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Prevalence and genetic diversity of porcine rotavirus A from diarrheic piglets in Northern Thailand

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Abstract

Background Rotavirus A (RVA) is an important pathogen causing acute viral gastroenteritis in young children and various animals. RVA is also recognized as a common cause of gastroenteritis in piglets. Epidemiological studies of porcine RVA (PoRVA) conducted in different settings worldwide reported that the prevalence of PoRVA infection ranged from 9.4% to 74.0% with the predominance of G4P[6], G4P[7], and G5P[7] genotypes. In Thailand, long-term epidemiological surveillance of PoRVA infection is limited. Continuous monitoring of PoRVA infection is required to gain a better understanding the prevalence and evolution of PoRVA. In this study, the prevalence and genetic diversity of PoRVA were investigated by screening of 1,260 stool samples collected from 0 to 5-week-old piglets with acute diarrhea during 2016 to 2023 by using real-time RT-PCR. The G- and P-genotypes of RVA were identified by characterization of the partial VP7 and VP4 genes by using multiplex-PCR, nucleotide sequencing, and phylogenetic analysis.

Results A total of 303 out of 1,260 (24.0%) samples were positive for PoRVA. Overall, the G5P[23] (28.7%) and G4P[23] (28.4%) were detected as the co-predominant PoRVA genotypes, followed by G5P[13] (9.9%), G3P[23] (9.6%), G9P[23] (8.2%), G4P[13] (7.9%), G9P[13] (3.3%), G3P[13] (1.7%), G4P[6] (1.7%), and G2P[23] (0.3%) genotypes. Additionally, a rare G2P[27] (0.3%) genotype re-emerged approximately 22 years after the initial detection in 2000 in Chiang Mai, Thailand.

Conclusion Our results revealed the prevalence of wide variety of PoRVA genotypes circulating in piglets with acute diarrhea in Thailand over a study period of seven years. Of these, G5P[23] and G4P[23] emerged as the most predominant genotypes, which were substantially different from previous reports in the same geographical area. The findings offer valuable contribution to a better understanding of molecular epidemiology and evolution of PoRVA in piglets with acute diarrhea.

Keywords Rotavirus, Porcine, Piglet, Diarrhea, Thailand

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Background

Rotaviruses (RVs) are the leading cause of acute diarrhea in young children and various animal species [1, 2]. RVs belong to the genus *Rotavirus* of the *Sedoreoviridae* family in the order *Reovirales.* The viral particle exhibits an icosahedral capsid containing three capsid protein layers of 65 to 75 nm in diameter. The RV genome consists of 11 segments of double-stranded RNA (dsRNA), which encode 6 structural viral proteins (VP1–VP4, VP6, and VP7) and 6 nonstructural proteins (NSP1–NSP6) [3]. RVs are classified into 9 groups (A to D and F to J) based on the molecular and antigenic characteristics of the VP6 protein. Among these, rotavirus A (RVA), RVB, RVC, and RVH have been identified in pig herds worldwide [4, 5]. Among the RV groups, RVA is considered the most common cause of enteric disease in humans and various animal species from a medical and veterinary health perspective, including suckling piglets within the first 8 weeks after birth [6–8]. The RVA strains have been classified by dual classification system based on antigenic and sequence differences of the outer capsid proteins, VP7 and VP4 proteins, into G (glycosylated) and P (protease-sensitive) genotypes, respectively [3].

For porcine RVA (PoRVA), the estimated prevalence of PoRVA infection in piglets with acute diarrhea reported from several countries worldwide, ranged from 9.4% to 74.0% [9–15]. The G3, G4, G5, G9, and G11 are the common G genotypes and frequently combine with the P[6], P[7], P[13], P[19], P[23], and P[26] genotypes. Globally, G5P[7], G4P[6], and G4P[7] were the most common PoRVA strains found in pigs [6]. During epidemiological surveillance of swines with acute diarrhea in Thailand from 2000 to 2016, the prevalence of PoRVA infection ranged from 9.5% to 23.0% [15–21]. In 2000 and 2001, The G3P[19] was detected as a co-predominant genotype with G4P[6] of PoRVA strains circulating in the Chiang Mai area [16], and a novel G2P[27] genotype was also detected for the first time in this area in 2000 [22]. Okitsu et al. [21] reported that the most widespread genotype observed in diarrheic piglets in Northern Thailand from 2006 to 2008 was G9P[23], followed by G3P[23] and G3P[13]. From 2009 to 2010, the PoRVA strains with a wide variety of G-P combinations were detected in two provinces (Chiang Mai and Lamphun) in Thailand, and the most predominant PoRVA strain was G4P[6], followed by G4P[23] and G3P[23]. Moreover, novel genotype combinations of G4P[19] and G9P[19] were also detected for the first time in piglets with diarrhea [19]. From 2011 to 2014, the G4P[13] genotype was reported as the most dominant PoRVA genotype circulating among piglets with diarrhea in the same area [17]. In addition, from 2011 to 2016, a multi-site surveillance study of PoRVA infection in swine reported the diversity of PoRVA strains in Thailand. The samples were chosen randomly from the RVA-positive samples obtained from commercial pig farms located throughout Thailand, and most of them were G9P[13] and G9P[23] genotypes [18].

According to the previous studies of PoRVA in Thailand, G3P[19], G4P[6], G9P[13], and G9P[23] were identified as dominant genotypes from 2000 to 2016. However, there is a lack of continuous long-term epidemiological surveillance of PoRVA to monitor the changing of PoRVA genotypes circulating in Thailand. Therefore, this study aimed to further investigate the epidemiology of PoRVA and conducted molecular characterization and phylogenetic analysis of PoRVA circulating in piglets with acute diarrhea across several farms in Northern Thailand from 2016 to 2023. Continuous monitoring of PoRVA infections in pigs can improve our understanding of the epidemiology and evolution of these PoRVA strains in the recent years.

Materials and methods

Specimen collection

A total of 1,260 fecal specimens were collected from piglets at the age of newborn to 5 weeks that exhibited acute diarrhea (indicated by watery or loose stools persisting for at least 12 h) raised in 41 commercial pig farms located in Chiang Mai, Lamphun, and Uttaradit provinces in Northern Thailand (Fig. 1). One specimen was collected from one pig pen, and it represented a sample of a litter. The specimen collection periods were from January 2016 to December 2017 and January 2019 to December 2023. Due to the global outbreak of African swine fever (AFS) in 2018, the access to pig farms was prohibited, therefore, the stool specimen of 2018 was not collected, and the time period was excluded from this study. All fecal samples were stored at -20°C until used.

Viral RNA extraction and detection of PoRVA by RT-qPCR

The viral RNA genome was extracted from 10% (w/v) stool suspension in 1X phosphate-buffered saline (PBS) at pH 7.4 by using Geneaid Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan) following the manufacturer's instructions. The extracted viral RNA was denatured by heating in a 50% dimethyl sulfoxide (DMSO) solution at 95 °C for 5 min, followed by cooling on ice. The conversion of viral RNA into complementary DNA (cDNA) was then performed using the RevertAid First Strand cDNA Synthesis kit with random primers (Thermo Scientific, MA, USA). The cDNA was detected by quantitative PCR (qPCR) and a cycle threshold (Ct) value less than 40 was considered positive for PoRVA. Nuclease-free water was used as a negative control. The qPCR reaction mixture (20 µl) consisted of 10 µl of 2×THUNDERBIRD® Probe qPCR Master Mix

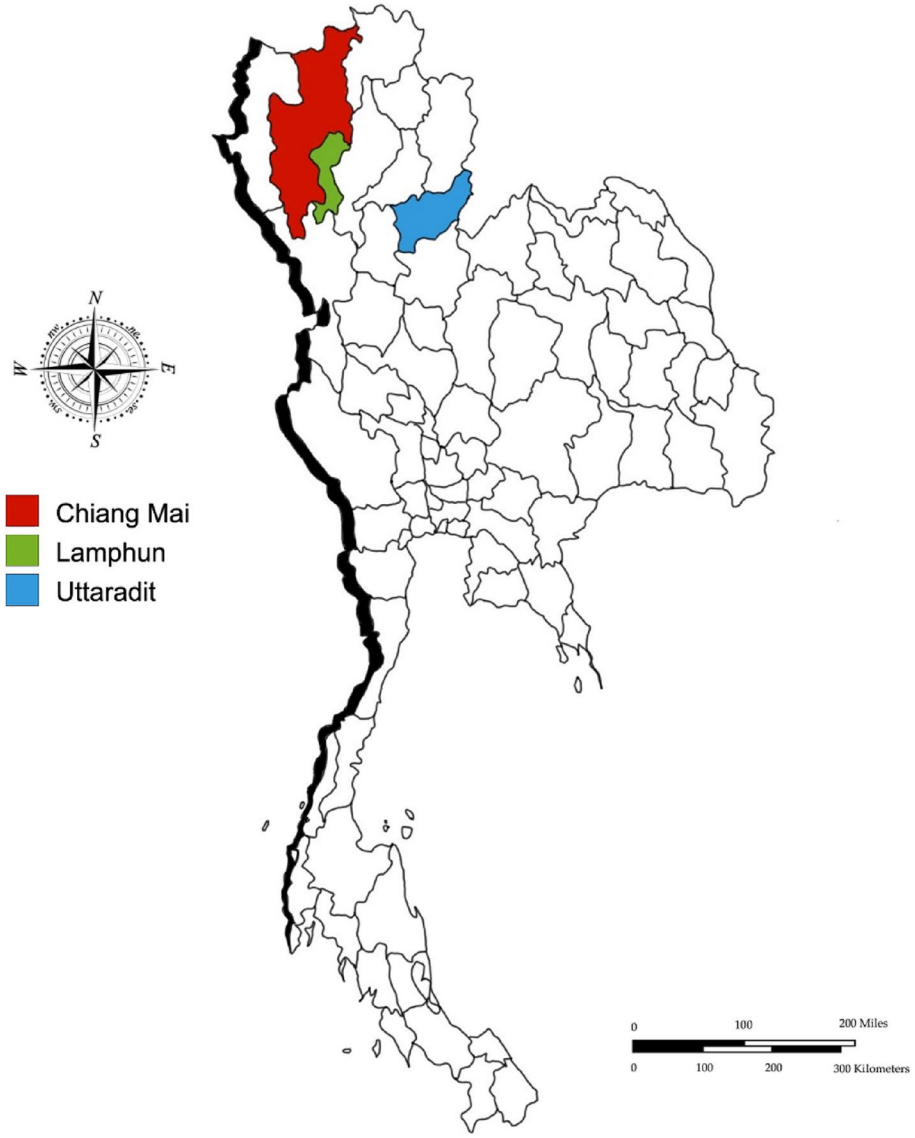

Fig. 1 Thailand map of the three provinces where the stool specimens were collected

(TOYOBO, Osaka, Japan), 10 nM (0.8 µl/reaction) of forward JVKF and reverse JVKR primers, 0.4 µl of JVKP probe and 6.0 µl of nuclease-free water, and 2 µl of cDNA template. The forward primer JVKF (5′- CAGTGGTTG ATGCTCAAGATGGA -3′), reverse primer JVKR (5′- TCATTGTAATCATATTGAATACCCA -3′), and probe JVKP (FAM- ACAACTGCAGCTTCAAAAGAAGWG T- MGB) were used for specific detection of PoRVA nucleic acid by targeting on the conserved region within the NSP3 gene [23]. The experiment was performed in a CFX Opus Real-Time PCR System (Bio-Rad, CA, USA). The thermocycling conditions were as follows: 95°C for 1 min, followed by 40 cycles at 95 °C for 15 s and 56 °C for 1 min. The results were analyzed using the CFX Maestro Software. The baseline was automatically set, and the threshold was manually placed during the exponential phase of the amplification reaction.

Identification of PoRVA G- and P-genotypes, nucleotide sequencing, and phylogenetic analysis

The semi-nested polymerase chain reaction (PCR) was used to identify G-genotypes (VP7) and P-genotypes (VP4) using specific primers as described previously [24–27]. For G-genotype, the initial PCR was performed by amplifying the partial VP7 gene using the sBeg9 forward primer in combination with the End9(s) reverse primer, yielding a product of 941 bp [26, 28]. The Con3 forward primer and the Con2 reverse primer were used

for the P-genotype to amplify the partial VP4 gene with a specific PCR product of 887 bp [29]. Subsequently, multiplex-PCR was performed by using the initial PCR product from semi-nested PCR as the template, and specific primers for G3-G5, and G9 [25, 26] and P[6], P[7], P[13], P[23], and P[19] [16, 17, 21, 26] were used for G- and P-genotyping, respectively. The RVA strains that their G- and/or P-genotypes could not be identified by specific primers were further characterized by nucleotide sequencing and phylogenetic analysis. The PCR products were purified using a Gel/PCR DNA Fragments Extraction Kit using the manufacturer's procedure (Geneaid, Taipei, Taiwan), and then sequenced (First BASE Laboratories Sdn Bhd, Selangor Darul Ehsan, Malaysia). The obtained nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with those of the reference strains available in the GenBank database. The phylogenetic trees of VP7 and VP4 genes of PoRVA were constructed by using Molecular Evolutionary Genetics Analysis version 10 (MEGA X) software based on the maximum likelihood method and selected the best-fit evolutionary model based on the Bayesian information score (BIC) [30]. The models used in this study were Hasegawa-Kishino-Yano + G + I (HKY + G + I) for VP7 and Tamura 3-parameter + G + I (T93 + G + I) for VP4. A bootstrap of 1000 replicates was used to assess the reliability of the branching order.

Statistical analysis

The statistical analyses were performed using IBM SPSS version 27.0 software. Categorical variables were analyzed using the Fisher's exact test. The *p*-values for each comparison were also calculated, and p-value less than or equal to 0.05 (≤ 0.05) was considered statistically significant.

Results

Prevalence of PoRVA and distribution of RVA strains in piglets with acute diarrhea

A total of 1,260 stool samples were screened for PoRVA by RT-qPCR and 303 (24.0%) were positive. As shown in Fig. 2, the prevalence of PoRVA infection in Thai swine gradually increased from 4.6% in 2016 to 8.8%, 11.6%, and 27.1% in 2017, 2019, and 2020, respectively. Then, there was a slight decline from 33.3% in 2021 to 29.4% in 2022, followed by a noticeable rise to 39.6% in 2023. Overall, the prevalence of PoRVA infection in piglets with acute diarrhea from 2016 to 2023 was 24.0%. To compare the prevalence trends and to identify the significant changes, the Fisher's exact test was performed in this study. The statistical analyses revealed that the prevalence of PoRVA in 2023 was significantly higher than those in 2016, 2017, 2019, 2020, 2021, and 2022 with the p-values of < 0.001, < 0.001, < 0.001, < 0.002, 0.001, and < 0.001, respectively. Similarly, the prevalence of PoRVA in each year of 2020, 2021, and 2022 was significantly higher than those of 2016, 2017, and 2019, respectively, with the *p*-values of ≤ 0.001. The remaining years did not show statistically significant differences. Moreover, the significant increase in PoRVA prevalence was also observed when comparing the early years (2016 to 2019) with the later years (2020 to 2023) with the *p*-values of 0.0024.

Distribution of PoRVA genotypes in piglets with acute diarrhea

The distributions of G- and P-genotype combinations of PoRVA in piglets with acute diarrhea are shown in

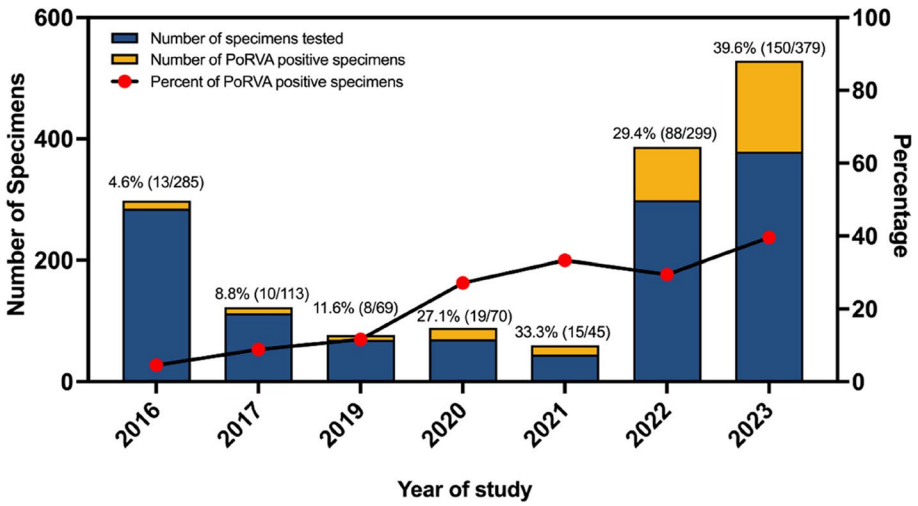

Fig. 2 Prevalence of porcine rotavirus A in piglets with acute diarrhea in Northern Thailand from 2016 to 2023

Table 1. The G5P[23] (28.7%) and G4P[23] (28.4%) were detected with high prevalence. Other genotypes including G5P[13], G3P[23], G4P[13], and G9P[23] were detected at the prevalent rates of 9.9%, 9.6%, 7.9%, and 8.2%, respectively. Moreover, G9P[13] (3.3%), G3P[13] (1.7%), G4P[6] (1.7%), and G2P[23] (0.3%) were detected with less prevalent rates. In addition, the unusual G- and P- genotype, G2P[27], was detected at the prevalence of 0.3%. Notably, even though overall prevalences of G5P[23] and G4P[23] were the most predominant genotypes detected in this study, G5P[23] was undetectable from 2017 to 2021, and suddenly emerged with exceptionally high prevalences from 2022 to 2023. Similarly, G4P[23] was also undetectable during the period of 2016 to 2020 and suddenly emerged with exceptionally high prevalences in 2021 to 2023.

Phylogenetic analysis of VP7 and VP4 genes

The PoRVA positive samples that their G-genotypes and P-genotypes could not be identified by multiplex PCR using genotype-specific primers, their genotypes were identified by nucleotide sequencing and phylogenetic analysis.

The phylogenetic tree of the VP7 gene of PoRVA strains is shown in Fig. 3. The phylogenetic tree of nucleotide sequences of a partial VP7 gene (375 nt) of 34 PoRVA representative strains detected in this study was analyzed together with those of the RVA reference sequences available in the GenBank database. The analysis revealed that 5 different PoRVA genotypes, including G2, G3, G4, G5, and G9 were detected in this study. A G2 PoRVA strain CMP200-22 shared the highest nucleotide sequence identity with the G2 PoRVA strain OH365-055 detected in Canada [31] at 85.5%. Furthermore, the CMP200-22 strain also shared a close genetic relationship with several G2 RVA reference strains detected in pigs and humans reported previously from Thailand [22] and USA [32], with the nucleotide sequence identities ranging from 79.4% to 84.6%. In addition, 8 strains of G3 detected in this study clustered closely with the G3P[19] PoRVA strains CMP096 and CMP099 [16] reported from Chiang Mai, Thailand in 2000 with nucleotide sequence identities of 89.4% and 97.8%, respectively. For G4 genotype, our 2 strains of G4 (CMP97-17 and CMP98-17) detected in 2017 were most closely related to the PoRVA G4P[6] strain SCGY-198 reported from China. The other 8 strains of G4 (CMP01-21, CMP04-21, CMP39-21, CMP176-22, CMP185-22, CMP304-23, CMP325-23, and CMP379-23) clustered closely with G4 reference strains detected in pigs, a wild boar, and humans from Thailand [20], Vietnam [33], and China [34] with the nucleotide sequence identities ranging from 92.9% to 96.4%. Six strains of G5 (CMP31-16, CMP107-17, CMP228-22, CMP238-22, CMP302-22, and CMP355-22) detected in this study were located most closely in the same branch with PoRVA G5P[13] strain CMP-001-12 reported previously from Thailand [17] with the nucleotide sequence identities ranging from 92.9% to 94.7%. The other 4 strains of G5 (CMP25-16, CMP40-17, CMP41-17, and CMP43-17) showed a close genetic relationship with 4 reference strains of G5 reported from China [35] with the nucleotide sequence identities ranging from 94.2% to 96.0%. One of our G9 PoRVA strain CMP106-17 detected in this study displayed high nucleotide sequence identity with the G9P[23] strain previously detected in a wild pig from Taiwan [36] at 85.9%. The other 4 strains of G9 (CMP64-19, CMP66-19, CMP15-20, and CMP17-20) were closely related to those of G9 reference strains circulating Thailand [18, 37, 38] with the nucleotide sequence identities ranging from 98.1% to 100%.

Table 1 Distribution of G- and P-genotype combinations of porcine rotavirus A strains circulating in piglets with acute diarrhea in Northern Thailand from 2016 to 2023

RVA genotypes	2016 (%)	2017(%)	2019(%)	2020(%)	2021(%)	2022(%)	2023(%)	Total (%)
G2P[23]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	1 (0.3)
G2P[27]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	1 (0.3)
G3P[13]	1 (7.7)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)	1 (1.1)	2 (1.3)	5 (1.7)
G3P[23]	9 (69.2)	2 (20.0)	0 (0.0)	12 (63.1)	0 (0.0)	0 (0.0)	6 (4.0)	29 (9.6)
G4P[6]	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.4)	0 (0.0)	5 (1.7)
G4P[13]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)	4 (4.6)	17 (11.3)	24 (7.9)
G4P[23]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (80.0)	27 (30.7)	47 (31.3)	86 (28.4)
G5P[13]	1 (7.7)	5 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (8.0)	17 (11.3)	30 (9.9)
G5P[23]	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	44 (50.0)	41 (27.3)	87 (28.7)
G9P[13]	0 (0.0)	1 (10.0)	1 (12.5)	1 (5.3)	0 (0.0)	0 (0.0)	7 (4.7)	10 (3.3)
G9P[23]	0 (0.0)	0 (0.0)	7 (87.5)	5 (26.3)	0 (0.0)	0 (0.0)	13 (8.8)	25 (8.2)
Total	**13 (4.3)**	**10 (3.3)**	**8 (2.6)**	**19 (6.3)**	**15 (5.0)**	**88 (29.0)**	**150 (49.5)**	**303 (100)**

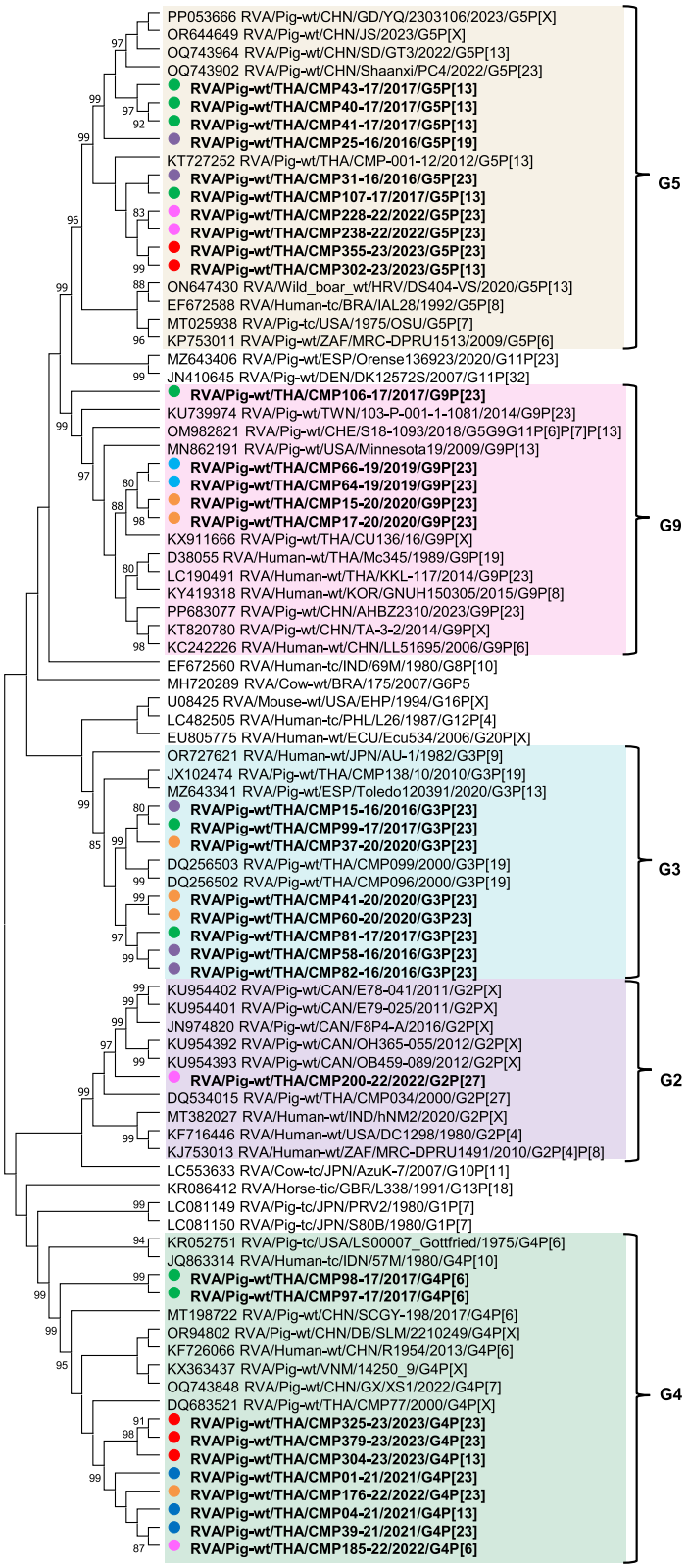

Fig. 3 Phylogenetic analysis of porcine rotavirus A based on partial nucleotide sequences of VP7 gene (375 nt). The tree was constructed using MEGA X software via the HKY + G + I as the best-fit evolutionary model. The representatives of porcine rotavirus A strains detected in this study are presented in boldface with purple (2016), green (2017), light blue (2019), orange (2020), dark blue (2021), pink (2022), and red (2023) colors filled circles. Bootstrap values above 80 percent are shown at separate nodes

The phylogenetic tree of the VP4 gene of PoRVA strains is shown in Fig. 4. The phylogenetic tree of the VP4 nucleotide sequence (759 nt) of 36 PoRVA representative strains detected in this study was analyzed together with the RVA reference strains. It was found that 5 P different genotypes, including P[6], P[13], P[19], P[23], and P[27] were detected. Three strains of P[6] (CMP97-17, CMP98-17, and CMP274-22) clustered closely together with P[6] human and PoRVA reference strains of P[6] reported from Thailand [18, 39, 40], and Russia [41], with high nucleotide sequence identities ranging from 91.3% to 97.8%. For P[13] genotype, 2 strains of P[13] (CMP43-17 and CMP273-22) were closely related to P[13] reference strains reported from Thailand [20, 40], and China [35] with the nucleotide sequence identities ranging from 92.2% to 96.1%. The other 3 strains of P[13] (CMP302-23, CMP304-23, and CMP361-23) were located closely in the same branch with P[13] reference strains reported from China [35] with nucleotide sequence identities ranging from 93.8% to 97.8%. Moreover, only one strain of P[19] (CMP25-16) PoRVA detected in this study clustered together with P[19] reference strains isolated from a human and pigs in Chiang Mai, Thailand [42] with nucleotide sequence identities ranging from 96.4% to 98.1%. Furthermore, the majority of PoRVA strains detected in this study belonged to the P[23] genotype, and they were closely related to P[23] strains detected in Thailand [18, 21] and other regions, including Vietnam [33] and China [35] with the nucleotide sequence identities sequencing from 83.5% to 96.5%. It was interesting to observe that the VP4 nucleotide sequence of one PoRVA strain CMP200-22 showed the highest nucleotide sequence identity (94.7%) with an uncommon P[27] PoRVA strain CMP034 detected previously in Chiang Mai, Thailand in 2000 [22].

Discussion

Rotaviruses can infect young animals of many species, including piglets [43]. The PoRVAs are commonly associated with weaning and post-weaning enteric infections in piglets at the age of 1 to 8 weeks. This infection can lead to diarrhea, dehydration, and retardation of growth rates among piglets [44]. Furthermore, PoRVA causes economic loss to farmers due to treatment costs related to high morbidity and mortality rates [8]. Here, we reported the prevalence and genetic diversity of PoRVA strains circulating in piglets with acute diarrhea in Northern Thailand during the period of 7 years from 2016 to 2017 and 2019 to 2023. It should be pointed out that a period of 2018 was not included in this study due to the global outbreak of AFS, access to the pig farms was prohibited. As a result, none of stool samples were collected in 2018, and therefore the prevalence and distribution of PoRVA genotypes in 2018 were missing. This is a limitation of this study. The overall prevalence of PoRVA infection during this study period was 24.0% (303 out of 1,260), which is obviously higher than those reported previously in Thailand from 2000 to 2016 (ranging from 9.5% to 23.0%) [16–21]. Even though the overall prevalence of PoRVA infection reported in the present study during a period of 2016 to 2023 was high at 24.0% (Table 1), the prevalences from 2016 to 2019 were relatively low, ranging from only 4.6% to 11.6%. The prevalence suddenly increased to as high as 27.1% in 2020 and continued rising to 33.3% in 2021 and up to 39.6% in 2023. The low prevalences of PoRVA infection from 2016 to 2019 (4.6% to 11.6%) observed in the present study are consistent with previous studies reported from several countries in Asia including Taiwan (6.3% in 2014 to 2017) [45], India (10.8% in 2016 to 2017) [46], South Korea (14.1% in 2014 to 2018) [47], and China (16.8% in 2017 to 2019) [48], and also in Africa such as Mozambique (11.8% in 2016) [49]. In addition, the increase in the prevalence of PoRVA to high level at 42% was reported most recently in China during a period of 2021 to 2024 [50]. Altogether, low prevalences of PoRVA infection during a period of 2016 to 2019 and abruptly increased to high prevalences in 2020 to 2023 observed in this study is in line with the global trend of PoRVA prevalence.

Our long-time (2016 to 2023) characterization of G- and P-genotypes of PoRVA strains circulating in piglets with acute diarrhea in Northern Thailand revealed that the G2, G3, G4, G5, and G9 genotypes, and P[6], P[13], P[23], and P[27] genotypes detected in this study (Table 1) were the common G- and P-genotypes circulating in several countries worldwide, including Thailand [17, 19], Japan [51], Vietnam [52], China [35], UK [27], Italy [53], Belgium [10], and Brazil [54]. Most PoRVA strains detected in this study carried P[23] (75.2%) and P[13] (22.8%) as the most predominant P-genotypes of 98.0%, while carried P[6] and P[27] at 1.7% and 0.3%, respectively, as shown in Table 1. The P[13] in combination with G4 and G5 genotypes were detected for the first time in this geographical area (Chiang Mai province, Thailand) in 2000 [16], while P[23] genotype was detected for the first time in this area in 2008 as the most predominant genotype [21]. The follow-up study during a period of 2011 to 2014 revealed that P[13] and P[23] continued to exist in this area as the most predominant genotypes at 40.6% and 34.4%, respectively and the P[13] combined with G3, G4, G5, and G11, whereas P[23] combined with G3, G4, G5, and G9 genotypes [17]. In the present study, the surveillance of 2016 to 2023, P[23] and P[13] continued circulating in this area as the most predominant genotypes at 75.2% and 22.8%, respectively. Altogether, the

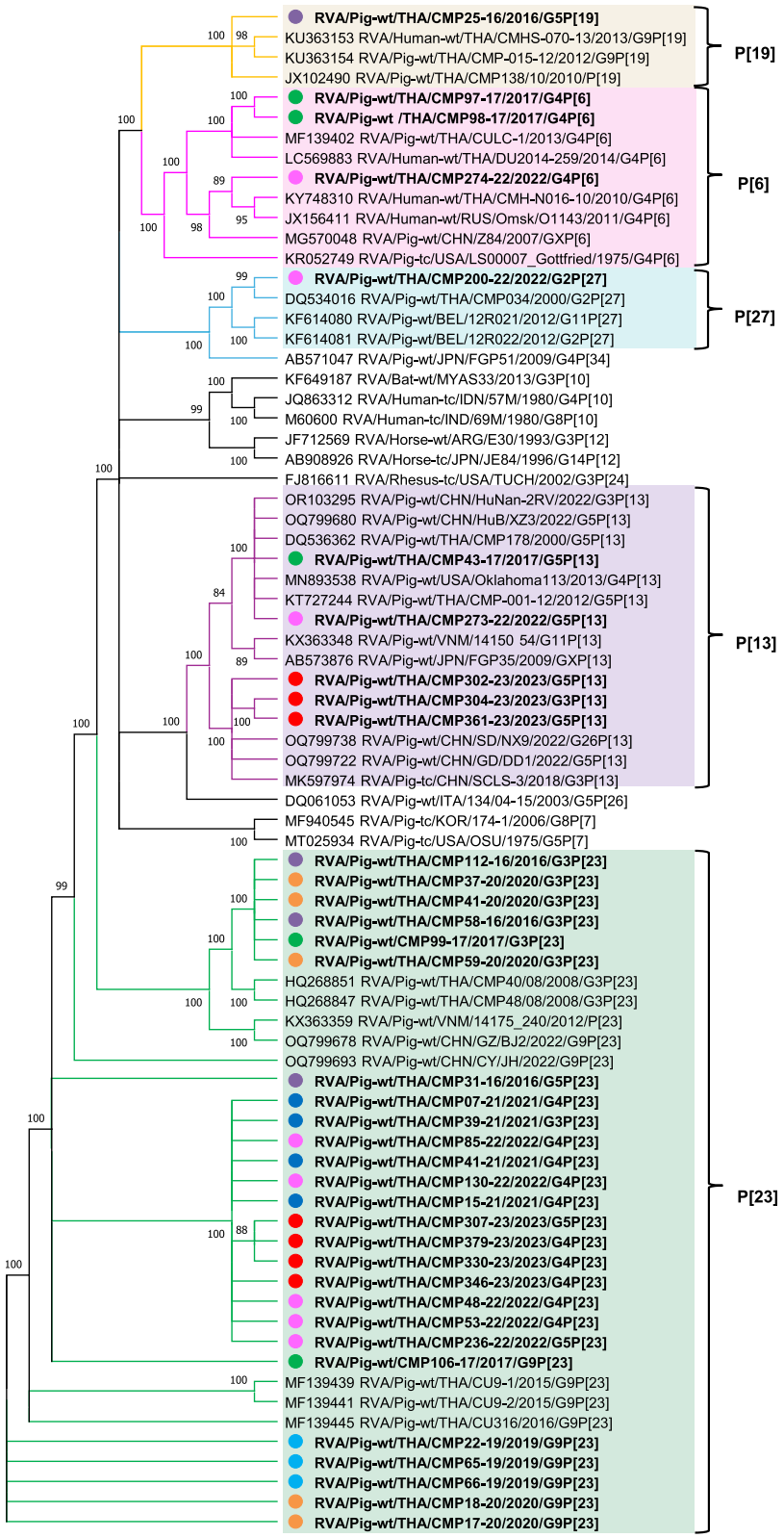

Fig. 4 Phylogenetic analysis of porcine rotavirus A based on partial nucleotide sequences of VP4 gene (759 nt). The tree was constructed using MEGA X software via the TN93 + G + I as the best-fit evolutionary model. The representatives of porcine rotavirus A strains detected in this study were presented in boldface with purple (2016), green (2017), light blue (2019), orange (2020), dark blue (2021), pink (2022), and red (2023) colors filled circles. Bootstrap values above 80 percent are shown at separate nodes

findings indicate that P[13] and P[23] in combination with various G-genotypes, including G2, G3, G4, G5, G9, and G11 remained circulating in piglets with acute diarrhea over two decades in this geographical area of Thailand. Previous studies revealed that P[13] and P[23] are related to high virulence strains. The PoRVA strains bearing P[13] and P[23] are host-restricted, predominantly found in pigs, and rarely identified in other animal sources or humans [20, 50, 55]. Therefore, the findings of P[13] and P[23] in acute diarrheic piglets in this study imply that P[13] and P[23] in combination with a great variety of G-genotypes of PoRVA are adapted well to maintain in piglets as the major PoRVA genotypes associated with diarrhea in piglets in this area. In addition, G3P[23], G4P[6], and G9P[23] were identified as the dominant genotypes from 2016 to 2020, then G5P[23] and G4P[23] became more prevalent during 2021 to 2023. Remarkably, G5P[23] emerged as the most prevalent genotype over seven years period in this study (Table 1). The emergence of G5P[23] and G4P[23] as the predominant genotypes have been reported in domestic pigs worldwide, especially in Asia, which is consistent with this study [6, 13]. The emergence of G5P[23] and G4P[23] may also be explained by lacking of herd immunity against these viral genotypes in pig population and probably linked to incomplete cross-protection from natural immunity [6].

The PoRVA strain CMP200-22 detected in this study was identified as G2P[27] genotype and closely related to the G2P[27] Thai strain CMP034, which was isolated previously in the same geographical area (Chiang Mai, Thailand) in 2000 to 2001 [22]. The PoRVA G2P[27] genotype was detected for the first time in a diarrheic piglet of 49 days old at a farm located in Mae Rim district, Chiang Mai province, in 2000. Since then, G2P[27] genotype was not detected in Thailand until approximately 22 years later, it was detected again in this study. To the best of our knowledge, G2P[27] genotype is a unique genotype isolated from pigs and seldom detected only in a few countries worldwide, such as Thailand, Italy, Japan, Slovenia, and Belgium [22, 51, 56–58]. The findings suggest that G2P[27] maintains in pig population in this geographical area with less replication efficiency compared to the other PoRVA genotypes. Although G2 strains are commonly associated with human infections, the P[27] genotype is seldom detected and has been predominantly identified in pigs. This uncommon combination of G2P[27] indicates that this genotype may have occurred from a zoonotic spillover event. Moreover, previous studies have shown that G2P[27] strains possess gene segments closely related to both human and animal RVAs [22, 59, 60]. This genetic diversity can lead to the emergence of novel strains with enhanced virulence and adaptability.

Nevertheless, to gain more information about the epidemiology and evolution of G2P[27], continued surveillance of PoRVA in this area is essential and full-genome nucleotide sequence of G2P[27] should be analyzed to gain the genetic backgrounds of unusual RVA strains and interspecies transmission between pigs and humans.

Conclusions

The present study conducted PoRVA surveillance over the period of seven years from 2016 to 2023 in acute diarrheic piglets in Thailand and a wide variety of G- and P-genotype combinations of PoRVA were detected. Most PoRVA strains detected in this study contained P[23] and P[13] genotypes in combination with various G-genotypes. In addition, a rare genotype of G2P[27], which was reported for the first time in 2000, was detected again in this study after disappearing for more than two decades.

Abbreviations

AFS	African swine fever
RVA	Rotavirus A
PoRVA	Porcine RVA
dsRNA	Double-stranded RNA
VP	Viral protein
NSP	Nonstructural protein
PBS	Phosphate-buffered saline
DMSO	Dimethyl sulfoxide
cDNA	Complementary DNA
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
Ct	Cycle threshold

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Authors' contributions

N.J. wrote manuscript, P.K., K.K. reviewed and edited manuscript, T.L., Z.X., A.Y. performed experiment, P.Y. A.K. collected specimens, Y.A., S.K., S.O., H.U. analyzed data, N.N. designed the experiment. All authors reviewed the manuscript.

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Data availability

The nucleotide sequences of PoRVA strains detected in this study were submitted to the GenBank database under accession numbers PQ281214-PQ281283.

Declarations

Ethics approval and consent to participate

This study received approval from Ethical Committee for human rights related to human experimentation, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (MIC-2567–0445).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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