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Issues with reducing the rotavirus-associated mortality by vaccination in developing countries

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ABSTRACT

More than 65% of the global rotavirus deaths were estimated to occur in 11 countries in Asia and Africa, claiming 345,000 children less than 5 years of age in 2004. While efficacious rotavirus vaccines are at hand, inequity in health delivery system within and between these countries was found to be the major hurdle against achieving the goal of rotavirus vaccine. When the coverage of currently used vaccines was applied to a rotavirus vaccine, a maximum of 202,550 deaths would be averted. Even if the coverage reached 80%, there would remain 96,841 children dying because of rotavirus diarrhea in these 11 countries. Studies are therefore encouraged to develop comprehensive strategies to resolve inequity in health delivery system enabling the increase in the immunization coverage.

1. Introduction

Gastroenteritis is a leading cause of childhood morbidity and mortality worldwide and rotavirus has been recognized as the single most important cause of severe diarrhea in children less than 5 years of age [1]. The annual burden of rotavirus disease is estimated at more than 110 million diarrhea episodes, 25 million clinical visits, 2 million hospitalizations and more than half a million deaths [2,3]. More than 90% of the rotavirus-associated deaths occur in developing countries [4], whereas morbidity due to rotavirus imposes considerable economic burden in the developed world. Despite improvement in hygiene and sanitation in recent years, the incidence of rotavirus infection remains unchanged [5]. Thus, developing a safe and effective vaccine to control rotavirus diarrhea in both developed and developing countries has been a high priority. The second generation rotavirus vaccines namely RotaTeq, a pentavalent human/bovine reassortant vaccine (Merck & Co., Whitehouse Station, NJ, USA) and Rotarix, a monovalent human attenuated vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) have recently been licensed in many countries including the United States of America and the European Union, after the efficacy and safety profile concerning intussusception for these vaccines were established by two large clinical trials, each involving more than 60,000 children [6,7].

A review of global burden of rotavirus infection shows that only a few countries, many of which are located in South Asia and sub-Saharan Africa, account for the majority of the rotavirus-associated deaths [4]. Hence, the implementation of rotavirus vaccine in these regions is an immediate priority not only as their national interest but as the commitment of the international society to achieving the goal of reducing two thirds of the global under-5 mortality rate until 2015, which is one of the targets of the millennium development goals (MDGs) [8]. Either of the new rotavirus vaccines most likely costs more than the sum of the vaccines used in the Expanded Program on Immunization (EPI) which cost an average of US$ 1 per dose [9]. Providing rotavirus vaccine is a crucial issue for developing countries where subsidization in the health sector is minimal. Rotavirus vaccine is to be integrated into the existing national immunization program being co-administered with diphtheria, tetanus toxoid and pertussis vaccine (DTP). Thus, the coverage of EPI vaccines especially that of third dose of DTP (DTP3) can be considered as an indicator of the future rotavirus vaccine coverage.

The aim of this study was to examine the factors potentially affect the implementation of a rotavirus vaccine into the national immunization program in those countries where the largest number of rotavirus-associated deaths occur. The factors we examined were the indicators of childhood mortality, the markers of health-care budget and the coverage of selected EPI vaccines to predict the uptake of the rotavirus vaccine when it is in use.

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2. Methods

2.1. Identification of countries where there were the largest number of rotavirus-associated deaths

The countries with the largest number of rotavirus-associated deaths were identified in two consecutive papers reviewing the global burden of rotavirus infection from 1986 to 2004 [2,3] and from the latest estimation of rotavirus attributed mortality by the world health organization (WHO) [10].

Since there are no direct data about the number of rotavirus-associated deaths in developing countries, the proportion of rotavirus infection in hospitalized children with severe diarrhea is commonly used as a proxy for the proportion of death due to rotavirus among children less than 5 years of age. The overall median of rotavirus infection in hospitalized children then was multiplied by the proportion of death due to diarrhea among children less than 5 years of age to calculate the number of rotavirus-associated deaths in low, middle (-low, -high) and high income countries [2,3].

2.2. Data collection and definition

For each of the countries with the largest number of rotavirus-associated death three categories of data (i.e., childhood mortality, the coverage of the national immunization and health economy) were obtained from each country's profile at the WHO web site [11]. The information thus obtained was then used to understand the current situation of childhood mortality and morbidity due to rotavirus and other vaccine-preventable diseases, the countries' achievement toward the control of vaccine-preventable diseases such as measles and poliomyelitis, and the competence of their health finances for introducing and maintenance of the new expensive rotavirus vaccines.

All indicators were selected and described based on the WHO definition, as follows.

2.3. Childhood mortality

The under-5 mortality rate, a main indicator of MDGs is defined as the probability of death per 1000 live births among children less than 5 years of age. The number of rotavirus-associated deaths and rotavirus mortality rate per 100,000 children less than 5 years of age reflect the current burden of the disease in each country.

2.4. Health economy

The total health expenditure (THE) is defined as the sum of government and private health expenditures (in international dollars) in a given year expressed as the proportion of gross domestic product (GDP) and per capita health expenditure. THE includes the sum of spending for health maintenance, restoration or enhancement of the health status in a country. General government expenditure on health (GGHE) is the sum of purchase and direct expenditure by all levels of government, social security agencies and public sectors to the health-care services and goods. External resources include all grants and loans for health goods and services, passing through governments or private entities in cash or in kind. Household out-of-pocket expenditure is defined as the household direct payments to public and private providers of health-care services, non-profit institutions and non-refundable cost-sharing such as deductibles, co-payments and fees for services.

2.5. Immunization coverage

The coverages of several EPI vaccines were assessed in this study such as DTP3, with which the rotavirus vaccine is supposed to be co-administered. The coverage of DTP3 in each county is also considered as an indicator of the performance and effectiveness of the national immunization program. The coverage of measles and poliomyelitis vaccines and the number of cases preventable by these vaccines were cited for all the countries to understand their situation to compare with the global distribution of the diseases and the countries' achievement regarding the international goals.

3. Results

The annual number of rotavirus-associated deaths was initially estimated by Parashar et al. at a median of 440,000 (range 352,000–592,000) between 1986 and 1999, and a median of 611,000 (454,000–705,000) between 2000 and 2004 [2,3]. The latest global estimate by the WHO was 527,000 deaths in 2004, which is considered as the baseline mortality attributed to rotavirus in the prevaccination era [10,12]. The majority of rotavirus-associated deaths occur in south Asia and sub-Saharan Africa (Fig. 1). Each of India, Nigeria, Democratic Republic (DR) of Congo, Ethiopia, China, Pakistan, Afghanistan, Bangladesh, Indonesia, Angola and Niger had more than 10,000 deaths per year. Approximately 346,000 rotavirus-associated deaths occurring in these countries alone

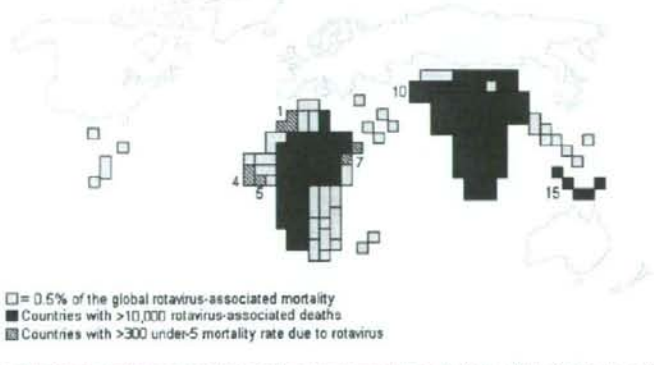

Fig. 1. The magnitude of the global distribution of rotavirus-associated deaths in pre-vaccination era (1. Mali, 2. Niger, 3. Ethiopia, 4. Sierra Leone, 5. Liberia, 6. Nigeria, 7. Somalia, 8. DR Congo, 9. Angola, 10. Afghanistan, 11. China, 12. Pakistan, 13. India, 14. Bangladesh, 15. Indonesia).

Table 1
The characteristics of childhood mortality and immunization in the countries with the largest number of rotavirus-associated deaths

Country	No. of under-5 deaths due to rotavirus	Mortality rate in		Under-5 mortality rate		DTP3		Averted deaths[d] by rotavirus vaccination with efficacy of	
		Under-5 due to rotavirus[a]	Under-5[b]	Ratio of rural/urban	Ratio of lowest/highest wealth quintile	Coverage[c] (%)	% of districts with <80% coverage	50%	90%
India	122,270	102	82	1.7	3.1	55	NG	33,624	60,524
Nigeria	49,974	228	191	1.6	3.3	54	60	13,493	24,287
D. R. Congo	30,444	281	200	NG	NG	77	54	11,721	21,098
Ethiopia	27,424	213	150	1.4	1.4	72	66	9,873	17,771
China	27,349	32	30	NG	NG	93	0	12,717	22,891
Pakistan	19,933	95	99	1.4	1.7	83	46	8,272	14,890
Afghanistan	17,992	338	241	NG	NG	77	56	6,927	12,468
Bangladesh	15,382	89	73	1.1	1.7	88	20	6,786	12,183
Indonesia	12,970	60	34	1.5	3.5	70	30	4,540	8,171
Angola	11,229	389	236	NG	NG	44	86	2,470	4,447
Niger	10,884	392	194	1.8	1.5	39	12	2,122	3,820

NG: data not given.
[a] Per 100,000 population <5.
[b] Per 1000 live birth.
[c] WHO-UNISEF estimates for 2006.
[d] Based on the current coverage of DTP3.

accounted for more than 65% of the global rotavirus-related mortality in 2004 (Table 1).

When it comes to the specific mortality rate due to rotavirus among 100,000 children less than 5 years of age, it varied more than 10-fold from 32 in China to more than 300 in Niger, Angola and Afghanistan. However, the countries where the rotavirus mortality rate was very high are not necessarily the countries where the number of rotavirus-associated deaths was very large; for instance Niger with the highest rotavirus mortality rate had the smallest number of rotavirus-associated deaths among these countries. In contrast, there were a large number of rotavirus-associated deaths in such countries as India, China, Bangladesh and Indonesia not because of higher mortality rates but because of the large cohort of children under 5 years of age. It is also apparent that the under-5 mortality (and consequently those of rotavirus) did not equally distribute within the different socioeconomical groups, and a higher childhood mortality was observed in children living in rural than in urban areas (≥1.5 in Indonesia, Nigeria, India and Niger) and in the poorest than in the richest children (>3 in Indonesia, Nigeria and India).

The relationships between under-5 mortality rate, rotavirus mortality rate and gross national income (GNI) per capita are shown in Fig. 2. The highest rates of mortality were observed in those countries including DR Congo, Niger, Ethiopia and Nigeria whose GNI was ≤1000 international dollars per capita. The lowest mortality rates were reported from China whose per capita GNI well exceeded 6600 international dollars. The rest of the countries with intermediate per capita GNIs showed intermediate mortality rates. Angola is an exception in this regard because the country had a middle-GNI, yet it had one of the highest mortality rates, presumably due to the long-term domestic unrest and political crisis [13]. The under-5 and rotavirus mortality rates also had an inverse relationship with THE per capita (Fig. 3). Generally, such countries as DR Congo, Afghanistan, Ethiopia, Niger, Angola and Nigeria which spent ≤US$ 55 as THE had the highest under-5 and rotavirus mortality rates, as indicated by circle in Fig. 3. However, Pakistan whose per capita THE was US$ 46 had a similar mortality rates as did middle-THE countries including Bangladesh, India and Indonesia. It was also revealed that China which spent the highest THE per capita had the lowest rates of under-5 and rotavirus mortality.

These countries were also divided into two groups according to the coverage of the EPI vaccines. Only China, Bangladesh and Pakistan were the countries where the coverages of DTP3, measles and poliomyelitis were more than 80%. It deserves mention that India and Nigeria where there occurred the highest number of rotavirus-associated deaths were among the countries where the DTP3 coverage was the lowest.

In addition to the DTP3 coverage, there is concern regarding the timing of rotavirus administration. We have already known from the experience of Rotashield that the incidence of intussusception

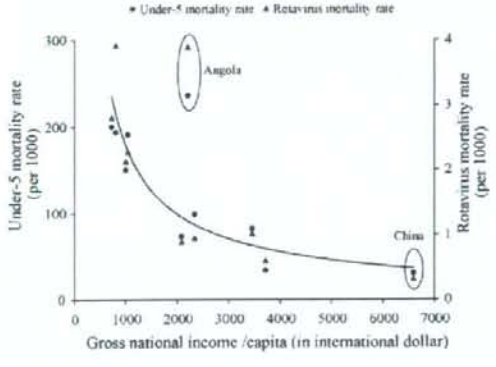

Fig. 2. The relationship between under-5 as well as rotavirus mortality rates and gross national income per capita.

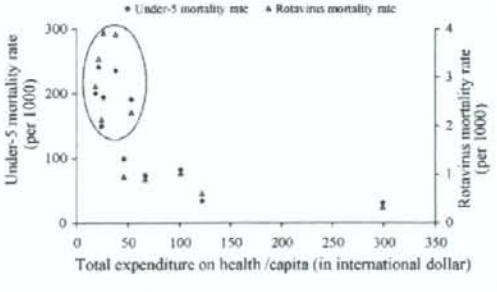

Fig. 3. The relationship between under-5 as well as rotavirus mortality rates and total expenditure on health per capita.

Table 2
The indicators of health economy in the countries with the highest number of rotavirus-associated death

Country	Gross national income (GNI)/capita[a]	Total expenditure on health (THE)		General government expenditure on health (GGHE)		External resources for health as % of THE	Out-of-Pocket expenditure[c] (%)	Population living below the poverty line[d] (%)
		Per capita[b]	% of GDP	Per capita[b]	% of THE			
India	3460	101	5	18	17.6	0.4	93.8	34.7
Nigeria	1040	53	4.3	17	32.4	6.4	90.4	70.8
D.R. Congo	720	18	4.2	6	33.6	22.6	100	NG
Ethiopia	1000	24	5.5	15	61	37.9	80.6	23
China	6600	299	4.7	117	39.1	0.1	86.7	16.6
Pakistan	2350	46	2	10	22.8	3.9	97.7	17
Afghanistan	NG	21	5	4	21	13.4	97.8	NG
Bangladesh	2090	67	3	19	28.6	15	88.3	36
Indonesia	3720	122	2.7	42	34.7	1.2	74.3	7.5
Angola	2210	38	2	30	81.8	7.3	100	NG
Niger	800	26	3.7	14	44.8	19	85.2	NG
Global	9420	777	8.7	434	55.9	0.3	52.2	NG

NG: data not given.
[a] PPP international US$ in 2005.
[b] At international US$ rate.
[c] As % of private expenditure on health.
[d] <US$ 1 per day.

associated with the first dose of vaccine increased with age and infants older than 90 days of age accounted for 80% of cases of intussusception associated with the first dose of RotaShield [14]. This means that for safety reasons, the first dose of currently available rotavirus vaccine should be administered between 6 and 12 weeks of age and immunization should not be initiated for infants older than 12 weeks of age [15]. Thus countries with EPI schedules that are not tightly controlled will potentially derive less benefit from rotavirus vaccines. In addition, delayed rotavirus vaccination may become a real safety concern in countries where prompt diagnosis and treatment of intussusception is difficult. Currently, there is little data to examine whether the first dose of DTP vaccine (DTP1) is given at a proper time in these countries, a critical issue which could influence the impact of a rotavirus vaccination programme on rotavirus-associated mortality.

Assuming that the coverage of rotavirus vaccine is the same as DTP3, that the first dose of the vaccine is given to all children at the proper time (which is very unlikely) and that the efficacy of the vaccine can vary between 50% and 90%, it is calculated that even the best scenario only predicts that 202,550 (59%) rotavirus-associated deaths will be averted in these countries, ranging from 25% in Niger to 84% in China (Table 1). A further 46,460 deaths may be averted if the vaccine coverage reaches 80% in these countries.

No direct relationship was found between the coverage of the routine vaccines and THE per capita. It was observed that even countries with low THE showed coverages more or less similar to countries with high and middle THE (data are not shown). It deserves mention that the total coverage of DTP3 in a country does not necessarily mean an equal coverage in all geographical settings. In fact, the DTP3 coverage varied from less than 50% to more than 90% within different districts in most of the countries (Table 1). Such urban-rural differences were also observed for the measles vaccine coverage. For instance, the coverage was 1.5–2.5 times higher in urban areas of India, Pakistan, Nigeria, Ethiopia and Niger than in rural areas of each of these countries.

Due to the low and unequal coverage of EPI immunization such as DTP3 and measles in most of these countries, there were a tremendous number of cases of the vaccines-preventable diseases. Globally, more than 84% of diphtheria, 44% of pertussis and 75% of measles cases were reported from these countries in 2006. A total of 1935 confirmed cases of poliomyelitis having occurred in these countries also accounted for approximately 96% of the global cases in the same year.

As to the health economy status in these countries (Table 2), the governmental expenditure on health was less than 20 international dollars per capita in India, Nigeria, DR Congo, Ethiopia, Pakistan, Afghanistan, Bangladesh and Niger whereas that of the global average was US$ 434 per capita. Social security had no investment on THE in most of the countries and external resources and donations accounted for less than 10% of THE in the majority of them. Therefore, out-of-pocket expenditures were spent to compensate 70–100% of the private expenditure on health in these countries, despite the fact that a substantial proportion of the people, ranging from 7% in Indonesia to 70% in Nigeria, lived below the poverty line (less than US$ 1 per day).

An extra financial load resulting from the implementation of rotavirus vaccine will dramatically increase the relative proportion of immunization expenditure which is obviously beyond the financial capacity of these countries. So far, no price for rotavirus vaccines is set for developing countries. However in the US, the Centers for Disease Control and Prevention (CDC) vaccine price list shows that each dose of RotaTeq costs US$ 55.05 and US$ 66.94 for public and private sectors respectively, until the end of March 2008. The countries' expenditures on health suggest that any rotavirus vaccine, even if it costs one-tenth of the US market price, would be unaffordable since a course of rotavirus vaccination will easily exceed the total general government expenditure on health per capita in most of the countries. A long-term and sustainable vaccination is a more challengeable task for these countries where subsidization in their health sector is severely limited.

4. Discussion

After several years of intermission following the withdrawal of RotaShield in 1999 [16], the hope to reduce rotavirus-associated mortality and the economic burden associated with it is now being restored by the introduction of new rotavirus vaccines [17]. However, none of the studies to date have had the sufficient sample size to detect the efficacy of rotavirus vaccines in preventing death.

This study focused on 11 countries with a large number of rotavirus-associated deaths, and it examined the indicators of childhood mortality, national immunization coverage and

the markers of health economy to figure out the potential challenges against the introduction of rotavirus vaccines into the childhood immunization schedule in the countries whose rotavirus-associated deaths account for approximately two-thirds of the global rotavirus mortality. This study also highlighted the desperate need for rotavirus vaccine as a real life-saver for tens of thousands of children living in such countries as DR Congo, Ethiopia, Afghanistan, Bangladesh, Angola and Niger which are among the least developed nations in the world [18].

Although rotavirus vaccine looks easier to develop than forthcoming malaria, HIV and new TB vaccines, the implementation of rotavirus vaccine is a crucial task in the areas where the burden of rotavirus mortality is the highest and reducing the number of deaths in socially deprived children is really difficult. Rotavirus immunization is also threatened in these areas by insufficient subsidization for rotavirus vaccine, a low coverage of immunization in general and, most importantly, the existence of horizontal inequity in childhood mortality and access to health care services. A fragile health system coupled with health inequity is a well known problem in developing countries and has previously been addressed in some of these countries [19–21]. However, the magnitude and characteristics of the inequity are different between and within the countries.

The introduction of rotavirus vaccine is only the beginning of long-term co-ordinated endeavor to achieve the goal; i.e., the reduction of the number of childhood hospitalization and deaths among different socioeconomic groups. Some indicators and methods therefore are needed for proper assessment of the effectiveness of the vaccine such as monitoring the reduction of severe diarrhea and death due to rotavirus infection. However, there are inherent difficulties in obtaining solid evidence. First, the detection of pathogens causing gastroenteritis requires laboratory assistance and more financial resources. Second, the vaccine's effect on the reduction of childhood mortality is much more difficult to measure in remote rural areas than in hospitals. Nevertheless, intensive laboratory-assisted surveillance is needed to observe the reduction of otherwise invisible rotavirus-associated deaths in remote communities, especially during the early period of vaccine introduction.

Improving the health delivery system is the other important issue which should be considered seriously in these countries. It is apparent that any attempt in these countries to overcome the weakness in their health system is not an easy task and it involves many factors including effective administration, changing traditional habits and misinformation inherent to certain local communities such as what was observed in northern Nigeria to boycott oral polio vaccine because of misconception about the spread of infertility and HIV by the vaccine [22,23]. More studies are needed to address such indigenous problems which can potentially threat the implementation of rotavirus vaccination in such societies.

Since there was no apparent association between DTP3 coverage and THE, simply increasing expenditure on health may not lead to an increasing immunization coverage. Focused studies therefore are needed to develop comprehensive strategies that can solve health inequity and improving immunization coverage.

To overcome some of these long-established problems, the Global Alliance for Vaccine and Immunization (GAVI) has created an innovative mechanism which is quite different from what the international community did before. The GAVI is taking action to support more than 70 low-income countries as well as China, India and Indonesia to expand their coverage for existing vaccines and introducing new emerging vaccines including for rotavirus. What the GAVI is doing is to establish a long-term sustainable market for the vaccines in developing countries and implementation of some financial and marketing innovation so-called Advance Market Commitments and International Finance Facility for Immunization [24]. These are new and much different approaches in which to bring a new business model to resolve complicated situation in the least developed nations. From the countries which are the subject of this study, the GAVI is supporting India, Nigeria, Ethiopia, Pakistan, Bangladesh, Indonesia and Niger in order to introduce rotavirus vaccine in their national immunization program from 2010, after the result of the vaccines efficacy are available from Africa and Asia in 2009 [25]. In a long-term prospective the countries are also committed to contribute to a co-financing scheme which for the first time will be run with the introducing of rotavirus vaccine in Africa and Asia, and require countries to co-finance for introducing of new rotavirus vaccine and under-used vaccines. The vaccines can be purchased with co-financing from 10 cents per dose by the poorest as well as fragile groups of countries including Afghanistan, Angola and DR Congo to 30 cents per dose for the least poor countries.

Whether what the GAVI is doing is a right approach or not, there is concern about the equity issue [26] and acceptability of the GAVI approach to commercialize immunization. The market of rotavirus vaccine may become competitive by the GAVI innovative but not necessarily co-operative. There is no guarantee that the vaccine market will function well in the future and uncertainty about the destiny of rotavirus vaccine in developing countries still exists.

In conclusion, this study draws attention to the global distribution of rotavirus-associated deaths which mostly occurred in 11 countries located in sub-Saharan Africa and South Asia. In most of these countries, uneven distribution in childhood mortality, coverage of routine immunization and indicators of health financing were observed not only between but also within the countries. This study shows that introducing a sustainable rotavirus vaccination program and improving the coverage of the immunization to a minimum 80% in all geographical settings and socioeconomical classes are among the most important issues and that they need to be undertaken by both the national government and international communities. Even if the coverage reaches 80%, there will remain 96,841 children who will die because of rotavirus diarrhea in these 11 countries. The goal of rotavirus vaccine, global reduction of rotavirus-associated deaths, will therefore be achieved only after the vaccine reaches the deprived children living in remote areas of the poorest nations in the world. While it is important to reinforce simultaneously other well-known interventions for preventing diarrhea deaths such as exclusive breast feeding [27], zinc [28] and vitamin A supplementation [29] as well as hygiene and family health measures, e.g., access to clean water [30], there is a desperate need for developing an innovative strategy to increase the vaccine coverage.

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BRIEF REPORT

Norovirus infection among children with acute gastroenteritis in Recife, Brazil: disease severity is comparable to rotavirus gastroenteritis

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Abstract *Norovirus* has captured increasing attention as an agent of childhood diarrhoea. However, it is not known whether norovirus causes as severe diarrhoea as rotavirus, particularly among children in developing countries. In a 1-year study conducted between May 2004 and April 2005 in Recife, Brazil, norovirus was detected by ELISA in 34/233 (15%) diarrhoeal children less than 5 years of age. The severity of clinical illness, as indicated by the presence of dehydration, the requirement for hospitalization, and the duration of hospital stay, was similar between children with norovirus and rotavirus infection. These data underscore the importance of norovirus as a cause of severe diarrhoea in children.

Norovirus is a genus in the family *Caliciviridae* whose members have long been established as major pathogens responsible for outbreaks of viral gastroenteritis among adults in various settings [6, 7]. More recently, norovirus has captured increasing attention as a significant etiological agent of acute diarrhoea in children, second only to rotavirus among the viruses associated with gastroenteritis [12]. Therefore, it is anticipated that noroviruses will have a relatively greater public health impact in countries that have introduced universal rotavirus immunization of infants, with consequent reduction of the burden of severe rotavirus diarrhoea. Brazil is the first country to introduce a rotavirus vaccine into the universal immunization schedule of its approximately 4 million annual birth cohort.

To date, sensitive detection assays for norovirus have consisted of time-consuming molecular methods such as reverse transcription (RT)-PCR [2, 7]. The availability of a recently released enzyme-linked immunosorbent assay (ELISA) for norovirus detection, with improved specificity (94–99%) compared with its precursor, SRSV (II)-AD, made by the same manufacturer [10, 20], prompted us to examine the role of norovirus in stored faecal specimens obtained from children with acute diarrhoea in Recife, Brazil, during a 1-year period (May 2004–April 2005) prior to the introduction of universal rotavirus vaccination. These specimens had previously been examined for rotavirus infection [11].

The aim of the current study was (i) to determine the rate of norovirus detection among children under five years of age with acute gastroenteritis and (ii) to compare the clinical characteristics and disease severity of norovirus gastroenteritis with that of rotavirus gastroenteritis.

A total of 290 children presenting with acute diarrhoea (defined as three or more liquid or loose stools in a 24-h period, lasting $\leq$ 14 days) to a 500-bed paediatric teaching hospital in Recife, Northeast Brazil, were enrolled between May 2004 and April 2005. The catchment area of this hospital was primarily within the poorest part of the city of Recife, and the hospital provided free medical care.

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Clinical data and stool specimens were collected after parental informed consent had been obtained, and the specimens were then frozen at −70°C until further testing. All faecal samples were tested for rotavirus by ELISA (Rotaclone, Meridian Bioscience, Inc., Cincinnati, OH, USA); 102 (35%) specimens contained rotavirus, with genotypes G1P[8] and G9P[8] accounting for 49 and 29% of rotavirus strains, respectively [11].

Norovirus infection was investigated using ELISA (NV-AD "Seiken", Denka Seiken, Tokyo, Japan) in 233 children whose faecal specimens were available in sufficient amounts. The results presented in this study include only those from patients whose specimens were tested for both norovirus and rotavirus.

Statistical analysis was performed with a software package, SPSS v. 11, and the Mann-Whitney U test was applied for the comparison of median ages.

Norovirus was detected as a sole agent in 28 (12%) children, whereas rotavirus was detected as a sole agent in 81 (35%) children. Both norovirus and rotavirus were detected in 6 (3%) children. Thus, 50% of acute gastroenteritis episodes occurring in children less than 5 years of age were accounted for by infection due to rotavirus and/or norovirus. Given the lower sensitivity of ELISA compared with RT-PCR for the detection of norovirus [2, 5, 7], it is expected that the contribution of norovirus as the etiological agent of acute gastroenteritis is even higher, and further efforts will be required to close the diagnostic gap [18].

While rotavirus infection tended to peak in June and July, norovirus infection occurred more or less year round (Fig. 1). A comparison of ages of children with single viral infections demonstrated that children with norovirus infection were significantly younger (median age 7.5 months; range 1–20 months) than children with rotavirus infection (median age 9.5 months, range 1–43 months) ($P = 0.03$) (Fig. 2). The observation that norovirus infection occurred from infancy is consistent with sero-epidemiological studies previously performed in Japan, China and Italy [9, 14, 17].

A set of clinical manifestations, together with the requirement for hospitalization, were used as parameters to compare the severity of illness between norovirus and rotavirus diarrhoea (Table 1). While fever and vomiting were more frequently observed in rotavirus diarrhoea cases, there was no difference in the occurrence of dehydration, which is the major indicator of severity of diarrheal illness. There was also no difference in the proportion of cases requiring hospitalization. Further comparison was made regarding the duration of hospital stay; children admitted with norovirus diarrhoea had a median hospital stay of 6.5 days (range 1–22) while children hospitalized with rotavirus diarrhoea had a median hospital stay of 5 days (range 1–57) ($P = 0.11$, not significant). The child who stayed for 57 days was a 6-month-old male infant who tested positive for rotavirus and presented with watery diarrhoea, severe dehydration and shock.

Using a newly available ELISA kit, we have detected norovirus in 15 % (34/233) of diarrhoea cases among children less than 5 years of age in Recife, Brazil. While the sensitivity of detection of norovirus using ELISA is expected to be lower than by RT-PCR [3, 10, 20], leading to an underestimate of disease burden, the ELISA format nevertheless facilitates large epidemiological studies of norovirus infection.

Previous studies of norovirus infections in tropical countries have focused on the genetic characterization of norovirus strains [1, 4, 8, 19]. In contrast, the current study, the first to compare in a tropical setting the clinical severity

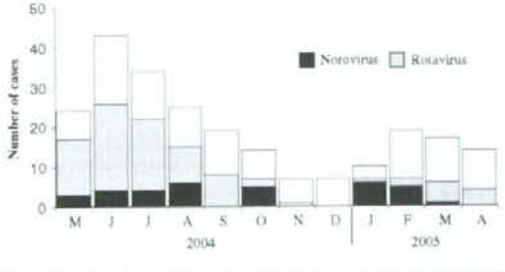

Fig. 1 Monthly distribution of cases according to viral agent. The columns filled in *black* represent cases with norovirus infection, those filled in *gray* represent cases with rotavirus infection, and the *open columns* represent cases in which neither agent was detected

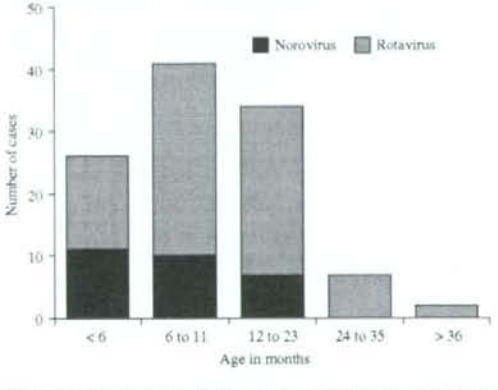

Fig. 2 Age distribution of cases of acute gastroenteritis according to viral agent. The columns filled in *black* represent cases with norovirus infection and those filled in *gray* represent cases with rotavirus infection

Table 1 Comparison of clinical manifestations and hospitalization status between norovirus diarrhoea and rotavirus diarrhoea

Parameters	Norovirus $n = 28$	Rotavirus $n = 82$	Odds ratio	95% confidence interval
Fever	19	72	0.29	0.41–0.82
Vomiting	23	78	0.24	0.06–0.95
Dehydration	18	46	1.41	0.53–3.76
Hospitalization	24	55	2.95	0.93–9.34

of norovirus and rotavirus diarrhoea, demonstrated that norovirus was detected in slightly younger children than rotavirus and that the disease severity associated with each virus was comparable (Table 1). Moreover, the observation that the median duration of hospital stay was 6.5 days for norovirus diarrhoea underscores the burden that paediatric norovirus infection can place on healthcare systems. In this regard, in a study of norovirus infection in the Netherlands, the median duration of illness in children less than one year of age was 6 days as opposed to 3 days in patients more than 12 years of age [15].

The limitations of this study include the lack of molecular characterization of norovirus strains. While such information provides a key to the molecular epidemiology of noroviruses, there is no difference between the clinical pictures of norovirus genogroup I or II, and typing to genogroup level is not sufficiently precise for molecular epidemiological studies [5].

Our observations, taken together with other studies showing that norovirus infection during infancy can be as severe as that of rotavirus [13, 16] and that norovirus is as frequently found as rotavirus in young children [6], leads us to offer a hypothesis that in countries employing universal rotavirus vaccination, norovirus could replace rotavirus as the main viral agent responsible for severe, acute gastroenteritis in infants and young children. To confirm this hypothesis, continued surveillance for norovirus and rotavirus infections among populations with high coverage of rotavirus vaccines will be required.

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Diversity of Human Rotavirus G9 Among Children in Turkey

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Between September 2004 and December 2005 a prospective study was conducted to understand the epidemiology of rotavirus infection among children with diarrhea attending two hospitals in Ankara, Turkey. Rotavirus was detected in 39.7% of the 322 stool samples and affected mainly children in the age group of 6–23 months. More than 70% and 39% of these cases occurred in children <2 and <1 year of age, respectively. In the temperate climate of Ankara rotavirus infection was prevalent throughout the year. Serotype G1P[8] was dominant followed by G9P[8]. In 38 samples a total of 5 electropherotypes were detected. All G9P[8] were of long electropherotype except one of short electropherotype. A proportion of G1 and G9 strains were in combination with P[6], P[4] or P nontypable. Mixed serotypes were responsible for 2.4% of the infections. A phylogenetic tree constructed with the deduced amino acid sequences of the VP7 gene showed that 16 Turkish G9 strains clustered with rotaviruses of lineage III. One G9 strain formed a new lineage, lineage IV with the Sri Lankan G9 rotaviruses. In the phylogenetic tree of the VP8* gene, the Turkish G9P[6] rotaviruses clustered with human strains of lineage Ia. Increased diversity of the G/P type combination and the presence of infection throughout the year in Turkey was a situation similar to developing countries. The occurrence of rotavirus infection at later age and low level of mixed infections in Turkey represented the situation of developed countries. This study suggests that diverse G9 rotaviruses are emerging in Turkey.

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KEY WORDS: rotavirus; serotypes; serotype G9; Turkey

INTRODUCTION

Recent estimates indicate that worldwide 611,000 deaths occur each year due to severe rotavirus diarrhea and approximately 85% of these deaths occur in the developing countries [Parashar et al., 2006]. Based on the diversity of the VP7 and VP4 antigens present on the outer capsid, rotavirus is classified into 15 G and 27 P types [Khamrin et al., 2007]. Among them types G1, G2, G3, G4, and G9 are associated mainly with human infection throughout the world [Glass et al., 2006]. On the other hand, several studies have identified large regional variations (e.g., India, Brazil, and Malawi) in

All authors had no conflicts of interest.

Accession numbers: The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB364368 for strain AHH41; AB364369, AHP102; AB364370, AHP74; AB364371, GUP102; AB364372, AHP85; AB364373, GUP180; AB364374, GUP30; AB364375, AHP66; AB364376, AHP28; AB364377, AHP32; AB364378, AHH11; AB364379, GUP148; AB364380, AHP22; AB364381, AHP58; AB364382, AHH12; AB364383, GUP54; AB364384, GUH13; AB306265, 05SLC051; AB306266, 05SLC056 and AB306267, 05SLC057.

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the serotype distribution of rotavirus strains [Gouvea et al., 1994; Leite et al., 1996; Ramachandran et al., 1996; Cunliffe et al., 1999]. Due to the vast variation in serotypes circulating globally and the imminent introduction of vaccines, continued typing of the rotavirus outer capsid proteins is essential to ensure the delivery of efficacious interventions.

In Turkey, diarrhea remains an important cause of illness among infants and young children; each year a significant number of children suffers from diarrheal diseases and is responsible for 8.4% of the death [Burden of diseases report, 2004]. With the geographical location of Turkey between Europe, Asia and Africa, the study of the molecular epidemiology of the circulating rotavirus strains may reveal unique genetic diversity. Limited epidemiological studies have been conducted in Turkey, including investigations in Ankara, Istanbul, Izmir and, Western and Southern regions of Turkey [Ceyhan et al., 1987; Turkoglu et al., 1993; Kurugol et al., 2003; Cataloluk et al., 2005; Karadag et al., 2005]. For the first time a study revealed that during 2000–2001 in Turkey serotype G1 was the most dominant (75.1%) followed by G4 (6.3%), G3 (3%) and G2 (0.8%) [Kurugol et al., 2003]. A recent study showed that during 2000–2002, G4 was the dominant serotype followed by G1, G2, G9, and G3 [Cataloluk et al., 2005]. In that study serotype G9 was present sporadically in only 3.4% of the samples collected from different areas of Turkey. The growing global divergence of rotavirus strains and the emergence of the G9 strains as an important cause of gastroenteritis emphasize the need for continuation of surveillance of rotavirus strains in developing countries [Armah et al., 2003]. The information generated by surveillance is important for the introduction of available rotavirus vaccines. Since the current available rotavirus vaccines are directed against type G1, G2, G3, and G4 rotaviruses. Emergence of other serotypes may affect the efficacy of the available vaccines. Therefore this prospective study was performed to understand the molecular epidemiology of rotavirus in two hospitals in Ankara, the capital of Turkey.

MATERIALS AND METHODS

Collection of Stool Specimens

Between September 2004 and December 2005, a prospective survey was conducted to determine the occurrence of rotavirus diarrhoea among children less than 5 years of age at Gazi University Hospital, and the Ministry of Health Ankara Training and Education Hospital in Ankara, Turkey. In general, most of the patients attending Ankara Training and Education Hospital are from the lower socioeconomic status while those attending the Gazi University Hospital are from the middle socioeconomic status. A case of diarrhea was defined as three loose stools during a 24 hr period. In this study, children who required hospitalization were classified as the inpatient group, and those who did not require hospitalization and treated only at the outpatient department of the same hospital were classified as the outpatient group. One stool specimen was collected from each patient and stored at −80°C until use.

Detection of Rotavirus

Using a 10% stool dilution in phosphate buffered saline, rotavirus antigens were detected by enzyme immunoassay (Rotaclone, Meridian Diagnostics, Inc., Cincinnati, OH), according to manufacturer's instruction.

Extraction of dsRNA and Determination of Electropherotypes

The genomic dsRNA was extracted from rotavirus-positive samples using the phenol-chloroform-isoamyl alcohol method [Watanabe et al., 2001]. The electropherotype was determined by running the extracted dsRNA through a polyacrylamide gel electrophoresis (PAGE), according to the method described previously [Koshimura et al., 2000]. Strains Wa (G1P[8]) and KUN (G2P[4]) were used as reference strains in each electrophoresis run.

Determination of G and P Types

For the VP7 and VP4 gene amplification, RT-PCR was done with AccessQuick RT-PCR (Promega Corporation, Madison, WI). G and P specific genotyping reactions were conducted using PCR Master Mix (Promega). The G and P genotypes of rotavirus-positive specimens were determined as follows; VP7 gene was amplified with consensus primers Beg9 and End9. The G type was identified with genotype specific primers for G1, G2, G3, G4, G8, and G9 [Gouvea et al., 1990]. The VP4 gene was amplified with consensus primers con-2 and con-3 [Gentsch et al., 1992], and the P type was identified with genotype specific primers for P[8], P[4], P[6], and P[9] as described previously [Gunasena et al., 1993; Uchida et al., 2006]. Samples which could not be typed were subjected to nucleotide sequencing.

Determination of Nucleotide Sequences

The nucleotide sequence of the VP7 and the VP8* part of VP4 genes were determined by BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA) according to the instruction of the manufacturer and the product was run into ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Phylogenetic Analysis

Sequence identity was searched with BLAST, multiple sequence alignment was done by ClustalW and phylogenetic tree was constructed by Mega 3.1 using neighbor-joining method [Kumar et al., 2004]. For the construction of phylogenetic trees deduced amino acid sequences were used. Bootstrap analysis of 1,000 replicates was done to find the significance of branching of the constructed tree.

Ethical Review of the Proposal and the Consent

The research proposal was approved by the ethical review board of the Faculty of Medicine, Gazi University and the local ethical committee of the Ankara Training and Education Hospital. The verbal consent of the mother or the guardian of the child was obtained prior to the sample collection.

RESULTS

Between September 2004 and December 2005 a total of 322 samples were collected from two hospitals, rotavirus was detected in 39.7% of the samples. Forty-four and 92 stool samples were collected from the inpatients and outpatients of the Ankara Training and Education Hospital, respectively. In the Ankara Hospital rotavirus was detected in 39% of the patients among them 40.9% and 38.0% were from inpatients and outpatients, respectively. From the inpatient and outpatients of Gazi University Hospital, 18 and 168 stool samples were collected. In Gazi University Hospital rotavirus was detected in 40.3% of the patients among them 22.2% and 39.7% were from inpatients and outpatients, respectively.

The age of the patients ranged from 15 days to 59 months. The age of the patients with and without rotavirus was 19.2 ± 15.5 (mean $\pm$ SD) and 21.4 ± 16.3 months old, respectively. Most of the rotavirus infection was found in the 6–23 months old children (Fig. 1) with the peak at 12–17 months old children. Number of rotavirus infection was lowest in patient <3 months of age. More than 70% and 39% cases of rotavirus diarrhea occurred in children <2 and <1 year of age, respectively. In the hospitalized patients with rotavirus infection, 81.8% of the children were <18 months old while in the outpatients 53.8% of the children were in the same age group.

Whether rotavirus infection had seasonal pattern was determined by the percentage of rotavirus cases detected among the diarrheal cases in each month. Rotavirus infection was found to be prevalent throughout the year (Fig. 2). However, it was most prevalent in the winter and autumn and lowest prevalence was found in the summer.

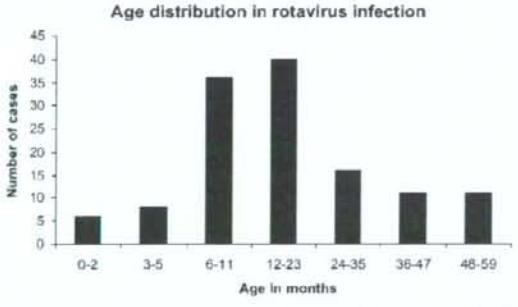

Fig. 1. The number of patients with rotavirus diarrhea distributed according to age.

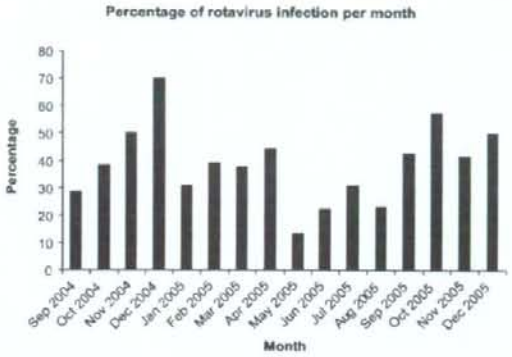

Fig. 2. The monthly occurrence of rotavirus diarrhea among the children in Turkey. The monthly occurrence is represented by the percentage of rotavirus cases detected among the diarrheal cases of each month.

To determine the diversity of the circulating rotavirus strains 128 samples were subjected to electropherotype. In 38 (29.7%) samples all the 11 segments of the rotavirus genome was visible. Based on the patterns, these were divided into 5 electropherotypes, E1–E5. A total of 26 samples belonged to electropherotype E2, 7 to E1, 3 to E3, 1 to E4 and 1 to E5. Electropherotypes E2 and E3 revealed a long configuration and were associated with serotype G1P[8] strains while pattern E4 displayed a short RNA profile and was associated with G2P[4] strains. Serotype G9P[8] and Gnontypable(nt)P[8] strains displayed the E1 long electropherotype and E5 short electropherotype was associated with G9P[8].

Among the samples typed successfully, serotype G1P[8] was predominant followed by G9P[8] (Table I). A proportion of G1 and G9 strains were in combination with P[6], or P nontypable (Pnt). The G1 and G9 strains which could be electropherotyped were in combination with P[8], therefore electropherotypes of strains in combination with other P types remain unknown. Mixed serotypes were responsible for infection in 2.4% of the cases. In 14 of the Gnt samples VP7 could not be amplified.

In the present study during the 4-month period, September to December 2004, only a single G9 strain was detected from a total of 19 rotavirus-positive samples. However during the whole period of 2005 among 109 samples with rotavirus infection serotype G9 was found in 19 samples, which was a shift of frequency of detection of serotype G9 from 5.3% to 17.4%. Out of 20 samples with serotype G9 rotavirus, 15 samples were from outpatients and the remaining samples were from inpatients.

Comparison of the VP7 antigenic regions A (aa 87-101), B (aa 143-152), C (aa 208-223), and F (aa 235-242) as defined by Dally-Smith and further developed by others [Dyall-Smith et al., 1986; Kirkwood et al., 1993; Martella et al., 2005]. Turkish G9 rotaviruses did not

TABLE I. Numbers and Percentages of Rotaviruses With Different G and P Serotypes Combination Detected in Turkey

Type	Number	Percentage of the total
G1P[8]	71	55.5%
G1P[6]	1	0.8%
G1P[4]	2	1.6%
G1P[8] and P[6]	1	0.8%
G1Pnt	1	0.8%
SubtotalG1	76	59.4%
G9P[8]	13	10.1%
G9P[6]	4	3.1%
G9P[4]	2	1.6%
G9P[8] and P[6]	1	0.8%
G9Pnt	2	1.6%
SubtotalG9	22	17.2%
G2P[8]	2	1.6%
G2P[4]	2	1.6%
G2P[8] and P[4]	1	0.8%
SubtotalG2	5	3.9%
G3P[8]	1	0.8%
G3P[4]	2	1.6%
SubtotalG3	3	2.3%
G4P[8]	4	3.1%
SubtotalG4	4	3.1%
GntP[8]	8	6.2%
GntP[6]	1	0.8%
GntP[4]	2	1.6%
GntP[nt]	7	5.5%
SubtotalGnt	18	14.1%
Total	128	100%

Gnt and Pnt indicate G nontypable and P nontypable, respectively.

reveal any amino acid substitutions. However amino acid substitutions were detected in other regions. The VP7 proteins had high identities among themselves ranging from 97% to 100%.

A phylogenetic tree was constructed with the deduced amino acid sequences of the VP7 gene of type G9 rotaviruses from Turkey and other countries. Of the 17 VP7 genes of the Turkish G9 strains analyzed, 16 clustered with contemporary global G9s, designated lineage III [Stupka et al., 2007] while a single Turkish G9 strain clustered with Sri Lankan G9 strains, forming a new lineage, designated IV (Fig. 3). Within lineage III, 14 Turkish G9 strains grouped together and two clustered separately with AT649, a G9 strain detected in the USA in 2000. The VP7 amino acid of Turkish strains showed a 95–99% identity with other strains of lineage III, 91–92% identity with strains of lineage I and 94–96% identity with strain of lineage II of G9 rotaviruses.

Phylogenetic analysis of the deduced amino acid sequence of the VP8* gene of Turkish G9P[6] strains, and other P[6] strains revealed that the Turkish strains clustered with human strains from different countries of lineage Ia (Fig. 4). At amino acid level the VP8* of the Turkish strains had 89–97% identity with the strains of the same lineage. While the VP8* of Turkish strains had 92%, 83–90%, 80%, 80–85%, 86–87%, and 84–85% identity with lineage Ib, Ic, II, III, IV, and V, respectively.

DISCUSSION

Several differences exist in the epidemiology of rotavirus infection between developing and developed countries. These differences include the earlier age of primary rotavirus infection, the high level of coinfection, the presence of maternally transferred antibodies in children of developing countries, and the circulation of atypical rotavirus strains bearing antigenic and genetic diversity [Armah et al., 2003]. In socioeconomic classification Turkey is categorized as a newly industrialized country; therefore it might be interesting to perceive the rotavirus epidemiology in a country which is transforming from a developing into a developed country. Using the combination of typing methods, a remarkable diversity of rotavirus strains circulating in Turkey was identified. Genetically and antigenically diverse rotavirus strains were co-circulating in the children. A wide range of strains with varied G/P type combination were detected in Turkish rotaviruses which might be comparable to that of other countries, such as India, Brazil, Bangladesh, and Nepal [Leite et al., 1996; Ramachandran et al., 1996; Unicomb et al., 1999; Uchida et al., 2006].

Similar to other studies [Kurugol et al., 2003; Karadag et al., 2005] the present study also revealed that rotavirus infection occurred throughout the year in the temperate climate of Turkey. This may be a distinctive aspect of the rotavirus epidemiology of that country. The circulation of viruses whole year round among the population may provide increase chance to the diversification of strains. This phenomenon has been observed in countries with tropical or subtropical climate such as in India where rotavirus infection occur throughout the year with some increase during winter [Bahl et al., 2005]. Unlike in developing countries where primary rotavirus infection occur at earlier age in Turkey rotavirus infection occur at relatively later age, which is a feature of rotavirus epidemiology of developed countries. For example in Iraq 75% of the rotavirus diarrhea occurred in children of <1 year of age [Ahmed et al., 2006], however, only 39% of rotavirus diarrhea occurred in Turkish children of that age group. In Iran 90% of the rotavirus infection occurs in children of <2 years of age [Khalili et al., 2004]. In Turkey 70% of rotavirus diarrhea occurred in this age group.

Like the present study, Kurugol et al. [2003] used ELISA and found comparable prevalence of rotavirus infection. Furthermore the prevalence of rotavirus infection in both hospitals was similar; therefore the prevalence found in the present study might be representing the correct situation in Turkey. Similar to the present study, in the Kurdistan region of Iraq 37% of diarrhea is caused by rotavirus [Ahmed et al., 2006]. There, serotype G1 is accounted for 38% of the infection followed by G4 (20%) and G9 (11%) [Ahmed et al., 2006]. In Iran 24.5–35% of the diarrhea is caused by rotavirus [Khalili et al., 2004; Farahtaj et al., 2007]. Similar to the present study the most prevalent serotype in Iran is G1P[8](59.2%) followed by G9P[8] (15.5%)[Farahtaj

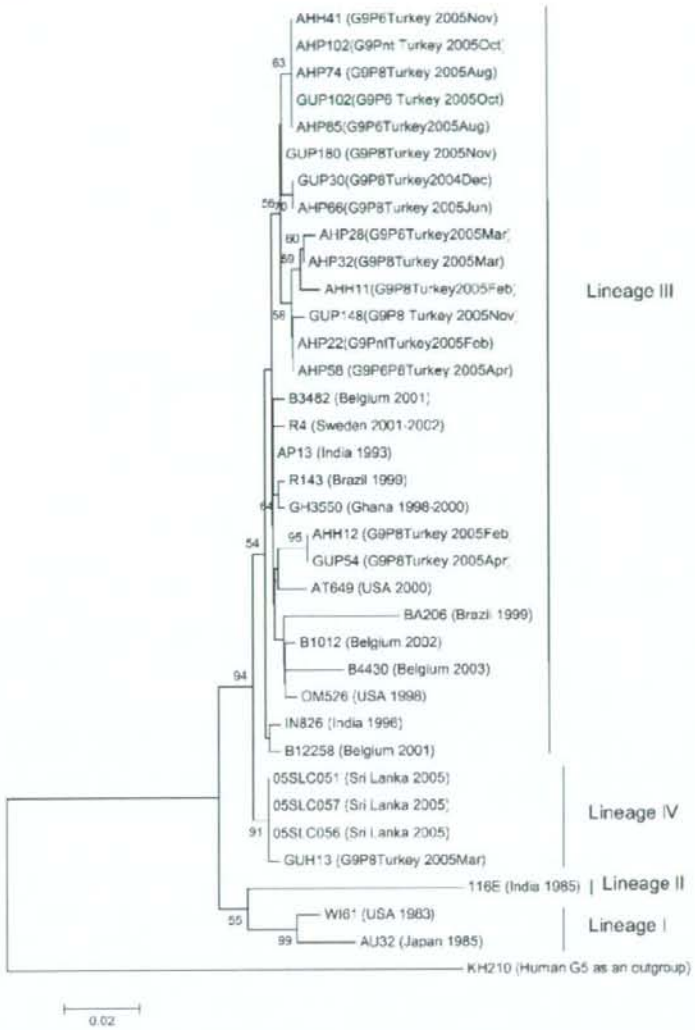

Fig. 3. Phylogenetic tree constructed with the deduced amino acid sequences of the VP7 gene of G9 strains from Turkey and global G9 strains. Human rotavirus KH210 (G5) was used as an outgroup. The number adjacent to the node represents the bootstrap value and values lower than 50% have not been indicated. Scale bar shows genetic distance expressed as amino acid substitutions per site.

et al., 2007]. A previous study in Iran also found G1 as the dominating serotype which was present in 82% of the samples followed by G2(13%) and Gnt (5%) [Khalili et al., 2004]. Of note, although in one sample only G12 was detected in Iran [Farahtaj et al., 2007], this serotype has not yet been detected in Turkey. Serotype G12 is emerging in several Asian countries. In Europe G12 has been reported from Slovenia [Steyer et al., 2007] and Hungary [Banyai et al., 2007].

In a previous study, serotype G9 was detected sporadically in only four samples out of 119 rotavirus-positive samples collected from nine different areas of Turkey during 2000–2002 [Cataloluk et al., 2005]. Possibly G9 rotaviruses emerged as a significant pathogen in Turkey during our study period. Compared to other countries this is a delayed emergence of G9 strain to cause significant number of infection. The earlier study from Turkey [Cataloluk et al., 2005] did not

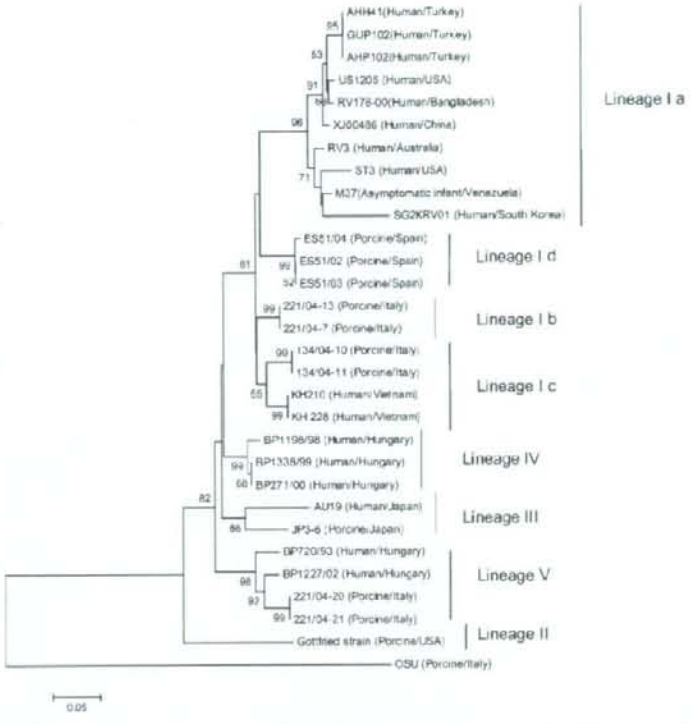

Fig. 4. Phylogenetic tree constructed with the deduced amino acid sequences of the VP8* gene of G9P[6] strains from Turkey and global P[6] strains. Porcine rotavirus OSU was used as an outgroup. The species and country of origin are shown in parentheses after the strain name. The number adjacent to the node represents the bootstrap values and values lower than 50% have not been indicated. Scale bar shows genetic distance expressed as amino acid substitutions per site.

mention the sites of sample collection and the region where serotype G9 rotaviruses were detected. In the present study the first case of G9 was detected in December 2004 in a 12 months old boy at Gazi University outpatient clinic. Comparing the electropherotype of this strain with other Turkish G9 rotaviruses showed that the strain spread rapidly and established itself in the population. Both in the United Kingdom and Ghana, G9 strains were reported to be emerged in sequential manner, G9 strains were first seen in one center and then spread to other [Armah et al., 2003]. If hospitalization is used as a proxy for the severity of rotavirus diarrhea we did not find that G9 rotaviruses cause more severe illness. This is consistent with the findings in Italy and the US where there was no difference in disease severity between patients with serotype G9 infection and patients with infection due to other G types [Arista et al., 1997; Clark et al., 2004].

Thus far, rotavirus serotype G9 has been associated with the VP4 type P[4], P[6], P[8], P[11], or P[19] [Martella et al., 2003]. Analysis of a global collection of G9 viruses showed that strains from different geographic locations may share the same constellation of genes and be virtually identical, while strains from the same country may have a highly conserved VP7 gene but a diverse assortment of other genes [Ramachnadran et al., 2000]. The P[8] specificity is thought to be a feature of the G9 strains of industrialized countries which are genetically stable because of less frequent reassortment events [Rahman et al., 2005]. In developing countries like India, Bangladesh and African countries, both G9P[6] and G9P[8] strains have been co-circulating because of frequent reassortment between different strains and by a high percentage of mixed infections in these countries [Rahman et al., 2005]. Several Turkish G9 strains were in combination with P[6], P[4], or P[nt] and mixed specificities. Although these combinations were also observed in G1 strains however the proportion was significantly less than in G9 strains. The association of G9 strains with mixed P type supports the idea that these strains display a "promiscuous" nature and reassort frequently. An earlier study [Cataloluk et al., 2005] did not find G9P[4] or G9P[6]. This finding may suggest either a recent reassortment, resulting in the emergence of strains in combination with different P types or a recent

invasion of strains with such combination. The VP4 type P[6] cause infection in both humans and pigs and, in the phylogenetic tree, Turkish P[6] strains clustered with the strains derived from human VP4. The human type has been reported to be originated from pigs [Banyai et al., 2004b]. Compared to India and Bangladesh [Unicomb et al., 1999; Jain et al., 2001], in Turkey there were less number of mixed infections with different serotypes.

In the UK and Nigeria G9P[6] strains with short electropherotype have been reported [Cubitt et al., 2000; Steele et al., 2002]. The genomic pattern of the dsRNA of the Turkish G9P[6] were not visible in PAGE therefore the electropherotype remains unknown. Since all the samples were stored in same conditions. Therefore, the lack of visualization in PAGE was possibly not due to the degradation rather indicates that there were less number of virus particles in the stool.

The G9 rotaviruses identified from different countries exhibited a great variety of genomic constellations, formed predominantly by reassortment of the VP7 and VP4 genes into both 'long' and 'short' electropherotype strains [Banyai et al., 2004a]. For example G9P[4] 'short' electropherotype strains were identified in Thailand; G9P[4] 'long' electropherotype strains were found in Brazil; G9P[6] 'short' electropherotype strains were detected in the USA, India, and Bangladesh; G9P[6] 'long' electropherotype strains were detected in India, Bangladesh and Kenya; and G9P[8] 'long' electropherotype strains circulated in the USA, Bangladesh, Libya, Cuba, Kenya, India, and Japan [Unicomb et al., 1999; Griffin et al., 2000; Oka et al., 2000; Ramachnadran et al., 2000; Araujo et al., 2001; Cunliffe et al., 2001; Jain et al., 2001; Zhou et al., 2001]. The present study identified a G9P[8] of 'short' electropherotype which to our knowledge is first reported in the world. The VP7 of this strain formed the new lineage with the Sri Lankan G9 strains which were of 'long' electropherotype [our observation].

Based on the amino acid sequences, G9 strains are divided phylogenetically into three lineages [Stupka et al., 2007]. Lineages I and II contain the sporadically detected strains of the 1980s and Lineage III contains the globally distributed modern lineage of the 1990s. The VP7 gene of most of the Turkish G9 strains were clustered in a large group with different VP4 combination and with different geographical origin encompassing Asian, North and South American, African and European contemporary G9 strains of lineage III. The G9 Turkish strains in this cluster were related to each other more closely than to those of other strains. Another group containing two strains clustered not with the largest group but with an American strain. The nucleotide sequences of the VP7 gene of earlier scattered Turkish G9 is not available in the GenBank, therefore it is difficult to conclude whether those strains spread or new varieties were introduced.

The divergence of the Turkish G9 strains suggests that all of the Turkish G9 strains were not evolved locally from one progeny but from different progenies which possibly occurred due to multiple introductions of G9 strains. The combination of evolution and reassortment of rotaviruses after its introduction to Turkey resulted in a distinct phylogenetic pattern. The segregation of new lineage was not evident with viruses from other parts of the world but with Sri Lankan rotaviruses. How spread of rotavirus in Turkey shaped its evolution deserves further studies.

Neutralizing antibody against the VP7 protein provide immunity for rotavirus infection [Chiba et al., 1986], the high identities among the VP7 protein of the Turkish G9 strain indicate that antibodies generated during the first infection by any of these strains may prevent subsequent infection by other strains of same serotype. The current rotavirus vaccines carry neither the G9 nor P[6] epitopes. It is imperative that surveillance of rotavirus continues to monitor effectively the emergence and spread of strains with unusual G and P type combinations. Since the current rotavirus vaccine was not designed for G9 strains there is a concern whether introduction of the vaccine will increase selectively G9 strains.

In conclusion, it was found that genetically diverse strains of serotype G9 rotavirus are emerging in Turkey. The epidemiology of rotavirus infection in Turkey has components similar to that of developing countries such as increase diversity of G/P serotype combinations and the presence of infection throughout the year. On the other hand occurrence of rotavirus infection at later age and low level of mixed infection with different serotypes are similar to the epidemiology of rotavirus infection of the developed countries. Relative late spread of serotype G9 may be a characteristic of rotavirus infection in Turkey. Surveillance is continuing to find out whether similar phenomenon also occurs in the case of the G12 strains.

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Norovirus Gastroenteritis Among Children in Iraqi Kurdistan

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Of 260 children under age 5 years who were hospitalized with acute gastroenteritis in Kurdistan, Iraq, between April and May 2005, 78 (30%) tested positive for norovirus by RT-PCR. These comprised genogroups GI (23%), GII (74%) and GI + GII (3%). Among 28 noroviruses sequenced, GII/4 was the predominant genotype. ***J. Med. Virol. 80:506–509, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: pediatric; diarrhea; norovirus; genotype

INTRODUCTION

Acute gastroenteritis is a leading cause of childhood morbidity and mortality among children in developing countries, and causes an estimated 2.5 million deaths each year in children less than 5 years of age [Kosek et al., 2003]. While rotavirus is firmly established as the major cause of severe gastroenteritis in infants and young children [Parashar et al., 2006], norovirus has gained increasing recognition as an important cause of severe gastroenteritis in this age group [Moreno-Espinosa et al., 2004]. Appreciation of the role of noroviruses in pediatric gastroenteritis has been facilitated by molecular methods which have enabled the detection of an expanding diversity of noroviruses in stool samples [Kojima et al., 2002].

Norovirus comprises a genus within the family *Caliciviridae*, and has a positive-sense, single stranded RNA genome ≈7.5 kb in length. Within the genus, two major genogroups, genogroup I (GI) and genogroup II (GII) are recognized among noroviruses recovered from humans, based on the sequences of the polymerase and capsid regions [Ando et al., 2000; Kageyama et al., 2004].

Within each genogroup, noroviruses are further divided into genetic clusters (or genotypes), and strains. While the genotype designation is yet to be agreed upon, classification and strain nomenclature has been proposed based upon the complete amino-acid sequences of the capsid region [Zheng et al., 2006].

In Iraq, as in many other developing nations, while diarrhea is a major cause of illness and death among children, little etiological information is available. We therefore carried out a survey to examine the burden of rotavirus diarrhea and the diversity of rotavirus strains among children admitted with acute gastroenteritis to Erbil Paediatric Hospital, Iraqi Kurdistan [Ahmed et al., 2006]. The current study was undertaken to complement the previous survey by examining stored stool specimens for the presence of noroviruses with a broadly reactive reverse-transcription (RT)-PCR assay [Kojima et al., 2002], and by determining the diversity of norovirus strains circulating in northern Iraq. While norovirus has been detected among U.S. and British troops in the country [Bailey et al., 2005; Thornton et al., 2005], to our knowledge this is the first report describing norovirus infections in Iraqi children.

MATERIALS AND METHODS

Study Population and Sample Collection

A total of 260 stool specimens were collected between April and May 2005 from children less than 5 years of age who were admitted with acute gastroenteritis (defined as three or more passages of liquid or semi-liquid stool within the preceding 24 hr) to the Emergency ward of Erbil Paediatric Hospital, Iraqi Kurdistan. Demographic and clinical data were collected through administration of a questionnaire [Ahmed et al., 2006]. All samples were frozen at −80°C until testing at the University of Liverpool, UK. Rotavirus detection and strain characterization in all samples was undertaken previously and the results have been published elsewhere [Ahmed et al., 2006].

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