**Introduction**

Rotavirus, the most common cause of severe diarrhea in infants and young children, remains a significant cause of morbidity and mortality worldwide [[1](#_ENREF_1)]. In 2010, ~200,000 global diarrhea deaths among children 5 years of age and younger were attributed to rotavirus, with ~25% occurring in Southeast Asia [[1](#_ENREF_1)]. In Bangladesh, single-site studies have demonstrated a considerable burden of rotavirus on healthcare systems; rotavirus was the etiology of gastroenteritis in 20% of children seeking care in a diarrhea-treatment facility in urban Dhaka [[2](#_ENREF_2)], and in 33% of the hospitalizations due to gastroenteritis in rural Matlab [[3](#_ENREF_3)].

Two live attenuated oral rotavirus vaccines, RotaTeq (RV5; Merck, Whitehouse Station, NJ, USA), a pentavalent (G1, G2, G3, G4, P[8]) bovine-human reassortant vaccine, and Rotarix (RV1; GSK Biologicals, Rixensart, Belgium), a monovalent (G1P[8]) human strain vaccine, are currently recommended by the World Health Organization (WHO) for introduction into national immunization programs worldwide [[4](#_ENREF_4)]. A key factor in the decision by countries to introduce rotavirus vaccines has been demonstrating high rotavirus disease burden through establishment of surveillance for rotavirus diarrhea. In addition, pre-vaccine data on disease burden has been crucial for monitoring vaccine impact after vaccine introduction. Assessing vaccine impact in resource-limited settings is particularly important given the lower efficacy of RV1 and RV5 shown in clinical trials in Africa and Asia, compared with that seen in Europe and America [[5-10](#_ENREF_5)].

In anticipation of introduction of a rotavirus vaccine into the routine immunization schedule in Bangladesh, this study aims to provide baseline data on the burden of rotavirus gastroenteritis and circulating strains at sentinel hospitals nationwide. Because the most recent verbal autopsy data from the Bangladesh Demographic and Health Survey noted an 85% reduction in diarrhea-specific mortality between 2004 and 2011 (from 7 to 1 per 1,000 live births) [[11](#_ENREF_11)], we specifically aimed at measuring the contribution of rotavirus to healthcare utilization.

**Methods**

Starting in July 2012, an active hospital-based rotavirus surveillance system, consisting of seven tertiary hospitals located in all seven divisions of Bangladesh (Figure 1), was initiated in three phases. These hospitals were chosen because they have a high number of pediatric gastroenteritis admissions each year. Rotavirus surveillance was started at the Dhaka, Rajshahi, and Sylhet sites in July 2012, extended to Chittagong and Rangpur in February 2013, and further extended to Khulna and Barisal in August 2013. Surveillance followed the WHO protocol for rotavirus surveillance in hospital settings [[12](#_ENREF_12)]. At each hospital, from 8:30 am to 4:00 pm each day (except for weekends and holidays), field assistants identified children 5 years of age and younger admitted to pediatric wards with diarrhoea by reviewing admission logbooks and screened them for acute gastroenteritis (AGE) symptoms. AGE was defined as the occurrence of ≥3 watery or looser-than-normal stools or ≥1 episode of forceful vomiting within a 24-hour period, with symptoms lasting ≤7 days. Surveillance physicians enrolled every 4th child listed who met the surveillance case definition. Compared to other sites, diarrhea-associated hospitalizations in Rangpur were low in number so, only at this site, the protocol was modified after 3 months to enroll every child admitted with AGE. Surveillance physicians collected demographic and clinical information of enrolled children from the parents and hospital records using a standard questionnaire and assessed the extent of dehydration following clinical criteria in WHO diarrhea treatment guidelines [[13](#_ENREF_13)]. In addition, discharge and death logbooks were reviewed to ascertain the outcome of children admitted with diarrhoea but not enrolled in the surveillance.

Field assistants collected bulk stool specimens (4 mL) from each child on the day of enrollment and immediately stored specimens in a -70°C liquid nitrogen dry shipper after collection. The study team shipped stool samples in these containers to the icddr,b virology laboratory in Dhaka every two weeks, where a commercially available enzyme-linked immunoassay (EIA) (Prospect™, Oxoid Diagnostics Ltd, United Kingdom) was used to test for rotavirus antigen. At icddr,b, stool samples were stored at -70°C. Every three months, G and P genotyping of ~25% of rotavirus positive specimens were done using methods described previously [[14](#_ENREF_14)]. EIA, genotyping and sequencing has been done at icddr,b virology laboratory in Dhaka.

We obtained written informed consent from the enrolled children’s parents or guardians. The study protocol was reviewed and ethics approval obtained from the ethical review committee of icddr,b.

We calculated the overall and site-specific proportions of rotavirus-associated AGE hospitalizations by dividing the number of rotavirus-positive stools by the total number of samples collected and tested for rotavirus. To estimate the number of rotavirus-associated hospitalizations, the overall proportion of rotavirus-associated AGE hospitalizations was applied to the total number of AGE admissions during the surveillance period. We compared clinical and demographic characteristics and outcomes between children testing positive versus negative for rotavirus. This included an assessment for differences in clinical severity of AGE between the two groups, using the 20-point Vesikari scale; illnesses with scores <7 were classified as mild, illnesses with scores ≥7 and ≤10 as moderate, and illnesses with scores ≥11 as severe. [[15](#_ENREF_15)] Proportions were compared using χ2 and Cochran-Armitage trend tests, and continuous variables were compared using the Wilcoxon rank sum test.

**Results**

During 212 hospital months of surveillance, 129,156 children 5 years of age and younger were admitted to the pediatric wards of participating hospitals, of whom 14,814 (12%) were hospitalized with AGE. Surveillance staff collected clinical data and stool specimens from 26% (3,783/14,814) of children admitted with AGE (Table 1). EIA testing identified rotavirus antigen in the stools of 64% (2,432/3,783) of enrolled children; detection rates varied by site from 59% to 69% (Table 1). Extrapolation of rotavirus-testing results to the untested AGE cases yielded an estimated total of 9,728 rotavirus AGE admissions. Thus, about 8% (9,728/129,156) of all pediatric admissions at participating hospitals were attributable to rotavirus-associated AGE during the study period.

Nearly all (96%) of the rotavirus hospitalizations were among children <2 years (Figure 2), with 57% occurring during the first year of life. Rotavirus hospitalizations occurred year-round, with rotavirus accounting for >10% of AGE admissions during every month of the year. Overall, rotavirus was detected in >80% of AGE cases during the rotavirus peak season (November-February) (Figure 3). There were no differences in the seasonal patterns among surveillance sites (data not shown).

The median Vesikari score among children with rotavirus-confirmed AGE was slightly higher for those with compared to those without laboratory-confirmed rotavirus infection (12 vs. 11; *P*<.001). The duration of illness before hospitalization was longer for children with rotavirus infection compared to children who did not have evidence of rotavirus infection (3 vs. 2 days; *P*<.001), but that there were no differences in the length of hospital stay (2 days) (Table 2). Among the children enrolled over the study period, 8 (0.25%) died in the hospital. Rotavirus was detected in the stool of 50% (4/8) of those who died from AGE, and three of the four rotavirus-associated deaths occurred in children aged 6-23 months (the remaining death was in a 3-month old infant). According to discharge and death logbooks, 104 children were admitted with diarrhea and died in hospital during the study period; 69% were not be screened or enrolled because they were admitted after surveillance hours, or because death occurred soon after arrival at the hospital (Table, Supplemental Digital Content 1, <http://links.lww.com/INF/C564>)

Genotyping was performed on 22% (543/2,432) of positive rotavirus stool samples (Table 3). Four G genotypes (G1, G2, G9 and G12) and three P genotypes (P4, P6 and P8) were identified. G12 (40%) and G1 (32%) were the most prevalent G types, and P[8] (76%) and P[6] (12%) were the most prevalent P types. G1P[8] (31%) and G12P[8] (29%) were the most commonly identified strains. Mixed rotavirus strains were seen in 13% of the children; 5 (1%) of specimens were untypeable. All detected G and P genotypes were observed in sites across the seven divisions of Bangladesh.

**Discussion**

This study confirms the considerable burden of rotavirus AGE among children 5 years of age and younger in Bangladesh. We found that rotavirus accounted for ~64% of all childhood hospitalizations for AGE or ~8% of all pediatric hospitalizations at participating hospitals during the study period. Rotavirus was detected year-round among children with AGE, and during the peak winter months from November through February, the detection rates of rotavirus among children hospitalized with AGE exceeded 80%. Children with rotavirus AGE had illness that was somewhat more severe than those with AGE from other causes. Children aged 6-23 months accounted for ~85% of all rotavirus AGE hospitalizations, indicating that rotavirus vaccines, which are administered in the first few months of life, have the potential to reduce the burden of rotavirus AGE substantially. Collectively, our findings demonstrate the potential value of rotavirus vaccination in reducing the tremendous morbidity from rotavirus AGE in Bangladeshi children.

Earlier single-site studies in Bangladesh have reported lower rotavirus prevalence in children admitted for AGE, ranging from 20% to 42% [[2](#_ENREF_2), [3](#_ENREF_3), [16](#_ENREF_16), [17](#_ENREF_17)]. The higher prevalence estimate for rotavirus we report may be attributed to a variety of factors, including the types of healthcare facilities where surveillance took place (e.g., urban vs. rural, referral vs. primary hospitals), as well as differences in laboratory procedures (PCR, EIA) and surveillance methodologies (case definitions, sample of children tested). Perhaps more importantly, improvements in sanitation and hygiene in Bangladesh [[18](#_ENREF_18)] may have disproportionately reduced the bacterial and parasitic causes of diarrhea (which are mainly transmitted through contaminated food and water), and may have had less of an impact on rotavirus diarrhea (which is largely spread person-to-person), resulting in an increase in the proportion of diarrhea attributable to rotavirus [[19](#_ENREF_19), [20](#_ENREF_20)]. This is supported by data from a longitudinal evaluation of hospitalizations data from icddr,b showing that the proportion of diarrhea cases attributable to rotavirus nearly doubled during 2002-2004 compared with 1993-1995 (42% versus 22%)[[21](#_ENREF_21)].

This study demonstrated substantial rotavirus strain diversity within Bangladesh. As opposed to previous years (1991-2012), when G1, G2, and G9 were the predominant strains detected in Bangladesh [[22](#_ENREF_22), [23](#_ENREF_23)], G12 was also commonly detected genotype in the last two and a half years, consistent with the recent global spread of this emergent genotype [[24](#_ENREF_24)]. We found that 31% of detected strains were homotypic to strains in both vaccines (i.e., matched in both the G and P types), 44% were partially heterotypic (matched only the G or P type), and 12% were fully heterotypic (i.e., did not match the G or P type). In addition, ~14% of the strains were mixed or untypeable using current primers. Although both natural and vaccine-induced protection against a range of genotypes has been shown in developed settings [[25](#_ENREF_25), [26](#_ENREF_26)], the considerable variability in circulating rotavirus strains in Bangladesh warrants an assessment of the effect prevalent and mixed genotypes may have on the overall effectiveness of the vaccine after it is introduced into Bangladesh’s immunization schedule, as well as close monitoring of the impact the vaccine could have on strain selection and the incidence of locally circulating strains.

Some limitations should be considered. First, because the surveillance system is based in tertiary hospitals, we likely captured a select group of children with severe disease, and the etiologic distribution of diarrheal illnesses may be different in other hospital settings in Bangladesh (e.g., in urban and primary care centers). However, at the same time, our surveillance systematically excluded children who died with diarrhea because these children were often admitted after-hours or died quickly after admission. Because of this, suggesting that our approach is not well-suited for studying deaths from rotavirus, and our data likely underestimate the total number of rotavirus related deaths, as well as case-fatality ratios. Second, year-to-year variation in rotavirus activity is known to occur, and the proportion of diarrhea attributable to rotavirus depends on the prevalence of other enteric pathogens and health-seeking behaviors and thus we may have captured a period of high rotavirus activity. However, rotavirus prevalence estimates were consistent across years and settings. Third, because controls were not included in this evaluation, we may have overestimated the proportion of cases attributable to rotavirus. However, any overestimation is expected to be small, as EIA rarely detects asymptomatic infections, and the attributable fraction of rotavirus detection with EIA has been shown to be >90% [[27-29](#_ENREF_27)].

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Legend for figure #2

* RV-positive children
* Cumulative%

Legend for figure #3

* RV-positive
* RV-negative
* Proportion of RV-positive children(%)
* Annualproportion(%)