Research Article

Epidemiology and Genetic Diversity of Group A Rotavirus in Acute Diarrhea Patients in Pre-vaccination Era in Southwest China†

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Abstract

Group A *rotavirus* (RVA) is one of the leading cause of acute diarrhea worldwide, the RVA-related disease burden and the genotypes of RVA is important reference to introduce RVA variance to national immunisation programmes, 1,121 diarrhea cases and 319 healthy controls were recruited from four sentinel hospital outpatient from July 2014 to June 2015. The prevalence of RVA was 244 (21.8%) in gastroenteritis cases and in 12 (3.8%) in healthy controls across all age group (OR=7.12, 95%CI=3.93–12.89); the detection rate of RVA in diarrhea patients under five years was more higher than in diarrhea cases over five years (26.1%, 222/850; 8.1%, 22/271, respectively, *P*=0.000), Of 244 RVA strains isolated from acute diarrhea cases, G9 (66.4%) was predominant G genotype, followed by G3 (18.7%), G1 (8.9%) and G1G3 (3.8%); P[8] was the overwhelming prevalence P genotype, followed by P[4] (4.7%); G9P[8] (54.9%) was most common G and P Combination, followed by G3P[8] (17.6%) and G1[8] (8.6%). The conclusion of the study was important to provide reference for introducing the RVA vaccine to prevent and control RVA-associated disease burden.

KEY WORDS: group A rotavirus; acute diarrhea; genotype; molecular epidemiology

INTRODUCTION

Acute diarrhea is important cause of morbidity and mortality in children under five years worldwide, which caused 1.7 billion diarrhea episodes and 700 thousand childhood deaths [Walker et al., 2013]. Group A *rotavirus* (RVA) related diarrhea was ubiquitous, RVA almost infected each children worldwide, which leaded 9.8 million severe diarrhea episodes and 193 thousand children death worldwide every year, it has been estimated worldwide, each year, RVA-associated diarrhea resulted in 9.8 thousand deaths in children less than 5 year, which account for 27% of all diarrhea-associated deaths and 3.0% of all childhood deaths [Walker et al., 2013], RVA-associated diarrhea also caused 57 thousand deaths in individuals over five age worldwide in 2010 [Lozano et al., 2012]. Diarrhea episodes and diarrhea-related deaths attributable to RVA infection was more severe in low-income and middle-income countries [Lozano et al., 2012; Walker et al., 2013], more than 80% RVA related deaths was occurred in poorest countries such Sub-Saharan Africa and Southern Asia [Parashar et al., 2003]. RVA-associated gastroenteritis is not decline with improvement of hygiene and sanitation [Duan et al., 2009]. The prevalence of RVA in acute diarrhea cases is still as high as 21%-43.6% in some countries, where was not introduced RVA vaccine [Durmaz et al., 2014], and the prevalence of RVA is up to 37.5% in diarrhea inpatients and 23.1% in diarrhea outpatients in some Asian countries [Kawai et al., 2012], in addition, RVA-related diarrhea caused substantial economic loss on society and individual [Alkoshi et al., 2014; Mendelsohn et al., 2008], the cost of RVA–associated diarrhea is as high as 80 dollar in each hospitalized diarrhea cases and 40 dollar in each community diarrhea children under five years respectively [Mendelsohn et al., 2008], therefore, RVA-related diarrhea was still one of serious public health problem.

RVA related diarrhea had a devastatingly impact on children, the prevalence of RVA in diarrhea children is 29.7% in China, which is the most commonly identified enteric pathogen in acute diarrhea cases [Yu et al., 2015], the RVA-related diarrhea was scanned with colloidal gold method in clinical setting, but RVA diagnostic confirmation and genotyping must need modern laboratory technology such reverse transcription polymerase chain reaction (RT-PCR), these method applied widely was hindered with expensive cost and the constraint of laboratory condition, especially in low-income and middle-income countries or regions [Duan et al., 2009]. Therefore, few data of RVA-related diarrhea was known in developing region such Yunnan province, China, in addition, the molecular epidemiological characteristic of RVA was not clarity. The objects of the study were to demonstrate RVA disease burden, to clarify RVA epidemiological characteristics and genotypes. These results of the study might be key suggestion to introduce RVA vaccine, and provide scientific reference to prevent and control RVA-related diarrhea.

MATERIALS AND METHODS

Study Subjects

Acute diarrhea patient is defined as who evacuated stool over three episodes within 24 hour and the stool form was abnormal (e.g. watery stool, liquid stool, mucous stool and bloody stool), which is accordance with WHO standard. Healthy control is defined as who had no diarrhea symptom for 7 days when recruited into the study.

Specimen and Information Collection

From July 2014 to June 2015, the study was conducted in four sentinel hospitals in Kunming city, stool specimens were collected at four sentinel hospitals: the first affiliated hospital of Kunming medical university, the first people's hospital of Yunnan province, the children's hospital of Kunming city and the Pushan community hospital. 1,121 stool specimens were obtained from acute gastroenteritis cases and 319 feces samples were collected from healthy controls, and then each stool sample was kept frozen at -40˚C and transported into the laboratory of center for disease control and prevention of Yunnan province every day, each stool specimen was diluted into 10% (w/v) suspension with phosphate-buffered saline (PBS), and then was centrifugated at 14,000 g for 10 min, the supernatant was collected for detecting the RVA. Structured questionnaire was used to collect the clinical symptom of each diarrhea patient, and demography information of every subject with nurse.

RNA Extraction

The RNA was extracted from each stool specimen with viral nucleic acid extraction kit (Geneaid, China) according to the manufacturer’s instructions, for each stool specimen, 150 μl of 10% supernatant was used to extracted RNA, the final viral genomes was 50 μl and kept frozen at -70˚C.

RVA Detection

Two-step reverse transcriptional polymerase chain reaction (RT-PCR) was used to detect the presence of RVA in each stool specimen, cDNA was synthetized with RNA template, inverse transcriptase (SuperScript II, Invitrogen, Carlsbad, CA) and random primer (Promegan, Madison, WI), the reaction condition was 42˚C for 90 min, 99˚C for 5 min and holding at 4˚C, and then the pair of primers as previously published was applied to amplify to VP6 gene of RVA [Phan et al., 2007], the amplification condition was 94˚C for 5 min, followed by 40 cycles at 94˚C for 1 min, 42˚C for 1 min, 72˚C for 1 min, and with final extension at 72˚C for 10 min, the length of amplification product was 379 bp. Suspicious positive of gel electrophoresis was further be sequenced to make definite diagnosis of RVA infection.

G and P Genotyping

Each RVA positive stool specimen confirmed with sequenced was conducted the semi-nested multiplex RT-PCR

to detect six G genotypes and six P genotypes, QIAGEN One-Step RT-PCR Kit (Qiagen, Hilden, Germany) and consensus primers was applied in genotyping (Table I), detailed explanation as follows: first-round PCR reaction for G genotypes was amplified with primers of VP7-forward (F) and VP7-reversed (R) described by Iturriza Go´mara et al [Iturriza-Gomara et al., 2004]; and then G genotypes was conducted with 2 μl first-round PCR product and a mixture of 20 μmol of each specific primer for G1-G4, G8, G9 and VP7-R primer described by Gouvea et al [Gouvea et al., 1990], the length of amplified produces were 618 bp, 521 bp, 682 bp, 452 bp, 754 bp and 179 bp for G1, G2, G3, G4, and G9, respectively. Similarly, first-round PCR reaction for P genotypes (VP4) was that VP4 was reverse transcribed with VP4-forward and VP4-reversed, and then P genotyping was conducted with 2 μl first-round PCR product and a mixture of 20 μmol each internal primer for P[4], P[6], P[8], P[9], P[10], P[11] and VP4-F primer described by Simmond et al [Simmonds et al., 2008], with the length of amplified produces were 362 bp, 146 bp, 224 bp, 270 bp, 462 bp and 191bp, respectively.

Thermal-cycling of G or P genotypes was performed as follows: 50˚C for 30 min, and then 94˚C for 15 min, followed by 40 cycles at 94˚C for 30 s, 42˚C for 30 s, and 72˚C for 1 min, with the final extension at 72˚C for 10 min, 1.5% agarose gel was conducted to observe these amplified products.

Statistical Analysis

Data analysis was performed with SPSS 19.0 (IBM, USA), odds ratios (ORs) and 95% confidence intervals (CIs) of categorical variables were calculated with chi-square test or fisher's exact test with two-tailed, the median or mean of quantitative variables were analyzed with rank-sum test, analysis of variance (ANOVA) or t test. The statistical method was applied according to the characteristic of data. $P<0.05$ was considered to significant differences.

RESULTS

Clinical Manifestations of the Diarrhea Cases Infected with RVA

From July 2014 to June 2015, feces samples were collected from 1,121 diarrhea cases and 319 healthy controls. These included 850 from diarrhea cases less than five years and 271 from gastroenteritis cases over five years; and 170 from healthy controls under five years and 149 from healthy controls over five years. The sex ratio (male/female) was 1.00 (559/562) in diarrhea cases and 1.07 (165/154) in healthy controls ($\chi^2=0.341$, $P=0.558$), respectively. The median age was 1.2 years in diarrhea cases (Interquartile range (IQR): 0.7-4.8 years) and 4.7 years (IQR: 0.8-40.0 years) in healthy controls. Various clinical symptoms were observed in RVA diarrhea cases across all age groups, vomiting was the most frequency symptom (n=104, 42.6%), followed by fever (n=55, 22.5%) and dehydration (n=41, 16.8%), among feces form, 73.3% (n=179) was presented with mucus stool, followed by watery stool (23.8%, n=58) and other form feces (1.6%, n=4) (Table I). The median age of RVA

positive diarrhea cases was younger than the median age of non-RVA diarrhea cases, and the diarrhea frequency of RVA infection patients was 8.0, which was higher than the frequency in non-RVA infection diarrhea cases (Table II). The average diarrhea frequency of patients with G9P[8], G3P[8], G1P[8] and G1G3P[8] genotype infection were 8, 6, 7 and 6 episodes per day in diarrhea cases across all age group, diarrhea frequency was no significant difference among diarrhea patients infected with various RVA genotypes (P>0.05).

The Prevalence of RVA in Diarrhea Cases and Healthy Controls

RVA was found in 244 (21.8%) in gastroenteritis cases and in 12 (3.8%) in healthy controls across all age group (OR=7.12, 95%CI=3.93–12.89). For subjects less than five years, RVA was detected in 222 (26.1%) diarrhea cases and 10 (5.9%) healthy subjects (OR=5.66, 95%CI=2.93-10.91); for subjects over five years, RVA was detected in 22 (8.1%) in diarrhea cases and 2 (1.3%) in healthy controls (OR=4.91, 95%CI=0.66-36.55); for diarrhea cases, the detection rate of RVA in diarrhea patients under five years was more higher than in diarrhea cases over five years (26.1%, 222/850; 8.1%, 22/271, respectively, χ^2=39.096, P=0.000); for healthy controls, the prevalence of RVA in healthy controls was not neglected, the detection rate of RVA in non-diarrhea children less than five years was higher than in healthy children over five years (5.9%, 10/170; 1.3%, 2/149, respectively, χ^2=4.507, P=0.034).

Common Genotypes among Diarrhea Cases and Healthy Controls

Among the 244 RVA positive samples identified by RT-PCR from diarrhea cases, 9 (3.7%) were partially typed. In one sample, P genotype was identified but G genotype was still not distinguished, and in eight RVA specimens, G genotype was defined but P genotype was remained negative, 235 samples were identified in both G and P genotypes, a total of 20 different G–P combinations were found with 5 different G genotypes and 4 different P genotypes. Among the G genotypes, G9 (n=156, 66.4%) was most common genotype detected, followed by G3 (n=44, 18.7%), G1 (n=3, 8.9%) and G2 (n=2, 8.5%); among the P genotypes, the predominant P genotype was P[8] (n=218, 92.8%), followed by P[4] (n=11, 4.7%), P[9] and P[6] (n=1, 2.1% equal). The three common G types (G9, G3 and G1) and the two most common P types (P[8] and P[4]) accounted for 94.0% and 97.5% of all RVA strains, respectively. For G and P combinations, G9P[8] (n=134, 57.0%) was the predominant G and P combination, followed by G3P8 (n=43, 18.3%) and G1P[8] (n=21, 8.9%), theses common three G and P combinations accounted for 84.2% in all RVA strains.

Among the 12 RVA strains isolated from healthy controls, 12 strains were determined in both G and P genotypes. For the G types, G9 was the most frequency (n=7, 58.7%), followed by G1 (n=3, 25.0%) and G3 (n=2, 16.7%); among P types, P[8] (n=11, 91.7%) was characterized as the most common genotype, followed by P[6] (n=1, 8.3%); G9P[8] (n=6, 50.0%) was the common often G and P combination detected in healthy controls,

followed by G1P[8] (*n*=3, 25.0%) and G3P[8] (*n*=2, 16.7%).

RVA and Common Genotypes distribution by Age, Sex and Season in Diarrhea Cases

In diarrhea cases, no significant difference of the prevalence of RVA was found among male and female diarrhea cases across all age groups (21.5%, 121/559; 21.9%, 123/562, respectively, χ^2=0.01, *P*=0.922). Similar frequencies of all common genotypes was observed in both males and females diarrhea cases with RVA positive, The proportion of G9P[8], G3P[8], and G1P[8] was 54.2%, 20.3% and 9.3%, respectively, in males, whereas these values were 57.4%, 15.6% and 8.2%, respectively, in females, (Fig. 1).

The seasonal distribution of RVA had strong seasonal pattern (χ^2=40.189, *P*=0.000), the prevalence of RVA had a peak in winter (32.2%, 127/394) and trough in summer (6.0%, 19/267), the prevalence of RVA in spring was 23.0% (46/200) and in autumn was 20.0% (52/260). No any large fluctuations in the proportion of G and P genotypes found in different months, for instance, G9P[8] was the most common genotype in each month, followed by G3P[8], (Fig. 2).

Diarrhea cases were further classified into five age groups, in which 718 (64.0%), 73 (6.5%), 59 (5.2%), 224 (20.0%) and 27 (2.4%) cases fall in the age group of 0-1 years, 1-2 years, 2-5 years, 5-65 years and ≥ 65 years, respectively. The prevalence of RVA had significant difference among these five groups (χ^2=54.94, *P*=0.000), the highest detection rate of RVA was in 0-1 years group (27.8%, 200/718), followed by over 65 years group (18.5%, 5/27), 2-5 years group (16.9%, 10/59) and 1-2 years (16.4%, 12/73) and the lowest prevalence was 5-65 years group (7.5%, 17/224). The proportion of each genotype was not huge changes in several age groups expect G1P[8], which was more detected in diarrhea cases in 5-65 years (Table III).

DISCUSSION

RVA is most common enteric pathogen in diarrhea cases under five years worldwide, not only in low-income and middle-income countries but also in industrialized countries [Walker et al., 2013]. RVA is still serious public problem in China, the prevalence of RVA was 29.7% in inpatient diarrhea cases under five years [Yu et al., 2015], but attribution to the constraint of fund and laboratory detection capacity, the laboratory-based surveillance study of RVA was seldom conducted in developing region in China. The study was to describe the epidemiologic feature of RVA and RVA-related disease burden among acute gastroenteritis patients across all age group. The prevalence of RVA in the study is slightly lower than that of five-years surveillance study conducted in China (29.7%) [Yu et al., 2015], which might be explained that the diarrhea cases were all from outpatient in this study, which was coincide with that the RVA was more prevalence in severe diarrhea cases, such inpatient diarrhea cases [Liu et al., 2014; Parashar et al., 2003; Sanchez-Padilla et al., 2009]; but the prevalence of RVA in the study was higher than that of other studies conducted in developed countries such Japan and Italy (11.9%, 2.6%, respectively), where

RVA vaccine was introduced to national immunization programmes [Ianiro et al., 2014; Tajiri et al., 2013]. Similar result was that, in Belgium, the detection rate of RVA in diarrhea case was various between 15.2% and 24.3% from 1987 to 2006, but the prevalence of RVA was quickly declined to 6.4% when RVA vaccine was introduced into the Belgian in 2006 [Zeller et al., 2010], and the detection rate of RVA was still as high as 23.0% in outpatient diarrhea case and 34.0% in hospitalized case in low-income and middle-income countries, where RVA vaccine was not obtained [Sanchez-Padilla et al., 2009]. In view of the social and economic development had significant difference between China and low-income region such sub-Saharan Africa, but the prevalence of RVA was resemble, it can be inferred that the prevalence of RVA was not affected by the improvement of water supply, hygiene and sanitation. Hence, safe, effective and affordable RVA vaccine was only effective way to reduce the RVA infection and RVA-related death [Grimwood and Buttery, 2007; Tamburlini et al., 2010].

In the study, significant difference was observed in diarrhea cases among different age group, the prevalence of RVA was 23.0% (53/227, data not shown) in diarrhea children under 6 months, but increased sharply to 30.3% in diarrhea case aged from 6 months to 1 year, and then detection rate of RVA declined continuously with the increase of age, this conclusion was in accordance with previous studies [Duan et al., 2009; Salim et al., 2014], it may be inferred that protective immunity against RVA might be obtained in children less than 6 month, this might be explained by several factors as follows: IgG acquired from mother can prevent children under 6 months from RVA infection [Levine and Robins-Browne, 2012]; in addition, breast milk might stop the RVA infection because which have substantial IgA and other non-specific immune factors such lactoferrin [Levine and Robins-Browne, 2012; Morrow et al., 2005].

In the study, G9 was the most common genotype in diarrhea cases, this result was correspond with the molecular epidemiology feature of RVA in China [Liu et al., 2014]. The predominance of G genotype had fluctuation in different periods and regions [Durmaz et al., 2014], G1 was predominant strain in China before 2000 year, and then G3 was most common genotype since 2000 to 2010, and then G9 became preponderant prevalence genotype in some regions of China since 2011 year, G genotypes variation might be attributed to natural variability [Li et al., 2014; Liu et al., 2014]. In addition, the RVA variance also had selective pressure to G genotype fluctuation, for instance, in Belgium, when RVA variance was introduced, G1 and G4 decreased with the G9 increased sharply [Zeller et al., 2010]; in Denmark, G9 became predominant genotype since 2011 [Midgley et al., 2014]. The summation of those G1, G2, G3 and G9 genotypes accounted for 94.8% in all G genotype in this study, this result was also similar to the other study conclusion, where the total of G1, G2, G3 and G9 accounted over 85% in all RVA strains in China [Li et al., 2014]. P[8] genotype accounted for 92.8% in all RVA strains in this study, and summation of P[8] and P[6] genotype reached 95% of all P genotype, this conclusion kept with the

result of other study conducted in China, which suggested that P[8] was persistent predominant genotype from 1980 to now [Liu et al., 2014], similar conclusion was that P[8] (80.8%) was characterized as predominant P genotype from 2007-2010 in Tunisia [Hassine-Zaafrane et al., 2011].

G9P[8] (55.4%) was predominant G and P combination, and the total proportion of G9P[8], G3P[8] and G1P[8] was as high as 81.8% in all RVA strains in the study, similar conclusion was obtained from other study [Durmaz et al., 2014]. The result of G and P combinations in study was different from other study, which showed G3P[8] (32.1%) and G1P[8] (24.5%) were the two most common genotype in diarrhea children in China [Li et al., 2014], this phenomenon might be attributed that G9 became dominant prevalence since 2011, that is why the combination of G9 and P genotypes became mainly G and P combinations. In Turkey, G9P[8] was also prominent genotype [Durmaz et al., 2014]; but some study showed that G1P[8] was prominent G and P combinations, followed by G3[P8] [Banyai et al., 2012; Wu et al., 2009].

Although RVA vaccine had low influence on reducing RVA-related diarrhea in developing countries, RVA variance can reduce obviously severe RVA-associated diarrhea and death worldwide. RVA variance is powerful way to prevent RVA infection with high cost-effectiveness [Danchin and Bines, 2009; Glass and Parashar, 2014; Santosham, 2010]. However, considering RVA genotypes were variation in spatial-temporal distribution, surveillance of G and P combinations genotypes was one of important reference factor to choose and introduce RVA variance in pre-vaccine era, Both safe, effective oral RVA vaccine, which named RotaTeq ® and Rotarix ®, are available widely in many countries except China. The former includes G1, G2, G3, G4 and P1A[8] genotypes; the latter contains G1P1A[8] genotype [Liu et al., 2014; Vesikari et al., 2006]. These two variance does not involve G9 genotype, however, Both of two RVA vaccines can prevent the RVA gastroenteritis caused by G9 genotype viruses, the reasons might be as follows: neutralizing antibody triggered by G1 genotype was not only prevent G1 genotype infection but also against G3 or G9 genotype because of cross immunization; in addition, one of neutralizing antibody of VP4 or VP7 could against RVA infection to some extent [Ward and Bernstein, 2009; Ward et al., 1993]. Therefore, it is very urgent and necessary to introduce RVA vaccine to reduce the RVA related disease burden.

In summary, this study clarified the RVA epidemiological characteristics in southwest, China; in addition, the study provided information on diversity of G/P genotypes before RVA vaccine be introduced, it is pivotal scientific evidence to introduce RVA vaccine to reduce RVA related disease burden.

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TABLE I. RVA detection, G/ P Genotypes Consensus and Type-Specific Primers.

Primers	Sequences	Nucleotide Position	Amplicon sizes
RVA detection			
VP6 F	GACGGVGCRACTACATGGT	747-1126	384
VP6 R	GTCCAATTCATNCCTGGTGG	1126-1106	
G-typing			
1st round (consensus)			
VP7 F	ATGTATGGTATTGAATATACCAC	51-71	881
VP7 R	AACTTGCCACCATTTTTTCC	932-914	
2nd round (type-specific)			
G1 (aBT1)	CAAGTACT AAATCAATGATG G	314-335	618
G2 (aCT2)	CAATGATATTAACACATTTTCTGTG	411-435	521
G3 (G3)	ACGAACTCAACACGAGAGG	250-269	682
G4 (aDT4)	CGTTTCTGGTGAGGAGTTG	480-499	452
G8 (aAT8)	GTCACACCATTTGTAAATTCG	178-198	754
G9 (G9)	CTTGA GTGACTAYAAATAC	757-776	179
VP7 R	AACTTGCCACCATTTTTTCC	932-914	
P-typing			
1st round (consensus)			
VP4 F	TATGCTCCAGTNAATTGG	132-149	661
VP4 R	ATTGCATTTCTTTCCATAATG	775-795	
2nd round (type-specific)			
P[4] (2T-1)	CTATTGTTAGAGGTTAGAGTC	492-474	362
P[6] (3T-1)	TGTTGATTAGTTGGATTCAA	278-259	146
P[8] (1T-1D)	TCTACTGGRTTRACNTGC	356-339	224
P[9] (4T-1)	TGAGACATGCAATTGGAC	402-385	270
P[10] (5T-1)	ATCATAGTTAGTAGTCGG	594-575	462
P[11] (P[11])	GTAAACATCCAGAATGTG	323-305	191
VP4 F	TATGCTCCAGTNAATTGG	132-149	

TABLE II. Comparison of Clinical Manifestations Between Diarrhea cases with and without RVA Infection

Clinical Manifestations	Patients infected with RVA, N = 244 (%)	Patients without infected with RVA, N = 877 (%)	Statistics	*P*-Value
Age (years, medium) [a]	0.92	1.33	5.672	0.000
Diarrhea frequency (⩾3 episodes per day, medium) [a]	8	5	10.575	0.000
Fever (>37˚C) [b]	55 (22.5)	83 (9.5)	30.239	0.000
Vomiting [b]	104 (42.6)	159 (18.1)	63.773	0.000
Dehydration [b]	41 (16.8)	23 (2.6)	71.308	0.000
Watery stool [b]	179 (73.4)	331 (37.1)	97.663	0.000
Mucus stool [b]	58 (23.2)	482(55.0)	74.378	0.000
Other stool [b]	4 (1.6)	71 (8.1)	9.314	0.002

[a] Rank-sum test with between two data groups.

[b] Chi-square tests between two data groups.

TABLE III. The Age Distribution of G/P Genotypes among Diarrhea Cases

G–P genotypes	＜1 years Patients infected with RVA, N = 200 (%)	1-2 years Patients infected with RVA, N = 12(%)	2-5 years Patients infected with RVA, N = 10 (%)	5-65 years Patients infected with RVA, N = 17 (%)	⩾ 65 years Patients infected with RVA, N = 5 (%)	χ^2	*P*-Value
G9P8	118 (59.0)	5(41.7)	4(40.0)	6(35.3)	1(16.7)	8.31	0.081
G3P8	33 (16.5)	1(8.3)	1(10.0)	6(35.3)	2(33.3)	5.89	0.208
G1P8	13 (6.5)	0(0.0)	2(20.0)	5(29.4)	1(16.7)	11.3	0.023
G3G1P8	7(3.5)	2(16.7)	2(20.0)	0(0.0)	0(0.0)	8.17	0.085
G/P un-typed	7(3.5)	2(16.7)	0(0.0)	0(0.0)	0(0.0)	5.56	0.234
Uncommon	22(11.0)	2(16.7)	1(10.0)	0(0.0)	1(16.7)	4.63	0.323

Uncommon genotypes included G9P[6], G9P[4], G8P[9], G4P[8], G4P[6], G3P[9], G2P[8], G2P[4] and G1P[1].

Figures:

Figure 1. Genotypes distribution between females and males diarrhea cases.

Figure 2. Seasonal distribution of G–P genotypes combinations in diarrhea cases. Uncommon genotypes included G9P[6], G9P[4], G8P[9], G4P[8], G4P[6], G3P[9], G2P[8], G2P[4] and G1P[1]. The seasons are divided into the Spring (Feb-Apr), Summer (May-Jul), Autumn (Aug-Oct) and Winter (Nov-Jan) in southwest, China.

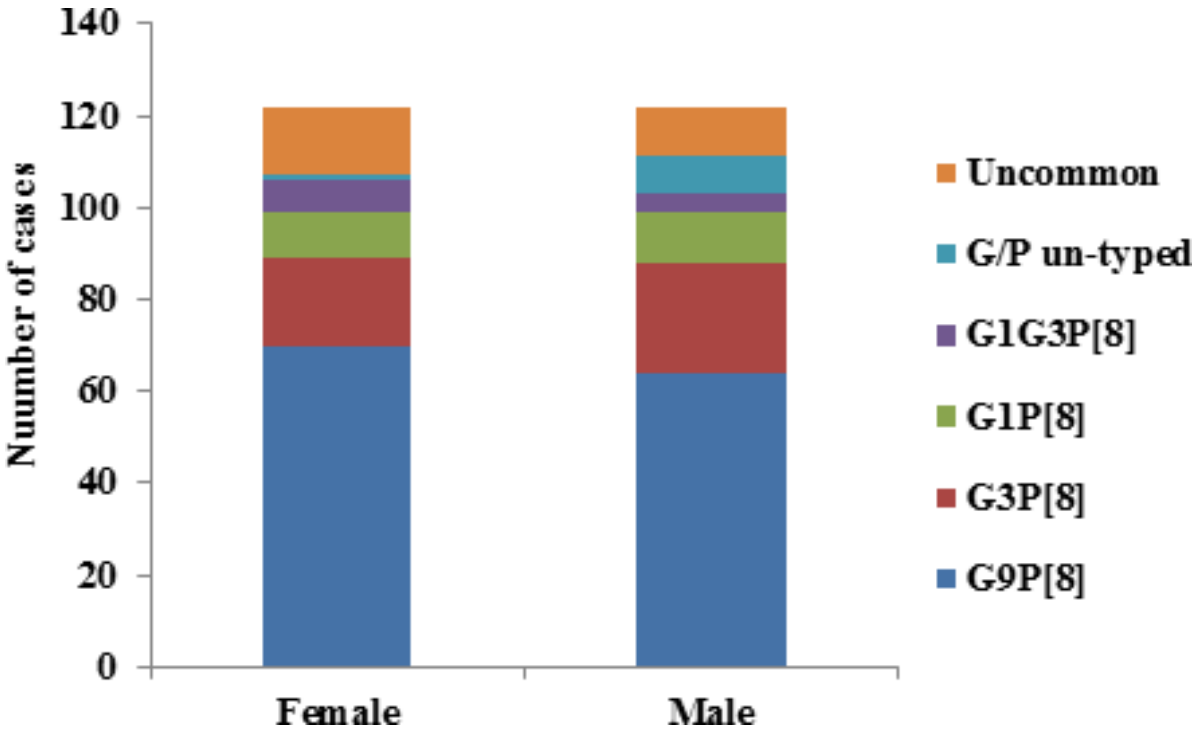

Figure 1

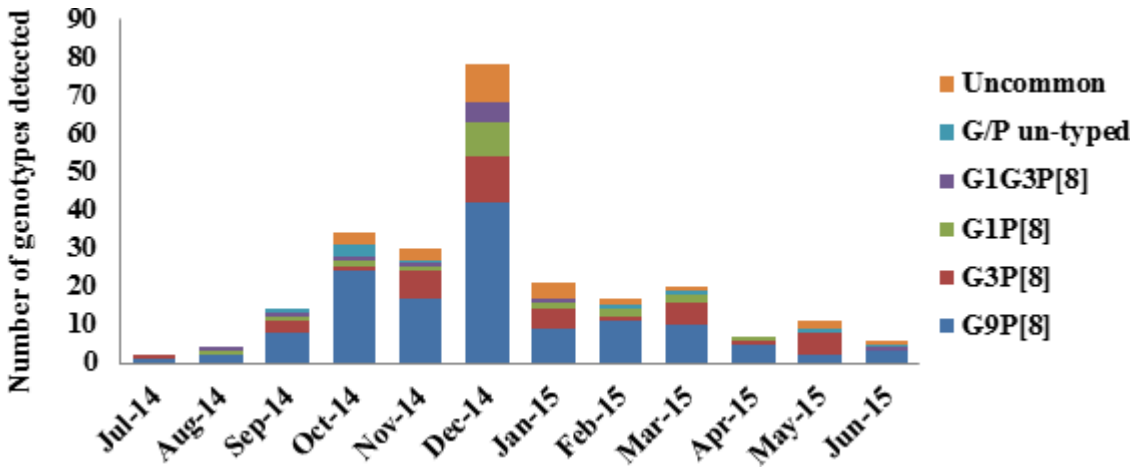

Figure 2