

Rotavirus associated gastroenteritis in Thailand

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Abstract Group A rotavirus is the leading cause of severe diarrhea in infants and young children, and in young animals of many species worldwide. Rotavirus is also the major cause of deaths of children younger than 5 years of age, particularly, in developing countries in Asia and Africa. In Thailand, the burden of rotavirus infection rate in children admitted to the hospitals with acute gastroenteritis ranged from 28.4 to 44.5 %. The seasonality of rotavirus gastroenteritis in Thailand was detected all-year-round with the peak from November to April of the following year. The distributions of G genotypes in pediatric patients during twelve-year surveillances of 2000–2011 were G1, G2, G3, G4, G9, and G12. The G9 was detected as the most predominant genotype in 2000–2004 while G1 and G3 were predominated in 2005–2009 and 2009–2011, respectively. The G4 was detected only in 2001–2003 and G12 only in 2007–2009 but was not detectable in any other years of surveillances. For P genotype, P[8] was the only P genotype that always existed as the most predominant with high prevalence. The G–P combination of human rotavirus strains circulated in Thailand were G1P[8], G2P[4], G2P[8], G3P[3], G3P[8], G3P[9], G3P[10], G3P[19], G9P[8], G12P[6], and G12P[8]. The G1P[8] was the most predominant strain followed by G9P[8], G2P[4], G3P[8], G12P[8], G3P[9], G3P[10], G3P[3], G2P[8], G3P[19], and G12P[6]. The studies of animal rotaviruses were performed mainly on porcine rotaviruses and a wide variety of porcine rotavirus strains have been reported, including G2P[27], G3P[6], G3P[13], G3P[19], G3P[23], G4P[6], G4P[13], G4P[19], G4P[23], G5P[6], G5P[13], G9P[7], G9P[13], and

G9P[19]. Several unusual strains of human rotaviruses that carried the genes with nucleotide sequences closely related to those of animal rotaviruses have been described in Chiang Mai, Thailand which provided evidences for interspecies transmission of rotaviruses between humans and animals, and also animals to animals are occurring in nature.

Keywords Rotavirus · Gastroenteritis · Children · Thailand

Introduction

Rotavirus infection is the leading cause of severe diarrhea in infants and young children both in developing and developed countries [27], and also in many species of young animals worldwide [5]. Globally, it was estimated that rotavirus gastroenteritis causes the deaths of 527,000 (range 475,000–580,000) in 2004 [26] and 453,000 (range 420,000–494,000) in 2008 [34] in children younger than 5 years. The majority of deaths were occurring in developing countries in Asia and Africa [26, 27, 34]. In Asia, the estimated total number of deaths due to rotavirus was 144,679 with the majority of deaths located in India (80,981), Pakistan (23,227), Indonesia (14,340) Myanmar (8,681), Bangladesh (5,724) and China (4,716) [7].

The epidemiology and distribution of rotavirus strains in Thailand during 1977–1996 have been comprehensively reviewed previously [18]. The prevalences of rotavirus infection in children with the age of under 5 years hospitalized with diarrhea in Thailand indicated in that review varied from study to study depending on the methods used for the detection of rotavirus, the inclusion and exclusion criteria for the specimen collection, and the study periods [1, 6, 7, 18].

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The two outer capsid proteins, VP7 and VP4, are the basis for a widely used dual classification system which defines G genotype and P genotype, respectively. Currently, 27 G genotypes and 37 P genotypes have been identified [20, 36].

The purpose of this study is to systematically review the epidemiology and distribution of rotavirus strains in humans and animals in Thailand. The unusual strains of rotaviruses detected recently were also analyzed to provide the evidences for interspecies transmission of rotaviruses between humans and animals, and animals to animals of different species as well.

We performed a literature search in PubMed and Scopus for the studies related to human and animal rotaviruses conducted in Thailand during the period of January 1998 through August 2013. We searched the term “rotavirus” and “Thailand”.

We included the studied that (1) fecal samples were collected from children with acute gastroenteritis, (2) examined the burden of rotavirus diarrhea in hospitalized patients with the age of 5 years old and under, (3) examined seasonality of rotavirus gastroenteritis and investigated the prevalence and distribution of group A rotavirus G and P genotypes.

Burden of rotavirus diarrhea

Rotavirus remains a major cause of severe diarrhea among children in Thailand, particularly those with the age of less than 5 years old. The incidence rate of rotavirus-associated hospitalization in children under 5 years of age has been estimated at 11.3 cases per 1,000 children per year [6, 7]. The number of deaths due to rotavirus gastroenteritis in children under 5 years of age in Thailand in 2008 was reported at 101 cases with the mortality rate of 2.2 per 100,000 children per year [7]. The rotavirus infection rates in pediatric patients hospitalized with acute gastroenteritis in Thailand during 1977–1996, reviewed from 15 studies, ranged from 27.0 to 34.0 % [18]. A comprehensive rotavirus surveillance performed in 2001–2002 by the Asian Rotavirus Surveillance Network (ARSN) reported that 45 % (range 38.0–49.0 %) of children with the age of 0–5 years old hospitalized with acute diarrhea were attributable to rotavirus and the rate of infection were higher than the previous estimates [1]. Similarly, hospital-based surveillance conducted in the same period (2001–2002) at 6 general hospitals located in different geographical regions of Thailand, including North, Northeast, East, Central, and South regions, demonstrated that 43.0 % (range 40.0–50.0 %) of children admitted to the hospitals with acute gastroenteritis caused by rotavirus [6]. Several rotavirus surveillances performed in Chiang Mai

province (North of Thailand) during 2000–2001 [11], 2002–2004 [10], 2005 [12], and 2007 [3] revealed the prevalence of rotavirus in children hospitalized with acute gastroenteritis at 34.0, 37.3, 29.3 and 29.4 %, respectively. Furthermore, studies in pediatric patients with diarrhea admitted to hospitals in five provinces located in different regions of Thailand in 2004–2006 in Bangkok (Central) and Buriram (Northeast) [35], and in 2007–2009 in Tak (North), Khon Kaen and Nakorn Rachasima (Northeast) provinces, and Bangkok (Central) [15] reported the prevalences of rotavirus infection at 42.3 and 28.4 %, respectively. In addition, the proportion of rotavirus-associated in children admitted to hospitals with acute gastroenteritis in Thailand, estimated from 8 studies, was 38.1 % (range 33.8–42.3 %) [7]. Most recently, the prevalence of rotavirus infection in infants and children with acute gastroenteritis admitted to two hospitals in Bangkok between June 2009 and May 2011 was estimated at 44.5 % [16].

In summary, the prevalence of rotavirus-associated gastroenteritis in children admitted to hospitals, reviewed from eight studies conducted during 2000–2011 in Thailand, ranging from 28.4 to 44.5 % [3, 7, 10–12, 15, 16, 35].

Seasonality of rotavirus gastroenteritis

The seasonal distribution of rotavirus-associated gastroenteritis in Thailand have been reviewed from a number of most relevant studies [1, 2, 6, 7, 15, 16]. In all studies, the rotavirus-associated diarrhea cases were detected all-year-round [1, 2, 6, 7, 16] except for one study [15] rotavirus diarrhea cases were detected only from October to May of the following year but not in any other months. The peak with high number of rotavirus diarrhea cases were detected in the winter months but the range of peak was slightly varied from study to study. The majority of studies [1, 6, 16] reported the peak from November to April while one study [7] reported the peak from November to February and another study reported the peak from December to April of the following year [15].

Distributions of rotavirus G and P genotypes in pediatric patients with diarrhea

In the previous review of rotavirus strains circulated in Thailand over the period of 15 years (1982–1997), reviewed from eight studies, only G but not P serotypes have been reported [18]. The overall distribution of G genotype from all studies reported during that period indicated that G1 was the most predominant genotype at the prevalent rate of 36.8 % followed by G2 at 21.0 %, G4 at 7.5 %, G3 at 2.5 %, and G9 at 0.4 %. There was no P

Table 1 Distribution of G genotypes of human group A rotaviruses circulated in pediatric patients in Thailand during the year 2000–2011

Year of study	No. specimen tested	No. group A rotavirus positive (%)	No. G-genotype detected							Predominant genotype (%)	References
			G1	G2	G3	G4	G9	G12	Untypeable		
2000–2001	315	107 (34.0)	–	3	6	–	98	–	–	G9 (91.6)	[11]
2001–2003	1,950	838 (43.0)	7	144	1	44	459	–	183	G9 (54.8)	[6]
2002–2004	263	98 (37.3)	33	23	2	–	40	–	–	G9 (40.8), G1 (30.6)	[10]
2005	147	43 (29.3)	27	12	2	–	2	–	–	G1 (62.8)	[12]
2004–2006	307	130 (42.3)	108	4	2	–	11	–	5	G1 (83.1)	[35]
2007	160	47 (29.4)	34	9	4	–	–	–	–	G1 (72.3)	[3]
2007–2009	557	158 (28.4)	78	32	2	–	35	11	–	G1 (49.4)	[15]
2009–2011	562	250 (44.5)	98	1	151	–	–	–	–	G3 (60.4)	[16]

Table 2 Distribution of P genotypes of human group A rotaviruses circulated in pediatric patients in Thailand during the year 2000–2011

Year of study	No. specimen tested	No. group A rotavirus positive (%)	No. P-genotype detected								Predominant genotype (%)	References
			P[3]	P[4]	P[6]	P[8]	P[9]	P[10]	P[19]	Untypeable		
2000–2001	315	107 (34.0)	1	3	–	103	–	–	–	–	P[8] (96.3)	[11]
2002–2004	263	98 (37.3)	–	23	–	73	2	–	–	–	P[8] (74.5)	[10]
2005	147	43 (29.3)	–	12	–	30	–	1	–	–	P[8] (69.8)	[12]
2004–2006	307	130 (42.3)	–	3	–	122	–	–	1	4	P[8] (93.8)	[35]
2007	160	47 (29.4)	–	9	–	38	–	–	–	–	P[8] (80.9)	[3]
2007–2009	557	158 (28.4)	–	32	1	124	1	–	–	–	P[8] (78.5)	[15]
2009–2011	562	250 (44.5)	–	1	–	249	–	–	–	–	P[8] (99.6)	[16]

serotype had been reported in those studies. In the present review, distributions of rotavirus G and P genotypes have been reviewed from eight studies conducted in Thailand between 2000 and 2011. The distribution of various G genotypes of human group A rotavirus circulated in pediatric patients hospitalized with acute gastroenteritis in Thailand during 2000–2011 are summarized in Table 1. During the period of twelve years of surveillances, G1, G2, G3, G4, G9, and G12 were detected in Thailand. The G1 was reported as the most predominant genotype in 2005–2009 at the prevalent rate ranging from 49.4 to 83.1 % while G3 was detected as the most predominant genotype only in 2009–2011 study at the prevalence of 60.4 %. The G4 was detected only in 2001–2003 in one study but not in others. The G9 was found to be the most predominant genotype in 2000–2004 with the prevalent rate ranging from 40.8 to 91.6 %. In addition, G9 was co-predominated with G1 in 2002–2004 with the prevalence of 40.8 % for G9 and 30.6 % for G1. The G12 was detected in this review only in 2007–2009 study [15].

The distribution of various P genotypes of human group A rotaviruses are also summarized in Table 2. During the period of 2000–2011, variety of P genotypes were detected in Thailand in children admitted to hospitals with diarrhea, including P[3], P[4], P[6], P[8], P[9], P[10], and P[19]. The

P[8] was the only P genotype that invariably existed as the most predominant genotype with very high prevalence, ranging from 69.8 to 99.6 % during 2000–2011. The P[4] was the second runner following P[8] genotype. It is interesting to note that several uncommon P genotypes, P[3], P[6], P[9], P[10], and P[19] have also been reported from Thailand during the studied period.

A number of G–P combinations of human group A rotavirus strains circulated in pediatric patients with diarrhea in Thailand during 2000–2011 are summarized in Table 3. Eleven strains of group A rotaviruses, G1P[8], G2P[4], G2P[8], G3P[3], G3P[8], G3P[9], G3P[10], G3P[19], G9P[8], G12P[6], and G12P[8] have been reported. The G1P[8] was the most common rotavirus strain detected in Thailand with the average frequency of detection at 49.01 %, followed by G9P[8], G2P[4] and G3P[8] at 24.0, 13.79, and 11.14 %, respectively. Additionally, the uncommon strains, G2P[8], G3P[3], G3P[9], G3P[10], G3P[19], G12P[6], and G12P[8] were also detected at 0.11, 0.14, 0.37, 0.33, 0.11, 0.09, and 0.90 %, respectively.

It should be noted that G2 is almost always in combination with P[4] except for one unusual strain G2P[8] which was detected in 2004–2006, the G2 was combined with P[8]. Moreover, the G3 detected in Thailand during

Table 3 Distribution of G–P combinations of human group A rotavirus strains circulated in pediatric patient in Thailand during the year 2000–2011

Year of study	Percentage of strains detected (%)											References
	G1P[8]	G2P[4]	G2P[8]	G3P[3]	G3P[8]	G3P[9]	G3P[10]	G3P[19]	G9P[8]	G12P[6]	G12P[8]	
2000–2001	–	2.8	–	1.0	4.6	–	–	–	91.6	–	–	[11]
2002–2004	33.7	23.5	–	–	–	2.0	–	–	40.8	–	–	[10]
2005	62.8	27.9	–	–	2.3	–	2.3	–	4.7	–	–	[12]
2004–2006	85.7	2.4	0.8	–	1.6	–	–	0.8	8.7	–	–	[35]
2007	72.3	19.2	–	–	8.5	–	–	–	–	–	–	[3]
2007–2009	49.4	20.3	–	–	0.6	0.6	–	–	22.2	0.6	6.3	[15]
2009–2011	39.2	0.4	–	–	60.4	–	–	–	–	–	–	[16]
Average (%)	49.01	13.79	0.11	0.14	11.14	0.37	0.33	0.11	24.0	0.09	0.90	

2000–2011 was found to combine with a wide variety of P genotypes including P[3], P[8], P[9], P[10], and P[19] to form G3P[3], G3P[8], G3P[9], G3P[10], and G3P[19] strains, respectively. The G12 was found to combine with P[6] and P[8] to form G12P[6] and G12P[8] strains, respectively.

Distributions of rotavirus G and P genotypes in animals

It is well-established that rotaviruses are enteric pathogens of humans and animals of many species, including bovine and swine. The distribution of group A rotavirus G and P genotypes across various animal species reported worldwide have been summarized by Martella et al. [19]. In Thailand, studies on rotavirus infection in animals are limited, only bovine and porcine rotaviruses have been reported.

For porcine rotaviruses, serotype G3 was initially reported in 1988 from piglets with diarrhea in Rachaburi province, the Central region of Thailand [31]. Later, serotypes G3 and G10 were reported from diarrheic piglets in Nakorn Pathom and Rachaburi provinces (Central region) and Chonburi province (East region) [29]. A number of studies on epidemiology and molecular characterization of porcine rotaviruses have been performed in Chiang Mai province, Northern region of Thailand since the year 2000 and the reports come out continually. In 2000–2001, the surveillance of porcine group A rotaviruses in diarrheic piglets revealed the prevalence of 22.3 % [17]. In this study, porcine rotavirus G2, G3, G4, G5, and G9 were detected. One isolate of G2 was found to combine with P untypeable and later it was identified as a novel P genotype P[27] [8]. The G3 and G4 genotypes were co-predominant genotypes with the prevalence of 43.6 and 46.2 %, respectively, followed by G5 (5.1 %) and G2 (2.6 %) genotypes. The majority of G3 genotypes were found in combination with P[19] whereas the rest were combined

with P[6] to form G3P[19] and G3P[6], respectively. Most of G4 were found to combine with P[6] to form G4P[6] and one G4 combined with P[13] to form G4P[13] strains. In addition, small proportion of G5P[13] and G9P[7] strains were also detected in this study.

The followed-up surveillance in diarrheic piglets carried out in 2002–2003 reported the prevalence of porcine rotavirus at 17.2 % and G3P[13], G4P[13], and G5P[13] strains were detected in this study [4]. For the surveillance in 2006–2008, the prevalence of porcine rotaviruses was 10.7 %, and G3P[13], G3P[23], G9P[23] were detected [25]. Recently, complete genome sequences of G3P[23], G9P[23], and G3P[13] porcine rotaviruses strains detected in Chiang Mai, Thailand in 2006–2008 have been reported [24]. Most recently, the surveillance of porcine rotaviruses in diarrheic piglets in Chiang Mai was performed and reported the prevalence at 19.8 % [32]. A wide range of porcine rotavirus strains were detected in that study, including G3P[6], G3P[13], G3P[19], G3P[23], G4P[6], G4P[19], G4P[23], G5P[6], G5P[13], G9P[13], and G9P[19] strains. The G4P[19] and G9P[19] detected in that study were reported for the first time as the strains with novel G–P combinations.

For bovine rotaviruses, the surveillance was also initially performed in 1988 in calves with diarrhea in Saraburi and Nakorn Rachasima provinces, Northeast region of Thailand [31]. A total of 25 out of 43 bovine fecal specimens were positive for group A rotaviruses by ELISA using rabbit anti-HRV serum. However, using mAbs specific to serotype 1–4 could not detect any bovine strains, even though all had the subgroup I specificity and long RNA migration pattern. The other study of bovine rotavirus surveillance was performed in diarrheic cows in 1988 and 1989. All the bovine rotavirus strains were determined as subgroup I but none of them were reactive with serotype 6-specific mAb, suggesting that they were not serotype 6 [30]. Later, by sequence analysis of VP7 gene, these bovine rotavirus strains were identified as serotypes 8 and 10 [33].

Detection of unusual strains leads to the discoveries of novel P[27] genotype and evidences for interspecies transmission

During epidemiological surveillance of porcine rotavirus infection in piglets in Chiang Mai, Thailand from June 2000 to July 2001, one unusual strain of porcine rotavirus, CMP034, was detected [17]. By multiplex PCR using type-specific primers, CMP034 was identified as G2 but P genotype was untypeable. The VP7 and VP4 genes of this strain were amplified, sequenced and analyzed in comparison with the reference sequences available in the GenBank database. VP4 sequence analysis showed a low amino acid sequence identity, ranging from 56.7 to 76.6 %, with 26 P genotypes reported previously. Phylogenetic analysis of the VP4 nucleotide sequence revealed that CMP034 was distantly related to other 26 P genotypes and located in a monophyletic branch of phylogenetic tree that separated from other P genotypes. It was, therefore, classified as a novel porcine rotavirus of genotype P[27] in the P genotype series [8]. Analyses of nucleotide and amino acid sequences of NSP4 gene of CMP034 strain also revealed a novel NSP4 genetic group [9] and now is classified by the Rotavirus Classification Working Group (RCWG) as NSP4 E9 genotype [20].

There are several unusual strains of human group A rotaviruses have been reported from the surveillances of rotaviruses in children admitted to the hospitals in Chiang Mai, Thailand. The first two human rotavirus strains, Mc323 and Mc345, were reported in 1988–1989 [37] that these two human rotavirus strains were genetically closely related to each other and to porcine rotaviruses than to human rotaviruses. Later, by nucleotide and amino acid sequence analyses of VP7 and VP4 genes of these strains confirmed that they were G9P[19] with genetically closely related to P[19] of porcine rotavirus [23]. Additionally, the surveillance of porcine rotaviruses in piglets with diarrhea in Chiang Mai which is the same geographical area where Mc323 and Mc345 had been detected, we could detect 13 strains of P[19] porcine rotaviruses [17]. By nucleotide sequence analysis of VP4 gene of these strains, they were found to be closely related to those of P[19] of Mc323 and Mc345 than to reference sequence of P[19] porcine rotaviruses. The finding implies that VP4 gene of Mc323 and Mc345 were more likely originated from VP4 gene of porcine rotavirus circulated in the same geographical area, probably via interspecies transmission and reassortment events that are occurring in nature.

Another rare unusual strains G3P[3] (CMH222) human rotavirus was isolated from a 2-year-old child during the epidemiological survey of rotaviruses in Chiang Mai, Thailand in 2000–2001 [14]. Characterization of VP4, VP6, VP7 and NSP4 genes of CMH222 revealed unusual

characteristics of those genes. The VP4 gene sequence was most closely related to P[3] rotavirus of caprine origin while VP7 sequence showed highest identity with those of simian G3 rotavirus. In addition, the VP6 and NSP4 sequences were most closely related to those of simian and caprine rotaviruses, respectively. The findings strongly suggest that multiple interspecies transmission of rotaviruses among caprine, simian, and human population might have been occurred in nature. The findings are also providing convincing evidences to support the notion that evolution of human rotaviruses is tightly intermingled with the evolution of animal rotaviruses.

Another two unusual and uncommon human rotavirus strains, G3P[9] (CMH120/04 and CMH134/04) were detected in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand, during an epidemiological survey of rotaviruses in 2004 [13]. Characterization and analysis of VP4, and VP7 genes of these two strains indicated that CMH120/04 and CMH134/04 were G3P[9] with the nucleotide and amino acid sequences closely related to P[9] of feline rotaviruses of cats and also closely related to human rotavirus AU-1 strain which was reported as a reassortant strain resulting from reassortment between the viruses of cat and human origins [21, 22].

Altogether, the findings of human rotaviruses with genetic characteristics of some genes that closely related with the genes of rotavirus of animals are suggestive of interspecies transmission of animal rotaviruses that cross the species barrier to infect human or vice versa.

Conclusion and future perspective

Group A rotavirus is a major cause of acute gastroenteritis, typically manifesting watery diarrhea, vomiting, and fever among infants and young children in both developed and developing countries around the world. By the age of 5 years, nearly all children have been experienced with rotavirus infection. The estimated data presented by the Asian Rotavirus Surveillance Network (ARSN) indicated that in Asian countries the median prevalence of rotavirus infection among children hospitalized with diarrhea is 43 %, which is as high as almost twice of the rate of estimated global disease. In Thailand, rotavirus associated with diarrhea remains an important cause of morbidity and mortality among children under 5 years of age. The accumulated data reviewed from 8 studies conducted in Thailand during 2000–2011 demonstrated that G1, G2, and G3 were almost always detected simultaneously in the same study while G4 was undetectable by 7 studies [3, 10–12, 15, 16, 34] but one which detected G4 in 2001–2003 [6]. However, a long-term survey on the distribution of human rotavirus G type in Thailand reported that G4 was

moderately prevalent (16.0 %) in 2001–2002 and could be detected with low prevalence (0.5–3.5 %) during 2002–2005 and undetectable in 2006–2007 [28]. The G9 was first detected in Chiang Mai, Thailand for the first time in 1988–1989 with low prevalence [36] and reached to high peak (82.6–91.6 %) in 2000–2001 [11, 28] and then gradually decreased to 40.8–54.8 % in 2002–2004 [6, 10, 28] and to low prevalence or undetectable from 2005 to 2011. The G12 was initially reported in Thailand in 1998–1999 and was undetectable since then, until 2004–2005 and 2006–2007 [28] and 2007–2009 [15] G12 was detected at low prevalence. The information on the distribution of rotavirus G and P genotypes among humans and various animal species is important to comprehend rotavirus evolution, and the mechanism by which rotaviruses evolve, cross the species barriers, exchange their genes during reassortment, and genetic divergence via accumulation of single-point mutations and/or via genetic rearrangements. Currently, two rotavirus vaccines (RotaRix and RotaTeq) that have been used widely are designed to provide antigenic coverage for globally important G and P genotypes (G1–G4, P[8]). However, recent surveillance studies demonstrated that G9 rotavirus has emerged as an important rotavirus genotype detected in human around the world and also in this area, therefore, the next generation of rotavirus vaccines may need to include a G9 rotavirus in addition to G1–G4 component.

In this review, information about the burden of rotavirus infection, diversity, and interspecies transmission of rotavirus genes between different animal species and humans are summarized. It is important, therefore, to maintain the surveillance of rotavirus strains, particularly in developing countries where mixed rotavirus infections have been shown to promote reassortment with high frequency. Such events may lead to the appearance of new strains or new variants that could escape from immune protection induced by an outdated vaccine.

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