 (IF: 2.371)
3. **Bányai K**, Gentsch JR, Új M, Mihály I, Glass RI, Szűcs G. 2004. Eight-year survey of human rotavirus strains demonstrates circulation of unusual G and P types in Hungary. Journal of Clinical Microbiology 42:393-397 (IF: 3.439)
4. **Bányai K,** Gentsch JR, Schipp R, Jakab F, Bene J, Melegh B, Glass RI, Szűcs G. 2004. Molecular epidemiology of P[8],G9 rotaviruses in Hungary between 1998 and 2001. Journal of Medical Microbiology 53:791-801 (IF: 2.484)
5. **Bányai K,** Martella V, Jakab F, Melegh B, Szűcs G. 2004. Sequencing and phylogenetic analysis of human genotype P[6] rotavirus strains detected in Hungary provides evidence for genetic heterogeneity within the P[6] VP4 gene. Journal of Clinical Microbiology 42:4338-4343 (IF: 3.439)

6. **Bányai K**, Sas Y, Varga L, Szűcs G. 2004. Survey of rotavirus infections in a Hungarian paediatric hospital. Acta Microbiologica et Immunologica Hungarica 51:431-435 (IF: –)
7. **Bányai K,** Szűcs G. 2005**.** Indokok és kérdések a rotavírusvakcina hazai bevezetésével kapcsolatban. Gyermekgyógyászat 56:196-208 (IF: –); *Hungarian*
8. **Bányai K,** Gentsch JR, Schipp R, Jakab F, Meleg E, Mihály I, Szűcs G. 2005**.** Dominating prevalence of P[8],G1 and P[8],G9 rotavirus strains among children admitted to hospital between 2000 and 2003 in Budapest, Hungary. Journal of Medical Virology 76:414-423 (IF: 2.331)
9. Gentsch JR, Laird AR, Bielfelt B, Griffin DD, **Bányai K,** Ramachandran M, Jain V, Cunliffe NA, Nakagomi O, Kirkwood CD, Fischer TK, Parashar UD, Bresee JS, Jiang B, Glass RI. 2005**.** Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. Journal of Infectious Diseases 192:S146-S159 (IF: 4.943)

- ***Oral presentations, posters and their abstract that appeared in scientific journals (Abst):***

1. **Bányai K**, Gentsch J, Holmes J, Griffin D, Glass R, Új M, Reuter G, Szűcs G – Rotavírus törzsek vizsgálata Magyarországon hat egymást követő rotavírus-szezonban (poster; Annual Meeting of the Hungarian Society for Microbiology, Keszthely, Hungary, 2000; Abst: Acta Microbiologica et Immunologica Hungarica, 2001, 48(2):224); *Hungarian*
2. **Bányai K**, Varga L, Sas Y, Új M, Reuter G, Szűcs G – Rotavírus fertőzések jelentősége a Baranya Megyei Gyermekkórház fertőző osztályának gasztroenterális anyagában négy rotavírus szezonban (1996-2000) (poster; National Congress of the Hungarian Society for Hygiene, Debrecen, Hungary, 2000); *Hungarian*
3. **Bányai K**, Gentsch J, Glass R, Szűcs G, Reuter G, Jakab F – G6 szerotípusú rotavírusok kimutatása Magyarországon – másodikként Európában (poster; Annual Meeting of the Hungarian Society for Infectology, Budapest, Hungary, 2000; Abst: Infektológia és Klinikai Mikrobiológia, 2000, Suppl. 1. S26); *Hungarian*
4. Gentsch J, Laird A, **Bányai K**, Griffin D, Cunliffe N, Shin G, Sen A, Jiang B, Glass R – Emerging rotavirus serotypes: implications for

global vaccination programs (poster; 12th International Congress of Virology, 2002; Paris, France)

5. **Bányai K**, Jakab F, Új M, Szűcs G – G9 szerotípusú humán rotavírusok azonosítása és filogenetikai elemzése (oral presentation; Annual Meeting of the Hungarian Society for Microbiology, Balatonfüred, 2002; Abst: Acta Microbiologica et Immunologica Hungarica, 2003, 51(1-2):179); *Hungarian*
6. **Bányai K,** Gentsch JR, Schipp R, Jakab F, Bene J, Melegh B, Glass RI, Szűcs G – Molecular epdemiology of human P[8],G9 rotaviruses in Hungary between 1998 and 2001 (poster; 8th International Symposium on dsRNA Viruses 2003; Castelvecchio Pascoli, Italy)
7. **Bányai K** – Rotavírus surveillance a vakcinációt megelőző időszakban Magyarországon (oral presentation; 11th National Congress on Vaccines, Eger, Hungary, 2005); *Hungarian*

Other publications

- ***Book chapters***:

1. Reuter G, Jakab F, **Bányai K,** Szűcs Gy. Gasztroenteritiszt okozó vírusok. In: Berencsi Gy (ed.) Orvosi molekuláris virológia. 22-39. old. 2005; *Hungarian*
2. **Bányai K,** Szűcs Gy. Acut enteritist okozó vírusinfekciók epidemiológiája, diagnosztikája, klinikuma és megelőzésének lehetőségei. In: Túri S (ed.) Gyermekgyógyászati továbbképző előadások. Tiszaparti esték 2004-2005; *Hungarian*

- ***Articles***:

1. **Bányai K**, Angyal M, Körmendi É, Lakatos F, Új M, Szűcs G. 2002. Humán rotavírus járvány felnőtt közösségben. Orvosi Hetilap 143:1347-1352 (IF: –); *Hungarian*
2. **Bányai K**, Máté Z, Ádám É, Új M, Nász I, Szűcs G. 2003. Screening adenoviruses in stool samples: evaluation of a genus-specific monoclonal antibody based enzyme immunoassay. Acta Microbiologica et Immunologica Hungarica 50:23-32 (IF: –)
3. Palya V, Glávits R, Dobos-Kovács M, Ivanics É, Nagy E, **Bányai K,** Reuter G, Szűcs G, Dán Á, Benkő M. 2003. Reovirus identified as cause of disease in young geese. Avian Pathology 32:129-138 (IF: 1.271)

4. Palya V, Glávits R, Dobos-Kovács M, Ivanics É, Nagy E, **Bányai K,** Reuter G, Szűcs G, Dán Á, Benkő M. 2003. Növendék ludak reovírus okozta betegsége (másodközlés). Magyar Állatorvosok Lapja 125:406-414 (IF: 0.089); *Hungarian*
5. **Bányai K,** Jakab F, Reuter G, Bene J, Új M, Melegh B, Szűcs G. 2003. Sequence heterogeneity among human picobirnaviruses detected in a gastroenteritis outbreak. Archives of Virology 148:2281-2291 (IF: 1.876)
6. Jakab F, Meleg E, **Bányai K,** Melegh B, Tímár L, Péterfai J, Szűcs G. 2004. One year survey of astrovirus infection in children with gastroenteritis in a large hospital in Hungary – Occurrence and genetic analysis of astroviruses. Journal of Medical Virology 72:71-77 (IF: 2.331)
7. Martella V, Ciarlet M, Baselga R, Arista S, Elia G, Lorusso E, **Bányai K,** Terio V, Madio A, Ruggeri F, Falcone E, Camero M, Decaro N, Buonavoglia C. 2005**.** Sequence analysis of the VP7 and VP4 genes identifies a novel VP7 gene allele of porcine rotaviruses, sharing a common evolutionary origin with human G2 rotaviruses. Virology 337:111-123 (IF: 3.071)
8. Jakab F, Péterfai J, Meleg E, **Bányai K,** Mitchell DK, Szűcs G. 2005**.** Clinical characteristics of human astrovirus-associated infections diagnosed in 1997 to 2002 in Hungary. Acta Paediatrica 94:667-671 (IF: 1.143)
9. **Bányai K,** Forgách P, Erdélyi K, Martella V, Bogdán Á, Hocsák E, Havasi V, Melegh B, Szücs G. 2005**.** Identification of the novel lapine rotavirus genotype P[22] from an outbreak of enteritis in a Hungarian rabbitry. Virus Research 113:73-80 (IF: 2.155)
10. **Bányai K,** Palya V, Benkő M, Bene J, Havasi V, Melegh B, Szűcs G. 2005**.** The goose reovirus genome segment encoding the minor outer capsid protein, σ1/σC, is bicistronic and shares structural similarities with its counterpart in muscovy duck reovirus. Virus Genes 31:285-291 (IF:1.25)
11. Jakab F, Péterfai J, Verebély T, Meleg E, **Bányai K**, Mitchell DK, Szűcs G. Human astrovirus infection associated with childhood intussusception. Pediatrics International (in press; IF: 0.58)
12. Martella V, **Bányai K,** Ciarlet M, Iturriza-Gomara M, Lorusso E, De Grazia S, Arista S, Decaro N, Elia G, Cavalli A, Corrente M, Lavazza A, Baselga R, Buonavoglia C. Relationships among porcine and human P[6] rotaviruses: evidence that the different human P[6]

lineages have originated from multiple interspecies transmission events. Virology (in press; IF: 3.071)

13. Martella V, Ciarlet M, **Bányai K,** Lorusso E, Cavalli A, Corrente M, Elia G, Arista S, Medici MC, Desario C, Decaro N, Lavazza A, Tempesta M, Buonavoglia C. Identification of a novel VP4 genotype carried by a G5 porcine rotavirus strain. Virology (accepted for publication; IF: 3.071)
14. PROTECT [**Banyai K,** Desselberger U, Franco E, Giaquinto C, Grimpel E, Huppertz HI, Meurice F, Meszner Z, Mrukowicz J, Rodrigo C, Soriano-Gabarro M, Tatochenko V, Vesikari T, De Vos B, Wolleswinkel-van den Bosch J]. The pediatric burden of rotavirus disease in Europe. Epidemiology and Infection (under revision)
15. Kowalska-Duplaga K, Mrukowicz JZ, Strus M, Heczko P, Krobicka B, Szűcs G, **Bányai K,** Kurowska-Baran D. LACTOBIF®, a marketed probiotic product containing *Bifidobacterium ruminantium*, was not effective in the treatment of acute rotavirus diarrhoea in infants. Pediatrics (under revision)

- ***Oral presentations, posters and their abstract that appeared in scientific journals (Abst):***

1. Szűcs G, Reuter G, **Bányai K**, Jakab F, Új M – A humán calicivírusok jelentősége, kimutatásuk és taxonómiai osztályozásuk (oral presentation; Annual Meeting of the Hungarian Society for Microbiology, Keszthely, Hungary, 2000; Abst: Acta Microbiologica et Immunologica Hungarica, 2001, 48(2):209-210); *Hungarian*
2. Reuter G, Kátai A, Kálmán M, Farkas T, Berke T, **Bányai K**, Jiang X, Matson DO, Szűcs G – Humán calicivírus fertőzés első igazolása élelmiszer-járványból Magyarországon (oral presentation; Annual Meeting of the Hungarian Society for Microbiology, Keszthely, Hungary, 2000; Abst: Acta Microbiologica et Immunologica Hungarica, 2001, 48(2):266-267); *Hungarian*
3. Jakab F, **Bányai K**, Reuter G, Szűcs G – A humán astrovírusok kimutatása tenyésztéssel és molekuláris biológiai módszerekkel (oral presentation; National Congress of the Hungarian Society for Hygiene, Debrecen, Hungary, 2000); *Hungarian*
4. Reuter G, Kucsera S, Somogyi G, Lencsés G, **Bányai K**, Szikra L, Szűcs G – Humán calicivírus járvány kórházi osztályon (oral presentation; National Congress of the Hungarian Society for Hygiene, Debrecen, Hungary, 2000); *Hungarian*

5. Szűcs G, Reuter G, **Bányai K**, Jakab F, Új M – A molekuláris vizsgálatok eredményei megváltoztatták a human calicivírusok klinikai jelentőségét és rendszertanát (oral presentation; Annual Meeting of the Hungarian Society for Infectology, Budapest, Hungary, 2000; Abst: Infektológia és Klinikai Mikrobiológia, 2000, Suppl. 1. S11); *Hungarian*
6. Reuter G, Farkas T, Berke T, **Bányai K**, Jiang X, Matson DO, Szűcs G – Human calicivírus fertőzések kimutatása hazánkban (oral presentation; Annual Meeting of the Hungarian Society for Infectology, Budapest, Hungary, 2000; Abst: Infektológia és Klinikai Mikrobiológia, 2000, Suppl. 1. S11); *Hungarian*
7. **Bányai K**, Reuter G, Új M, Szűcs G – Picobirnavírus kimutatása emberi székletmintából (poster; Annual Meeting of the Hungarian Society for Microbiology, Balatonfüred, Hungary, 2001; Abst: Acta Microbiologica et Immunologica Hungarica, 2003, 50(2-3):281); *Hungarian*
8. Jakab F, Tímár L, **Bányai K,** Szűcs G – A humán astrovírus fertőzések jellemzői és klinikai vonatkozásai (oral presentation; Annual Meeting of the Hungarian Society for Microbiology, Balatonfüred, Hungary, 2002; Abst: Acta Microbiologica et Immunologica Hungarica, 2003, 51(1-2):178); *Hungarian*
9. Martella V, Baselga R, Ciarlet M, Lorusso E, Decaro N, Buonavoglia D, **Bányai K,** Buonavoglia C – Idenification of an atypical porcine rotavirus with a VP7 gene resembling human G2 rotaviruses (oral presentation; European Society for Virology Meeting, Madrid, Spain, 2004)
10. Meleg E, Jakab F, Kocsis B, **Bányai K,** Melegh B, Szűcs G – Humán astrovírusok első kimutatása szennyvízmintákból Magyarországon (oral presentation; PhD Scientific Days, Budapest, Hungary, 2005); *Hungarian*
11. Martella V, **Bányai K,** Ciarlet M, Lorusso E, De Grazia S, Camero M, Corrente M, Lavazza A, Ricci D, Buonavoglia C – Analysis of porcine P[6] rotaviruses identifies human-like strains and provides evidence that the different human P[6] lineages have originated from multiple interspecies transmission events (poster; 24th Annual Meeting of the American Society for Virology, Penn State University, USA, 2005)
12. Meszner Z, **Banyai K,** Pazdiora P, Mrukowicz J, Molnar G, Gorelov AV, Avdicova M, Kraigher A – Retrospective analysis of

childhood viral gastroenteritis in seven(six) countries in Central and Eastern Europe (CEE) (oral presentation; 23rd Meeting of the European Society for Paediatric Infectious Diseases, Valencia, Spain, 2005)

13. Martella V, **Bányai K,** Ciarlet M, Iturriza-Gómara M, Lorusso E, De Grazia S, Ricci D, Medici MC, Tempesta M, Buonavoglia C – Relationships among porcine and human P[6] rotaviruses (poster; 1st European Rotavirus Biology Meeting Paris, France, 2005)
14. Szűcs Gy, **Bányai K** – A rotavírus fertőzés nagyságrendje és költségkihatásai (oral presentation; 12th Scientific Meeting of the Pediatric Gastroenterology Section of the Society of Hungarian Pediatricians and the Society of Hungarian Gastroenterologists, Eger, Hungary, 2005); *Hungarian*

No. of book chapters: 2
No. of articles: 22 (+ 2 under revision)
No. of conference presentations: 21
Cumulative impact factor: 40.424
No. of independent citations: 20