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Prevalence of Rotavirus and Rapid Changes in Circulating Rotavirus Strains among Children with Acute Diarrhea in China, 2009-2015

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Highlights

- The rotavirus universal immunization program has not been introduced in China.
- The burden of diarrhea attributed to rotavirus is substantially high.
- Children living in urban areas of lower-middle-income regions had the highest burden of rotavirus.
- Genotype G9P[8] has rapidly evolved to be the dominant rotavirus strain in recent years.
- The study highlights the importance of conducting rotavirus strain surveillance.

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Running Title: Rotavirus surveillance in China

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Background

Rotavirus is a leading cause of morbidity and mortality in young children worldwide. In China, the universal immunization of children with the rotavirus vaccine has not been introduced, and the two globally distributed vaccines (RotaTeq and Rotarix) are not licensed in the country. We aim to determine the prevalence and strain diversity of rotavirus in children with diarrhea aged ≤ five years across China.

Materials and methods

Sentinel-based surveillance of acute diarrhea was conducted at 213 participating hospitals in China from January 1, 2009, through December 31, 2015. Group A rotavirus (RVA) was tested by using enzyme-linked immunosorbent assays, and G- and P-genotype of RVA were tested by RT-PCR methods.

Results

Of 33,616 children with diarrhea, 10,089 (30%) were positive for RVA; RVA-associated diarrhea was identified in 2247 (39.5%, n=2247/5685) inpatients and 7842 (28.1%, n=7842/27931) outpatients. Children living in low-middle-income regions suffered from the highest burden of rotavirus, with 40.7% of diarrhea cases attributed to rotavirus infection, followed by 31.3% in upper-middle-income and 11.2% in high-income regions. The majority of children (88.9%, n=8976/10089) who tested positive for RVA were children aged ≤2 years. The seasonal peak of RVA was in the winter. Among all 2533 RVA strains genotyped, five strain combinations, G9P[8], G3P[8], G1P[8], G2P[4] and G3P[4], contributed to 71.3% (1807/2533) of the RVA-associated diarrhea cases. The predominant

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strain of RVA has rapidly evolved from G3P[8] and G1P[8] to G9P[8] in the recent years, with the proportion of G9P[8] having increased remarkably from 3.4% in 2009 to 60.9% in 2015.

Conclusions

The burden of diarrhea attributed to rotavirus is high in China, highlighting the potential value of vaccination. The rapid shift of RVA strains highlights the importance of conducting rotavirus surveillance to ensure that currently marketed vaccines provide protective efficacy against the circulating strains.

Introduction

Diarrhea is the second leading infectious cause of fatal childhood disease worldwide, causing half a million deaths among children aged <5 years in 2015.[1] Rotavirus is the single most common cause of severe and fatal diarrhea in this age group, estimated at ~10 million severe episodes and 118,000–183,000 deaths each year.[1-4] Nearly half of all rotavirus deaths occur in Asia.[3] Currently, two safe and effective rotavirus vaccines (RotaTeq, Merck & Co, Inc, USA and Rotarix, GlaxoSmithKline Biologicals SA, Belgium) are available globally,[5] and the World Health Organization (WHO) recommends that rotavirus vaccination for infants should be included in all national immunization programs as a comprehensive strategy to control diarrheal diseases.[6] Over 90 countries have since added rotavirus vaccine into their national immunization programs,[7] and a substantial decrease in childhood mortality and hospitalization due to severe diarrhea has been documented following rotavirus vaccine

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introduction.[8, 9] However, fewer than 20% of the countries in Asia have introduced rotavirus vaccines into their national immunization programs.[10, 11]

China is both one of the high-burden countries for diarrhea[12] and one of the countries that delayed the introduction of rotavirus vaccination into the national immunization program. The two international rotavirus vaccines (RotaTeq and Rotarix) have not been licensed for use in China. However, a domestic Jennerain vaccine (the Lanzhou lamb rotavirus vaccine [LLR], the Lanzhou Institute of Biological Products, China) has been licensed since 2000. At the end of 2014, over 60 million doses of LLR had been distributed to children, and a moderate-to-low efficacy of 30-50% and a coverage rate of 15%-30% were reported for the vaccine by several postmarketing observational studies.[13-16] Unfortunately, LLR has not been included in the Expanded Program on Immunization (EPI) since its licensing, partly due to the lack of reliable data on childhood rotavirus infections at a national level to support vaccination activities.

To inform policy makers on rotavirus diarrhea prevention, a nationwide, multicenter, prospective study, the National Key Science and Technology Project on Infectious Disease Surveillance Technique Platform (IDSTP), has been ongoing since 2009,[17] in which the activity and strain diversity of rotavirus have been continuously monitored. The primary objectives of the IDSTP are (i) to monitor the prevalence of rotavirus over time to identify priorities for intervention; (ii) to assist timely identification of antigenically distinct novel or rare strains, which emerged rapidly and spread widely in the community; and (iii) to inform policy makers on future vaccine development, enhance recommendations and improve implementation in national vaccination activities. In this study, we determined the prevalence

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and strain diversity of rotavirus among diarrheal children aged <5 years in China using data generated by the IDSTP from 2009-2015.

Materials and Methods

Sentinel-based Surveillance

IDSTP is a nationwide sentinel-based surveillance system that has been conducting syndromic surveillance at 213 participating hospitals across the country since January 1, 2009 (Figure 1 & supplementary table 1). These hospitals were selected based on three principles: i) each of the 31 provinces of mainland China would include a minimum of one hospital; ii) various types of hospitals (e.g., children's, general, urban and rural community health service centers) would be included; and iii) hospitals would have the capability and resources to conduct ongoing surveillance.

Patients visiting these hospitals' outpatient (e.g., emergency room and clinics) and/or inpatient (e.g., wards and intensive care unit) departments were registered and screened for eligibility of inclusion year-round by an attending practitioner. Diarrhea was defined as ≥3 passages of watery, loose, mucoid, or bloody-stools within a 24-h period. Patients meeting the case definition of diarrhea were eligible for inclusion. A total sample size of ~10,000 persons per year was selected for the whole country. The total sample size was then allocated and assigned to each sentinel hospital (median=54 cases/hospital/year) after weighting using the recorded outpatient numbers from the previous year. Convenience sampling was used to recruit cases among eligible patients. Attending doctors in the hospital would decide when, whom, in what hospital setting and how many patients to enroll. To account for seasonal variations of diarrheal illness onset, it was determined that each hospital was to recruit ≥5%

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of the allocated number on a monthly basis.[17] Between 2009 and 2011, both inpatients and outpatients were included. Since 2012, the enrollment of inpatients has been suspended because of the overlap of the study with another national surveillance program conducting surveillance for diarrhea among hospitalized children. Verbal consents were obtained from parents/guardians before enrollment. The study protocol was approved by the ethical review committee at the Chinese Center for Disease Control and Prevention (China CDC, Beijing, China).

Data and Specimen Collection

Patients or children's guardians were interviewed with the use of a standardized case reporting form at the time of recruitment, and additional medical information was abstracted from medical charts by trained staff. The collected information included demographics (e.g., sex, date of birth, and address) and clinical characteristics (e.g., signs/symptoms, date of symptom onset, date of diagnosis). After recruitment, fresh, whole stool was collected in a sterilized container without preservative and stored at -20 °C until rotavirus screening and strain characterization. All collected stool samples were packed and transported in iceboxes within 48 h to a network laboratory according to UN3373 transportation requirements.[18, 19] There were 92 network laboratories for this project in the country, and each of them served a specific number of hospitals in the same catchment area.

Laboratory Testing

A standardized method and operation procedure were adopted by all laboratories for rotavirus testing and characterizing of strains and validated before initiating surveillance. Group A rotavirus (RVA) antigens were detected directly in stool samples by using enzyme-linked

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immunosorbent assay (ELISA, ProSpecTTM Rotavirus kit, Oxoid Ltd, Basingstoke, UK), according to the manufacturer's instructions. For ELISA-positive samples, multiplex reverse-transcription polymerase chain reaction assays were used for further genotyping of strains that were received at 33 network laboratories, due to the capacity restriction.[17]

Viral nucleic acid was extracted from each specimen using the Viral RNA/DNA Kit (Geneaid Biotech, Taipei, Taiwan) or the QIAamp® Viral RNA Mini Kit (Qiagen, Valencia, CA), according to the manufacturers' instructions. The primers used in G- and P- genotyping PCRs (Supplementary Table 2) and procedures have been described in previous studies.[20-22]

Statistical Analysis

For this paper, data from children under five years old were analyzed. Prevalence was calculated by dividing the number of children that tested positive for rotavirus by the total number of children tested. To study the seasonal pattern of rotavirus activity, we scaled the prevalence of rotavirus for different months and weeks from 0 to 1 according to percentile rank and further represented data as thermodynamic diagrams. To quantify seasonal characteristics, multiple linear regression models using a binomial distribution and a log link were also fitted to individual patient data collected from 2009-2015, including sine and cosine functions of the illness onset week to represent annual and semiannual periodic cycles as described previously.[23, 24] Since the efficacy of rotavirus vaccines was shown to be lower in low-resource regions than in high-resource regions in previous studies,[5] we measured rotavirus prevalence by economic regions according to the World Bank's classification of economies[25] to help identify priorities for future intervention. We divided the country into three regions based on the Gross Regional Product (GRP) per capita of the province from

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2009-2015,[26] namely, lower-middle-income regions (GRP $1,056-3,955), upper-middle-income regions (GRP $3,956-12,235) and high-income regions (GRP ≥$12,236). Cases were also classified into rural and urban, depending on the administrative classification of the residence. We used Chi-square tests or Fisher's exact tests for categorical variables, and Wilcoxon rank-sum or Kruskal–Wallis tests for continuous variables, as appropriate. All analyses were conducted in R version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria),[27] and the geographic map was produced using ArcGIS 10.2.2 (ESRI, Redlands, CA, USA).

Results

Characteristics of patients

Between January 1, 2009, and December 31, 2015, a total of 33,616 children with diarrhea aged <5 years across the country were included in our analysis, of which 27,931 (83.1%) were recruited at outpatient settings and 5,685 (16.9%) at inpatient settings. No patients were recruited at inpatient settings from 2012-2015. The median age of children was 0.9 years (interquartile range: 0.5-1.4 years) and 21,683 (64.5%) were male (Table 1). Among the three economic regions, upper-middle-income regions had the most number of patients (n=24,110, 71.7%), followed by lower-middle-income regions (n=5,002, 14.9%) and high-income regions (n=4,504, 13.4%). Of the total enrolled, 17,930 (54.0%) children lived in urban areas and 15,276 (46.0%) lived in rural areas.

Prevalence of rotavirus

Overall, RVA was identified in 10,089 children (30.0%). Children aged 6-23 mo had the highest positive percentage of RVA tests compared to other age groups (34.7%, n=7124/20506,

p<0.001). Moreover, RVA was more frequently identified among children attending an inpatient setting than children visiting an outpatient department (39.5% vs. 28.1%, p<0.001), it was more common among children in lower-middle-income regions than in upper-middle and high-income regions (40.7%, 31.3% and 11.2%, respectively; p<0.001), and it was more common among children living in rural areas than in those living in urban areas (34.9% vs. 26.0%, p<0.001) (Table 2). However, when stratified by economic region, RVA infection was more common among children living in urban areas than those living in rural areas in lower-middle-income regions (45.2% vs. 37.5%, p<0.001). The seasonal peak of RVA activity was in winter months, i.e., in week 50 (mid-December) for outpatients and in week 46 (mid-November) for inpatients (Figure 2).

Clinical presentations of rotavirus-associated diarrhea

The median age of RVA infection was 1.0 year (IQR=0.6-1.3 years), and 88.9% (n=8967) of RVA infection occurred among children aged ≤2 years. The median illness duration before seeing a doctor was 2 days (IQR=1-3 days). Symptom/signs including fever, vomiting, dehydration, and watery-stool were more frequent among children who tested positive for RVA (28.6%, 39.4%, 4.1%, and 72.6%, respectively) than among children who tested negative for RVA (21.9 %, 21.8%, 1.9% and 60.1%, respectively, p<0.001). Antibiotic usage before seeing a doctor was also more frequent among children with diarrhea associated with RVA (14.9% vs. 11.5%, p<0.001) (Table 3). Among children with rotavirus-associated diarrhea, fever (41.9% vs. 20.3%, p<0.001) and dehydration (20.5% vs. 0.9%, p<0.001) were more common in inpatient than outpatient, while vomiting (51.2%% vs. 34.6%, p<0.001) and respiratory symptom (13.2% vs.9.8%, p<0.001) were more common in outpatient than

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inpatient (Supplementary table 3).

Distribution of rotavirus strains

A total of 2,533 isolates were genotyped during 2009-2015. For the G-types studied, 2,232 (88.1%) had a single G specificity, 151 (6.0%) had mixed G specificities and 150 (5.9%) were unable to be assigned any G-type specificity. Meanwhile, for P-types, 2,338 (92.3%) isolates had a single P specificity, 103 (4.1%) had mixed P specificities and 92 (3.6%) were unable to assign any P-type specificity.

Among the 2,232 isolates with a single G specificity, the most common G-type identified was the G9 strain (n=922, 41.3%), while the four globally common strains, G1, G2, G3 and G4, constituted 57.3% (n=1,278) of the single G-type identified isolates. Among 2,338 isolates with a single P specificity, P[8] (n=1,785, 76.3%) was the most common P-type. Together, P[8] and P[4] constituted 89.5% (n=2,093) of these single P-type identified isolates. Regardless of mixed infections, five strain combinations, G9P[8], G3P[8], G1P[8], G2P[4] and G3P[4], were associated with 71.3% (n=1807/2533) of all RVA-associated diarrhea in children, with 794 (31.3%), 478 (18.9%), 365 (14.4%), 115 (4.5%), and 55 (2.2%) strains, respectively, during the whole period of 2009-2015 (Supplementary table 4).

During the seven-year study period, RVA strains circulating in children changed from year to year, with no one single strain persistent in the population. G3P[8] was predominant in the first couple of years, whereas genotype P[11] gained predominance in 2011. In 2012, the G1P[8] strain dominated, but in the most recent years, G9P[8] became the predominant circulating strain across the whole country, raising from 3.4% (2009) to 60.9% (2015) of all strains isolated (Figure 3).

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Discussion

Using large-scale, up-to-date national surveillance data, this study examined rotavirus infection and strain diversity in China and highlighted the importance of using routine surveillance data to identify priorities for intervention and detect emergent or predominant strains to guide vaccination activity. Nationally, one-third of childhood diarrhea was associated with rotavirus infection, and the prevalence of rotavirus was higher in inpatients than in outpatients, accounting for 28% of outpatient visits and 40% of hospitalizations attributed to diarrhea in children. These numbers were very close to 32.5% of outpatients and 42.6% of inpatients reported in a 2011 review and to 30% of outpatients and 40% of inpatients reported in a 2016 review,[16, 28] suggesting that the burden of diarrhea attributed to rotavirus in the country has not changed very much over these years. To reduce the burden of diarrheal disease, the inclusion of rotavirus vaccine into the national immunization program should be seriously considered. Of the total rotavirus infections that occurred in children, 89% occurred in children aged ≤23 mo, and children aged 6-23 mo had the highest prevalence of rotavirus among all age groups in the study. The WHO recommends that the first dose of the rotavirus vaccine should be administered starting at age 6 weeks and the last dose at a maximum age of two years.[6] Vaccination of children >24 months of age is not recommended. In China, the one domestic available vaccine, LLR, is administrated to children aged between 2-35 mo, with one dose per year for three consecutive years,[13] which is unfavorable for the vaccine to confer full protection for young children before their risk of infection is high. Our analysis of the relative frequency of rotavirus infection in children of different age groups suggested that the immunization procedure of LLR should be better optimized.

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Our results showed that, as the region's per capita income increased from less than $3,955 to $12,236 or more per year, the observed prevalence of rotavirus in children decreased from 40.7% to 11.2%. A region's economic development seemed to have an impact on the burden of rotavirus disease Moreover, rotavirus was detected more commonly in children living in rural areas rather than in urban areas, yet children living in urban area of lower-middle-income regions had the highest burden of rotavirus infection, suggesting that population over-crowdedness had an effect on the burden of rotavirus illnesses when the population was stratified by region's economic development. However, good hygiene and sanitation seemed unlikely to reduce the occurrence of rotavirus disease in previous studies[29], and the prevalence of rotavirus was reportedly not associated with population density in previous studies.[16, 28] We postulated that people living in high-income regions might be more affluent and have more access to rotavirus vaccines that conferred protection to some of the children in these regions. The observed association between population density or economic development and rotavirus infection might have been biased to some extent by the vaccination status of children, which was not measured in our current study. Future studies measuring vaccine coverage and efficacy in this group of children with diarrhea are warranted.

Globally, five strains, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], have caused most of the severe diarrheal illness in children in most countries during the last 20 years.[30] This was also true in our study; the top five strain combinations (G9P[8], G3P[8], G1P[8], G2P[4] and G3P[4]) were associated with 71.3% of rotavirus-associated diarrhea from 2009-2015, except G4P[8] was less common in our study (<1% of all isolates). Moreover, the dominant rotavirus

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strains circulating in the pediatric population changed unpredictably from year to year. No persistence of a single rotavirus strain was observed. From 2009-2015, the most dramatic change observed was that the previously predominant strains, G3P[8] and G1P[8], were rapidly replaced by genotype G9P[8] across all regions in the country in the most recent years, increasing from 3.4% in 2009 to 61.3% of all isolates in 2015. The newly emerged genotype G9P[8] has spread worldwide since its first emergency in the 1980s[31] and has gained predominance in several countries, including Australia, Ghana and Thailand,[32-34] as well as China.[35] Although the two globally licensed vaccines, RotaTeq and Rotarix, both demonstrate protective efficacy against G9P[8] via their P[8] component contained in the vaccines,[36-38] the one rotavirus vaccine in China, LLR, a lamb strain of rotavirus vaccine characterized as G10P[12], has no available data studying vaccine efficacy against the G9P[8] heterotypic strain. Previous studies conducted with other Jennerian vaccines reported inconsistent results,[39, 40] suggesting that animal strain vaccines might confer less heterotypic protection than homotypic protection to different G/P strains. The efficacy of this domestically available vaccine on other heterotypic strains needs to be assessed by well-designed clinical trials or observational studies in the future.

Our study had limitations. First, our surveillance protocol has undergone several modifications during the study period. Inclusion of children in inpatient settings was suspended since 2012. This affected comparison of our results between different study periods and between children subgroups. Second, the convenience sampling method used in our study can introduce unpredictable biases. For example, different health-seeking behaviors of patients, subjective judgment of clinicians on when and whom to sample, and a sampling

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scheme that was not pre-determined could have yielded patterns in the data that do not represent the true burden and epidemiological features of rotavirus. Nevertheless, the large sample size collected in our study might, to some extent, offer some relief to these concerns. Further improvement of the surveillance system attributes based on these aspects is welcomed. Third, we did not collect the historical information of vaccine usage for the diarrheal children in our study and were thus unable to evaluate the vaccine efficacy or to relate high prevalence of rotavirus in low-middle-income regions or rural areas to vaccine usage in the region. Fourth, population-based rates for rotavirus-associated diarrhea were not readily available, owing to the lack of population denominators in our study.

Conclusions

The burden of diarrhea attributed to rotavirus is high in China, especially in urban areas of lower-middle-income regions, where the per capita gross regional income is less than $4,000 per year. The inclusion of the rotavirus vaccine in the national immunization program to mitigate rotavirus burden should be considered. No persistence of any single rotavirus strain was observed during 2009-2015, and the G9P[8] strain has shifted to gain dominance in recent years. Continuous surveillance of rotavirus strain evolution is needed to ensure that currently marketed vaccines provide protective efficacy against emergent or antigenically distinct novel strains in China and the global health community.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Disclaimers

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency or the official position of the China CDC.

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Tables

Table 1. Patient characteristics of children with diarrhea in China, 2009-2015

Characteristics	All patients, (n=33,616)	Hospital settings		
		Outpatient, (n=27,931)	Inpatient, (n=5,685)	*p-value*
Sex, male	21683 (64.5)	17845 (63.9)	3838 (67.5)	<0.001
Age, median (IQR), yr	0.9 (0.5-1.4)	0.9 (0.5-1.4)	0.7 (0.4-1.0)	<0.001
Age group				<0.001
1-5 mo	8186 (24.4)	6258 (22.4)	1928 (33.9)	
6-11 mo	12249 (36.4)	9822 (35.2)	2427 (42.7)	
12-23 mo	8257 (24.6)	7526 (26.9)	731 (12.9)	
24-59 mo	4924 (14.6)	4325 (15.5)	599 (10.5)	
Residential Area*				<0.001
Rural	15276 (46%)	11494 (41.4)	3782 (69.3)	
Urban	17930 (54%)	16253 (58.6)	1677 (30.7)	
Year of surveillance				<0.001
2009	4319 (12.8)	2455 (8.8)	1864 (32.8)	
2010	5576 (16.6)	3801 (13.6)	1775 (31.2)	
2011	4427 (13.2)	2381 (8.5)	2046 (36.0)	
2012	4573 (13.6)	4573 (16.4)	-	
2013	5631 (16.8)	5631 (20.2)	-	

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2014	4634 (13.8)	4634 (16.6)	-
2015	4456 (13.3)	4456 (16.0)	-

Note: Data are presented as no. (%) of patients unless otherwise indicated. No patients were recruited at inpatient settings during years 2012-2015.

* The no. of patients in rural and urban areas did not equal the total no. of patients due to missing data.

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Table 2. Frequency of Group A rotavirus detected in children with diarrhea in China, 2009-2015, stratified by economic regions.

Characteristics	All patients		*p-value*	Lower-middle-income regions		*p-value*	Upper-middle-income regions		*p-value*	High-income regions		*p-value*
	No. tested	No. identified, (%)		No. tested	No. identified, (%)		No. tested	No. identified, (%)		No. tested	No. identified, (%)	
Total	33616	10089 (30.0)		5002	2034(40.7)		24110	7552(31.3)		4504	503 (11.2)	
Hospital settings			<0.001			<0.001			<0.001			-
Outpatient	27931	7842 (28.1)		3682	1356 (36.8)		19745	5983 (30.3)		4504	503 (11.2)	
Inpatient *	5685	2247 (39.5)		1320	678 (51.4)		4365	1569 (35.9)		0	-	
Sex			0.006			0.153			0.104			0.808
Male	21683	6618 (30.5)		3298	1365 (41.4)		15611	4946 (31.7)		2774	307 (11.1)	
Female	11933	3471 (29.1)		1704	669 (39.3)		8499	2606 (30.7)		1730	196 (11.3)	
Age group			<0.001			<0.001			<0.001			<0.001
1-5 mo	8186	1843 (22.5)		1001	381 (38.1)		6238	1393 (22.3)		947	69 (7.3)	
6-11 mo	12249	4329 (35.3)		1899	931 (49.0)		9244	3273 (35.4)		1106	125 (11.3)	
12-23 mo	8257	2795 (33.9)		1263	490 (38.8)		5551	2095 (37.7)		1443	210 (14.6)	

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24-59 mo	4924	1122 (22.8)		839	232 (27.7)		3077	791 (25.7)		1008	99 (9.8)	
Residential Area #			<0.001			<0.001			<0.001			<0.001
Rural	15276	5336 (34.9)		2408	902 (37.5)		11795	4272 (36.2)		1073	162 (15.1)	
Urban	17930	4653 (26.0)		2426	1097 (45.2)		12076	3215 (26.6)		3428	341 (9.9)	

Regions were classified according to the World Bank's classification of economies: Lower-middle-income regions=Gross Regional Product (GRP) in the province $1,006-3,955 per capita per year; upper-middle-income regions=GRP $3,956-12,235 per capita per year; and high-income regions=GRP ≥$12,236 per capita per year.

Note: * No patients were recruited at inpatient settings during the years 2012-2015; # The no. of patients in rural and urban areas did not equal the total no. of patients due to missing data; an em dash denotes that data are currently unavailable.

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Table 3. Clinical presentations of children with and without rotavirus-associated diarrhea in China, 2009-2015

Clinical Characteristics	Children with rotavirus-associated diarrhea (n=10,089)	Children without rotavirus-associated diarrhea (n=23,527)	*p-value*
Illness duration before seeing a doctor, median days, (IQR)	2 (1-3)	2 (1-3)	<0.001
Symptoms/signs, no. (%) *			
Fever (body temperature >38.0 °C)	1414 (28.6)	3148 (21.9)	<0.001
Vomiting	3773 (39.4)	4924 (21.8)	<0.001
Dehydration	419 (8.5)	470 (3.3)	<0.001
Respiratory symptom	1284 (12.7)	3242 (13.8)	0.01
Antibiotic usage before visiting, no. (%) *	1108 (14.9)	2080 (11.5%)	<0.001

Note: * No. of patients in the column did not sum up to equal the total no. of patients due to missing data. Respiratory symptoms included cough, sore throat, or rhinorrhea. Dehydration included thirst, decreased skin turgor, sunken eyes, or sunken fontanelle (in infants). IQR= interquartile range.

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Figure Legends

Figure 1. Locations of the 213 hospitals and 92 network laboratories participating in the diarrheal illnesses surveillance of China, 2009-2015.

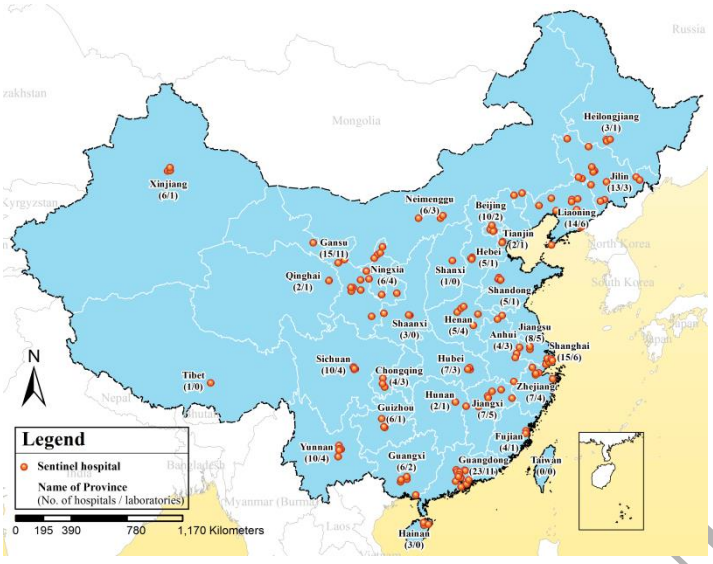

Figure 2. Temporal trends of rotavirus infection in children with diarrhea in China, 2009-2015. (A) Time series of weekly positive percentage of rotavirus, by hospital setting; (B) Heat map of monthly positive percentage of rotavirus, scaled from 0 to 1 according to percentile rank, by province; (C) Heat map of average weekly positive percentage of rotavirus, scaled from 0 to 1 according to percentile rank, by province; (D) The weekly positive percentage of rotavirus in outpatients, with a fitted seasonal curve superimposed; (E) The weekly positive percentage of rotavirus in inpatients, with a fitted seasonal curve superimposed.

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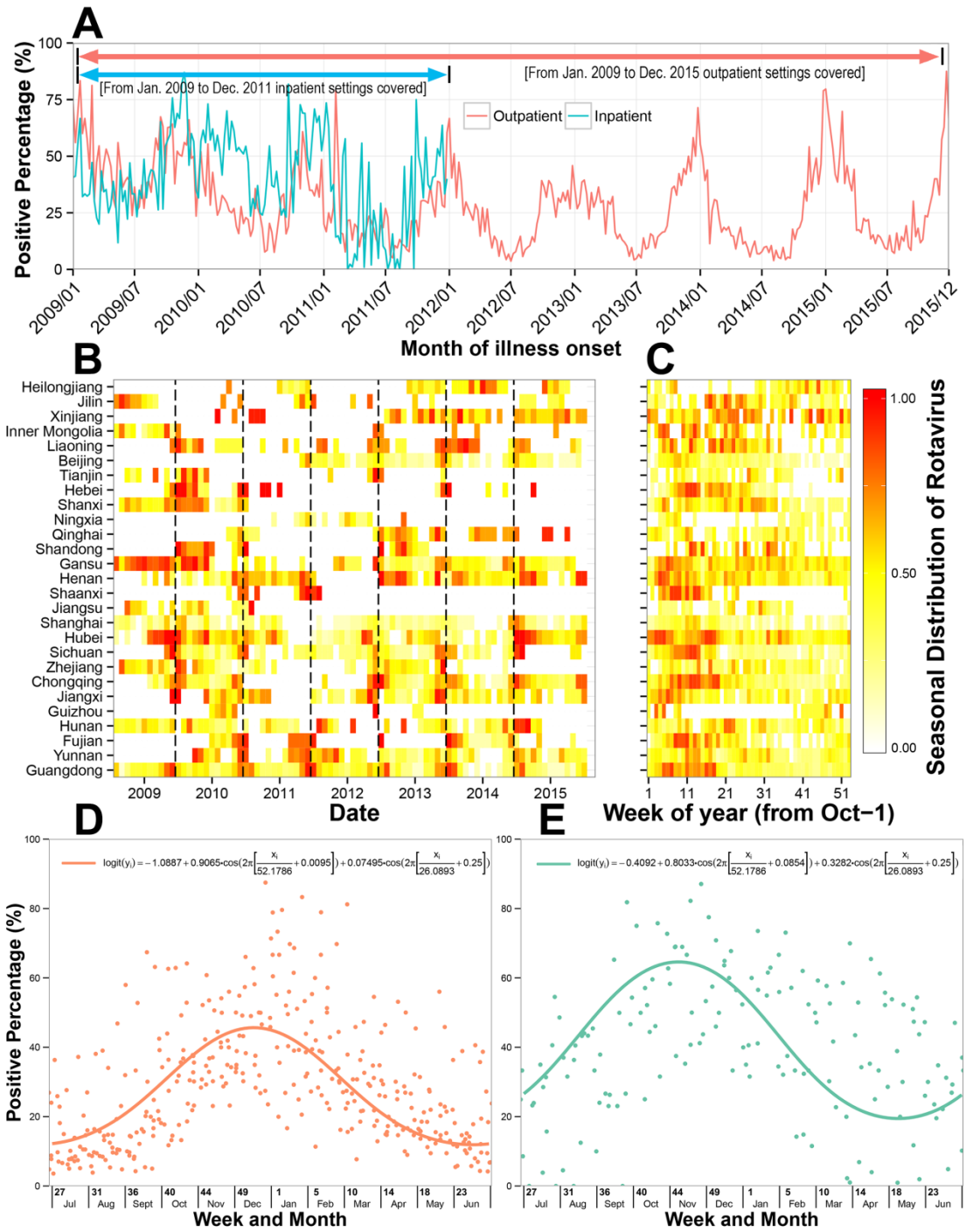
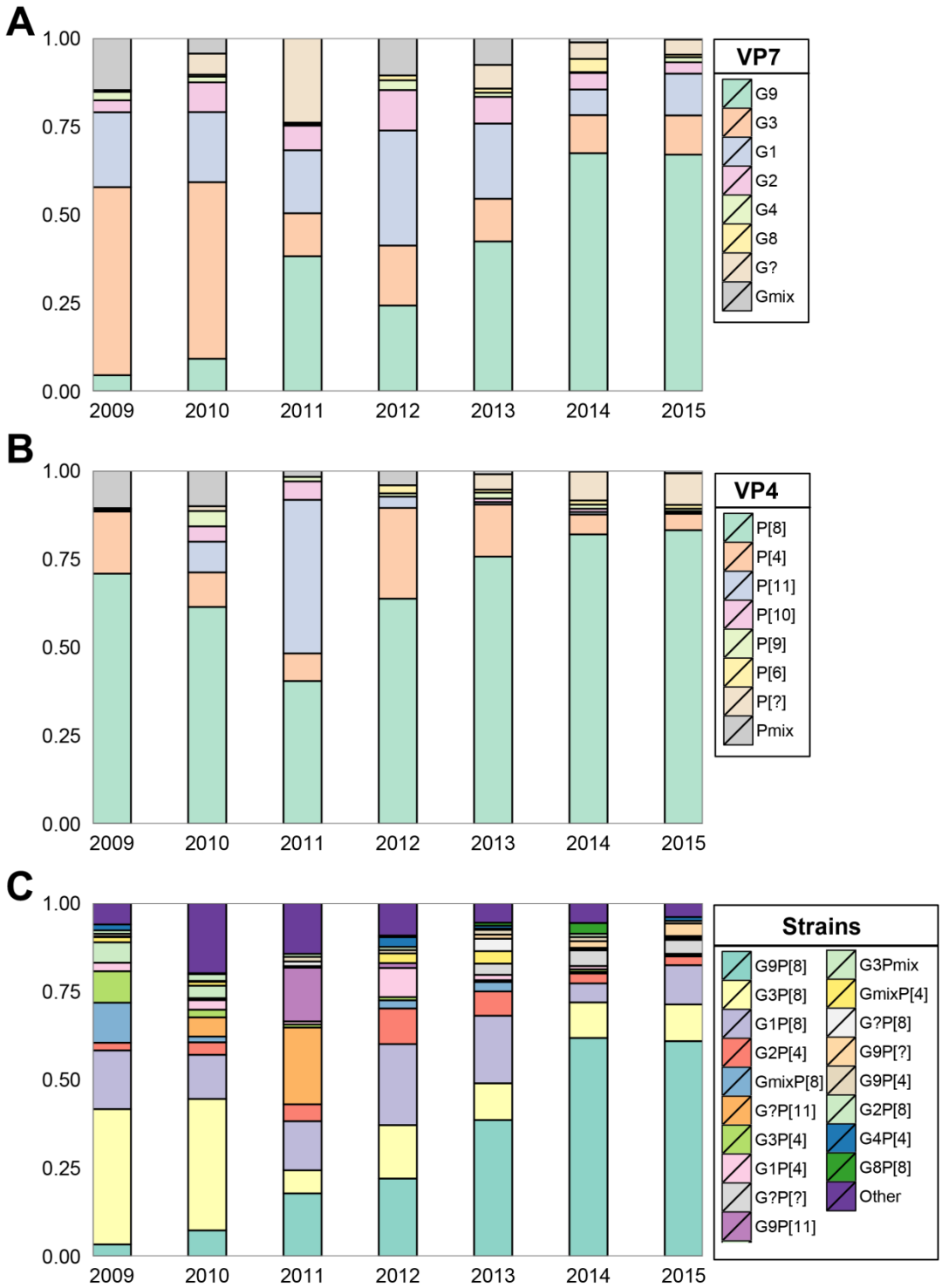


Figure 3. Rotavirus strains distribution in China, 2009-2015. (A) Strains classified by VP7 genotype; (B) Strains classified by VP4 genotype; (C) Strains classified by G-P genotype combinations. [?]=non-typeable; mix=two or more G-P-types identified.


A
1.00
0.75
0.50
0.25
0.00
2009
2010
2011
2012
2013
2014
2015
VP7
G9
G3
G1
G2
G4
G8
G?
Gmix
B
VP4
P[8]
P[4]
P[11]
P[10]
P[9]
P[6]
P[?]
Pmix
C
Strains
G9P[8]
G3P[8]
G1P[8]
G2P[4]
GmixP[8]
G?P[11]
G3P[4]
G1P[4]
G?P[?]
G9P[11]
G3Pmix
GmixP[4]
G?P[8]
G9P[?]
G9P[4]
G2P[8]
G4P[4]
G8P[8]
Other