Burden of Rotavirus-Associated and Non-Rotavirus-Associated Diarrhea among Nonhospitalized Individuals in Central Italy: A 1-Year Sentinel-Based Epidemiological and Virological Surveillance

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A community sentinel pediatrician–based epidemiological and virological surveillance study was conducted to estimate the incidence of gastroenteritis and laboratory-confirmed rotavirus-associated disease. The 1-year cumulative incidence of gastroenteritis in the cohort of children aged 0–5 years was 21%, with the highest rates in the 7–12-month and 13–18-month age groups (41.1% and 41.7%, respectively). Approximately one-third of gastroenteritis cases requiring an office visit or telephone consultation were attributable to rotavirus infection.

Rotavirus infection is the most common cause of gastroenteritis in infants and young children. According to recent estimates, >600,000 deaths attributable to rotavirus infection occur annually among children <5 years of age, with the majority of the deaths occurring in developing countries [1]. In developed countries, the public health burden is measured in infant hospitalizations, health care costs, and loss of time from work for the parents, and the epidemiological and virological picture is mainly based on data and specimens collected from hospitalized children [2–6]. Although it is clear that hospitalizations account for significant health care costs, little is known about rotavirus disease among children who do not require hospitalization. Nevertheless, the majority of gastroenteritis cases are managed by pediatricians during office visits or by telephone consultation.

To estimate the incidence of gastroenteritis and laboratory-confirmed rotavirus-associated disease in the community, we conducted a 1-year sentinel-based surveillance of a cohort including children who were 0–5 years of age living in a medium-sized town in Italy. The epidemiological, virological, and clinical features of gastroenteritis were characterized, including morbidity rates by age group, temporal distribution, genotype distribution of rotavirus strains, and presenting symptoms.

Patients, materials, and methods. In Italy, primary health care is provided by community pediatricians who monitor physical and psychosocial growth and development, promote age-appropriate screening, establish the first contact with the patient for diagnosis and treatment of acute and chronic disorders, and coordinate the management of health problems requiring multiple professional services. Each pediatrician surveys ~800 children who range in age from newborns to 14 years. Community-based prospective surveillance was conducted by an ad hoc sentinel-based network, including 10 primary care pediatricians who surveyed 3611 children who were 0–5 years of age. When stratified by age range, the number of children in each age group was as follows: 0–12 months of age, 754; 13–24 months of age, 714; 25–36 months of age, 714; 25–36 months of age, 610; and >36 months of age, 1533. The cohort of 3611 children was recruited from a medium-sized urban area in central Italy (Leghorn, Italy) during April 2005–April 2006. Participating primary care pediatricians were randomly selected from among members of the Italian Federation of Paediatricians working in the Leghorn area. The study population corresponds to 24% of the entire district population of children aged 0–5 years. Pediatricians reported on a weekly basis to the surveillance center the number of new cases of gastroenteritis, defined as the occurrence of ≥3 watery stools within a period of 24 h [7]. New cases included cases in patients who required ambulatory or domiciliary visits or telephone consultations. Every sentinel transmitted demographic data, familial status (i.e., whether the patient had siblings), child care facility or preschool attendance, presenting symptoms, and presence of dehydration in new cases. Dehydration was defined as the presence of ≥3 signs or symptoms, including impairment of general condition (e.g., lethargy, drowsiness, limp, and cyanotic extremities), quality of radial pulse (weak, thread, or feeble impalpable), quality of respiration (deep or rapid), skin elasticity (pinch retract slowly or very slowly), the appearance of the eyes...
FIGURE 1. Weekly incidence of gastroenteritis (GE) and rotavirus-positive samples according to genotype

(sunken or very sunken) and mucous membrane (dry or very dry), and reduced presence of tears and urine output reported by parents [8]. Furthermore, pediatricians asked each parent for a stool sample within 6 days after clinical onset (mean interval between clinical onset and stool sample collection ± SD, 2.8 ± 1.8 days).

One stool sample was collected from each child with gastroenteritis, was stored at −20°C, and was sent to the Department of Health Sciences, University of Genoa (Genoa, Italy), where all specimens were tested for rotavirus using a real-time PCR (Fastset Rotavirus; Arrows Diagnostics). Samples with positive results were P- and G-genotyped using a primer-specific semi-nested multiplex PCR, as previously described and recently modified [9].

**Statistical analysis.** The χ² test was used for the comparison of frequencies. Logistic regression was performed to evaluate the relationship between rotavirus positivity and certain epidemiological features (e.g., age, presence of siblings, and attendance of child care facility or preschool) and presenting clinical symptoms of gastroenteritis that were found to be statistically significant in univariate analysis.

**Results.** A total of 684 children (18.9%) in the cohort presented with 757 episodes of diarrhea. During the 1-year surveillance period, 620 children had 1 episode, 55 had 2 episodes, and 9 had 3 episodes. Ten children were ultimately hospitalized for gastroenteritis. The 1-year cumulative incidence of gastroenteritis in the cohort of children who were 0–5 years of age was 21%, with a higher rate in the 0–24-month age group (32.9%) than in the 25–71-month age group (12.6%; P < .01). In particular, the cumulative incidence of gastroenteritis was higher in the 7–12-month age group (41.1%) and the 13–18 month age group (41.7%), and it decreased progressively in the older age groups (cumulative incidence of 33.2% in the 19–24-month age group, 20.3% in the 25–30-month age group, and 15.3% in the 31–36-month age group), reaching rates of 9%–14% among subjects >37 months of age. Low cumulative incidence (17.5%) was observed in the 0–6-month age group. The incidence of gastroenteritis by age group (0–24 months of age vs. 25–71 months of age) and virological data, including rotavirus-positive samples and genotyping characterization, are reported in figure 1.

With respect to temporal distribution, a peak in gastroenteritis incidence was observed between week 14 (4 April) and week 20 (22 May) of 2005, with twice the relative mean weekly incidence, compared with that for summer and autumn of 2005 and winter of 2005–2006. In fact, for children who were 0–24 months of age, the mean weekly incidence was 12.4 cases per 1000 children-weeks during the period of peak incidence and decreased to 5.3 cases per 1000 child-weeks during weeks 21–50 (23 May–18 December) of 2005 and to 4.9 cases per 1000
child-weeks during the period from week 51 of 2005 through week 14 of 2006. For children 24–71 months of age, the weekly incidence was 6.4 cases per 1000 child-weeks during the period of peak incidence, 1.7 cases per 1000 child-weeks in the summer and autumn of 2005, and 1.8 cases per 1000 child-weeks in the winter of 2005–2006.

Stool samples were collected for 79.7% of the episodes of diarrhea. Of these stool samples, 2 were not sufficient for RNA extraction; therefore, 595 (99.7%) of the specimens were tested. A total of 203 (34.1%) of 595 specimens had test results positive for rotavirus (95% CI, 0.3%–37.9%). Approximately one-third of collected samples had test results that were positive for rotavirus in each 6-month age group (range, 28.1%–37.3%), with the exception of the 0–6-month age group, which had the lowest proportion of rotavirus-positive samples (22.2%), and the 31–36-month and 37–42-month age groups, which had the highest proportions of rotavirus-positive samples (47.2% and 43.3%, respectively). Of 203 rotavirus-positive samples, 148 (72.9%) were typeable; the most prevalent types identified included P[8]G9 (42.6% of the typeable samples), P[8]G1 (16.2%), P[8]G3 (14.2%), and P[8]G8 (8.8%). Less common types included P[4]G2, P[8]G4, and P[8]G10, which were found in 5.4%, 5.4%, and 2.7% of rotavirus-typeable samples, respectively. Seven (4.7%) of the 148 typeable rotavirus-positive samples revealed mixed infection (G1 plus G9 and G1 plus G3).

During the peak period of gastroenteritis incidence, the mean (± SD) number of rotavirus-positive samples collected was 15.6 ± 5.6, compared with 1.4 ± 1.6 and 3.3 ± 2.2 rotavirus-positive samples registered in summer and autumn 2005 and winter 2005–2006, respectively. The strain distribution did not show statistically significant variations during the surveillance period, although the proportion of P[8]G9 strains appeared to be higher during the peak period of the epidemic (53.4% of typeable samples) than during summer and autumn 2005 (33.3%) and winter 2005–2006 (28.1%); likewise, P[1]G8 circulated more frequently during winter 2005–2006 (31.3% of typeable samples) than during the previous periods (week 14–20 2005, 11.4%; week 21–50 2005, 19%).

With respect to the demographic and epidemiological features presented in table 1, patients with rotavirus-positive gastroenteritis attended child care facilities more frequently than did children with rotavirus-negative gastroenteritis (39.4% vs. 24.4%; P < .01, by χ² test); other factors, such as age, preschool attendance, and number of siblings, did not seem to be associated with rotavirus positivity.

Fever and dehydration at presentation were more frequently associated with rotavirus-associated gastroenteritis than with non–rotavirus-associated gastroenteritis using both univariate analysis (fever, 31.8% vs. 56.2%; P < .01; dehydration, 9.7% vs. 18.7%; P < .01) and multivariate analysis. Fever and dehydration at initial presentation were associated with a higher likelihood of rotavirus gastroenteritis, with ORs of 2.6 (95% CI, 1.8–3.7; P < .02) and 1.8 (95% CI, 1.1–3; P = .02), respectively. Other clinical features at presentation, such as abdominal pain (51.6% in rotavirus-positive cases vs. 46.9% in rotavirus-negative cases), number of stools (mean number of stools ± SD, 5.1 ± 1.7 vs. 4.6 ± 1.5), and presence of blood (0.5% vs. 1%) and/or mucus (11.8% vs. 10.3%) in stools, were not significantly different between rotavirus-positive and rotavirus-negative patients (table 1).

**Discussion.** By coupling a sentinel pediatrician–based epidemiological surveillance for the detection and documentation of gastroenteritis cases with a virological surveillance for laboratory confirmation of the etiology and characterization of rotavirus, our study defined the impact on pediatric practices...
of rotavirus-associated and non–rotavirus-associated gastroenteritis in the community. Some potential limitations of this investigation should be mentioned. First, the study focused on only a single health care setting (primary care facilities) and disregarded the burden of gastroenteritis on hospitals and emergency departments. Second, only children seeking health care were identified, resulting in an underestimation of incidence rates and in potential bias (because study selection may have been influenced by factors such as the socioeconomic status of the parents or the severity of the child’s disease).

In our study, one-third of infants and one-fifth of children who were 0–5 years of age required at least 1 visit or contact with pediatricians for gastroenteritis. The burden was particularly heavy during the period of peak incidence in the spring, which lasted only 7 weeks, when ~9% of the infants in our study had gastroenteritis. The seasonality of gastroenteritis, characterized by peak incidence in the first weeks of spring, was also observed in other studies performed in Italy or in countries with a similar climate, such as France [10]. Comparing the impact of gastroenteritis with that of another syndrome that dramatically affects infants and young children, such as influenza-like illness, the cumulative incidence of the former appears to be higher than the annual incidence of influenza-like illness in children who were 0–4 years of age, which was estimated to be 15.7% during the past 3 seasons with use of a sentinel pediatrician approach [11]. The etiologic role of rotavirus among hospitalized children with gastroenteritis is relatively well-documented in high-income countries, such as Italy, causing ~44% (range, 40%–50%) of childhood diarrhea hospitalizations for childhood diarrhea [1]. In our community surveillance, approximately one-third of gastroenteritis cases requiring an office visit or telephone consultation were attributable to rotavirus infection, and the proportion of rotavirus-positive samples reached 53.9% and 49.1% during the 2005 spring peak and the winter of 2004–2005, respectively (data not shown). In contrast with the findings of several other investigators [12–14], the proportion of rotavirus-positive samples did not tend to decrease among children >2 years of age and reached the highest rate among the 31–36-month and 37–42-month age groups. One possible reason for this persistently high incidence of rotavirus infection among children >2 years of age is that it could be related to the type of surveillance used—sentinel pediatrician–based surveillance, rather than hospital-based surveillance—leading to a relatively higher sensitivity in case detection, skewed towards the recording of mild cases and, thus, taking into account gastroenteritis occurring in relatively older children, which generally does not require hospitalization. Overall, gastroenteritis due to rotavirus infection is associated with more-severe clinical presentations, compared with other forms of gastroenteritis, and is generally associated with higher rates of fever and dehydration, as has also been reported by others [15].

The virological characterization clearly pointed out 3 interesting facts: first, the persistent circulation of rotavirus outside of the typical epidemic season (e.g., summer and autumn); second, the high heterogeneity of strains during the 1-year surveillance period, with co-circulation of P[8]G1, P[8]G3, P[8]G8, and P[8]G9 genotypes; and third, the very high incidence of P[8]G9 infections attributable to strains belonging to a closely related genetic cluster that was distinct from the other G9 strains recently isolated in other European countries, America, and Asia [16]. During the same seasons, a higher incidence of infections due to G9 strains was observed in northern Italy (with G9 strains accounting for 84.4% of the typeable strains), and similar genotype distributions were reported in France (54.7%) and Sweden (38.2%) [10]. In other countries, such as Germany and the United Kingdom, and in southern Italy, G9 strains represented <15% of the typed strains [10, 17, 18].

In conclusion, sentinel pediatrician–based epidemiological and virological surveillance allowed us to estimate the impact of rotavirus-associated and non–rotavirus-associated gastroenteritis in terms of age-specific incidence and to define the high heterogeneity of circulating strains. These data contribute to better defining the epidemiological picture in a European country, fundamental information in light of the recent introduction of rotavirus vaccines, including a monovalent live attenuated vaccine and a pentavalent bovine-human reassortant vaccine. Continued surveillance will be crucial to further understand the mechanisms underlying immune protection against rotavirus infections and, in particular, the degree of homotypic and heterotypic protection conferred by vaccines.

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