UNIVERSITY OF VETERINARY
Postgraduate School of Veterinary Science

COMPARATIVE PHYLOGENETICAL ANALYSIS OF MAMMALIAN AND AVIAN ROTAVIRUSES

Brief Summary of the PhD Thesis

Hajnalka Harami-Papp

2017

Brief Summary of the PhD Thesis

COMPARATIVE PHYLOGENETICAL ANALYSIS OF MAMMALIAN AND AVIAN ROTAVIRUSES

Hajnalka Harami-Papp

Supervisor: Dr. Krisztián Bányai

UNIVERSITY OF VETERINARY
Postgraduate School of Veterinary Science

2017, Budapest

Introduction

The *Rotavirus* genus –member of the *Reoviridae* family– has great importance in public and animal health. They are one of the most prevalent viruses that cause acute gastroenteritis in animal and human hosts.

Almost 95% of the children under the age of five years have been affected by rotavirus infection worldwide. Diarrhea caused by rotavirus infection is causes leads to hospitalization of nearly two million children and to the death of half a million infants per year. Rotavirus infections also inflict severe economic losses in the livestock sector due to secondary symptoms as weight loss, developmental retardation and ultimately mortality due to severe fluid loss.

Human and mammalian rotaviruses are extensively investigated due to their major involvement in enteric diseases, however, only limited data about rotaviruses of avian hosts are available. Rotaviruses have a linear, segmented dsRNA genome. This constellation allows the combination of segment between different strains (reassortment events). This evolutionary mechanism is thought to be one of the main reason of the high genetic variability found in rotaviruses.

During my PhD period in the *Discovery of New Diseases Group* (MTA Institute for Veterinary Medical Research) our main goal was to determine and phylogenetically investigate numerous rotavirus group A (RVA) genome sequences from various, rarely investigated mammalian and avian hosts. Via comparative genome analysis of the investigated RVA strains we would like to further explore the mechanisms of rotavirus evolution. The obtained data could contribute to the geological mapping of rotavirus populations in time, and thus will help to follow the migration of RVA strains causing gastroenteritis epidemics. Hopefully, these epidemiological observations could aid in the development of effective vaccination programs in the future.

For extensive data acquisition our laboratory introduced a next generation sequencing method in addition to the classical molecular methods. The obtained genome sequence data were analyzed by phylogenetic and comparative analysis for scientific publication.

Aims of the study

1. Genome sequencing of novel Group A rotavirus (RVA) strains derived from various, rarely investigated host species originating from Hungary and international collaborations using classical molecular methods.
2. Introduction of a high-throughput next generation sequencing method (IonTorrent PGM) to determinate rare unidentified Group A rotavirus (RVA) strains.
3. Comparison of the determined RVA strains to international references and synthesis of sequence data in systematic reviews to explore the genetic diversity of Group A rotavirus (RVA) strains in various animal host.

Materials and methods

Samples

The 12 rotavirus positive samples used in our experiments were provided to us in the forms of tissue pieces or faeces by the Hungarian National Food Chain Safety Office (NÉBIH), CEVA Phylaxia Zrt., Public Authority for Agriculture and Fish Resources is Kuwait, and the Irish Equine Centre in Ireland. The samples were derived from equine, old world camel, canine, chicken, pheasant and domesticated turkey host species and were collected in Hungary, Ireland and Kuwait.

Molecular methods

Total RNA extraction from stool samples has been performed using QIAamp viral RNA Mini Kit (QIAgen). The complete or partial genome of rotaviruses has been determined with classical molecular biology methods in the case of eight rotavirus strains. The viral RNA was translated to cDNA with a one-step or two-step RT-PCR method. Further amplification by PCR was performed with specific primers. Nucleotide sequences were determined using the traditional Sanger sequencing method. The

ends of genome segments were determined by an RNA ligation based method.

In the case of four RVA strains complete genome analysis has been done via a newly introduced high-throughput next generation sequencing method. Briefly, the virus genome was amplified by a modified SISPA (sequence independent single primer amplification) method, followed by complete genome sequencing by a semiconductor IonTorrent Personal Genome Machine® (Life Technologies).

Nucleotide sequences obtained with the classical method were checked, corrected and aligned to homologous sequences alignments with the BioEdit and GeneDoc software. Data obtained from Ion Torrent sequencing were analysed with the CLC Genomics Workbench (www.clcbio.com) software.

The MEGA 5 software was used for generation of phylogenetic trees. The maximum likelihood tree-reconstruction algorithm was used along with the best fit substitution model that was selected by the Bayesian information criterion. The reliability of the phylogenetic tree has been validated by bootstrap analysis repeated 500 times.

Results

Mammalian rotaviruses

We have determined whole genome sequences of **equine RVAs** by classical molecular methods in part of an international research collaboration for the first time. Both investigated equine RVA strains originated from Ireland, from the same collection, with nearly the same collection date. A similar genome constellation was determined for the two strains, however, they belonged to distinct phylogenetic lineages. One of the equine RVA strains showed high sequence similarity with an equine RVA strain from South-Africa, whereas the other equine RVA strain was slightly, but considerably differed from other equine RVA strains determined by the collaboration or others. Some genes were found to be related to equine RVAs from Argentina, whereas other genes were related to equine RVA strains from Japan and Great Brittan. In addition to sequence determination, we have synthesized our data with previously published results in a systematic review about equine RVA global distribution to explore equine RVA epidemiology.

We have also determined the partial genome sequence of a **camel RVA** strain originated from Kuwait

by classical molecular methods. We have obtained the sequence information of a set of camel RVA genes that have not been investigated yet. Analyses revealed that this RVA strain is of high similarity to ovine, bovine, porcine and human RVA strains, further formed different lineage from other camel RVA strains from Egypt and Kuwait. Based on phylogenetical analysis we assumed that the camel RVA strain originated from reassortment events between animal and human RVA strains. Strikingly, the camel RVA strain had a unique NSP4 genotype that's sequence similarity was lower than 80% to other reference NSP4 genotypes. Due to this difference, this gene sequence was designated as a novel genotype (E15) by the Rotavirus Classification Working Group.

We have also determined the complete genome sequence of a Hungarian **canine RVA** strain with a next generation sequencing method. Its complete genome analysis revealed high similarity to an already specified human RVA strain from a children with gastroenteritis in Italy.

Both strains had a unique NSP1 genotype (A15). However, the canine RVA strain also showed close relation to other canine and feline RVA strains. Based on

the close genealogical relationship with human and canine/feline RVA strains we assumed a canine-human zoonotic potential of rotaviruses.

Avian rotaviruses

In addition to the investigation of mammalian RVA strains, we have determined the genomes of eight Hungarian avian RVA strains. The complete genome sequence of a laboratory control chicken RVA strain and complete or partial genomes from pheasant and turkey hosts were determined by classical molecular or next generation sequencing methods.

The genotype constellation of the **chicken RVA** strain was divergent form other previously published chicken RVA strains; interestingly it was more similar to RVA strains previously found in turkeys.

The five examined **pheasant RVA** strains had variations in genotype constellation. One of the strains was highly similar to a published pheasant RVA strains from Germany and Hungary, two strains were highly similar to turkey RVA strains and two other strains showed a close phylogenetic relationship with chicken RVA strains. In summary, these results suggest that

reassortment between pheasant and other RVA strains can effectively and frequently occur.

In addition to the above strains, we have determined the complete genome sequence of two **turkey RVA** strains. These strains showed close relation with other turkey and Hungarian pheasant RVA strains.

For the determination of complete or partial genome of domestic bird RVA strains, we partially used classical molecular methods and a new generation sequencing method.

New scientific achievements and conclusions

1. We have determined complete genome sequences of equine derived rotaviruses for the first time in a national collaboration. The phylogenetic analysis revealed a conservative genome constellation of equine RVA strains.
2. We have determined partial nucleotide sequences of VP7, VP4, VP1, VP2, NSP2, NSP3, NSP4, and NSP5 genes from a novel camel RVA strain. Based on the phylogenetic analysis we suggested the introduction of a new NSP4 genotype (E15), which has been accepted by the Rotavirus Classification Working Group.
3. We have determined the complete genome sequence of a Hungarian canine RVA strain. Results of the phylogenetic analysis revealed a unique A15 NSP1 genotype that has not been observed in other canine RVAs previously, and suggested the direct transmission of a rotavirus strain between canine and human hosts.
4. We have determined complete or partial genome segments of eight avian rotaviruses from

Hungarian chicken, pheasant and turkey hosts. Phylogenetic analysis of the obtained data indicated that avian RVA strains generally possess a mosaic genome constellation in which different genes originated for various avian hosts. The observed mosaic genome constellations likely reflect the high frequency of reassortment events, between RVA strains infecting avian hosts.

Scientific publications

Articles published in peer-reviewed scientific journals with impact factor

Papp H., Mihalov-Kovács E., Dóró R., Marton S., Farkas S.L., Giammanco G.M., De Grazia S., Martella V., Bányai K. **Full-genome sequencing of a Hungarian canine G3P[3] Rotavirus A strain reveals high genetic relatedness with a historic Italian human strain** Virus Genes 50: pp. 310-315. (2015)

IF: 1,285

Papp H., Malik Y.S., Farkas S.L., Jakab F., Martella V., Bányai K. **Rotavirus strains in neglected animal species including lambs, goats and camelids** Virusdisease 25: pp. 215-222. (2014)

IF: 0,364

Papp H., Marton S., Farkas S.L., Jakab F., Martella V., Malik Y.S., Palya V., Bányai K. **Classification and characterization of a laboratory chicken rotavirus strain carrying G7P[35] neutralization antigens on the genotype 4 backbone gene configuration** Biologicals 42: pp. 299-304. (2014)

IF: 1,209

Papp H., Rigó D., Dán Á., Farkas S.L., Oldal M., Jakab F., Bányai K. **Szokatlan rotavírus antigén kombináció azonosítása fiatal fácánban** Magyar Állatorvosok Lapja 136: pp. 729-735. (2014)

IF: 0,185

Papp H., Matthijnssens J., Martella V., Ciarlet M., Bányai K. **Global distribution of group A rotavirus strains in horses: A systematic review** Vaccine 31: pp. 5627-5633. (2013)

IF: 3,49

Matthijnssens J., Mino S., Papp H., Potgieter C., Novo L., Heylen E., Zeller M., Garaicoeachea L., Badaracco A., Lengyel G., Kisfali P., Cullinane A., Collins P., Ciarlet M., O'Shea H., Parreno V., Banyai K., Barrandeguy M., Van Ranst M. **Complete molecular genome analyses of equine rotavirus A strains from different continents reveal several new genotypes and a largely conserved genotype constellation** Journal Of General Virology 93: pp. 866-875. (2012)

IF: 3,13

Papp H.*, Al-Mutairi L.Z.*, Chehadeh W., Farkas L.S., Lengyel Gy., Jakab F., Martella V., Szűcs Gy., Bányai K. **Novel NSP4 genotype in a camel**

G10P[15] rotavirus strain Acta Microbiologica et Immunologica Hungarica 59: pp. 411-421. (2012)

*authors contributed equally

IF: 0,65

Abstracts or proceedings published in international conference handbooks

Papp H., Borzák R., Farkas Sz., Kisfali P., Lengyel Gy., Molnár P., Melegh B., Matthijnssens J., Jakab F., Martella V., Bányai K. **Independent zoonotic transmission of porcine G4P[6] rotaviruses identified sporadically in hungarian pediatric patients over a 15 year period**; 5th European Rotavirus Meeting, 2013.10.6-9., Valencia, Spain (2013)

Papp H., Dán Á., Ursu K., Bányai K. **Reassortant rotavirus strains identified in Hungarian pheasants**; FEMS 2013, 5th Congress of European Microbiologists, 2013.07.21-25., Leipzig, Germany (2013)

Papp H., Al Muair L.Z., Lengyel G., Grósz G., El-Sayed F.H., Esam A.A., Szűcs G., Bányai K. **Molecular characterization of a Kuwaiti camel rotavirus strain**; 4th European Rotavirus Meeting, 2011.10.2-5., Altafiumara, Italy (2011)

László B., Papp H., Dandár E., Deák J., Gray J., Iturriza-Gomara M., Jakab F., Juhász Á., Kovács J., Kónya J., Lengyel Gy., Martella V., Mészáros J., Mészner Zs., Mihály I., Molnár P., Nyúl Z., Pátri L., Puskás E., Schneider F., Tóth A., Tóth E., Szűcs G., Bányai K. **Prevalence of human rotavirus strains in Hungary, 2007-2011**; 4th European Rotavirus Meeting, 2011.10.2-5., Altafiumara, Italy (2011)

László B., Papp H., Dandár E., Deák J., Gray J., Iturriza-Gomara M., Jakab F., Juhász Á., Kovács J., Kónya J., Lengyel Gy., Martella V., Mészáros J., Mészner Zs., Mihály I., Molnár P., Nyúl Z., Pátri L., Puskás E., Schneider F., Tóth A., Tóth E., Szűcs Gy., Bányai K. **Emerging human rotavirus strains, 2007-2010, Hungary**; International Meeting on Emerging Diseases and Surveillance 2011.02.4-7., Vien, Austria (2011)

Scientific publications not related to the doctoral thesis

Pongor L., Harami-Papp H., Méhes E., Czirók A., Győrffy B. **Cell Dispersal Influences Tumor Heterogeneity and Introduces a Bias in NGS Data Interpretation.** Scientific Reports. 2017 Aug 4;7(1):7358.

IF: 4,259 (2016)

Menyhárt O., Harami-Papp H., Sukumar S., Schäfer R., Magnani L., de Barrios O., Győrffy B. **Guidelines for the selection of functional assays to evaluate the hallmarks of cancer**. Biochim Biophys Acta. 2016 Dec;1866(2):300-319.

IF: 5,083

Harami-Papp H., Pongor L.S., Munkácsy G., Horváth G., Nagy Á.M., Ambrus A., Hauser P., Szabó A., Tretter L., Győrffy B. **TP53 mutation hits energy metabolism and increases glycolysis in breast cancer**. Oncotarget. 2016 Oct 11;7(41):67183-67195.

IF: 5,008

Papp H., László B., Jakab F., Ganesh B., De Grazia S., Matthijnssens J., Ciarlet M., Martella V., Bányai K. **Review of group A rotavirus strains reported in**

swine and cattle Veterinary Microbiology 165: pp. 190-199. (2013)

IF: 3,13

Ndze V.N., Achidi E.A., Papp H., Kovács E., Farkas S.L., Adiogo D., Kisfali P., Ngeng M.B., Abena M.T.O., Martella V., Esona M.D., Bányai K. **Shared G12 VP7 gene among human and bovine rotaviruses detected in Cameroonian villages** Acta Microbiologica et Immunologica Hungarica 60:(1) pp. 21-28. (2013)

IF: 0,65

Ndze V.N., Papp H., Akum A.E., Hortense G.K., László B., Farkas S., Kisfali P., Melegh B., Esona M.D., Bowen M.D., Bányai K., Gentsch J.R., Obama Abena M.T. **One Year Survey of Human Rotavirus Strains Suggests the Emergence of Genotype G12 in Cameroon** Journal of Medical Virology 85:(8) pp. 1485-1490. (2013)

IF: 2,37

Papp H., Borzák R., Farkas S., Kisfali P., Lengyel G., Molnár P., Melegh B., Matthijnssens J., Jakab F., Martella V., Bányai K. **Zoonotic transmission of reassortant porcine G4P[6] rotaviruses in Hungarian pediatric patients identified**

sporadically over a 15 year period Infection, Genetics and Evolution 19: pp. 71-80. (2013)

IF: 2,77

László B., Kónya J., Dandár E., Deák J., Farkas Á., Gray J., Grósz G., Iturriza-Gomara M., Jakab F., Juhász Á., Kisfali P., Kovács J., Lengyel Gy., Martella V., Melegh B., Mészáros J., Molnár P., Nyúl Z., Papp H., Pátri L., Puskás E., Sántha I., Schneider F., Szomor K., Tóth A., Tóth E., Szűcs Gy., Bányai K. **Surveillance of human rotaviruses in 2007-2011, Hungary: exploring the genetic relatedness between vaccine and field strains** Journal of Clinical Virology 55:(2) pp. 140-146. (2012)

IF: 3,29

Mladenova Z., Papp H., Lengyel G., Kisfali P., Steyer A., Steyer A.F., Esona M.D., Iturriza-Gómara M., Bányai K. **Detection of rare reassortant G5P[6] rotavirus, Bulgaria** Infection, Genetics and Evolution 12: pp. 1676-1684. (2012)

IF: 2,77

Ursu K., Papp H., Kisfali P., Rigó D., Melegh B., Martella V., Bányai K. **Monitoring of group A rotaviruses in wild-living birds in Hungary** Avian Diseases 55:(1) pp. 123-127. (2011)

IF: 1,46

Bányai K., Papp H., Dandár E., Molnár P., Mihály I., Van Ranst M., Martella V., Matthijnssens J. **Whole genome sequencing and phylogenetic analysis of a zoonotic human G8P[14] rotavirus strain** Infection, Genetics and Evolution 10: pp. 1140-1144. (2010)

IF: 3,09