Rotavirus Surveillance in Europe, 2005–2008: Web-Enabled Reporting and Real-Time Analysis of Genotyping and Epidemiological Data

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Background. The first European rotavirus surveillance network, EuroRotaNet, comprising 16 laboratories in 15 European countries, has been established.

Methods. Fecal samples from gastroenteritis cases positive for group A rotavirus antigen were collected from multiple European countries from 2005 to mid-2008 and were subjected to G and P genotyping. Epidemiological data collected included age, sex, geographical location, setting, dates of onset and sample collection, and clinical symptoms.

Results. A total of 8879 rotavirus-positive samples were characterized: 2129 cases were from the 2005–2006 season, 4030 from the 2006–2007 season, and 2720 from the ongoing 2007–2008 season. A total of 30 different G and P type combinations of strains circulated in the region from 2005 through 2008. Of these strains, 90% had genotypes commonly associated with human infections—G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]—and 1.37% represented potential zoonotic introductions. G1P[8] remained the most prevalent genotype in Europe as a whole, but the incidence of infection with G1P[8] rotavirus strains was <50% overall, and all 3 seasons were characterized by a significant diversity of cocirculating strains. The peak incidence of rotavirus infection occurred from January through May, and 81% of case patients were aged <2.5 years.

Conclusions. Data gathered through EuroRotaNet will provide valuable background information on the rotavirus strain diversity in Europe before the introduction of rotavirus vaccines, and the network will provide a robust method for surveillance during vaccine implementation.

The European Rotavirus Network (EuroRotaNet) was established in January 2007 to gather comprehensive information on the rotavirus genotypes cocirculating throughout Europe. Surveillance is achieved through the establishment of national networks for sampling and the use of standardized methods for strain characterization.

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Potential conflicts of interest: none reported.

The Journal of Infectious Diseases 2009; 200:S215–221

0022-1899/2009/20009S1-0028$15.00
DOI: 10.1086/605049

Financial support: The activities of EuroRotaNet are funded by the collaborating institutes and an unrestricted educational grant jointly from GlaxoSmithKline Biologicals and Sanofi Pasteur MSD. The grant is administered through the Centre for Infections, Health Protection Agency, London, United Kingdom; the World Health Organization; and the Rotavirus Vaccine Program, and representatives of the funding bodies and representatives of the study participants are represented on the EuroRotaNet steering committee.

Supplement sponsorship: This article was published as part of a supplement entitled "Global Rotavirus Surveillance: Preparing for the Introduction of Rotavirus Vaccines," which was prepared as a project of the Rotavirus Vaccine Program, a partnership between PATH, the World Health Organization, and the US Centers for Disease Control and Prevention, and was funded in full or in part by the GAVI Alliance.

The specific aims of EuroRotaNet are the following: (1) to develop methods and algorithms for effective rotavirus typing (G and P) and characterization (including VP6 and NSP4 genotypes); (2) to monitor the effectiveness of current genotyping methods and to respond to changes associated with genetic drift and shift; (3) to describe in detail the molecular epidemiology of rotavirus infections in Europe during consecutive rotavirus seasons, through genotyping of rotavirus-positive samples collected throughout each country; (4) to monitor the emergence and spread of novel rotavirus strains throughout Europe; and (5) to develop the infrastructure that may inform future surveillance activities and nested studies for evaluation of (a) the effectiveness of rotavirus vaccines in the general population, through monitoring the reduction in disease associated with common rotaviruses, and (b) the possible vaccine-induced emergence of antibody-escape mutants, genotypes other than those included in the vaccine, and/or reassortants of vaccine and naturally circulating wild-type strains. This article reports the data collected through EuroRotaNet to date.

MATERIALS AND METHODS

Study area. Currently, the EuroRotaNet database holds data from 19 European countries. In 2007, the study area consisted initially of 11 European countries, including Denmark, Finland, France, Germany, Hungary, Italy, The Netherlands, Slovenia, Spain, Sweden, and the United Kingdom. An additional 3 European countries, Belgium, Bulgaria, and Lithuania, joined in 2008, and Greece and Romania joined in January 2009. In addition, data from countries that are not formal members of EuroRotaNet, including Azerbaijan, Georgia, Portugal, Tajikistan, and Ukraine, were uploaded to the database. The database contains data from hospitalized cases and from cases in the community, collected from rural and urban populations. The minimum number of samples that each country needs to test to be able to detect strains circulating with an incidence of 1% has been calculated from data obtained in previous studies [1, 2] and has been set as a target for rotavirus characterization in each country.

Sample collection. Fecal samples submitted for investigation of gastroenteritis that tested positive for group A rotavirus antigen were collected for genotyping. Hospitals, pediatricians, and general medical practitioners in the community who were willing to participate in the surveillance over several years were identified in each of the participating countries to allow valid comparisons to be made between each rotavirus season and within each member country. The denominator of the number of children aged <5 years in each catchment area is provided whenever possible. Although rotavirus is investigated as a cause of gastroenteritis mainly in children aged <5 years, no age limit has been set. This is important because the emergence of novel strains is often associated with infection and disease in older individuals [1].

Epidemiological data. Data including age, sex, geographical location, setting (hospital or community), dates of onset and sample collection, and clinical symptoms (diarrhea and/or vomiting or other symptoms) were collected and linked to the genotyping data.

Genotyping and strain characterization. Rotavirus strains were genotyped to identify their G and P types [1, 2]. Rotavirus VP6 subgroups and NSP4 genotypes were determined for uncommon or novel strains identified through G and P typing, to identify possible zoonotic transmission [3]. The testing algorithm and genotyping methods are available on the EuroRotaNet Web site (http://www.eurorota.net/).

The majority of EuroRotaNet participants have ample experience with rotavirus characterization, and genotyping is performed by a designated laboratory in each country (there are 2 designated laboratories in Spain). Quality assurance was determined by circulation of blinded panels of well-characterized rotavirus strains to all participants on a yearly basis to ensure comparability of data.

Data sharing and analysis. A Web-accessible database (http://www.eurorota.net/) was developed by the Health Protection Agency to provide a central portal for dissemination of network information and for submission and interrogation of disparate surveillance data. The public interface provides unrestricted access to information on EuroRotaNet, as well as on rotavirus, rotavirus characterization methods, and relevant publications and links to other related sites of interest. Participants in the network have password-protected access to rotavirus strain types, epidemiological data, analysis tools, and a bulletin board to facilitate communication among network members. Analytical tools have been developed to query the online database, which allows real-time analysis of the distribution of rotavirus genotypes temporally, geographically (by country), or by age, sex, or setting (hospital or community). The data can be analyzed and compared according to calendar year or rotavirus season (September–August). Other tools, such as geographical analysis, are currently being developed.

RESULTS

The database contains 14,315 entries from a total of 19 European countries, and more than one-half (62%) of the entries are rotavirus strains collected from 2005 through 2008. Historic data go back to 1991 and are mostly from the United Kingdom. A total of 2129 cases were from the 2005–2006 season, 4030 were from the 2006–2007 season, and 2720 were from the ongoing 2007–2008 season (see Table 1 for a detailed breakdown of the countries included each season). Data from 2007–2008 were preliminary at the time that the manuscript of this

Table 1. Distribution of Rotavirus Genotypes in the EuroRotaNet Database during 3 Consecutive Rotavirus Seasons from 2005 through 2008 in 19 Countries

Strain group, genotype	No. (%) of strains for season			
	2005–2006[a] (n = 2129)	2006–2007[b] (n = 4030)	2007–2008[c] (n = 2720)	Total (n = 8879)
Common human strains	1939 (91.08)	3552 (88.14)	2514 (92.43)	8005 (90.16)
G1P[8]	922 (43.31)	**1686 (41.84)**	**1690 (62.13)**	4298 (48.41)
G2P[4]	**51 (2.40)**	**598 (14.84)**	**175 (6.43)**	824 (9.28)
G3P[8]	81 (3.80)	125 (3.10)	128 (4.71)	334 (3.76)
G4P[8]	**90 (4.23)**	**377 (9.35)**	**190 (6.99)**	657 (7.40)
G9P[8]	**795 (37.34)**	**766 (19.01)**	**331 (12.17)**	1892 (21.31)
Reassortants of common human strains	25 (1.17)	41 (1.02)	16 (0.59)	82 (0.92)
G1P[4]	2 (0.09)	13 (0.32)	8 (0.29)	23 (0.26)
G2P[8]	17 (0.80)	22 (0.55)	6 (0.22)	45 (0.51)
G3P[4]	1 (0.05)	1 (0.02)	0 (0)	2 (0.02)
G4P[4]	3 (0.14)	1 (0.02)	1 (0.04)	5 (0.06)
G9P[4]	2 (0.09)	4 (0.10)	1 (0.04)	7 (0.08)
Potential zoonotic strains	40 (1.88)	56 (1.39)	26 (0.96)	122 (1.37)
G1P[6]	3 (0.14)	0 (0)	0 (0)	3 (0.03)
G2P[6]	1 (0.05)	4 (0.10)	1 (0.04)	6 (0.07)
G3P[6]	1 (0.05)	0 (0)	0 (0)	1 (0.01)
G3P[9]	0 (0)	1 (0.02)	1 (0.04)	2 (0.02)
G4P[6]	6 (0.28)	5 (0.12)	0 (0)	11 (0.12)
G4P[14]	1 (0.05)	0 (0)	0 (0)	1 (0.01)
G6P[8]	0 (0)	0 (0)	2 (0.07)	2 (0.02)
G6P[9]	0 (0)	4 (0.10)	0 (0)	4 (0.04)
G6P[11]	0 (0)	1 (0.02)	0 (0)	1 (0.01)
G8P[4]	2 (0.09)	0 (0)	1 (0.04)	3 (0.03)
G8P[6]	0 (0)	4 (0.10)	1 (0.04)	5 (0.06)
G8P[8]	1 (0.05)	2 (0.05)	1 (0.04)	4 (0.04)
G8P[14]	2 (0.09)	0 (0)	0 (0)	2 (0.02)
G9P[6]	4 (0.19)	5 (0.12)	0 (0)	9 (0.10)
G9P[9]	0 (0)	1 (0.02)	0 (0)	1 (0.01)
G10P[6]	1 (0.05)	1 (0.02)	0 (0)	2 (0.02)
G10P[8]	2 (0.09)	0 (0)	2 (0.07)	4 (0.04)
G10P[14]	0 (0)	5 (0.12)	0 (0)	5 (0.06)
G10P[4]	0 (0)	1 (0.02)	1 (0.04)	2 (0.02)
G12P[6]	0 (0)	4 (0.10)	3 (0.11)	7 (0.08)
G12P[8]	16 (0.75)	18 (0.45)	13 (0.48)	47 (0.53)
Partially typed strains	100 (4.7)	150 (3.72)	104 (3.82)	354 (3.99)
Mixed types	25 (1.17)	231 (5.73)	60 (2.21)	316 (3.56)

NOTE. Data for 2007–2008 include only entries received up to the end of May 2008; entries for which epidemiological data were not available were excluded. Statistically significant ($P < .001$) changes in the genotype distribution from one season to the next are shown in bold font.

[a] Includes data from Bulgaria, Denmark, France, Germany, Lithuania, The Netherlands, Portugal, Slovenia, Spain, and the United Kingdom.

[b] Includes data from Azerbaijan, Bulgaria, Denmark, Finland, France, Georgia, Hungary, Italy, The Netherlands, Slovenia, Spain, Sweden, Tajikistan, Ukraine, and the United Kingdom.

[c] Includes data from Belgium, Denmark, France, Hungary, Italy, The Netherlands, Slovenia, Spain, Sweden, and the United Kingdom.

article was prepared, and we include entries submitted up to the end of May 2008.

Temporal distribution of rotavirus infections in Europe. The 2006–2007 rotavirus season was delayed in relation to the previous and subsequent seasons (Figure 1). In 2005–2006 and 2007–2008, the highest number of rotavirus infections was detected in February, whereas in 2006–2007, the peak was in April, and a significant number of infections was detected in May.

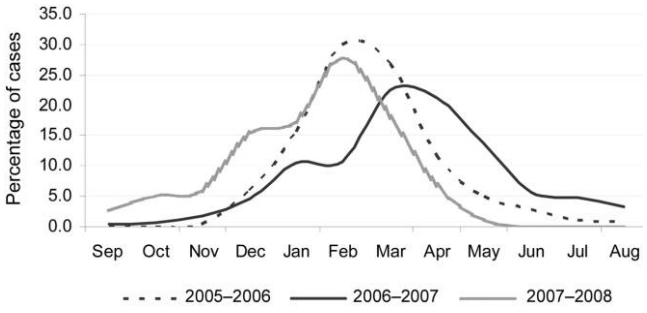

Figure 1. Temporal distribution of rotavirus infections during 3 consecutive rotavirus seasons, 2005–2008. Total numbers of cases analyzed were 2129, 4030, and 2720 for the 2005–2006, 2006–2007, and 2007–2008 seasons, respectively (data for 2007–2008 represent entries uploaded to the database by the end of May 2008).

Differences in the month in which rotavirus infections peaked were observed across the EuroRotaNet countries. The earliest peaks were detected in Spain in January, and in Italy in March. In France and the United Kingdom, the incidence of infection peaked in April, and in the Scandinavian countries and in Hungary and Slovenia, the peak of infection was in May (Table 2).

Genotype distribution. The most common rotavirus genotype detected during the 3 rotavirus seasons from 2005 through 2008 was G1P[8] (Table 2). However, in 2005–2006 and 2006–2007, G1P[8] represented <50% of the strains cocirculating in Europe, and this increased significantly to 62% in 2007–2008 ($P < .001$). All 3 seasons were characterized by a high diversity of cocirculating strains, resulting in a relatively high percentage of mixed infections (3.5%). The second most common genotype during the 3 seasons was G9P[8]; however, the percentage of this genotype decreased significantly from season to season ($P < .001$). The percentage of G2P[4] strains increased significantly in 2006–2007 and then decreased significantly in 2007–2008 ($P < .001$). The incidence of infection with G3P[8] strains remained stable during the 3 consecutive seasons, whereas significant differences were observed from season to season in the incidence of infection with G4P[8] strains ($P < .001$). G12P[8] strains were detected in several European countries, with a relatively low incidence of infection with these strains during the 2005–2006 season, and the incidence remained similar through 2007–2008 (Table 2).

The relative incidence of infection with the various genotypes differed among the participating countries and among seasons (Table 3). G1P[8] was the most common genotype in Denmark, France, the United Kingdom, and Slovenia during each of the 3 rotavirus seasons, although the relative incidence of infection with G1P[8] strains varied from season to season (Table 3). In addition, the incidence of infection with G2P[4] strains increased significantly in 2006–2007 in these 4 countries, and decreased again in 2007–2008. In Spain, G9P[8] strains were the most prevalent in the 2005–2006 and 2006–2007 seasons, but the incidence of infection with these strains decreased significantly in 2007–2008, whereas G1P[8] became the most prevalent genotype in 2007–2008, representing 84.7% of strains. G2P[4] strains were present at constant low levels during the 3 seasons in Spain.

Epidemiological characteristics. Most of the symptomatic rotavirus infections in Europe for which samples were collected occurred in children aged <2.5 years (4263 [81.5%] of 5228 case patients for whom age was available), with the peak between 6 and 18 months of age (2463 [47.1%]). Smaller numbers of infections were reported among children aged 2.5–7 years (736 [14.1%]), young adults (59 [1.1%]), and elderly persons (114 [2.2%]). No significant differences were seen in the distribution of genotypes according to age (data not shown).

No significant differences were seen in the incidence of rotavirus infections reported in male and female individuals (55% and 45% of 5805 individuals, respectively), and no differences were observed in the distribution of genotypes between the sexes (data not shown).

Of 7081 rotavirus infections in 2005–2008 for which the setting was recorded, 65% were hospitalized cases and 35% were community cases. The percentages of the different genotypes detected in hospital or community cases followed the same distribution, with the exception of G2P[4] strains, which were found significantly more often in hospitalized cases ($P < .001$; data not shown).

DISCUSSION

EuroRotaNet provides consistent and comparable data on rotavirus strain diversity across Europe through national networks for sampling and the use of standardized methods for strain characterization. Rotavirus infections are nonnotifiable, and this network has no influence on the national surveillance of rotavirus infections in the different European countries. However, the data obtained in this study should be representative of the strains circulating in each of the countries and provide a more robust comparison among countries and seasons than do data from small, isolated, and country-centric studies published previously.

Analysis of the EuroRotaNet database revealed that, for the 3 rotavirus seasons from 2005 through 2008, G1P[8] remained the most prevalent genotype in Europe as a whole, but the incidence of infection with G1P[8] strains remained <50%. All 3 seasons were characterized by a significant diversity of cocirculating strains. Recently, an increase in the incidence of infection with G2P[4] rotavirus strains has been reported in Brazil, where the monovalent G1P[8] rotavirus vaccine is currently being used [4, 5]. This finding raises questions about

Table 2. Distribution of Rotavirus Infections by Month in Different European Regions during the 2006–2007 Season

Month	No. (%) of rotavirus infections					
	Spain (n = 601)	Italy (n = 317)	France (n = 521)	United Kingdom (n = 831)	Hungary and Slovenia (n = 629)	Scandinavia[a] (n = 323)
September	5 (0.8)	0 (0.0)	1 (0.2)	2 (0.2)	2 (0.3)	3 (0.9)
October	18 (3.0)	1 (0.3)	3 (0.6)	0 (0.0)	0 (0.0)	2 (0.6)
November	31 (5.2)	7 (2.2)	2 (0.4)	8 (1.0)	2 (0.3)	5 (1.5)
December	91 (15.1)	11 (3.5)	23 (4.4)	2 (0.2)	12 (1.9)	2 (0.6)
January	**154 (25.6)**	30 (9.5)	39 (7.5)	42 (5.1)	6 (1.0)	7 (2.2)
February	49 (8.2)	40 (12.6)	95 (18.2)	95 (11.4)	70 (11.1)	20 (6.2)
March	90 (15.0)	**176 (55.5)**	139 (26.7)	265 (31.9)	113 (18.0)	49 (15.2)
April	32 (5.3)	21 (6.6)	**169 (32.4)**	**281 (33.8)**	120 (19.1)	87 (26.9)
May	76 (12.6)	13 (4.1)	44 (8.4)	91 (11.0)	**204 (32.4)**	**92 (28.5)**
June	29 (4.8)	5 (1.6)	3 (0.6)	28 (3.4)	53 (8.4)	41 (12.7)
July	19 (3.2)	3 (0.9)	3 (0.6)	8 (1.0)	21 (3.3)	10 (3.1)
August	7 (1.2)	10 (3.2)	0 (0.0)	9 (1.1)	26 (4.1)	5 (1.5)

NOTE. Entries for which epidemiological data were incomplete were excluded. The highest number of infections for each geographic region is shown in bold font. Data for the 2007–2008 season were not included because they were not yet complete.

[a] Data for Scandinavia include data for Denmark, Faero Islands, and Finland.

the ability of this vaccine to confer cross-protection against G2P[4] rotavirus disease and the possibility that the increased incidence of infection with rotavirus of this genotype is being driven by the introduction of the vaccine. However, data from the EuroRotaNet network indicated a significant increase in the incidence of infection with G2P[4] rotavirus strains during the 2006–2007 season, compared with the 2005–2006 season, in the absence of a vaccination program. Therefore, the findings in Brazil may represent normal fluctuation in the interseasonal diversity of strains rather than vaccine-driven selection, as has been reported previously [1, 2]. Interestingly, G2P[4] rotavirus infections in Europe were significantly associated with hospital cases and are possibly associated with more-severe disease. The reemergence of G2P[4] strains has previously been associated with mutations within antigenic sites on the VP7 gene leading to the emergence of antibody escape mutants [6–8], and this may also explain the association of G2P[4] strains with more-severe disease.

Significant differences were also observed in the relative incidences of infection with the various rotavirus genotypes among countries (Table 3). The incidence of infection with strains of zoonotic origin fluctuated between 0% and 7.8% among these 5 countries. Remarkably, Denmark reported the highest incidence of infection with such strains in 2005–2006, but none were reported in the subsequent seasons. Further analysis of these data should clarify whether the high incidence reported in 2005–2006 was limited to a particular location and/or represented an outbreak. Interestingly, 1.34% of all strains in the database from 2005 through 2008 had unusual G and/or P types, suggesting either direct zoonotic transmission or reassortment between zoonotic and human strains, as previously documented [1, 3, 9–15].

If extrapolated across the 14 European countries, with an estimated 3,579,070 cases of rotavirus infection, this would account for a mean of 47,959 cases of infection with rotavirus strains of possible zoonotic origin each year. Among these, it is of note that G9P[6] strains have reemerged in Hungary. G9 strains first emerged in Europe in 1995 as G9P[6] strains and were likely introduced through zoonosis [1]. Subsequently, the G9P[8] genotype originated through reassortment with human strains and spread throughout Europe and the rest of the world to become a common human strain, displacing G9P[6] strains [1]. This finding may suggest zoonotic reintroduction of G9P[6] into Europe, and further investigation suggested that these strains were imported from India [16]. G12 in combination with P[6], P[8], or P[9] has been detected among strains in Europe, although the incidence of infection with these strains remained relatively low.

The data being gathered through EuroRotaNet will provide valuable background information on the rotavirus strain diversity in Europe before the introduction of rotavirus vaccines, and the network will provide a robust method for surveillance during vaccination implementation. Furthermore, because rotavirus vaccination is not being introduced equally across Europe, comparisons of genotype distributions between neighboring countries with and without universal rotavirus vaccination programs will be possible. Although still preliminary, the data collected through EuroRotaNet suggest a south-to-north and west-to-east pattern of rotavirus spread in Europe. Continued monitoring in consecutive seasons though this net-

Table 3. Rotavirus Genotype Distributions in 5 EuroRotaNet-Participating Countries Selected on the Basis of Significant Data Available for 3 Consecutive Rotavirus Seasons, 2005–2008

	Denmark			France			Slovenia			Spain			United Kingdom		
Genotype	2005–2006	2006–2007	2007–2008	2005–2006	2006–2007	2007–2008	2005–2006	2006–2007	2007–2008	2005–2006	2006–2007	2007–2008	2005–2006	2006–2007	2007–2008
G1P[8]	41.18	48.63	48.4	71.36	57.38	63.6	66.67	52.74	75.4	9.42	38.26	84.7	57.24	65.41	72.3
G2P[4]	12.75	17.81	1.6	0.47	10.95	1.8	0.00	18.60	6.0	0.00	0.92	1.1	5.04	14.34	7.3
G3P[8]	1.96	4.11	0.0	9.86	2.86	1.6	1.11	6.10	0.0	0.45	3.14	4.5	9.21	6.04	9.4
G4P[8]	0.98	4.79	7.0	0.94	0.95	0.0	10.00	17.68	9.7	0.00	0.37	0.5	1.10	1.51	5.7
G9P[8]	34.31	19.86	1.6	16.43	26.43	31.7	17.78	1.83	9.0	89.69	53.23	6.9	24.12	9.18	4.6
G12P[8]	0.00	4.11	0.8	0.00	0.24	1.1	2.22	0.00	0.0	0.00	0.00	0.3	2.41	1.01	0.1
Human rotavirus reassortant	0.98	0.68	1.6	0.00	0.00	0.0	0.00	0.00	0.0	0.45	2.59	0.3	0.66	1.76	0.3
Zoonotic origin	7.84	0.00	0.0	0.94	1.19	0.2	2.22	3.05	0.0	0.00	1.48	1.9	0.22	0.75	0.3

NOTE. Data are percentages.

work will provide evidence as to whether this is a true and repetitive phenomenon.

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