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Molecular chracterisation of porcine group A rotaviruses: Studies on the age-related occurrence and spatial distribution of circulating virus genotypes in Poland

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ABSTRACT

Rotaviruses of group A (RVAs) commonly occur in farm animals. In pigs, they cause acute gastrointestinal disease which is considered as significant factor of economic losses in pig farming. The aim of the study was an assessment of the prevalence of rotavirus (RV) infections in farmed pigs in Poland, genotype identification of the virus strains in conjunction with their age-related occurrence and regional (province) distribution pattern in pig herds. In total, 920 pig faecal samples were collected from pigs between the ages of one week and two years old from 131 farms. RVAs were detected using ELISA and molecular methods followed by a sequence-based identification of G (VP7) and P (VP4) virus genotypes. RV antigen was found in 377 (41%) of pig faecal samples. The correlation between pig age and frequency of RV infections was shown. In the Polish pig population, 145 RVA strains representing 33 GP genotypes were identified. Subsequent molecular analysis revealed an age-dependent and regional diversity in distribution of genotypes and virus strains. Besides typical pig RVA strains, novel strains such as G5P [34], G9P[34], and human G1P[8] were identified in this animal host. Findings from this study showed a change over time in the genotype occurrence of circulating pig RVAs in Poland. The high genetic variability of RV strains and acquisition of new virus genotypes have led to the emergence of novel, genetically distinct RVAs. The changes in the genotype occurrence of RVA strains in pigs indicate the need for their continuous epidemiological surveillance.

1. Introduction

Rotavirus (RV) infections commonly occur in humans and farm animals. In pigs, they cause acute gastroenteritis manifested by a decrease in appetite, apathy and diarrhoea of varying degrees of severity (Pejsak and Truszczyński, 2005). This gastrointestinal disease is considered a significant factor of economic losses in pig farming (Truszczyński and Pejsak, 2012; Papp et al., 2013). RVs are non-enveloped viruses containing segmented, double-stranded RNA genome (Parashar et al., 2003). Each genome segment encodes six structural viral proteins (VP1, VP2, VP3, VP4, VP6 and VP7) as well as, depending on the virus strain, five or six non-structural proteins (NSP1-NSP5/6) (Matthijnssens et al., 2008b). Based on the antigenic properties of the VP6 capsid protein, animal and human RVs have been classified into eight serogroups (A–H) (Matthijnssens and Van Ranst, 2012; Midgley et al., 2012). Further classification of RVA strains into G (VP7) and P (VP4) genotypes depends on nucleotide sequence differences of genes 9 and 4, encoding the outer capsid proteins VP7 and VP4, respectively (Matthijnssens et al., 2011). The segmented structure of the virus genome favours the occurrence of reassortation events associated with the exchanges of viral genome segments between different strains co-infecting a single cell (Dennehy et al., 2008). It may result in formation of novel, antigenically different RV strains able to cross a species barrier (Matthijnssens et al., 2008; Martella et al., 2010; Matthijnssens et al., 2010).

Group A rotaviruses (RVA) are the most epidemiologically important RV serogroup in pigs, as they are responsible for almost 90% of RV infections in these animals (Gentsch et al., 2005; Martella et al., 2010; Matthijnssens et al., 2011). The virus strains from other serogroups (i.e. B, C, E and H) are less frequently identified in pigs (Collins et al., 2008; Marthaler et al., 2013; Molinari et al., 2014) although strains from serogroup H are wide-spread in commercially raised pigs in the USA (Marthaler 2014). So far, 12 G and 17 P RVA genotypes have been identified in pigs (Tate et al., 2012; Papp et al., 2013). However,

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the majority of detected virus strains in the worldwide pig population mainly belong to G5, G3, G4, P[7] and P[6] genotypes. Despite spatial and seasonal variation in the distribution of G RVA genotypes, their occurrence in pigs in Europe, North and South America is similar (Papp et al., 2013). Nevertheless, some regional differences exist, for example, in Europe, besides G5, the G4 and G3 RVA strains have often been detected in pigs, while G11 in Americas, G3 and G9 genotypes in Asia (Papp et al., 2013). Additionally, G5 RVA and occasionally detected in other regions of the world G26 were found in Africa (Amimo et al., 2015). In the case of P RVA genotypes circulating in pigs there are also differences observed in worldwide genotype distribution. For example, the P[6] and P[13] strains were more prevalent in pigs in Europe (Papp et al., 2013). Other genotype such as P[7] was the most common in the Americas and Asia in which P[23] was also frequently detected (Papp et al., 2013). Differences not only in the geographical distribution of RVA genotypes were observed, but also in the frequency of their occurrence in the same pig population over years (Papp et al., 2013).

The aim of the study was an assessment of the prevalence of RV infections in farmed pigs in Poland and genotype identification of the circulating virus strains in conjunction with their age-related occurrence and regional distribution pattern in pig herds.

2. Materials and methods

2.1. Sample collection

Faecal samples from 920 pigs at the age of one week to two years were collected from 2011 to 2015. Most of the sampled pigs were in good health (87.4%), as only 116 (12.6%) faeces samples were derived from diarrheic animals. The animals were housed on 131 small and large-scale commercial farms located in 107 districts in 16 provinces in Poland. The number of monitored farms was proportional to the pig population in a given province (Table 1) (Central Statistical Office, 2011). On each farm, individual faecal samples (˜ 30 g) were randomly collected from 6 to 8 animals of different ages with only two samples per animal age group. Animals were divided into four age groups: 1–4 weeks (nursing piglets), 5–8 weeks (suckling piglets), 9–16 weeks (weaned pigs) and > 17 weeks (porkers). In the case when on the farm animals representing one of the studied age group were not present, then a smaller number of samples was taken respectively. Samples were stored at −20 °C until analysis.

2.2. Detection of RV antigens

The ELISA RIDASCREEN Rotavirus Kit (R-biopharm, Germany) was used for initial screening of faecal samples for the presence of RV antigen. The test was carried out according to the manufacturer's instructions. Only RV-positive samples were subjected to further molecular investigation aiming to determine P and G RVA genotypes.

2.3. RNA extraction and RT-PCR amplification of VP7 and VP4 RVA gene fragments

Supernatants obtained from 10% faecal suspensions were used for RNA extraction using a QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instruction. The nucleic acids were directly used for molecular analyses or stored at −80 °C.

Amplification of RVA gene fragments encoding VP7 and VP4 proteins was performed by the RT-PCR method using a OneStep RT-PCR Kit (Qiagen, Germany) and previously described primer sets VP7F/VP7R (Park et al., 2011), Beg9/End9 (Gouvea et al., 1990), Gen-VP4F/Con2 (Gentsch et al., 1992; Matthijnssens et al., 2008a) and Con3/Con2 (Gentsch et al., 1992). Before amplification, viral RNA was denatured at 97 °C for 5 min. and quickly chilled on ice. The following thermal-cycling conditions were used: reverse transcription at 45 °C for 60 min., and thermal activation of HotStar*Taq* DNA Polymerase at 95 °C for 15 min. followed by 40 cycles consisting of a denaturation step at 94 °C for 1 min., annealing of the gene-specific primers encoding the VP7 and VP4 proteins at 47 °C and 50 °C respectively for 2 min., and extension at 72 °C for 2 min. The final extension was carried out at 72 °C for 7 min. The correct performance of the molecular analyses at the nucleic acid extraction was monitored by the inclusion of positive (tissue culture supernatant containing porcine RVA OSU strain, VR-892, ATCC, USA) and negative controls. PCR products (10 µl) were analysed in 1.7% agarose gel stained with SimplySafe (EURx, Poland). The electrophoresis was carried out in 1xTBE buffer at 100 V (5 V / cm) for 90 min. The sizes of obtained amplicons were compared to the DNA molecular weight marker (Gene Ruler 100 bp Plus DNA Ladder, Thermo Fisher Scientific, USA) and visualized under UV light.

2.4. Sequence analysis and genotype determination of RVA in pigs

The VP7 and VP4 amplicons were subsequently excised from the agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen,

Table 1
Herd size, number of monitored farms and sampled animals in particular provinces of Poland.

Herd size (animals in thousands)*	Province	Number of						Total
		districts	farms	sampled animals in age groups (weeks)				
				1 - 4	5 - 8	9 - 16	> 17	
> 2000	Wielkopolska (WP)	18	22	26	21	40	56	143
1000 - 2000	Lublin (LB)	11	14	41	18	27	22	108
	Pomerania (PM)	9	13	18	32	30	7	87
	Kujawy-Pomerania (KP)	11	11	27	17	20	21	85
	Mazovia (MZ)	10	11	14	25	20	14	73
	Łódź (LD)	7	7	19	4	8	11	42
500 - 1000	Warmia-Masuria (WM)	5	5	6	13	13	2	34
	Opole (OP)	5	5	12	12	14	2	40
	Podlasie (PL)	3	3	–	4	13	3	20
< 500	West Pomerania (ZP)	8	19	34	35	10	45	124
	Silesia (SL)	5	5	6	16	13	2	37
	Świętokrzyskie (SK)	4	4	3	2	10	14	29
	Podkarpacie (PK)	3	4	11	4	5	14	34
	Lower Silesia (DS)	3	3	11	5	7	1	24
	Lubuskie (LS)	3	3	10	5	8	1	24
	Małopolska (MP)	2	2	7	3	5	1	16
Total		107	131	245	216	243	216	920

* Central Statistical Office, 2011.

Germany) according to the manufacturer's instructions. The purified PCR products were sequenced in both directions on a 3730XL DNA Analyzer (Applied Biosystems, USA) by Genomed, Poland. Raw data of the nucleotide sequences of both strands from each amplicon were corrected independently and compared. The consensus sequence was established with the use of the CLC Genomic Workbench v. 7.5 nucleotide sequence editor (Qiagen, Bioinformatics, USA). For identification of the virus G and P genotypes, nucleotide sequences were analysed using the RotaC automated RV genotyping tool (http://www.regatools.be/rota) (Maes et al., 2009). GenBank accession numbers of the nucleotide sequences for VP7 (MK239708 – MK239752) and VP4 (MK239662 – MK239707) gene fragments of RVA strains found in pigs are provided in a supplemental table.

2.5. Statistical analysis

The multiple range test was used to determine the confidence intervals (NIR) of the least significant differences (LSD) between the percentage of particular RVA genotypes and occurrence of GXP[X] RVA strains detected in pigs. A two-way analysis of variance (ANOVA) without interactions was used to show the differences in the frequency of genotypes and strain occurrence in pig population in Poland. Additionally, to assess differences in the prevalence of RV infections, in the frequencies of the occurrences of G and P genotypes and in the RVA strains in pigs from a particular province, a two-way ANOVA with interaction was applied. The percentage of detected genotypes and RVA strains in provinces was an interaction. This method was also used for an assessment of the relationship between pig age (animals age group) and the frequency of occurrence of RV infections, G and P genotypes and virus strains in the tested animal population. The adopted significance level was $\alpha = 0.05$. All calculations were performed with the Statgraphics Centurion v. XV package (Statpoint Technologies, USA).

3. Results

3.1. Prevalence of RV infections in pigs

The presence of RV antigen was detected in 377 (41%) out of 920 pig faecal samples. The relationship between pig's age and frequency of RV infections in the tested animals was elucidated ($p < 0.0001$) (Table 2). In piglets at age 1–4 and 5–8 weeks respectively 57.9% and 54.1% of animals were infected. The number of infected pigs from these age groups was significantly higher than it was among older animals from other groups ($p < 0.0001$) (Table 2). There were no statistically significant differences observed ($p > 0.05$) in the frequency of RV infections in pigs at the same age housed in different provinces.

3.2. Identification of G genotype of pig RVA strains

G genotype was identified for 217 (57.6%) out of 377 virus strains detected by ELISA in pig faeces. In the Polish pig population infections were caused by RVA strains belonging to 8 G genotypes (Table 3). G5 was the most frequently detected (31.3%), while infections caused by G6 RVAs were less common in pigs (0.5%). In the case of G1, G2, G3, G4, G9 and G11 RVA, differences in the frequency of their occurrence in pigs were not statistically significant ($p > 0.05$). Analysing the relationship between the occurrence of a particular G RVA genotype and the animal's age, infections in piglets up to 4 weeks old were caused by G5 (12.4%) and G4 (13.8%) RVA strains ($p < 0.0001$). In animals in the 5–8 week age group, G5 RVAs (10.6%) were dominant. In pigs older than 9 weeks none of the detected G genotypes prevailed ($p > 0.05$). In addition, statistically significant differences in the frequency of the occurrence of G RVA genotypes in pigs by Polish province were observed. Among pig RVA cases in Pomerania (PM) and Lubuskie (LS) provinces, infections caused by G5 strains accounted for 8.5% and 4.1% respectively. They occurred more frequently in pigs than infections with other G genotypes ($p < 0.0001$). However, this relation was not shown for other provinces in which differences between the occurrences of particular G RVA genotypes was not statistically significant (Table 4). Nevertheless, regional differences in the number of G RVA genotypes detected in pigs were observed. The greatest diversity of G genotypes was found in animals from farms in Kujawy-Pomerania (KP) (8 genotypes), Wielkopolska (WP), West Pomerania (ZP), Lublin (LB) (7 genotypes) and PM (6 genotypes) provinces. In contrast to these results, infections solely caused by RVA strains belonging to 2 different G genotypes were observed in pigs from farms in Małopolska (MP) and Świętokrzyskie (SK) provinces. Identification of virus genotypes responsible for infections in pigs from Podlasie (PL) province was not

Table 3
Number of detected pig RVA strains with particular G genotype in different age groups.

Genotype	Animal age (weeks)								
	Number of RVA strains (%)								
	1-4		5-8		9-16		> 17		Total (%)
G1	9	(4.1)^{b}	3	(1.4)^{b}	2	(0.9)^{b}	1	(0.5)^{a}	15 (6.9)^{B}
G2	2	(0.9)^{b}	4	(1.8)^{b}	8	(3.7)^{b}	3	(1.4)^{b}	17 (7.8) ^{B}
G3	19	(8.8)^{b}	5	(2.3)^{b}	4	(1.8)^{b}	2	(0.9)^{b}	30 (13.8) ^{B}
G4	30	(13.8)^{c}	13	(6)^{b}	2	(0.9)^{b}	2	(0.9)^{b}	47 (21.7) ^{C}
G5	27	(12.4)^{c}	23	(10.6)^{c}	12	(5.5)^{b}	6	(2.8)^{b}	68 (31.3)^{C}
G6	–	–	1	(0.5)^{a}	–	–	–	–	1 (0.5)^{A}
G9	7	(3.2)^{b}	11	(5.1)^{b}	3	(1.4)^{b}	6	(2.8)^{b}	27 (12.5) ^{B}
G11	3	(1.4)^{b}	5	(2.3)^{b}	1	(0.5)^{a}	3	(1.4)^{b}	12 (5.5) ^{B}
Total	97		65		32		23		217 (100)

a, b, c – different letters indicate age group of animals in which the percentage of RVA strains with specific G genotype differs significantly ($LSD_{0.05}$).
A,B,C - different letters indicate genotypes which prevalence differ significantly ($LSD_{0.05}$).
The numbers in brackets are the percentage of particular virus genotype detected to all virus strains with identified G genotype.

Table 2
Age, and health status of tested pigs and ELISA results for the occurrence of RVA infections in animals.

Animal age (weeks)	Production group	Number of pigs				Total
		non-diarrhoeic	diarrhoeic	ELISA		
				positive (%)	negative (%)	
1 - 4	nursing piglets	173	72	144 (57.9)^{b}	101 (42.1)	245 (100)
5 - 8	suckling piglets	195	21	120 (54.1)^{b}	96 (45.9)	216 (100)
9 - 16	weaned pigs	230	13	72 (27.2)^{a}	171 (72.8)	243 (100)
> 17	porkers	206	10	41 (17.5)^{a}	175 (82.5)	216 (100)
Total		804	116	377 (41)	543 (59)	920 (100)

a, b – different letters indicate age group of animals in which the percentage of RVA infected pigs differs significantly ($LSD_{0.05}$). The numbers in brackets are the percentage of infected or non-infected animals within each analysed age group.

Table 4
Number of detected pig RVA strains with particular G genotypes in different provinces.

Genotype		Province															
		DS	KP	LB	LS	LD	MP	MZ	OP	PK	PL	PM	SL	SK	WM	WP	ZP	Total
Number of RVA strains (%)	G1	–	1 (0.5)[a]	2 (0.9)[a]	–	–	–	–	–	1 (0.5)[a]	–	–	–	–	–	2 (0.9)[a]	9 (4.1)[a]	15
	G2	–	2 (0.9)[a]	1 (0.5)[a]	–	–	1 (0.5)[a]	1 (0.5)[a]	3 (1.4)[a]	–	–	1 (0.5)[a]	–	–	2 (0.9)[a]	3 (1.4)[a]	3 (1.4)[a]	17
	G3	2 (0.9)[a]	2 (0.9)[a]	4 (1.8)[a]	2 (0.9)[a]	–	–	–	5 (2.3)[a]	–	–	1 (0.5)[a]	6 (2.9)[a]	–	1 (0.5)[a]	3 (1.4)[a]	4 (1.8)[a]	30
	G4	2 (0.9)[a]	2 (0.9)[a]	6 (2.9)[a]	–	2 (0.9)[a]	–	6 (2.9)[a]	2 (0.9)[a]	4 (1.8)[a]	–	2 (0.9)[a]	2 (0.9)[a]	3 (1.3)[a]	1 (0.5)[a]	11 (5.1)[b]	4 (1.8)[a]	47
	G5	–	4 (1.8)[a]	8 (3.7)[a]	9 (4.1)[b]	5 (2.3)[a]	1 (0.5)[a]	4 (1.8)[a]	2 (0.9)[a]	2 (0.9)[a]	–	18 (8.5)[b]	2 (0.9)[a]	1 (0.5)[a]	1 (0.5)[a]	7 (3.2)[a]	4 (1.8)[a]	68
	G6	–	1 (0.5)[a]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
	G9	3(1.4)[a]	5 (2.3)[a]	3 (1.4)[a]	–	2 (0.9)[a]	–	1 (0.5)[a]	1 (0.5)[a]	1 (0.5)[a]	–	2 (0.9)[a]	–	–	–	3 (1.4)[a]	6 (2.9)[a]	27
	G11	–	1 (0.5)[a]	1 (0.5)[a]	1 (0.5)[a]	1 (0.5)[a]	–	–	–	2 (0.9)[a]	–	1 (0.5)[a]	–	–	–	4 (1.8)[a]	1 (0.5)[a]	12
Total																		217 (100)

a, b – different letters indicate statistically significant differences ($LSD_{0.05}$) in genotype prevalence in particular provinces.
The numbers in brackets are the percentage of particular virus genotype detected to all virus strains with identified G genotype.

Table 5
Number of detected pig RVA strains with particular P genotypes in different age groups.

Genotype	Animal age (weeks)								Total (%)
	Number of RVA strains (%)								
	1-4		5-8		9-16		> 17		
P[6]	28	(17.8)[b]	23	(14.6)[b]	3	(1.9)[a]	4	(2.5)[a]	58 (36.9)[B]
P[7]	10	(6.4)[a]	6	(3.9)[a]	4	(2.5)[a]	–	–	20 (12.7)[A]
P[8]	–	–	2	(1.3)[a]	–	–	–	–	2 (1.3)[A]
P[11]	–	–	1	(0.6)[a]	–	–	–	–	1 (0.6)[A]
P[13]	26	(16.6)[b]	7	(4.5)[a]	5	(3.3)[a]	4	(2.5)[a]	42 (26.7)[B]
P[14]	1	(0.6)[a]	–	–	–	–	–		1 (0.6)[A]
P[22]	2	(1.3)[a]	–	–	–	–	1	(0.6)[a]	3 (1.9)[A]
P[23]	1	(0.6)[a]	1	(0.6)[a]	–	–	–	–	2 (1.3)[A]
P[26]	–	–	2	(1.3)[a]	2	(1.3)[a]	–	–	4 (2.5)[A]
P[27]	–	–	2	(1.3)[a]	2	(1.3)[a]	1	(0.6)[a]	5 (3.2)[A]
P[32]	–	–	5	(3.3)[a]	1	(0.6)[a]	3	(1.9)[a]	9 (5.7)[A]
P[34]	2	(1.3)[a]	1	(0.6)[a]	3	(1.9)[a]	4	(2.5)[a]	10 (6.4)[A]
Total	70		50		20		17		157 (100)

a, b, c – different letters indicate age group of animals in which the percentage of RVA strains with specific P genotype differs significantly ($LSD_{0.05}$).
A,B,C - different letters indicate genotypes which prevalence differ significantly ($LSD_{0.05}$).
The numbers in brackets are the percentage of particular virus genotype detected to all virus strains with identified P genotype.

successful (Table 4).

3.3. Identification of P genotype of pig RVA strains

P genotype was determined for 157 (41.6%) RVA strains detected in pigs (Table 5). They belonged to 12 P genotypes, among which P[6] (36.9%) and P[13] (26.7%) were the most common. There were no significant differences observed in the frequency of prevalence of RVA strains having other P genotypes ($p > 0.05$). P[6] (17.8%) and P[13] (16.6%) RVA prevailed in piglets up to 4 weeks of age, whereas in the piglet group of 5–8 weeks of age, P[6] RVA was the most frequently detected (14.6%). In animals older than 9 weeks none of the detected P genotypes was dominant ($p > 0.05$). In the tested pig population, the P[8] and P[14] RVA genotypes were identified, which are not specific to this host. In the LB province, the percentages of the P[6] (5.7%) and P[13] (3.8%) strains infecting pigs were significantly higher ($p < 0.0001$) than to other detected virus P genotypes (Table 6). P[13] RVA strains were frequently identified in pigs from the LS province. However, infections caused by P[6] RVA dominated in animals from WP (10.8%) and Podkarpacie (PK) (3.8%) provinces, while P[7] RVAs were highly prevalent in pigs from Silesia (SL) province (3.8%, $p < 0.0001$). The highest number of different P genotypes among the detected RVA strains was identified in PM (7 genotypes) and ZP (6 genotypes). In addition, a single strain of P[6] genotype was detected in the PL region (Table 6).

3.4. Geographical distribution of RVA strains

In total, 145 RVA strains representing 33 GP genotypes were identified in the Polish pig population. For 72 strains only the G genotype was identified, whereas the P genotype was successfully determined for 12 strains (Table 7). In pigs, G4P[6] RVA (13.8%) strains were the most frequently detected. They were highly prevalent compared to other GP RVA genotypes. The G4P[6] strains were found in animals from 12 out of 16 provinces (except LS, Łódź (LD), MP and PL) (Fig. 1). Other RVA strains often found in Poland were G5P[13] (8.2%) and G3P[13] (5.1%). They occurred in pigs farmed in western (ZP, LS, WP, Lower Silesia (DS) and Opole (OP), northern Warmia-Masuria (WM) and eastern (LB) parts of Poland. Besides typical pig RVA genotypes, novel

Table 6
Number of detected pig RVA strains with particular P genotypes in different provinces.

Genotype		Province															
		DS	KP	LB	LS	LD	MP	MZ	OP	PK	PL	PM	SL	SK	WM	WP	ZP	Total
Number of RVA strains (%)	P[6]	2 (1.3)[a]	3(1.9)a	9(5.7)[b]	–	1 (0.6)[a]	–	5 (3.2)[a]	2 (1.3)[a]	6 (3.8)[b]	1 (0.6)[a]	3 (1.9)[a]	2 (1.3)[a]	1 (0.6)[a]	1 (0.6)[a]	17 (10.8)[b]	5 (3.2)[a]	58
	P[7]	1 (0.6)[a]	1 (0.6)a	2 (1.3)[a]	–	2 (1.3)[a]	–	–	–	1 (0.6)[a]	–	2 (1.3)[a]	6 (3.8)[b]	–	–	2 (1.3)[a]	3 (1.9)[a]	20
	P[8]	–	–	–	–	–	–	–	–	–	–	1 (0.6)[a]	–	–	–	–	1 (0.6)[a]	2
	P[11]	–	–	–	–	–	–	–	–	–	–	–	1 (0.6)[a]	–	–	–	–	1
	P[13]	2 (1.3)[a]	2 (1.3)[a]	6 (3.8)[b]	6 (3.8)[b]	1 (0.6)[a]	–	3 (1.9)[a]	1 (0.6)[a]	2 (1.3)[a]	–	6 (3.8)[b]	–	1 (0.6)[a]	1 (0.6)[a]	5 (3.2)[a]	6 (3.8)[b]	42
	P[14]	–	–	–	–	–	–	–	–	–	–	1 (0.6)[a]	–	–	–	–	–	1
	P[22]	–	1 (0.6)[a]	–	–	–	–	–	2 (1.3)[a]	–	–	–	–	–	–	–	–	3
	P[23]	–	–	–	–	–	–	–	–	1 (0.6)[a]	–	1 (0.6)[a]	–	–	–	–	–	2
	P[26]	–	–	–	1 (0.6)[a]	2 (1.3)[a]	–	–	–	–	–	–	–	–	–	–	1 (0.6)[a]	4
	P[27]	–	–	1 (0.6)[a]	–	–	–	–	2 (1.3)[a]	–	–	–	–	–	2 (1.3)[a]	–	–	5
	P[32]	–	–	–	–	–	–	–	–	–	–	3 (1.9)[a]	–	–	1 (0.6)[a]	–	5 (3.2)[a]	9
	P[34]	–	2 (1.3)[a]	–	–	–	–	–	1 (0.6)[a]	–	–	–	–	–	–	7 (4.5)[a]	–	10
Total																		157 (100)

a, b – different letters indicate statistically significant differences ($LSD_{0,05}$) in genotype prevalence in particular provinces.
The numbers in brackets are the percentage of particular virus genotype detected to all virus strains with identified P genotype.

strains such as G5P[34] and G9P[34] were identified in pigs. They infected animals from WP, PM, ZP and OP in which the largest population of pigs and number of farms are present. A strain rare for the swine host but often detected in humans, G1P[8] RVA, was found on a pig farm in the ZP province. In this study a significant relationship between the occurrence of particular RVA strains in pigs and animal age was shown. In the group of piglets at the age of 1–4 weeks, the number of animals infected with G4P[6] RVA was higher than the number infected with other virus strains detected in this group. However, in piglets at the age of 5–8 weeks, G4P[6] and G5P[13] occurred more frequently than other virus strains. In animals older than 9 weeks none of the detected RVA strains was dominant.

4. Discussion

The use of RV vaccines and the improvement of breeding conditions have significantly reduced piglet mortality, but RV diarrhoea is still considered an important health problem on pig farms (Barreiros et al., 2004; Lorenzetti et al., 2011). The worldwide prevalence of RVA infections in pigs is age dependent and ranges from 0.8% in Europe to 72% in South America (Barreiros et al., 2003; Collins et al., 2010; Lachapelle et al., 2014; Machnowska et al., 2014). In this study, the prevalence of RVA infections in the population of Polish pigs was estimated at 41% (Elisa based results), which was higher than observed in Germany 0.8% (Machnowska et al., 2014), Ireland 6.5% (Collins et al., 2010), Spain 7% (Halaihel et al., 2010), Denmark 10% and Slovenia 20% (Midgley et al., 2012). A higher (71.5%) prevalence of RVA infections in pig herds was only observed in Italy and the United Kingdom (Martella et al., 2007; Chandler-Bostock et al., 2015).

The results of molecular genotyping of Polish pig RVA strains showed a large variation among G and P genotypes (33 genotypes). It was found that RVA strains belonged to 8 G (G1, G2, G3, G4, G5, G6, G9, G11) and 12 P (P[6], P[7], P[8], P[11], P[13], P[14], P[22], P[23], P[26], P[27], P[32], P[34]) genotypes respectively. A similar panel of circulating G and P RVA in pigs was observed in Slovenia (Midgley et al., 2012) and Great Britain (Chandler-Bostock et al., 2014). In other European countries, RV infections in pigs were caused by a less diverse panel of the strains encompassing 4–6, different G and P genotypes (Martella et al., 2005; Collins et al., 2010; Midgley et al., 2012; Machnowska et al., 2014; Monini et al., 2014; Theuns et al., 2014, 2015; Wenske et al., 2018). This low strain diversity could result from differences in piglet age at weaning, the length of the fattening period, and the scale of national and international animal trade in particular countries (Collins et al., 2010; Monini et al., 2014; Theuns et al., 2014, 2016; Wenske et al., 2018). When a temporal analysis of the occurrence in pigs in Poland of particular G RVA genotypes was performed, it became clear that since 2002 infections caused by G5 and G4 RVA have dominated uninterruptedly. The G5 RVA genotype is considered the most widespread virus genotype in pigs in the world (Kim et al., 2010; Papp et al., 2013; Chandler-Bostock et al., 2014; Theuns et al., 2016). In addition to the G5, G3 and G4 virus genotypes which are often identified in the Polish pig population, it is noteworthy that G9 RVA strains appeared in pigs in Poland, and constituted a large (12.4%) proportion of the strains circulating in this animal species. Viruses with this genotype were found in Asia in the late 1990s and since then they have spread worldwide causing infections in both humans and animals (Santos et al., 1999; Matthijnssens et al., 2010; Wu et al., 2017). In Europe, G9 RVA in pigs was first reported in 2005 in Ireland (Collins et al., 2010). In the subsequent years strains possessing this genotype appeared in Danish, Spanish (Midgley et al., 2012), British (Chandler-Bostock et al., 2014), Belgian (Theuns et al., 2014), Italian (Monini et al., 2014) and German (Wenske et al., 2018) pigs with different prevalences ranging from 6.2% to 44%. G1 RVA with its worldwide prevalence not exceeding 2%, belongs to the group of virus strains rarely detected in pigs (Papp et al., 2013). Prior to this study in Europe only in Slovenia (Steyer et al., 2008) and Belgium (Theuns et al., 2015)

Table 7
Occurrence of G/P RVA strains in pigs.

	P[X]													
GX (%)	P[6]	P[7]	P[8]	P[11]	P[13]	P[14]	P[22]	P[23]	P[26]	P[27]	P[32]	P[34]	P[X]	Total
G1	–	8 (3.5)	1 (0.4)	–	3 (1.4)	–	–	–	–	–	–	–	3 (1.4)	15
G2	–	–	–	–	2 (0.9)	–	–	–	–	4 (1.7)	2 (0.9)	2 (0.9)	7 (3.0)	10
G3	2 (0.9)	6 (2.6)	–	–	12 (5.1)	–	1(0.4)	–	–	–	–	–	9 (3.9)	30
G4	31 (13.8)	–	–	–	2 (0.9)	–	–	–	2 (0.9)	–	–	–	12 (5.1)	47
G5	8 (3.5)	4 (1.7)	–	1 (0.4)	19 (8.2)	–	1 (0.4)	–	1 (0.4)	–	2 (0.9)	5 (2.2)	27 (11.6)	68
G6	–	–	–	–	–	–	–	–	–	–	–	–	1 (0.4)	1
G9	6 (2.6)	2 (0.9)	–	–	3 (1.4)	–	–	1 (0.4)	–	–	3 (1.4)	2 (0.9)	10 (4.3)	27
G11	5 (2.2)	–	–	–	–	–	1 (0.4)	1 (0.4)	1 (0.4)	–	–	1 (0.4)	3 (1.4)	12
GX	6 (2.6)	–	1 (0.4)	–	1 (0.4)	1 (0.4)	–	–	–	1 (0.4)	2 (0.9)	–	–	12
Total	58	20	2	1	42	1	3	2	4	5	9	10	72	229 (100)

The numbers in brackets are the percentage of particular G/P RVA strain detected to all virus strains with identified genotypes.

had single G1 RVA strains been detected in pigs. It is worth making mention of the first identification in a pig in Poland of bovine G6 RV reassortant strain, which rarely occurs in this animal (Martella et al., 2001; Midgley et al., 2012; Theuns et al., 2016). The prevalence of other genotypes such as G2 and G11 in pigs in Poland was comparable to that in other European countries (Midgley et al., 2012; Vlasova et al., 2017).

In this study infections caused by P[6] RVA (36.9%) and P[13] (26.7%) were most frequently detected. In comparison to the results of previous studies conducted by Winiarczyk et al. (2002), an increase in the number of P[6] RVA infections in pigs was observed in Poland. As demonstrated by other authors, this RV genotype has been shown as dominant in pig herds in Denmark (56%) (Midgley et al., 2012), Slovenia (41%) (Midgley et al., 2012) and Great Britain (33%) (Chandler-Bostock et al., 2014). The incidence of P[13] RVA infections in the Polish pig population was twice as high as in other European countries,

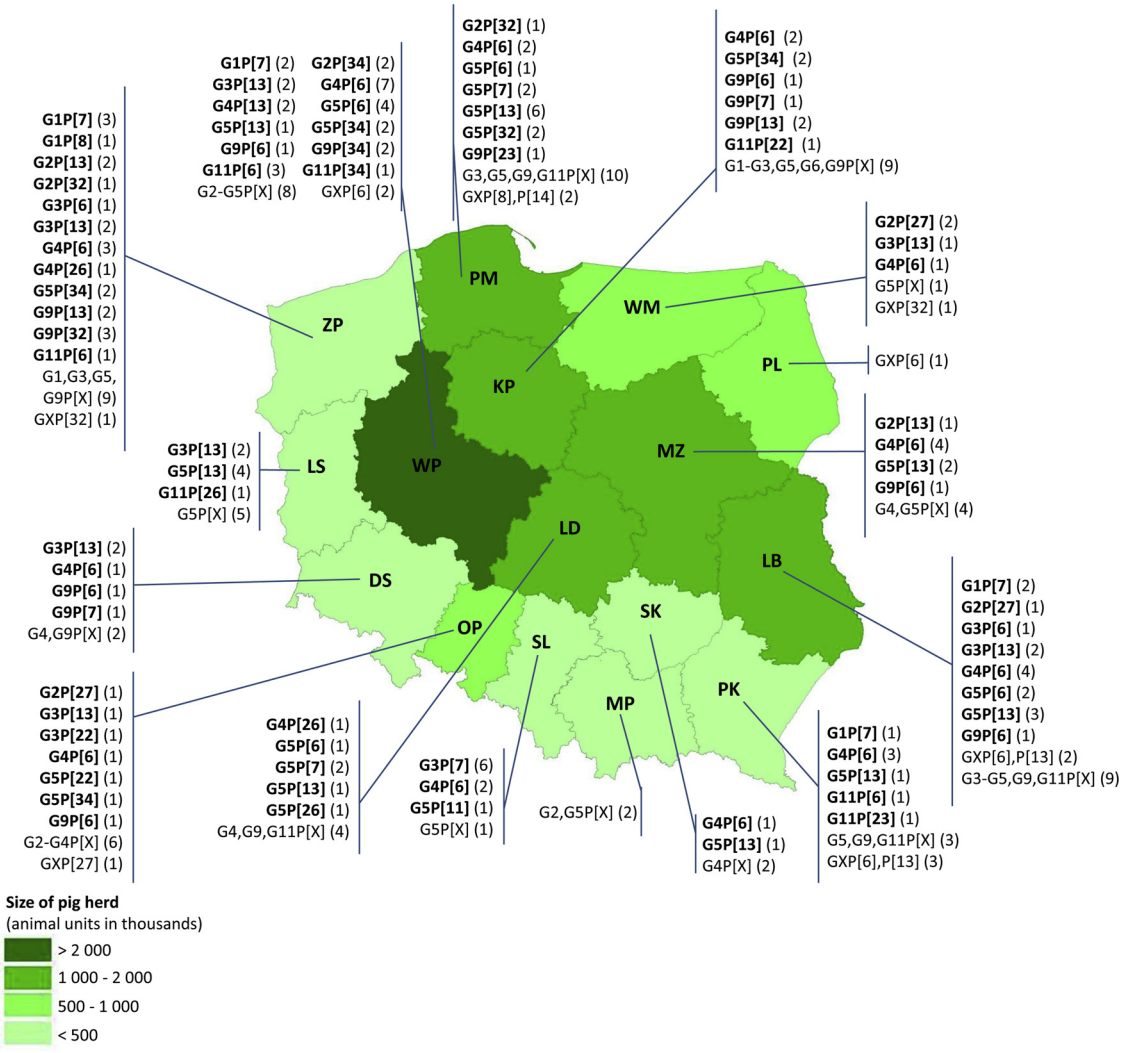

Fig. 1. Geographical distribution of pig RVA strains in Poland. Values in brackets indicate number of detected RVA strains of particular genotype. GX, P[X] – undetermined genotype.

where single cases of infections were noticed (Collins et al., 2010; Midgley et al., 2012; Chandler-Bostock et al., 2014; Monini et al., 2014; Theuns et al., 2014). In contrast to Europe, in North America P[13] RVA strains were the most commonly identified in pigs (Amimo et al., 2013). Another virus genotype important in the epidemiology of RVA infections in pigs in Poland is P[7] with 12.7% prevalence, which is similar to that observed in elsewhere in Europe (Papp et al., 2013). A much higher spread of this virus genotype was found in animals kept in Asia (41.7%) and in North and South America (77.2%) (Amimo et al., 2013).

Animal age might have a significant influence on the occurrence frequency of particular G and P genotypes in pigs in Poland. In piglets up to 8 weeks of age, the infections were mainly caused by prevailing G4, G5 and P[6], P[13] RVA genotypes. There were no differences observed in the prevalence of G and P genotypes in older animals, which may be associated with the lower number of RVA infections in this animal group. Geographical differences in the occurrence of RVA genotypes were also shown. The greatest strain diversity was found in pigs from the north-western part of Poland and in Lublin province, where the pig population and the number of large-scale commercial farms were the highest.

In our studies, more heterogeneous circulation of G and P RVA genotypes in pigs has been seen in Poland over the last 15 years. It is evidence for a genetic reassortment having occurred between virus strains having different genotypes as well as between strains originating from different hosts. As seen in previous studies P[11], P[22], P[23], P[26], P[27], P[32], and P[34] RVA are newly emerging virus strains in pigs (Steyer et al., 2008; Collins et al., 2010; Midgley et al., 2012; Papp et al., 2013; Chandler-Bostock et al., 2014, 2015; Monini et al., 2014; Theuns et al., 2015, 2016). Besides detection of the typical RVA genotypes for pigs, the novel strains G5P[34], G9P[34] and GXP[14] were detected in the swine host. Until now, the P[14] genotype of RVA had been associated only with infections in ruminants and rabbits (De Leener et al., 2004; Matthijnssens et al., 2006, 2009; Bonica et al., 2015). Further evidence of genetic reassortment of RVA strains is provided by the presence of P[8] RVA which has previously been detected in pigs only sporadically (Chandler-Bostock et al., 2014). Indeed, RVAs of this genotype are associated with human infections (Iturriza-Gómara et al., 2009; László et al., 2012; Usonis et al., 2012; Konstantopoulos et al., 2013; Roczo-Farkas et al., 2016).

The emergence of new, genotypes rarely detected in pigs in Poland could be associated with importation of asymptomatically infected animals from neighbouring countries. For example, strains with G1P[7], G2P[27], G2P[32], G2P[34], G9P[13], G9P[23] and G9P[32] genotypes were detected in Poland and Germany. In this light, a rise in G4P[6] RVA infections in Polish pigs could be also linked to significant movement of live animals from Denmark and the Netherlands to Poland occurring in the years 2011–2015 (Statistics Poland, 2018). G4P[6] RVAs come among the most frequently found strains in pigs in these countries (van der Heide et al., 2005; Midgley et al., 2012).

Although molecular methods were used for identification of RVA strains, the number of detected strains may not fully reflect the pattern of circulating genotypes as the products of analysed genes for some strains were not always obtained. This could be considered as a major limitation of the study. Failures in the identification of pig RVA genotypes have also been reported by other authors (Parra and Espinola, 2006; Simmonds et al., 2008; Solberg et al., 2009). Generally, they resulted from mismatches of the primers to sequences of the amplified gene fragments (Simmonds et al., 2008) or an insufficient amount of viral RNA available for molecular analyses (Solberg et al., 2009). In addition, unreadable chromatograms at the stage of sequencing and difficulties in assembling the consensus nucleotide sequences were also observed. Furthermore, the presence of mixed infections caused by different virus strains could affect reverse transcription as the consensus sequences could not be established for 17 (4.5%) samples containing a mixture of closely related matrices.

5. Conclusion

Findings from this study showed diversity of G and P RVA genotypes circulating in pigs in Poland. The variability and acquisition of new virus genotypes has led to the emergence of novel, genetically distinct RVAs. The emergence of RVAs genotypes that were not detected previously in the swine host is probably influenced by the observed country-to-country differences in breeding conditions of pigs, population density and scale of animal trade. In comparison to other European countries a higher genetic variability of pig RVA strains in Poland was observed. The changes in the genotype occurrence of RVA strains in pigs indicate the need for continuous epidemiological surveillance of circulating strains combined with the identification of virus genotypes.

Conflict of interests

The authors have no conflict of interest to declare.

Authors' contributions

IK performed the serological and molecular assays, compiled and analysed results and drafted the manuscript. AD visited studied farms and collected pig faecal samples. JK performed statistical analyses, interpreted the results and prepared their graphical presentation. AR conceived the original idea, interpreted the results and corrected the manuscript. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.03.026.

References

Amimo, J.O., Vlasova, A.N., Saif, L.J., 2013. Detection and genetic diversity of porcine group A rotaviruses in historic (2004) and recent (2011 and 2012) swine fecal samples in Ohio: predominance of the G9P[13] genotype in nursing piglets. J. Clin. Microbiol. 51 1142 LP-1151.

Amimo, J.O., Junga, J.O., Ogara, W.O., Vlasova, A.N., Njahira, M.N., Maina, S., Okoth, E.A., Bishop, R.P., Saif, L.J., Djikeng, A., 2015. Detection and genetic characterization of porcine group A rotaviruses in asymptomatic pigs in smallholder farms in East Africa: Predominance of P[8] genotype resembling human strains. Vet. Microbiol. 175, 195–210. https://doi.org/10.1016/J.VETMIC.2014.11.027.

Barreiros, M.A.B., Alfieri, A.A., Alfieri, A.F., Médici, K.C., Leite, J.P.G., 2003. An outbreak of diarrhoea in one-week-old piglets caused by group A rotavirus genotypes P[7],G3 and P[7],G5. Vet. Res. Commun. 27, 505–512. https://doi.org/10.1023/A:1025797809680.

Barreiros, M.A.B., Alfieri, A.F., Médici, K.C., Leite, J.P.G., Alfieri, A.A., 2004. G and P genotypes of group A rotavirus from diarrhoeic calves born to cows vaccinated against the NCDV (P[1],G6) rotavirus strain. J. Vet. Med. Ser. B 51, 104–109. https://doi.org/10.1111/j.1439-0450.2004.00737.x.

Bonica, M.B., Zeller, M., Van Ranst, M., Matthijnssens, J., Heylen, E., 2015. Complete genome analysis of a rabbit rotavirus causing gastroenteritis in a human infant. Viruses 7, 844–856. https://doi.org/10.3390/v7020844.

Central Statistical Office, 2011. Statistical Yearbook of the Republic of Poland.

Chandler-Bostock, R., Hancox, L.R., Nawaz, S., Watts, O., Iturriza-Gomara, M., Mellits, K.M., 2014. Genetic diversity of porcine group A rotavirus strains in the UK. Vet. Microbiol. 173, 27–37. https://doi.org/10.1016/J.VETMIC.2014.06.030.

Chandler-Bostock, R., Hancox, L.R., Payne, H., Iturriza-Gomara, M., Daly, J.M., Mellits, K.H., 2015. Diversity of group A rotavirus on a UK pig farm. Vet. Microbiol. 180, 205–211. https://doi.org/10.1016/J.VETMIC.2015.09.009.

Collins, P.J., Martella, V., O'Shea, H., 2008. Detection and characterization of group C rotaviruses in asymptomatic piglets in Ireland. J. Clin. Microbiol. 46, 2973–2979. https://doi.org/10.1128/JCM.00809-08.

Collins, P.J., Martella, V., Sleator, R.D., Fanning, S., O'Shea, H., 2010. Detection and characterisation of group A rotavirus in asymptomatic piglets in southern Ireland. Arch. Virol. 155, 1247–1259. https://doi.org/10.1007/s00705-010-0713-1.

De Leener, K., Rahman, M., Matthijnssens, J., Van Hoovels, L., Goegebuer, T., van der Donck, I., Van Ranst, M., 2004. Human infection with a P[14], G3 lapine rotavirus.

Virology 325, 11–17. https://doi.org/10.1016/J.VIROL.2004.04.020.
Dennehy, P.H., Bertrand, H.R., Silas, P.E., Damaso, S., Friedland, L.R., Abu-Elyazeed, R., 2008. Coadministration of RIX4414 oral human rotavirus vaccine does not impact the immune response to antigens contained in routine infant vaccines in the United States. Pediatrics 122 e1062 LP-e1066.
Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K., Bhan, M.K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. 30, 1365–1373.
Gentsch, J.R., Laird, A.R., Bielfelt, B., Griffin, D.D., Bányai, K., Ramachandran, M., Jain, V., Cunliffe, N.A., Nakagomi, O., Kirkwood, C.D., Fischer, T.K., Parashar, U.D., Bresee, J.S., Jiang, B., Glass, R.I., 2005. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J. Infect. Dis. 192, S146–S159.
Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276–282.
Halaihel, N., Masía, R.M., Fernández-Jiménez, M., Ribes, J.M., Montava, R., De Blas, I., Gironés, O., Alonso, J.L., Buesa, J., 2010. Enteric calicivirus and rotavirus infections in domestic pigs. Epidemiol. Infect. 138, 542–548. https://doi.org/10.1017/S0950268809990872.
Iturriza-Gómara, M., Dallman, T., Bá, K., Bö, B., Buesa, J., Diedrich, S., Fiore, L., Johansen, K., Korsun, N., Kroneman, A., Lappalainen, M., László, B., Maunula, L., Matthinjnssens, J., Midgley, S., Mladenova, Z., Poljsak-Prijatelj, M., Pothier, P., Ruggeri, F.M., Sanchez-Fauquier, A., Schreier, E., Steyer, A., Gray, J., 2009. Rotavirus surveillance in Europe, 2005–2008: web-enabled reporting and real-time analysis of genotyping and epidemiological data establishment of national networks for sampling and the use of standardized methods for strain characterization. J. Infect. Dis. 200, 215–221. https://doi.org/10.1086/605049.
Kim, H.-J., Park, S.-I., Ha, T.P.M., Jeong, Y.-J., Kim, H.-H., Kwon, H.-J., Kang, M.-I., Cho, K.-O., Park, S.-J., 2010. Detection and genotyping of Korean porcine rotaviruses. Vet. Microbiol. 144, 274–286. https://doi.org/10.1016/J.VETMIC.2010.01.019.
Konstantopoulos, A., Tragiannidis, A., Fouzas, S., Kavaliotis, I., Tsiatsou, O., Michailidou, E., Spanaki, A., Mantagos, S., Kafetzis, D., Papaevangelou, V., Gopala, K., Holl, K., 2013. Burden of rotavirus gastroenteritis in children < 5 years of age in Greece: hospital-based prospective surveillance (2008–2010). BMJ Open 3, e003570. https://doi.org/10.1136/bmjopen-2013-003570.
Lachapelle, V., Sohal, J.S., Lambert, M.-C., Brassard, J., Fravalo, P., Letellier, A., L'Homme, Y., 2014. Genetic diversity of group A rotavirus in swine in Canada. Arch. Virol. 159, 1771–1779. https://doi.org/10.1007/s00705-013-1951-9.
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, G., 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, G., Bányai, K., 2012. Surveillance of human rotaviruses in 2007–2011, Hungary: exploring the genetic relatedness between vaccine and field strains. J. Clin. Virol. 55, 140–146. https://doi.org/10.1016/J.JCV.2012.06.016.
Lorenzetti, E., da Silva Medeiros, T.N., Alfieri, A.F., Alfieri, A.A., 2011. Genetic heterogeneity of wild-type G4P[6] porcine rotavirus strains detected in a diarrhea outbreak in a regularly vaccinated pig herd. Vet. Microbiol. 154, 191–196. https://doi.org/10.1016/J.VETMIC.2011.06.026.
Machnowska, P., Ellerbroek, L., Johne, R., 2014. Detection and characterization of potentially zoonotic viruses in faeces of pigs at slaughter in Germany. Vet. Microbiol. 168, 60–68. https://doi.org/10.1016/J.VETMIC.2013.10.018.
Maes, P., Matthijnssens, J., Rahman, M., Van Ranst, M., 2009. RotaC: A web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 9, 238. https://doi.org/10.1186/1471-2180-9-238.
Martella, V., Pratelli, A., Greco, G., Tempesta, M., Ferrari, M., Losio, M.N., Buonavoglia, C., 2001. Genomic characterization of porcine rotaviruses in Italy. Clin. Diagn. Lab. Immunol. 8, 129–132.
Martella, V., Ciarlet, M., Baselga, R., Arista, S., Elia, G., Lorusso, E., Banyai, K., Terio, V., Madio, A., Ruggeri, F.M., 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. https://doi.org/10.1016/j.virol.2005.03.031.
Martella, V., Ciarlet, M., Bányai, K., Lorusso, E., Arista, S., Lavazza, A., Pezzotti, G., Decaro, N., Cavalli, A., Lucente, M.S., Corrente, M., Elia, G., Camero, M., Tempesta, M., Buonavoglia, C., 2007. Identification of group A porcine rotavirus strains bearing a novel VP4 (P) genotype in Italian swine herds. J. Clin. Microbiol. 45, 577–580.
Martella, V., Bányai, K., Matthijnssens, J., Buonavoglia, C., Ciarlet, M., 2010. Zoonotic aspects of rotaviruses. Vet. Microbiol. 140, 246–255. https://doi.org/10.1016/J.VETMIC.2009.08.028.
Marthaler, D., Rossow, K., Culhane, M., Collins, J., Goyal, S., Ciarlet, M., Matthijnssens, J., 2013. Identification, phylogenetic analysis and classification of porcine group C rotavirus VP7 sequences from the United States and Canada. Virology 446, 189–198. https://doi.org/10.1016/J.VIROL.2013.08.001.
Matthijnssens, J., Van Ranst, M., 2012. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr. Opin. Virol. 2, 426–433. https://doi.org/10.1016/J.COVIRO.2012.04.007.
Matthijnssens, J., Rahman, M., Martella, V., Xuelei, Y., De Vos, S., De Leener, K., Ciarlet, M., Buonavoglia, C., Van Ranst, M., 2006. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. J. Virol. 80, 3801–3810.
Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S.M., Palombo, E.A., Iturriza-Gómara, M., Maes, P., Patton, J.T., Rahman, M., Van Ranst, M., 2008a. Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J. Virol. 82, 3204–3219.
Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Kirkwood, C.D., Martella, V., Mertens, P.P.C., Nakagomi, O., Patton, J.T., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U., Van Ranst, M., 2008b. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153, 1621–1629. https://doi.org/10.1007/s00705-008-0155-1.
Matthijnssens, J., Potgieter, C.A., Ciarlet, M., Parreño, V., Martella, V., Bányai, K., Garaicoechea, L., Palombo, E.A., Novo, L., Zeller, M., Arista, S., Gerna, G., Rahman, M., Van Ranst, M., 2009. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? J. Virol. 83, 2917–2929.
Matthijnssens, J., Joelsson, D.B., Warakomski, D.J., Zhou, T., Mathis, P.K., van Maanen, M.-H., Ranheim, T.S., Ciarlet, M., 2010. Molecular and biological characterization of the 5 human-bovine rotavirus (WC3)-based reassortant strains of the pentavalent rotavirus vaccine, RotaTeq®. Virology 403, 111–127. https://doi.org/10.1016/J.VIROL.2010.04.004.
Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Bányai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P.C., Nakagomi, O., Parreño, V., Rahman, M., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., van Ranst, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch. Virol. 1397–1413. https://doi.org/10.1007/s00705-011-1006-z.
Midgley, S.E., Bányai, K., Buesa, J., Halaihel, N., Hjulsager, C.K., Jakab, F., Kaplon, J., Larsen, L.E., Monini, M., Poljšak-Prijatelj, M., Pothier, P., Ruggeri, F.M., Steyer, A., Koopmans, M., Böttiger, B., 2012. Diversity and zoonotic potential of rotaviruses in swine and cattle across Europe. Vet. Microbiol. 156, 238–245. https://doi.org/10.1016/J.VETMIC.2011.10.027.
Molinari, B.L.D., Lorenzetti, E., Otonel, R.A.A., Alfieri, A.F., Alfieri, A.A., 2014. Species H rotavirus detected in piglets with diarrhea, Brazil, 2012. Emerg. Infect. Dis. 20, 1019–1022. https://doi.org/10.3201/eid2006.130776.
Monini, M., Zaccaria, G., Ianiro, G., Lavazza, A., Vaccari, G., Ruggeri, F.M., 2014. Full-length genomic analysis of porcine rotavirus strains isolated from pigs with diarrhea in Northern Italy. Infect. Genet. Evol. 25, 4–13. https://doi.org/10.1016/J.MEEGID.2014.03.024.
Papp, H., László, B., Jakab, F., Ganesh, B., De Grazia, S., Matthijnssens, J., Ciarlet, M., Martella, V., Bányai, K., 2013. Review of group A rotavirus strains reported in swine and cattle. Vet. Microbiol. https://doi.org/10.1016/j.vetmic.2013.03.020.
Parashar, U.D., Hummelman, E.G., Bresee, J.S., Miller, M.A., Glass, R.I., 2003. Global illness and deaths caused by rotavirus disease in children. Emerg. Infect. Dis. 9, 565–572. https://doi.org/10.3201/eid0905.020562.
Park, S.-I., Matthijnssens, J., Saif, L.J., Kim, H.-J., Park, J.-G., Alfajaro, M.M., Kim, D.-S., Son, K.-Y., Yang, D.-K., Hyun, B.-H., Kang, M.-I., Cho, K.-O., 2011. Reassortment among bovine, porcine and human rotavirus strains results in G8P[7] and G6P[7] strains isolated from cattle in South Korea. Vet. Microbiol. 152, 55–66. https://doi.org/10.1016/J.VETMIC.2011.04.015.
Parra, G.I., Espinola, E.E., 2006. Nucleotide mismatches between the VP7 gene and the primer are associated with genotyping failure of a specific lineage from G1 rotavirus strains. Virol. J. 3, 35. https://doi.org/10.1186/1743-422X-3-35.
Pejsak, Z., Truszczyński, M., 2005. Biegunki prosiąt ssących i odsadzonych. Życie Weter. 3, 151–154.
Roczo-Farkas, S., Kirkwood, C.D., Bines, J., A.R.S.G, 2016. Australian rotavirus surveillance program annual report, 2015. Commun. Dis. Intell. Q. Rep. 40, E527–E538.
Santos, N., Lima, R.C.C., Nozawa, C.M., Linhares, R.E., Gouvea, V., 1999. Detection of porcine rotavirus type G9 and of a mixture of types G1 and G5 associated with Wa-like VP4 specificity: evidence for natural human-porcine genetic reassortment. J. Clin. Microbiol. 37, 2734–2736.
Simmonds, M.K., Armah, G., Asmah, R., Banerjee, I., Damanka, S., Esona, M., Gentsch, J.R., Gray, J.J., Kirkwood, C., Page, N., Iturriza-Gómara, M., 2008. New oligonucleotide primers for P-typing of rotavirus strains: Strategies for typing previously untypeable strains. J. Clin. Virol. 42, 368–373. https://doi.org/10.1016/J.JCV.2008.02.011.
Solberg, O.D., Hasing, M.E., Trueba, G., Eisenberg, J.N.S., 2009. Characterization of novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus. Virology 385, 58–67. https://doi.org/10.1016/J.VIROL.2008.11.026.
Statistics Poland, 2018. Analytical Platform - SWAiD - Knowledge Databases - Foreign Trade. [WWW Document]. URL. http://swaid.stat.gov.pl/HandelZagraniczny_dashboards/Raporty_konstruowane/RAP_SWAID_HZ_3_2.aspx.
Steyer, A., Poljsak-Prijatelj, M., Barlic-Maganja, D., Marin, J., 2008. Human, porcine and bovine rotaviruses in Slovenia: evidence of interspecies transmission and genome reassortment. J. Gen. Virol. 89, 1690–1698. https://doi.org/10.1099/vir.0.2008/001206-0.
Tate, J.E., Burton, A.H., Boschi-Pinto, C., Steele, A.D., Duque, J., Parashar, U.D., 2012. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect. Dis. 12, 136–141. https://doi.org/10.1016/S1473-3099(11)70253-5.
Theuns, S., Desmarets, L.M.B., Heylen, E., Zeller, M., Dedeurwaerder, A., Roukaerts, I.D.M., Van Ranst, M., Matthijnssens, J., Nauwynck, H.J., 2014. Porcine group A rotaviruses with heterogeneous VP7 and VP4 genotype combinations can be found together with enteric bacteria on Belgian swine farms. Vet. Microbiol. 172, 23–34. https://doi.org/10.1016/J.VETMIC.2014.04.002.
Theuns, S., Heylen, E., Zeller, M., Roukaerts, I.D.M., Desmarets, L.M.B., Van Ranst, M., Nauwynck, H.J., Matthijnssens, J., 2015. Complete genome characterization of recent and ancient Belgian pig group A rotaviruses and assessment of their evolutionary

relationship with human rotaviruses. J. Virol. 89, 1043–1057.
Theuns, S., Vyt, P., Desmarets, L.M.B., Roukaerts, I.D.M., Heylen, E., Zeller, M., Matthijnssens, J., Nauwynck, H.J., 2016. Presence and characterization of pig group A and C rotaviruses in feces of Belgian diarrheic suckling piglets. Virus Res. 213, 172–183. https://doi.org/10.1016/J.VIRUSRES.2015.12.004.
Truszczyński, M., Pejsak, Z., 2012. Biegunki świń wywołane przez wirusy warunkowo chorobotwórcze. Med. Weter. 68, 9–14.
Usonis, V., Ivaskeviciene, I., Desselberger, U., Rodrigo, C., 2012. The unpredictable diversity of co-circulating rotavirus types in Europe and the possible impact of universal mass vaccination programmes on rotavirus genotype incidence. Vaccine 30, 4596–4605. https://doi.org/10.1016/J.VACCINE.2012.04.097.
van der Heide, R., Koopmans, M.P.G., Shekary, N., Houwers, D.J., van Duynhoven, Y.T.H.P., van der Poel, W.H.M., 2005. Molecular characterizations of human and animal group A rotaviruses in The Netherlands. J. Clin. Microbiol. 43, 669–675.
Vlasova, A.N., Amimo, J.O., Saif, L.J., 2017. Porcine rotaviruses: epidemiology, immune responses and control strategies. Viruses. https://doi.org/10.3390/v9030048.
Wenske, O., Rückner, A., Piehler, D., Schwarz, B.-A., Vahlenkamp, T.W., 2018. Epidemiological analysis of porcine rotavirus A genotypes in Germany. Vet. Microbiol. 214, 93–98. https://doi.org/10.1016/J.VETMIC.2017.12.014.
Winiarczyk, S., Paul, P.S., Mummidi, S., Panek, R., Gradzki, Z., 2002. Survey of porcine rotavirus G and P genotype in Poland and the United States using RT-PCR. J. Vet. Med. B 49, 373–378. https://doi.org/10.1046/j.1439-0450.2002.00572.x.
Wu, F.-T., Bányai, K., Jiang, B., Liu, L.T.-C., Marton, S., Huang, Y.-C., Huang, L.-M., Liao, M.-H., Hsiung, C.A., 2017. Novel G9 rotavirus strains co-circulate in children and pigs. Taiwan. Sci. Rep. 7, 40731. https://doi.org/10.1038/srep40731.