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Genomic Characterization of a Rare Human G5P[6] Rotavirus in Brazil: Evidence of Longstanding Zoonotic Connections with Porcine RVA

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Introduction

Rotavirus (RVA) genotype G5 is frequently detected in swine but is only sporadically identified in humans



Hotspot for RVA genetic diversity

- **Brazil was the first** to detect G5P[8] strains in humans;
- **Sustained circulation** of these strains occurred in the country;
- **Period:** between the 1980s and early 2000s;
- **Rare event:** few other countries have reported it.

Phylogenetic studies confirmed a porcine origin for VP7 (G5) and a human origin for VP4 (P[8]) and other segments, indicating reassortment events between porcine and human strains

Introduction



Brazil

Date: May 2013

Strain: (RVA/Human-wt/BRA/IAL-R406/2013/G5P[6]

Location: Goiânia city, Goiás State, Midwestern, Brazil

Patient: 11-year-old male

- The P[6] genotype is commonly found in porcine RVA strains;
- Human infections with G5P[6] strains are extremely rare, reported only in a few countries, including **China (2001-2003)**, **Vietnam (2004)**, **Bulgaria (2008)**, **Taiwan (2009)**, **Japan (2011)** and **Zambia (2014)**.

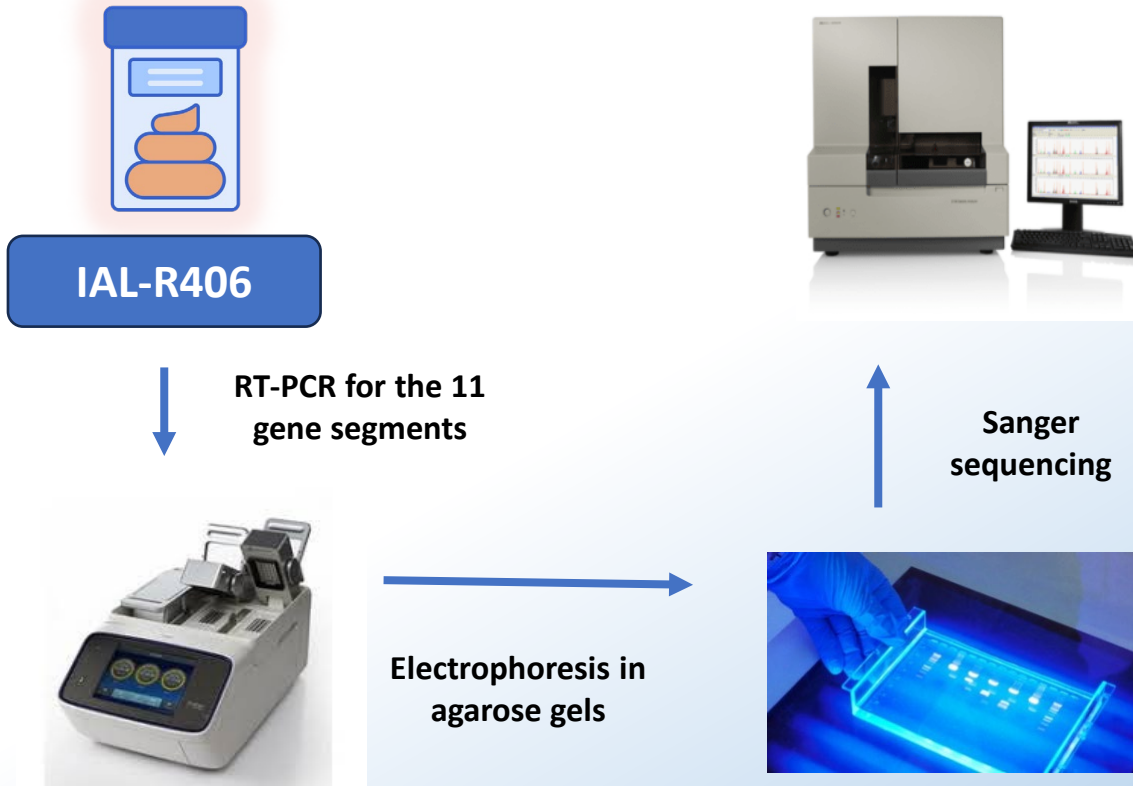
Objectives



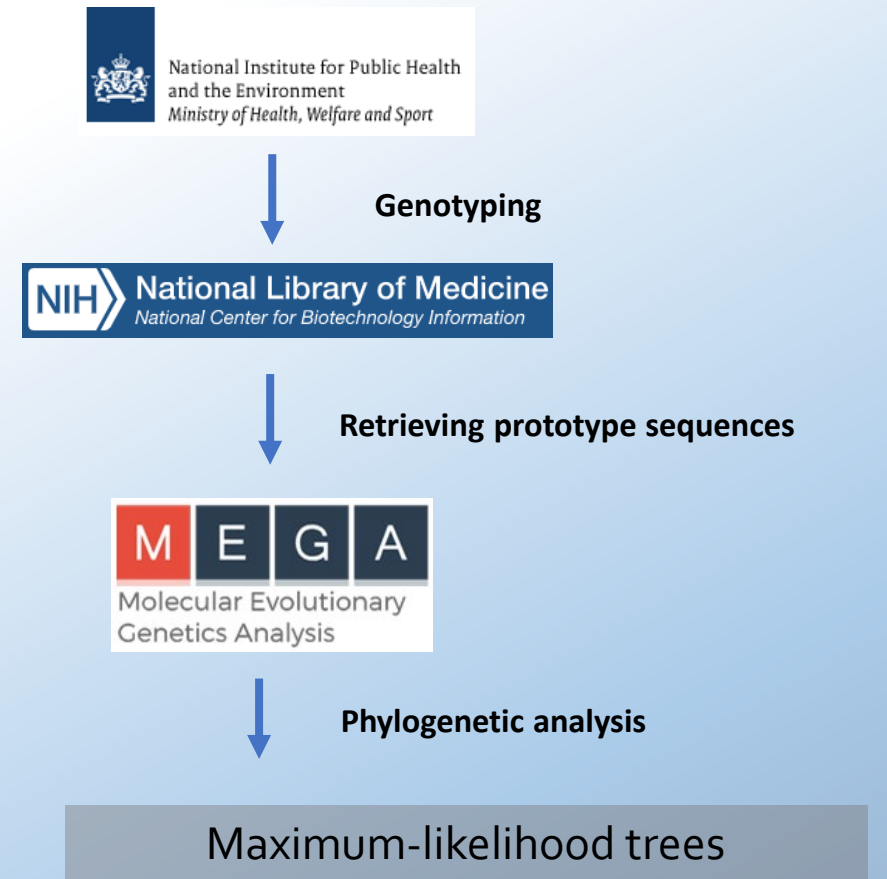
The aims of the present study were to:

- Perform full-genotype characterization and phylogenetic analysis of the RVA/Human-wt/BRA/IAL-R406/2013/G5P[6] strain to elucidate its evolutionary origin;
- Assess its genomic relationship with both human and animal RVA strains;
- Investigate interspecies transmission dynamics;
- Highlight the importance of molecular surveillance in detecting and monitoring emerging or rare RVA genotypes.

Material and Methods



- **VP4 P[6] genotype:** Five lineages (I–V) following Martella et al. (2006)
- **VP7 G5 genotype:** Three lineages (I–III) following da Silva et al. (2011)

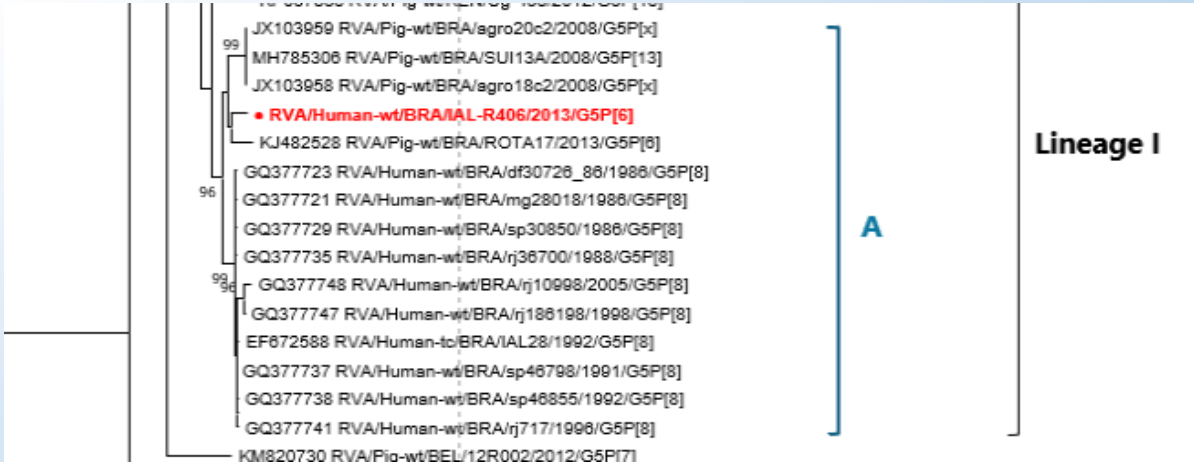


Substitution models used:

- GTR + G + I: NSP1, VP2, VP3
- GTR + G: VP1
- T92 + G + I: NSP2, NSP5, VP4, VP6, VP7
- T92 + G: NSP3
- HKY + G: NSP4

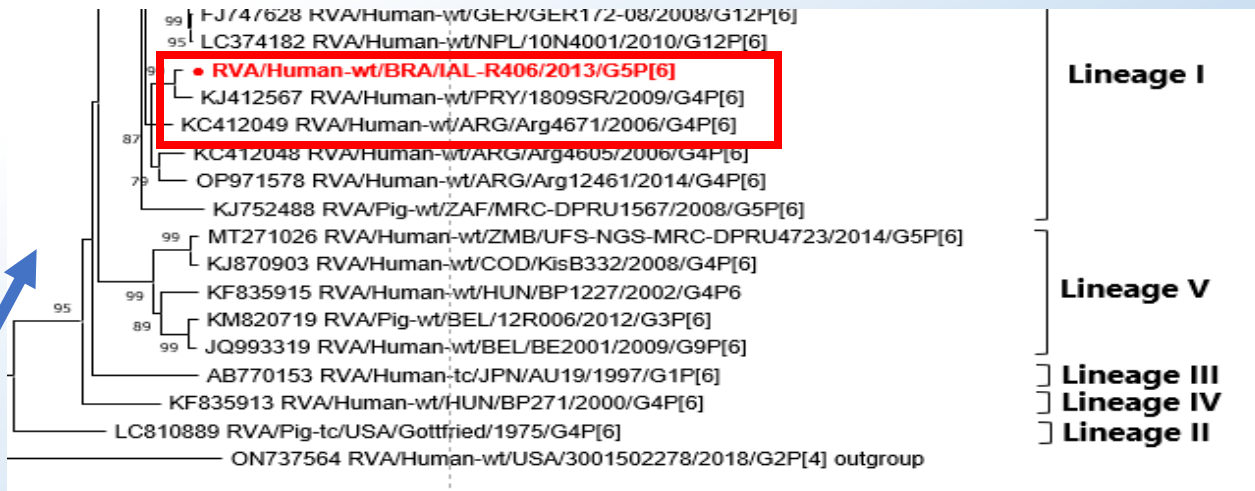
Branch support: 1000 bootstrap replicates

Results and Discussion



- **G5 establishment in Brazil:** Adapted to humans since the 1980s;
- **Circulation:** Detected in both humans and animals for decades;
- **Lineage I:** Comprises Brazilian human and porcine strains collected between 1986 and 2013.

Results and Discussion



- Lineage: Brazilian P[6] clustered within Lineage I;
- Closest relationships: Most related to G4[6] porcine-like human strains from Paraguay (2009) (96.8% nt) and Argentine (2006) (93.7% nt);
- Similarity with swine strains (Lineage I): 88.9%–93.1%;
- Limitation: VP8*-coding region of BRA/IAL-R406/2013/G5P[6] not sequenced, preventing broader comparison with other Brazilian human and animal P[6] strains.

Strain	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Human-wt/BRA/IAL-R406/2013/G5P[6] ^a	G5	P[6]	I1	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-wt/CHN/LL36755/2003/G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-wt/CHN/LL3354/2000/G5P[6]	G5	P[6]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/BGR/BG260/2008/G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/ZMB/UFS-NGS-MRCDPRU4723/2014/G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/PRY/1809SR/2009/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-wt/ARG/Arg4671/2006/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/ARG/Arg4605/2006/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-wt/ARG/Arg12461/2014/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-tc/BRA/IAL28/1992/G5P[8]	G5	P[8]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/GBR/ST3/1974/G4P[6]	G4	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/CHN/R479/2004/G4P[6]	G4	P[6]	I5	R1	C1	M1	A1	N1	T7	E1	H1
RVA/Human-wt/CHN/E931/2008/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/BRA/COD379/1991/G4P[6]	G4	P[6]	I1	R1	Cx	M1	A1	N1	T1	E1	H1
RVA/Human-tc/BRA/R49/1997/G1P[9]	G1	P[9]	I1	R1	C1	M2	A1	N2	T2	E1	H1
RVA/Human-wt/BEL/BE2001/2009/G9P[6]	G9	P[6]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Human-wt/BRA/rj24598/2015/G26P[19]	G26	P[19]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/BEL/12R005/2012/G4P[7]	G4	P[7]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/JPN/BU2/2014/G5P[7]	G5	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/BEL/12R002/2012/G5P[7]	G5	P[7]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-tc/USA/OSU/1975/G5P[7]	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-wt/BRA/SUI13A/2008/G5P[13]	G5	P[13]	I5	R1	C1	Mx	A8	Nx	T7	E1	H1
RVA/Pig-wt/BRA/SUI24A/2008/G3P[13]	G3	P[13]	I5	R1	C1	M2	A8	N1	T7	E1	H1
RVA/Pig-wt/BRA/ROTA17/2013/G5P[6]	G5	P[6]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-wt/BRA/ROTA24/2013/G5P[6]	G5	P[6]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-wt/IRL/R2WTA79/2014/G5P[13]	G5	P[13]	I5	R1	C1	M1	A8	N2	T7	E9	H1
RVA/Pig-wt/ITA/3BS/2009/G9P[23]	G9	P[23]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CMP-011-09/2009/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-tc/VEN/A131/1988/G3P[7]	G3	P[7]	I5	R1	C2	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/KOR/K71/2006/G5P[7]	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-wt/KNA/ET8B/2015/G5P[13]	G5	P[13]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-wt/ITA/2CR/2009/G9P[23]	G9	P[23]	I5	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Wildboar-wt/HRV/DS229-Z/2020/G3P[13]	G3	P[13]	I5	R1	C1	M1	A8	N1	T7	E1	H1

Results and Discussion

G5-P[6]-I1-R1-C1-M1-A8-N1-T7-E1-H1

- **Rarity:** constellation only once reported in humans (China, 2003, strain LL36755)
- **Genetic:** Brazilian IAL-R406 and Chinese LL36755 strains grouped separately ► independent evolution rather than direct transmission

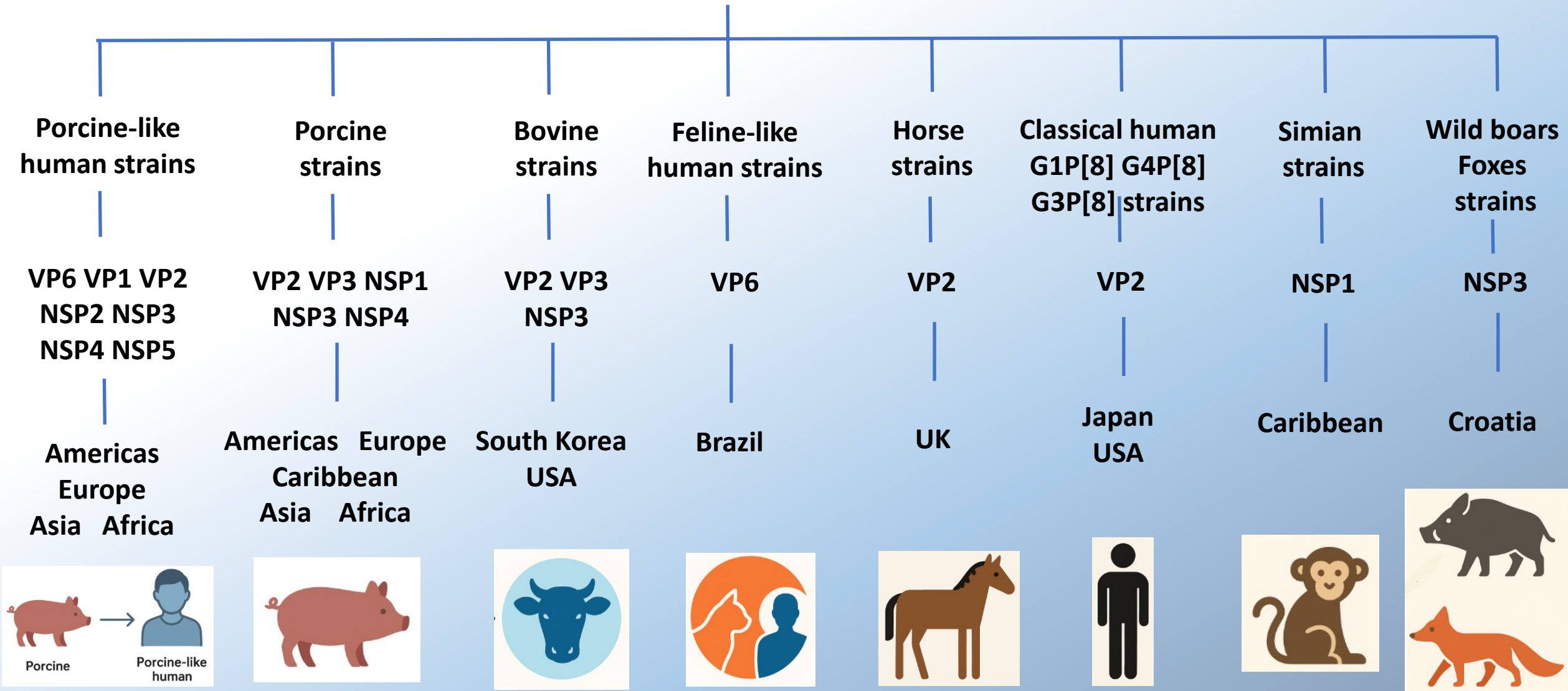
Constellations

- **South American porcine-like human strains:** Paraguay and Argentina strains differ in VP7 (G4 vs. G5)
- **Other countries:** Zambia, Japan, Bulgaria differ in NSP3 (T1 vs. T7)
- **Brazilian pigs (2013):** Differ in VP6 (I5 vs. I1)

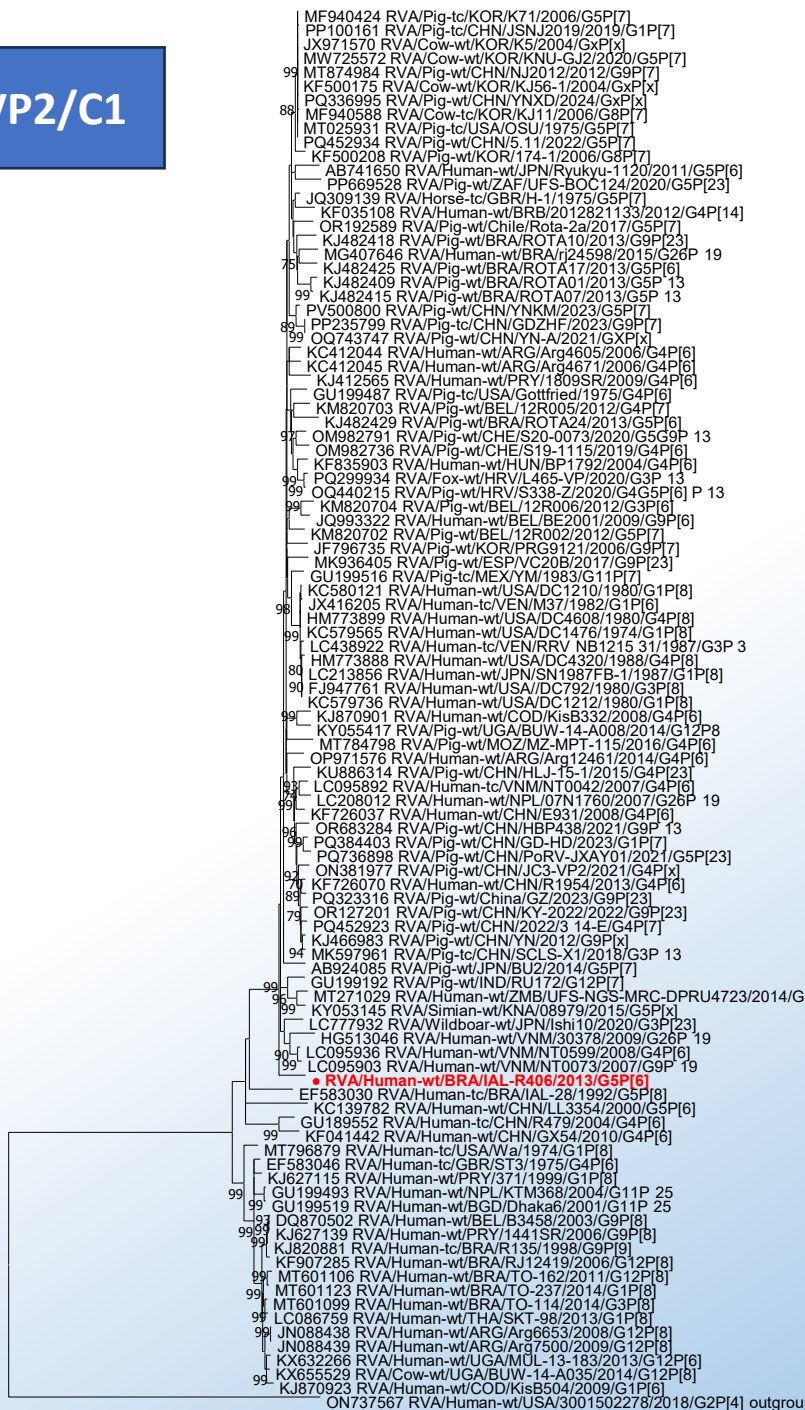
Conclusion: Similar genomic backbones have arisen independently worldwide, driven by zoonotic events and local evolutionary dynamics

Results and Discussion

RVA/Human-wt/BRA/IAL-R406/2013/G5P[6]



Results and Discussion



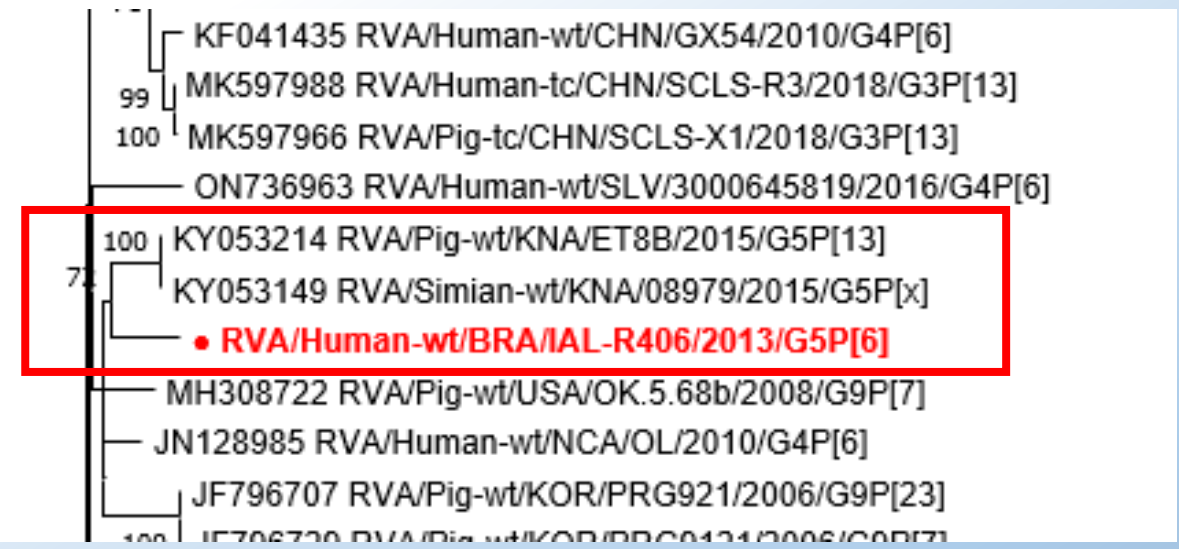
VP2: comparable nucleotide homology (91.3–91.9% nt) with several strain sets:

- **Porcine-like human strains:**
Vietnam: NT0599/2008/G4P[6], NT0073/2007/G9P[19]
Venezuela: M37/1982/G1P[6]
Argentina: Arg12461/2014/G4P[6]
Barbados: 2012821133/2012/G4P[14]
- **Classical human strains:**
G1P[8], G4P[8], G3P[8] from USA and Japan (1974–1987)
- **Porcine strains:**
China, Korea, USA (1975–2024)
- **Bovine strains:**
Korea (2004–2020)
- **Equine strain:**
Great Britain: H-1/1975/G5P[7]

NSP1/A8



Results and Discussion



NSP1 A8: highest similarity (90.7% nt) with:

- Porcine strain: RVA/Pig-wt/KNA/ET8B/2015/G5P[13] (St. Kitts, Caribbean, 2015)
- Primate strain: RVA/Simian-wt/KNA/08979/2015/G5P[x] (St. Kitts, Caribbean, 2015)

Li et al., 2008; Stupka et al., 2009; Degiuseppe et al., 2013; Martinez et al., 2014; Navarro et al., 2017; Maringa et al., 2020; Tacharoenmuang et al., 2021; Akari et al., 2023)

Closest similarity:

- Animal-like human strains (91.1–92.8% nt) – Europe, Asia, Africa, Americas
- Domestic animals (mainly pigs) (91.1–92.2% nt) – Russia, Canada, South Africa, Japan, India, Italy, Ireland, Switzerland, Belgium
- Bovine strain: USA (91.3% nt)
- Wild animals (wild boars, foxes): Croatia (91.3–92.1% nt)



Conclusions



Complete genome analysis of RVA/Human-wt/BRA/IAL-R406/2013/G5P[6] reveals insights into RVA genetic diversity, evolution, and interspecies transmission in Brazil

Identification of the rare G5-P[6]-I1-R1-C1-M1-A8-N1-T7-E1-H1 constellation highlights swine as a long-standing reservoir for sporadic zoonotic infections



These findings highlight the importance of a One Health approach, combining human and animal RVA surveillance to understand cross-species transmission

Sporadic human G5P[6] infections suggest limited human adaptability, emphasizing the need to monitor animal reservoirs for emerging public health threats



A main limitation is the lack of patient epidemiological data, especially animal contact, which limits interpretation of RVA trends from animal populations

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