Zoonotic potential of rotaviruses detected in the Czech pig farms

Romana Moutelikova

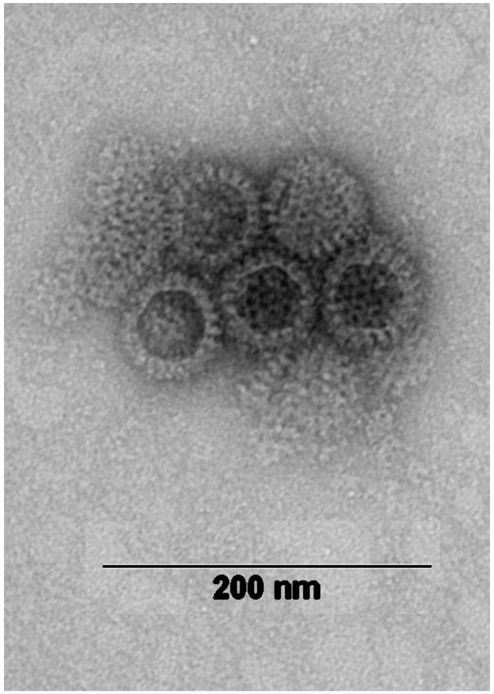
VRI

„Know thy self, know thy enemy...“ (Sun Tzu, The Art of War)

family: *Reoviridae,* genus: *Rotavirus*

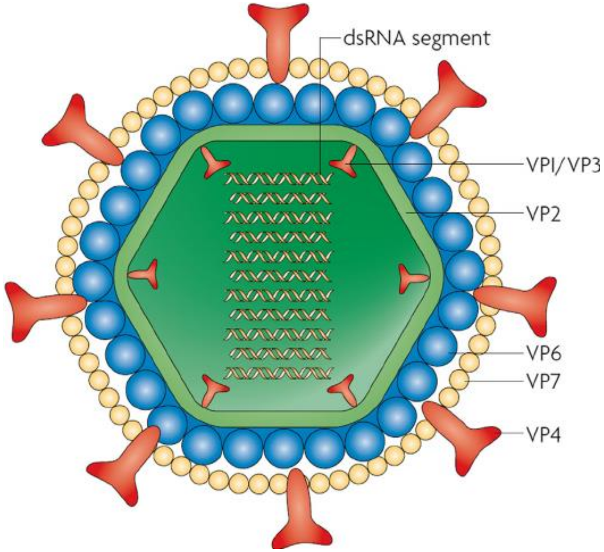
- 9 species – *Rotavirus A – D, F – J* (RVA-RVD, RVF-RVJ) (RVE was excluded by ICTV in 2019 – its rare finding 40 years ago was not further confirmed)
- Infections of men and pigs: RVA, RVB, RVC, RVH
  (only in birds: RVD, RVF a RVG)
- Zoonotic species: **RVA** (RVB/RVC – possibly)

- non-enveloped virus → resistence to common desinfectants (quarternary ammonium, amphotheric surfactants), stable at pH 3-9 (effective desinfections – chlorine, ethanol 60-80%, iodine, aldehydes)
- up to 9 months survival in the environment (RT)
- infectivity of RVA in pig excrements at **10°C** was demonstrated after **32** months

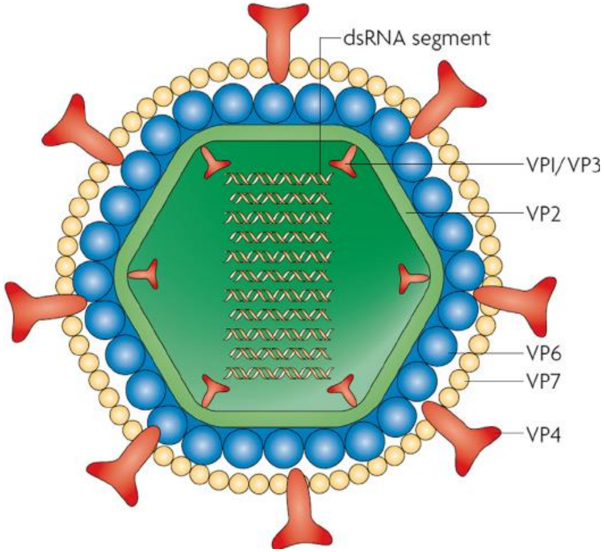


Negatively stained preparation of rotavirus particles captured by a transmission electron microscope (picture by Pavel Kulich, MVD, Ph.D., VRI Brno)

- major neutralizing antigens **VP7** (G-genotypes) and **VP4** (P-genotypes), group antigen **VP6** (I-genotypes)

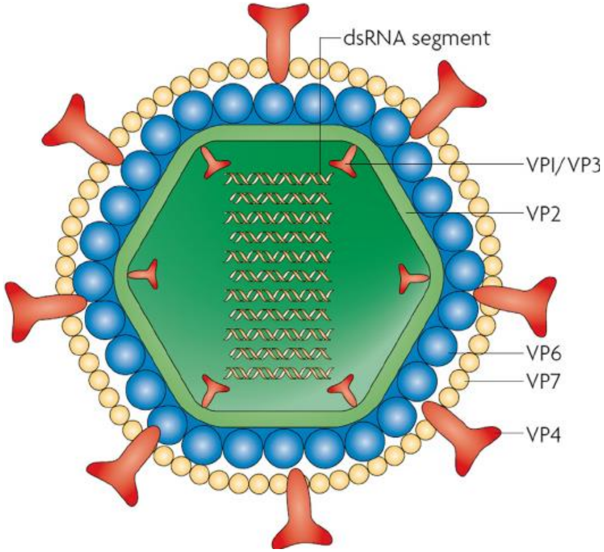


- major neutralizing antigens **VP7** (G-genotypes) and **VP4** (P-genotypes), group antigen **VP6** (I-genotypes)



- Typing of rotaviruses – sequencing of segments encoding **VP7** and **VP4** proteins → classification of RVA into G- and P-types → characterization of circulating pathogenic strains (evaluation of vaccination effectiveness), up to date **42 G**-types, **58 P**-types, **32 I**-types

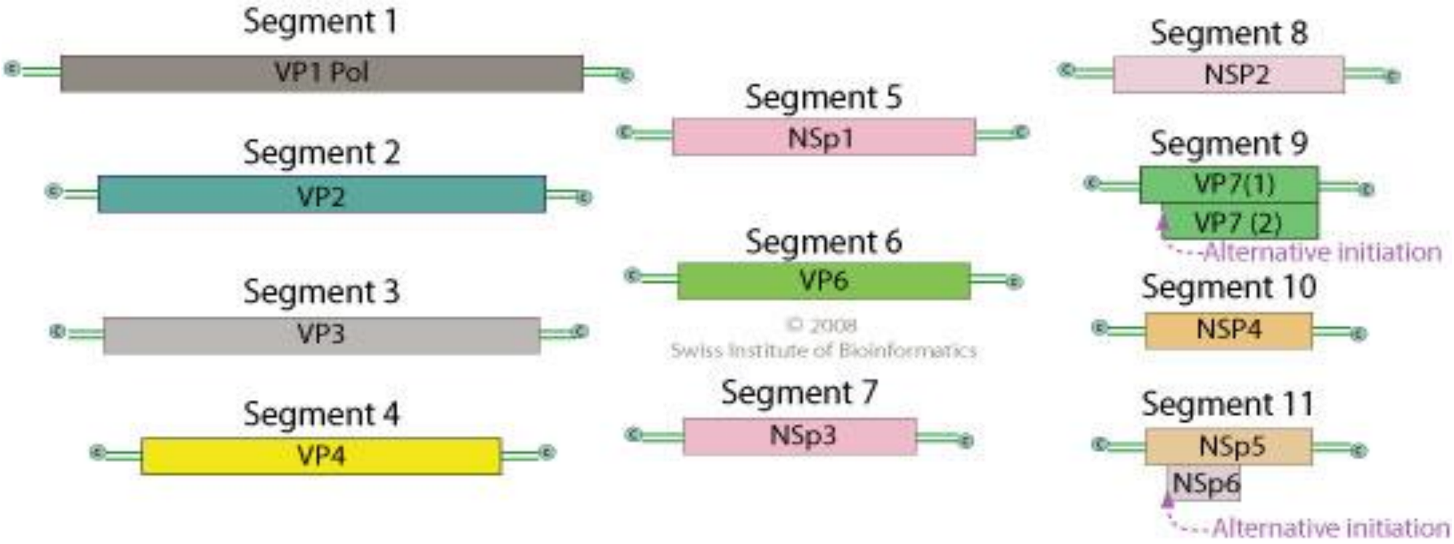
- major neutralizing antigens **VP7** (G-genotypes) and **VP4** (P-genotypes), group antigen **VP6** (I-genotypes)



- Typing of rotaviruses – sequencing of segments encoding **VP7** and **VP4** proteins → classification of RVA into G- and P-types → characterization of circulating pathogenic strains (evaluation of vaccination effectiveness), up to date **42 G**-types, **58 P**-types, **32 I**-types

- Sequence and phylogenetic analysis – detailed information about the RVA isolate → monitoring of possible sources or routes of infection transmission

- genome of RV – dsRNA – 11 segments → possible reassortment during infection with 2 or more strains → high variability of strains + interspecies transmission

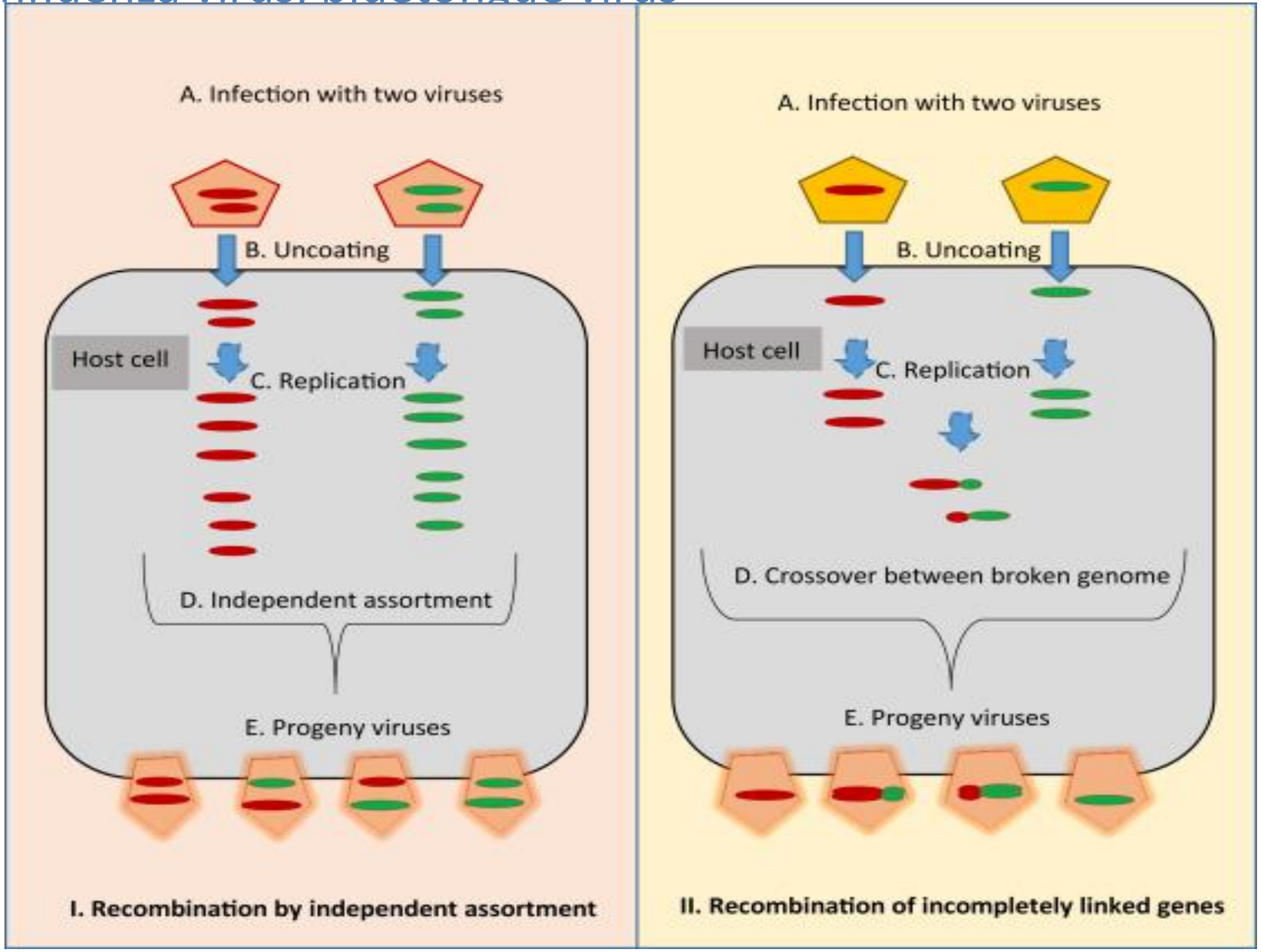


© 2008 Swiss Institute of Bioinformatics

Re-assortment x Re-combination

viruses with segmented genomes x all other viruses

rotavirus, influenza virus, bluetongue virus



Domingo E., Virus as Populations, 2020

Methods of RV detection

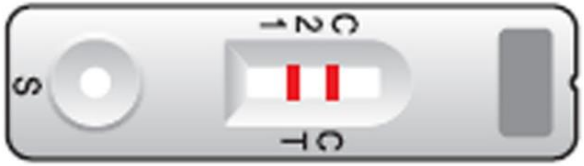
- Electron microscopy (sensitivity > 10^4 virions/µl, cannot distinguish different RV species)

Methods of RV detection

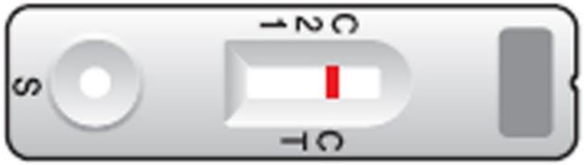
- Electron microscopy (sensitivity > 10^4 virions/µl, cannot distinguish different RV species)
- Cultivation on cell-lines (very labourious; just some of wild strains)

Methods of RV detection

- Electron microscopy (sensitivity > 10^4 virions/µl, cannot distinguish different RV species)
- Cultivation on cell-lines (very labourious; just some of wild strains)
- Detection of RV antigen
  - ✓ Immunochromatographic assay
  - ✓ Latex aglutination
  - ✓ ELISA



Positive



Negative

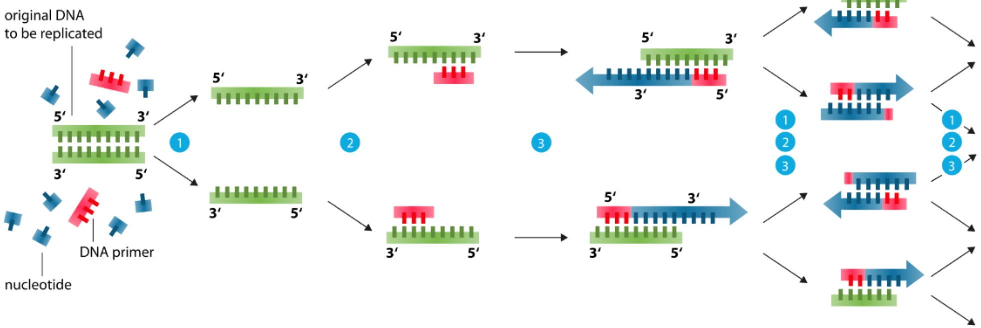
Methods of RV detection

- Electron microscopy (sensitivity > 10^4 virions/µl, cannot distinguish different RV species)
- Cultivation on cell-lines (very labourious; just some of wild strains)
- Detection of RV antigen
- Molecular-biological methods (detection of virus genome – RNA)
    - Non-amplifying (hybridization)
    - Amplifying (different types – LAMP, PCR, qPCR, multiplex…)

Detection of viral RNA

- RT-PCR

Polymerase chain reaction - PCR



① **Denaturation** at 94-96°C

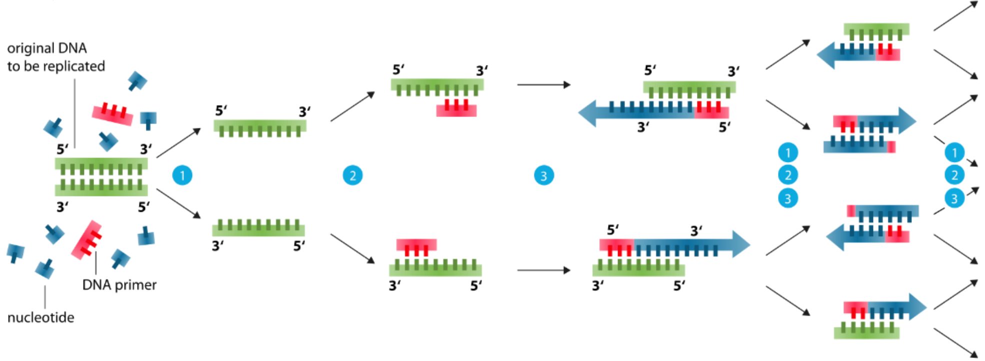
② **Annealing** at ~68°C

③ **Elongation** at ca. 72 °C

Detection of viral RNA

- RT-PCR

Polymerase chain reaction - PCR

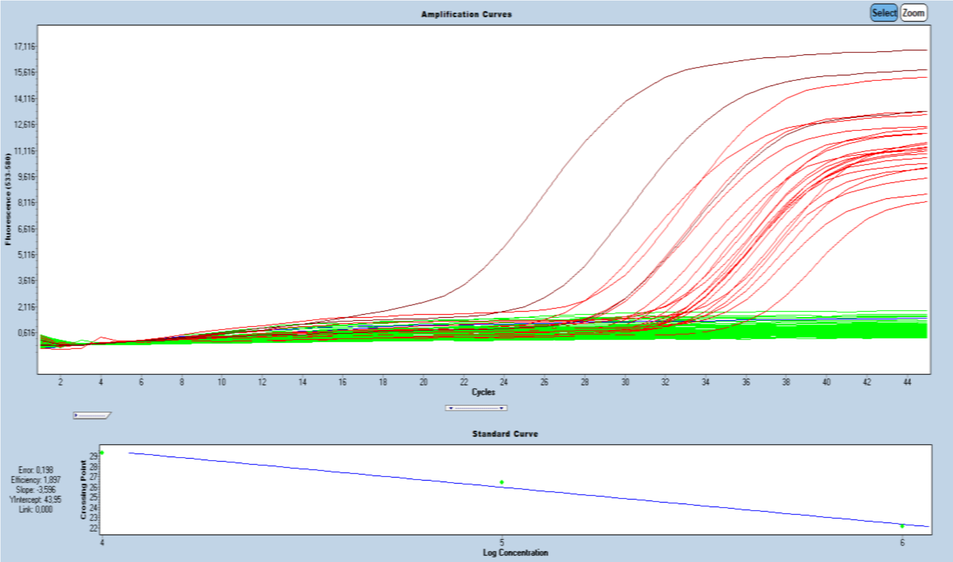


① **Denaturation** at 94-96°C

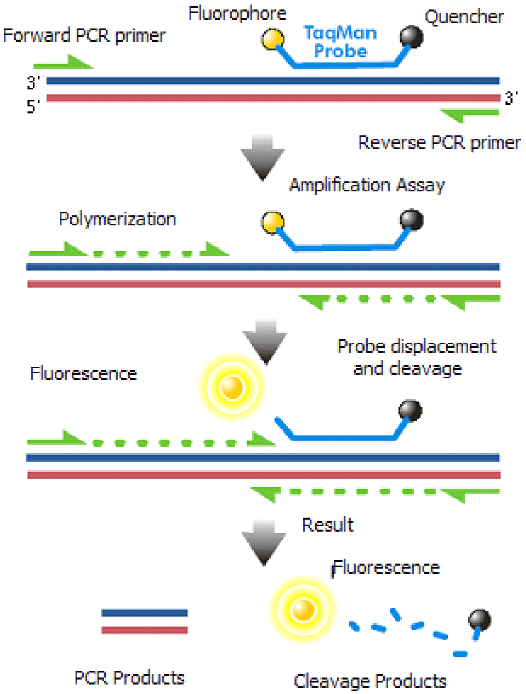
② **Annealing** at ~68°C

③ **Elongation** at ca. 72 °C

- RT-qPCR (possibility of virus quantification, sensitivity < 10 copies)



Error: 0,198
Efficiency: 1,897
Slope: -3,596
YIntercept: 43,95
Link: 0,000

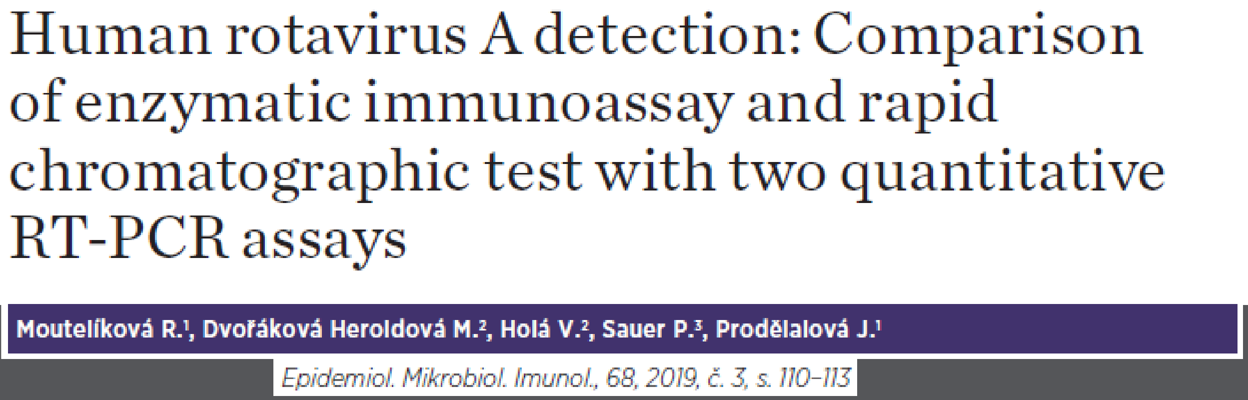


One-step RT-qPCR for poRVA + huRVA detection

- primers + TaqMan® probe detecting both human and porcine RVA strains
- detection of 2 targets on conserved sequence of NSP3-coding gene – higher sensitivity and specificity of reaction
- quantification of the reaction with the use of dsRNA transcript

One-step RT-qPCR for poRVA + huRVA detection

- primers + TaqMan® probe detecting both human and porcine RVA strains
- detection of 2 targets on conserved sequence of NSP3-coding gene – higher sensitivity and specificity of reaction
- quantification of the reaction with the use of dsRNA transcript



Human rotavirus A detection: Comparison of enzymatic immunoassay and rapid chromatographic test with two quantitative RT-PCR assays

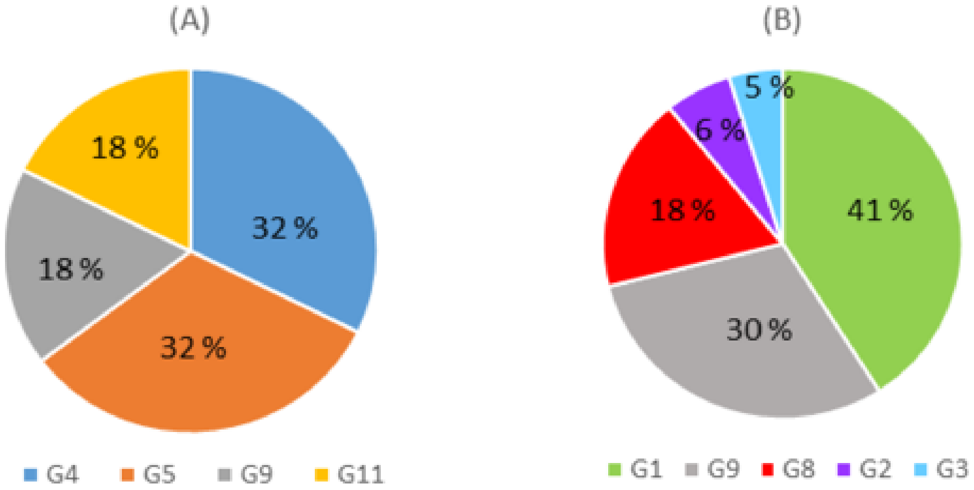
Moutelíková R.[1], Dvořáková Heroldová M.[2], Holá V.[2], Sauer P.[3], Prodělalová J.[1]

Epidemiol. Mikrobiol. Imunol., 68, 2019, č. 3, s. 110–113

Detected RVA genotypes

- genotype G9 very frequent (in pigs as well as humans)
- genotypes G9 rare in humans until the 90th, now common
- thanks to sequencing, the way of introduction of G9 among human patients was determined – interspecies transmission from pigs

RVA genotypes
in pigs (A) (n=44)
and humans (B) (n=122)



New RVA genotype G8 in humans

- whole-genome sequencing of RVA by NGS
- RVA backbone – human (DS-1-like)

New RVA genotype G8 in humans

- whole-genome sequencing of RVA by NGS
- RVA backbone – human (DS-1-like)

G8 P[8] I2 R2 C2 M2 A2 N2 T2 E2 H2

VP7- VP4- VP6- VP1- VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6

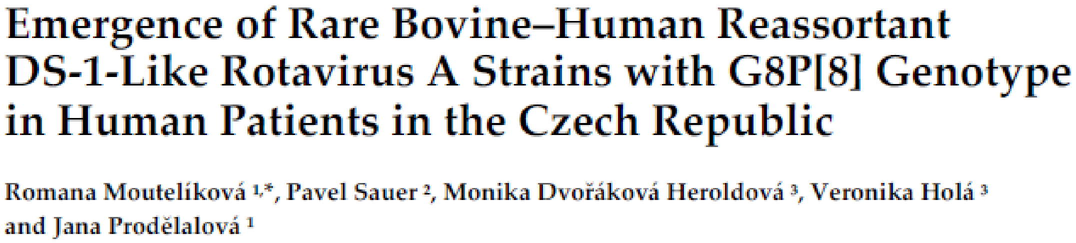
New RVA genotype G8 in humans

- whole-genome sequencing of RVA by NGS
- RVA backbone – human (DS-1-like)

G8 P[8] I2 R2 C2 M2 A2 N2 T2 E2 H2

VP7- VP4- VP6- VP1- VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6

- segment VP7 – 99% homology with a Vietnamese RVA strain (**reassortant** between **bovine** RVA from India and **human** RVA)
- different from G8 segments from Africa and southern Europe



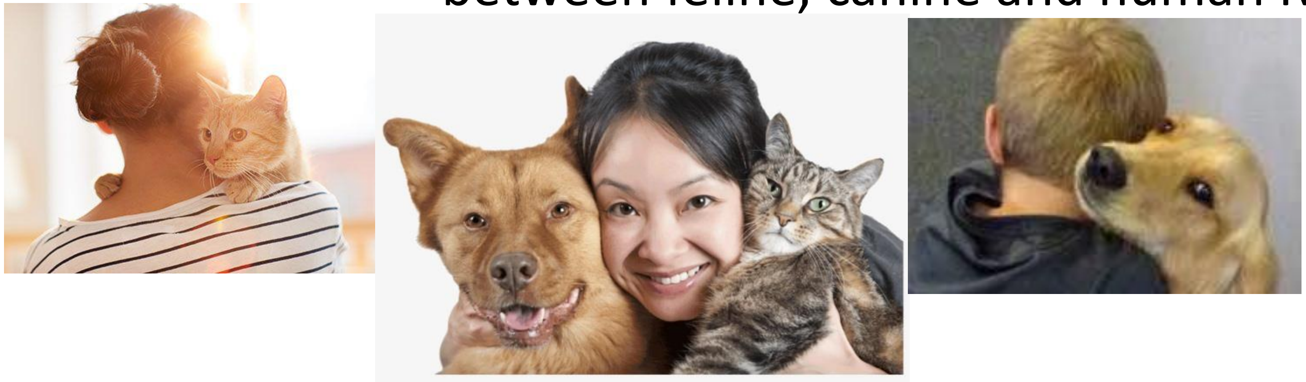
Emergence of Rare Bovine–Human Reassortant DS-1-Like Rotavirus A Strains with G8P[8] Genotype in Human Patients in the Czech Republic

Romana Moutelíková [1,*], Pavel Sauer [2], Monika Dvořáková Heroldová [3], Veronika Holá [3] and Jana Prodělalová [1]

Viruses **2019**, *11*(11), 1015; https://doi.org/10.3390/v11111015

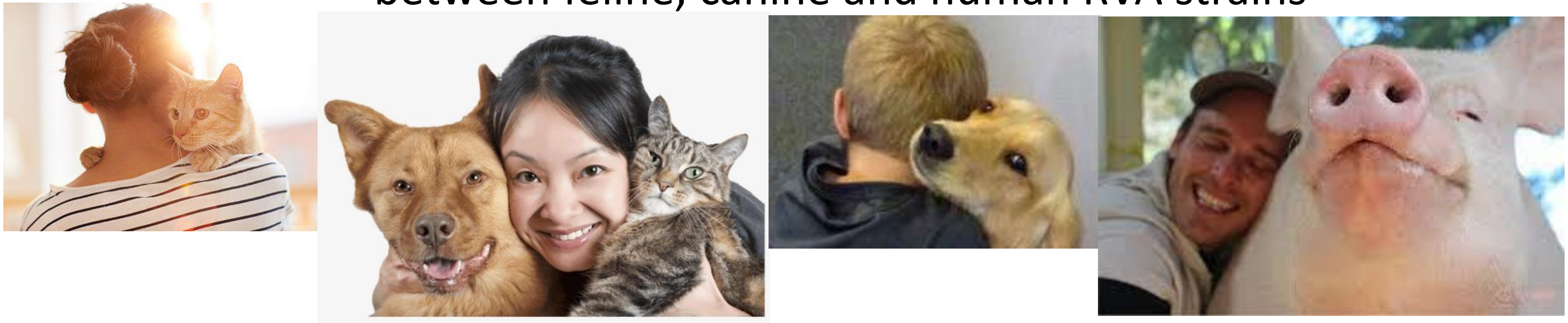
Zoonotic potential of rotaviruses

- **G3P[9]** – 99% similarity (nt) with **RVA/Cat-wt/ITA/BA222/2005/G3P[9]** – its origin – multiple re-assortment of genomic segments between feline, canine and human RVA strains



Zoonotic potential of rotaviruses

- **G3P[9]** – 99% similarity (nt) with **RVA/Cat-wt/ITA/BA222/2005/G3P[9]** – its origin – multiple re-assortment of genomic segments between feline, canine and human RVA strains



- **G9** – in humans one of the most common genotypes worldwide, its probable origin – the domestic pig

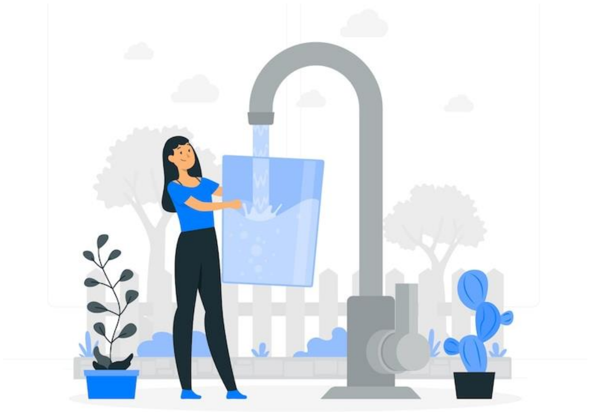
Zoonotic potential of rotaviruses

- **G3P[9]** – 99% similarity (nt) with **RVA/Cat-wt/ITA/BA222/2005/G3P[9]** – its origin – multiple re-assortment of genomic segments between feline, canine and human RVA strains

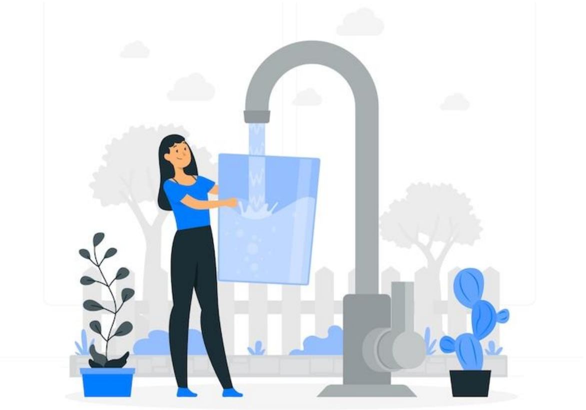
- **G9** – in humans one of the most common genotypes worldwide, its probable origin – the domestic pig
- **G3P[8]** – similarity between VP7 segments of equine RVAs; its origin - east Asia

!!!Viruses are transmitted in all directions!!!

How **not** to catch a virus…



How **not** to catch a virus…



- **RotaTeq®** - 98% protection against severe RVGE
  - 96% less likely to be hospitalized through the first two years after vaccination
- **Rotarix®** - 85 to 96% protection against severe rotavirus gastroenteritis

Summary

- The epidemiology of porcine as well as human RVs is very complex and highly dynamic
- Characterization of RV strains based on molecular biological methods is necessary to ensure accuracy of diagnostical kits
- Due to zoonotic potential of porcine RVs it is necessary to monitor the circulating genotypes both in human as well as in swine population

Summary

- The epidemiology of porcine as well as human RVs is very complex and highly dynamic
- Characterization of RV strains based on molecular biological methods is necessary to ensure accuracy of diagnostical kits
- Due to zoonotic potential of porcine RVs it is necessary to monitor the circulating genotypes both in human as well as in swine population

Thank you for your attention and stay healthy!!!