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Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network

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SUMMARY

EuroRotaNet, a laboratory network, was established in order to determine the diversity of co-circulating rotavirus strains in Europe over three or more rotavirus seasons from 2006/2007 and currently includes 16 countries. This report highlights the tremendous diversity of rotavirus strains co-circulating in the European population during three years of surveillance since 2006/2007 and points to the possible origins of these strains including genetic reassortment and interspecies transmission. Furthermore, the ability of the network to identify strains circulating with an incidence of $\geqslant$1% allowed the identification of possible emerging strains such as G8 and G12 since the beginning of the study; analysis of recent data indicates their increased incidence. The introduction of universal rotavirus vaccination in at least two of the participating countries, and partial vaccine coverage in some others may provide data on diversity driven by vaccine introduction and possible strain replacement in Europe.

Key words: Rotavirus.

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INTRODUCTION

Rotaviruses are a major cause of gastroenteritis in children aged <5 years worldwide [1, 2] and of acute diarrhoea in the young of many other mammalian species (calves, piglets, lambs, rabbits, etc.). They cause more than 600 000 deaths each year, mostly of infants and young children in developing countries [1], and are a significant cause of morbidity and hospitalizations in developed countries.

Rotaviruses are classified according to the serological cross-reactivity of the inner capsid protein VP6 into five groups (A–E) and two more groups (F, G) are likely to exist. Within group A rotaviruses, there are subgroups (I, II, I+II, non-I, non-II) characterized by their reactivities with two VP6-specific monoclonal antibodies [3]. More recently, RT–PCR has been applied to the subgroup classification of human rotaviruses, and only two major subgroups have been identified [4].

In order to characterize strains on the basis of their surface proteins VP4 and VP7, a binary classification scheme distinguishing types has been established for group A rotaviruses. The system differentiates G-types (VP7-specific, G for glycoprotein) and P-types (VP4-specific, P for protease-sensitive protein). To date, 23 different G-types and 31 P-types have been described [5–9], indicating extensive genomic diversity within group A rotaviruses.

Rotaviruses possess a genome of 11 segments of dsRNA and VP4 and VP7 are coded for by different RNA segments (segments 4 and 7, 8 or 9 depending on strain, respectively). As rotaviruses were found to reassort readily in doubly infected cells *in vitro* and *in vivo* [7, 10–12], various combinations of VP4 and VP7 types have been observed in natural rotavirus isolates [13–16].

The European Rotavirus Network (EuroRotaNet), was established in January 2007 [17], and has conducted rotavirus strain surveillance in Europe for three consecutive years. Participation in EuroRotaNet is voluntary, and the network activities are funded between the collaborating institutes and GlaxoSmith-Kline Biologicals (GSK) and Sanofi Pasteur-MSD (SPMSD). The participating institutes provide expertise, laboratory space, equipment and supervision, and funding for labour and consumable costs associated with strain characterization is provided in the form of an unrestricted collaborative grant in equal parts from GSK and SPMSD, and administered through the Centre for Infections, Health Protection Agency, London, UK.

The study was undertaken in order to gather comprehensive information of the rotavirus types co-circulating throughout Europe, including both urban and rural settings, and encompassing at least three consecutive rotavirus seasons. The aims of the study were (i) to develop methods and algorithms for effective rotavirus typing (G and P) and characterization (including VP6 and NSP4 genotypes) and to monitor the effectiveness of current genotyping methods and respond to changes associated with genetic drift and shift; (ii) 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; and (iii) to monitor the emergence and spread of common and novel rotavirus strains within Europe and develop the infrastructure that may serve as a platform for future surveillance activities and nested studies. These studies will be used to monitor the effectiveness of a rotavirus vaccine in the general population, through monitoring the reduction in disease associated with common rotavirus types; to detect the possible vaccine-induced emergence of antibody escape mutants and the possible emergence in the general population of genotypes other than those included in the vaccine and reassortants between vaccine and naturally circulating wild-type strains.

MATERIALS AND METHODS

Samples

Hospitals, paediatricians and general medical practitioners that were willing to participate in the study over several years were identified in each of the participating countries in order to allow valid comparisons to be made between each rotavirus season and within each member country. Faecal samples submitted for the routine laboratory investigation of infantile gastroenteritis and positive for group A rotavirus antigen were collected for genotyping. A total of 19 140 rotavirus-positive samples were genotyped between September 2006 and August 2009 in 15 European countries (Table 1).

Rotavirus genotyping

Rotavirus strains were characterized through genotyping using standardized methods to identify their G- and P-types [Manual of rotavirus detection and characterization methods, WHO (http://www.who.int/nuvi/rotavirus/WHO_IVB_08.17_eng.pdf); European

Table 1. *Number of rotavirus strains in the EuroRotaNet database per country and rotavirus season (includes data for September to August in each of the three season from 2006 to 2009; entries for which date of sample collection was incomplete were excluded)*

Country	2006/ 2007	2007/ 2008	2008/ 2009	Study target	Total	% target achieved
Belgium		607	214	940	821	87·3
Bulgaria	338	328	299	940	965	102·7
Denmark	185	270	245	1410	700	49·7
Finland	142	266	226	1410	634	45·0
France	578	764	620	2193	1962	89·5
Germany	39	964	752	2877	1755	61·0
Greece	ns	ns	96	470	96	20·4
Hungary	388	586	436	1410	1410	100·0
Italy	347	1290	748	2103	2385	113·4
Lithuania	1	564	446	940	1011	107·6
The Netherlands	16	139	838	1410	993	70·4
Romania	n.s.	n.s.	t.b.u.	470	—	0·0
Slovenia	353	631	234	1410	1218	86·4
Spain	544	662	530	2352	1736	73·8
Sweden	32	578	115	1410	725	51·4
UK	844	910	975	2115	2729	129·0
Total	**3807**	**8559**	**6774**	**23 860**	**19 140**	**80·2**

n.s., No sampling; t.b.u., to be uploaded.

Belgium, Bulgaria and Lithuania joined the network in 2008, Greece and Romania in 2009. Only data uploaded up to 31 December 2009 is included in the analysis.

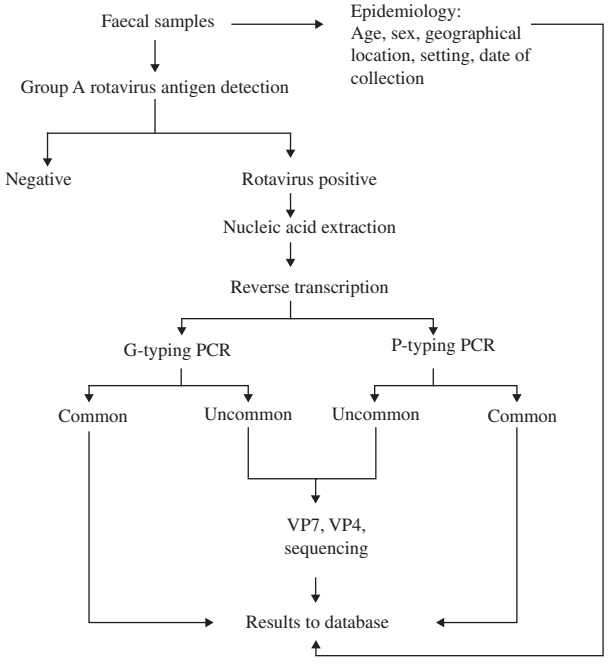

Fig. 1. Algorithm describing the testing and reporting of rotavirus-positive samples.

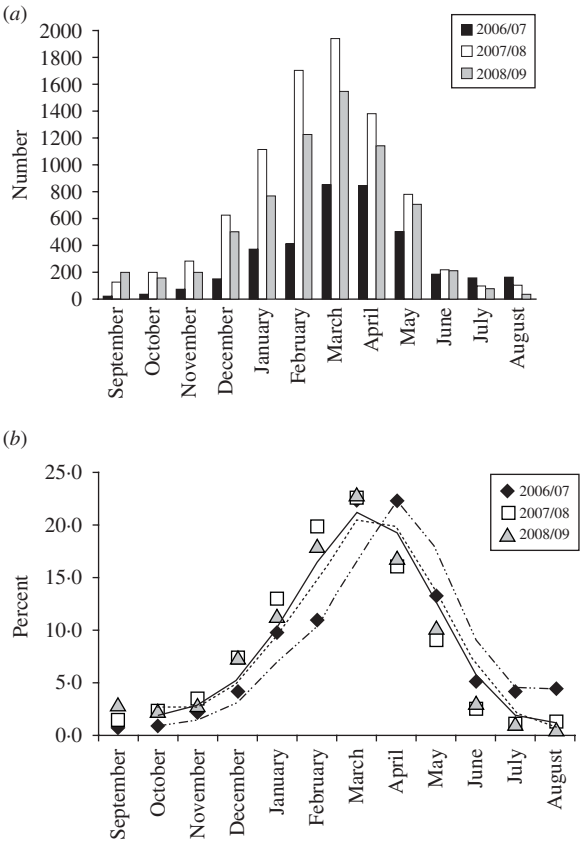

Fig. 2. Temporal distribution of rotavirus infections in the EuroRotaNet database in three consecutive seasons, 2006/2007, 2007/2008 and 2008/2009. (*a*) Numbers of strains each month in three consecutive seasons. (*b*) Percent of the total rotavirus strains in a season each month showing the moving-average trend analysis.

rotavirus detection and characterization methods v4 (http://www.eurorota.net/docs.php)] and following the algorithm described in Figure 1. Rotavirus subgroups (VP6) and NSP4 genotypes were determined for uncommon or novel strains identified through G- and P-typing in order to identify possible zoonotic transmission [18, 19] (European rotavirus detection and characterization methods v4).

Epidemiological data

Epidemiological data including age, sex, geographical location, including postcode in some countries, setting (hospital or community, urban or rural), symptoms (diarrhoea, vomiting, or diarrhoea and vomiting, or other) and date of onset and date of sample collection were entered into a web-accessible database and linked to the genotyping data (http://www.eurorota.net/).

Table 2. *Possible origins of rotavirus strains circulating within Europe. Common and reassortant human rotavirus strains, zoonotic strains and animal-human hybrid rotavirus strains are identified*

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
Common human rotavirus genotypes								
G1P[8]	1637	43·00	4532	52·95	3101	45·78	9270	48·43
G2P[4]	584	15·34	747	8·73	597	8·81	1928	10·07
G3P[8]	113	2·97	324	3·79	381	5·62	818	4·27
G4P[8]	282	7·41	1282	14·98	1318	19·46	2882	15·06
G9P[8]	768	20·17	900	10·52	544	8·03	2212	11·56
Reassortment among common human rotavirus genotypes								
G1P[4]	11	0·29	30	0·35	15	0·22	56	0·29
G2P[8]	21	0·55	46	0·54	23	0·34	90	0·47
G3P[4]	1	0·03	1	0·01	5	0·07	7	0·04
G4P[4]	1	0·03	6	0·07	17	0·25	24	0·13
G9P[4]	4	0·11	16	0·19	16	0·24	36	0·19
Potential zoonotic rotavirus genotypes								
G3P[3]	1	0·03	0	0·00	0	0·00	1	0·01
G3P[6]	0	0·00	0	0·00	9	0·13	9	0·05
G3P[9]	0	0·00	2	0·02	3	0·04	5	0·03
G6P[9]	4	0·11	3	0·04	1	0·01	8	0·04
G6P[14]	1	0·03	3	0·04	0	0·00	4	0·02
G8P[6]	4	0·11	1	0·01	1	0·01	6	0·03
G8P[14]	0	0·00	1	0·01	2	0·01	3	0·01
G9P[6]	5	0·13	0	0·00	4	0·06	9	0·05
G9P[9]	2	0·05	3	0·04	1	0·01	6	0·03
G9P[10]	0	0·00	1	0·01	1	0·01	2	0·01
G10P[14]	5	0·13	1	0·01	0	0·00	6	0·03
G12P[9]	0	0·00	4	0·05	4	0·06	8	0·04
Possible human-animal hybrid rotavirus genotypes								
G1P[6]	0	0·00	0	0·00	4	0·06	4	0·02
G2P[6]	5	0·13	17	0·20	2	0·03	24	0·13
G2P[9]	1	0·03	1	0·01	0	0·00	2	0·01
G2P[10]	1	0·03	0	0·00	0	0·00	1	0·01
G4P[6]	5	0·13	0	0·00	3	0·04	8	0·04
G4P[9]	0	0·00	1	0·01	1	0·01	2	0·01
G4P[10]	0	0·00	0	0·00	1	0·01	1	0·01
G6P[4]	0	0·00	1	0·01	0	0·00	1	0·01
G6P[8]	0	0·00	2	0·02	0	0·00	2	0·01
G6P[11]	1	0·03	0	0·00	0	0·00	1	0·01
G8P[4]	0	0·00	3	0·04	63	0·93	66	0·34
G8P[8]	2	0·05	2	0·02	6	0·09	10	0·05
G10P[4]	1	0·03	2	0·02	0	0·00	3	0·02
G10P[6]	0	0·00	1	0·01	0	0·00	1	0·01
G10P[8]	0	0·00	6	0·07	15	0·22	21	0·11
G10P[10]	0	0·00	1	0·01	0	0·00	1	0·01
G12P[4]	0	0·00	0	0·00	3	0·04	3	0·02
G12P[6]	1	0·03	4	0·05	41	0·61	46	0·24
G12P[8]	21	0·55	46	0·54	52	0·77	119	0·62
Total	**3482**	**91·46**	**7990**	**93·35**	**6234**	**92·03**	**17 706**	**92·51**

RESULTS

Temporal distribution

Rotavirus infections peak in the winter months in temperate climates, and the analysis of the data in EuroRotaNet reflects this seasonality. The peak of rotavirus infections in Europe occurred in March in all three seasons between 2006 and 2009 (Fig. 2). The moving-average trend analysis indicated that rotavirus infections peaked in April in

Table 3 (*a*). *Co-infections with multiple rotavirus strains* (*single G-type with two or more P-types*)

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
G1P[4] + P[8]	10	0·26	19	0·22	22	0·32	51	0·27
G2P[4] + P[8]	6	0·16	6	0·07	12	0·18	24	0·13
G9P[4] + P[8]	6	0·16	10	0·12	8	0·12	24	0·13
G9P[6] + P[8]	1	0·03	6	0·07	2	0·03	9	0·05
G4P[4] + P[8]	1	0·03	2	0·02	3	0·04	6	0·03
G3P[4] + P[8]	3	0·08	1	0·01	1	0·01	5	0·03
G8P[4] + P[6]	0	0·00	0	0·00	5	0·07	5	0·03
G1P[6] + P[8]	1	0·03	1	0·01	1	0·01	3	0·02
G10P[4] + P[8]	0	0·00	1	0·01	1	0·01	2	0·01
G12P[4] + P[8]	0	0·00	1	0·01	1	0·01	2	0·01
G1P[8] + P[10]	0	0·00	1	0·01	1	0·01	2	0·01
G2P[4] + P[6]	1	0·03	0	0·00	1	0·01	2	0·01
G2P[6] + P[8]	2	0·05	0	0·00	0	0·00	2	0·01
G8P[6] + P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G12P[8] + P[9]	0	0·00	0	0·00	1	0·01	1	0·01
G12P[9] + P[11]	0	0·00	0	0·00	1	0·01	1	0·01
G1P[4] + P[6] + P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G1P[8] + P[9]	0	0·00	1	0·01	0	0·00	1	0·01
G2P[4] + P[9]	1	0·03	0	0·00	0	0·00	1	0·01
G3P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G4P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G8P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G9P[8] + P[9]	1	0·03	0	0·00	0	0·00	1	0·01
Total	**33**	**0·87**	**50**	**0·58**	**65**	**0·96**	**148**	**0·77**

2006/2007 and in March in 2007/2008 and 2008/2009 (Fig. 2).

Differences were observed across EuroRotaNet participating countries in the month in which rotavirus infections peaked. The earliest peaks of infection were detected in Spain in 2006/2007 and 2008/2009, in January and December, respectively, and in 2007/2008 infection peaked in February. Late season peaks were associated with countries in the East or North of Europe with infection peaking in Hungary in May 2007 and in Finland in May 2008 and 2009.

Genotype distribution

Total dataset

The total number of rotavirus-positive samples included were 19 140 for the 3 years and included 3807 in 2006/2007, 8559 in 2007/2008, and 6774 in 2008/2009. These numbers were used as denominators for calculating percentages. A total of 141 different combinations of G- and P-types were found between 2006 and 2009 and included those with combinations either singly or as multiple infections of G1, G2, G3, G4, G6, G8, G9, G10, G12 and P[3], P[4], P[6], P[8], P[9], P[10], P[11], P[14] genotypes (Table 2).

Common rotavirus strains

G1P[8] rotavirus strains were predominant in all three seasons between 2006 and 2009. Strains circulating with a prevalence of >1% included G1P[8] (48·43%), G4P[8] (15·06%), G9P[8] (11·56%), G2P[4] (10·07%) and G3P[8] (4·27%) (Table 2).

Possible origins of rotavirus strains

Rotavirus strains found circulating within the European population between 2006 and 2009 can be stratified according to their possible origins. Human rotavirus strains are the most prevalent, making up 89·4% of circulating strains. Strains whose derivation may be associated with reassortment between animal and human strains make up 1·7% and reassortants of the common human strains make up 1·1%. Infection with rotavirus strains of animal origin may represent 0·3% of strains found (Table 2).

Table 3 (*b*). *Co-infections with multiple rotavirus strains* (*multiple G-types with a single P-type*)

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
G1 + G9P[8]	70	1·84	103	1·20	34	0·50	207	1·08
G1 + G4P[8]	5	0·13	36	0·42	36	0·53	77	0·40
G1 + G2P[4]	5	0·13	22	0·26	9	0·13	36	0·19
G4 + G9P[8]	1	0·03	12	0·14	19	0·28	32	0·17
G3 + G9P[8]	8	0·21	12	0·14	10	0·15	30	0·16
G1 + G3P[8]	5	0·13	10	0·12	7	0·10	22	0·11
G1 + G2P[8]	9	0·24	4	0·05	2	0·03	15	0·08
G1 + G10P[8]	0	0·00	9	0·11	2	0·03	11	0·06
G1 + G12P[8]	2	0·05	2	0·02	5	0·07	9	0·05
G2 + G4P[8]	4	0·11	0	0·00	5	0·07	9	0·05
G1 + G2 + G9P[8]	8	0·21	0	0·00	0	0·00	8	0·04
G2 + G4P[4]	0	0·00	3	0·04	5	0·07	8	0·04
G2 + G9P[4]	4	0·11	2	0·02	2	0·03	8	0·04
G3 + G8P[8]	0	0·00	0	0·00	6	0·09	6	0·03
G9 + G12P[8]	0	0·00	1	0·01	4	0·06	5	0·03
G2 + G9P[8]	3	0·08	0	0·00	1	0·01	4	0·02
G3 + G4P[8]	1	0·03	0	0·00	3	0·04	4	0·02
G1 + G3 + G9P[8]	3	0·08	0	0·00	0	0·00	3	0·02
G1 + G9P[4]	2	0·05	1	0·01	0	0·00	3	0·02
G4 + G12P[8]	0	0·00	2	0·02	1	0·01	3	0·02
G1 + G4 + G9P[8]	1	0·03	1	0·01	0	0·00	2	0·01
G1 + G9P[6]	1	0·03	0	0·00	1	0·01	2	0·01
G2 + G12P[4]	0	0·00	0	0·00	2	0·03	2	0·01
G2 + G3P[4]	0	0·00	0	0·00	2	0·03	2	0·01
G3 + G9 + G12P[8]	0	0·00	2	0·02	0	0·00	2	0·01
G4 + G8P[4]	0	0·00	0	0·00	2	0·03	2	0·01
G9 + G10P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G1 + G12P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G12P[6]	1	0·03	0	0·00	0	0·00	1	0·01
G1 + G2 + G3P[8]	1	0·03	0	0·00	0	0·00	1	0·01
G1 + G2 + G4P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G2 + G9P[4]	1	0·03	0	0·00	0	0·00	1	0·01
G1 + G6P[9]	0	0·00	1	0·01	0	0·00	1	0·01
G1 + G8 + G12P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G8P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G8P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G10 + G12P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G2 + G10P[4]	0	0·00	1	0·01	0	0·00	1	0·01
G2 + G9P[6]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G10P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G8P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G9P[4]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G9P[6]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G9P[9]	1	0·03	0	0·00	0	0·00	1	0·01
G4 + G10P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G8 + G12P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G9 + G3P[6]	0	0·00	1	0·01	0	0·00	1	0·01
Total	**136**	**3·57**	**230**	**2·69**	**168**	**2·48**	**534**	**2·79**

Mixed rotavirus infections

A total of 809 mixed rotavirus infections were detected between 2006 and 2009. These could be classified as single G-type with two or more P-types (148, 0·8 %), multiple G-types with a single P-type (534, 2·8 %) or multiple G- and P-types (127, 0·7 %). These strains are representative of those genotypes

Table 3 (*c*). *Co-infections with multiple rotavirus strains* (*multiple G- and P-types*)

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
G1 + G2P[4] + P[8]	14	0·37	18	0·21	10	0·15	42	0·22
G2 + G9P[4] + P[8]	7	0·18	9	0·11	6	0·09	22	0·11
G2 + G4P[4] + P[8]	0	0·00	2	0·02	6	0·09	8	0·04
G1 + G2 + G9P[4] + P[8]	5	0·13	1	0·01	1	0·01	7	0·04
G1 + G9P[4] + P[8]	3	0·08	3	0·04	1	0·01	7	0·04
G2 + G3P[4] + P[8]	2	0·05	0	0·00	4	0·06	6	0·03
G1 + G4P[4] + P[8]	0	0·00	0	0·00	5	0·07	5	0·03
G1 + G9P[6] + P[8]	3	0·08	0	0·00	0	0·00	3	0·02
G4 + G9P[4] + P[8]	0	0·00	0	0·00	3	0·04	3	0·02
G1 + G2P[4] + P[6]	0	0·00	1	0·01	1	0·01	2	0·01
G1 + G3P[4] + P[8]	0	0·00	1	0·01	1	0·01	2	0·01
G2 + G12P[4] + P[8]	1	0·03	1	0·01	0	0·00	2	0·01
G8 + G12P[6] + P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G1 + G2 + G12P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G2 + G3P[4] + P[8]	1	0·03	0	0·00	0	0·00	1	0·01
G1 + G2 + G4P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G2 + G4P[8] + P[10]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G3P[6] + P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G1 + G9P[8] + P[9]	0	0·00	0	0·00	1	0·01	1	0·01
G2 + G12P[6] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G2 + G4P[6] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G2 + G9P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G3 + G4 + G9P[8] + P[9]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G4P[4] + P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G3 + G9P[4] + P[8]	1	0·03	0	0·00	0	0·00	1	0·01
G4 + G12P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G8 + G12P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G9 + G12P[4] + P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G9 + G12P[8] + P[9]	1	0·03	0	0·00	0	0·00	1	0·01
Total	**38**	**1·00**	**40**	**0·47**	**49**	**0·72**	**127**	**0·66**

seen in single infections and include mixtures of human and of animal rotavirus strains (Table 3*a–c*).

Partially typed rotavirus strains

A total of 625 strains were partially typed, in 330 (1·72%) and 295 (1·54%) strains, only the P-type or G-type, respectively, was obtained (Table 4).

Emerging rotavirus strains

Rotavirus strains emerging in Europe between 2006 and 2009 included G12 and G8 strains (Table 5). These genotypes were found circulating in each of the three seasons and both G12 ($\chi^2 = 22{\cdot}83$, $P < 0{\cdot}0001$) and G8 ($\chi^2 = 69{\cdot}66$, $P < 0{\cdot}0001$) showed significant increases in the third season.

Changing molecular epidemiology

G1P[8] strains were most prevalent in the 2007/2008 season with the exception of Lithuania where only 5·5% of strains were of this genotype. Over the three seasons, and in the majority of countries, the number of G1P[8] strains rose in the second season and fell in the third. The highest incidences of infection with G2P[4] strains in any season were in Belgium, Bulgaria and Greece where this genotype was found in >30% of the strains. In season 1, G2P[4] was the most prevalent strain in Bulgaria and in seasons 1 and 2 in Belgium. In the majority of countries, this genotype accounted for <20% of strains in any season. G3P[8] accounted for <20% of strains in all countries in any season. This strain was not found in the first season in Bulgaria, Italy or Hungary, in the second in Slovenia and in the third in Bulgaria

Table 4. *Partially typed rotavirus strains*

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
G-UDP[8]	40	1·05	98	1·14	129	1·90	267	1·39
G-UDP[4]	6	0·16	8	0·09	16	0·24	30	0·16
G-UDP[4]+P[8]	3	0·08	4	0·05	4	0·06	11	0·06
G-UDP[6]	1	0·03	0	0·00	9	0·13	10	0·05
G-UDP[9]	0	0·00	0	0·00	6	0·09	6	0·03
G-UDP[10]	1	0·03	1	0·01	2	0·03	4	0·02
G-UDP[11]	0	0·00	0	0·00	1	0·01	1	0·01
G-UDP[8]+P[14]	0	0·00	0	0·00	1	0·01	1	0·01
Total	**51**	**1·34**	**111**	**1·30**	**168**	**2·48**	**330**	**1·72**
G1P-UD	24	0·63	85	0·99	40	0·59	149	0·78
G9P-UD	17	0·45	11	0·13	13	0·19	41	0·21
G2P-UD	15	0·39	13	0·15	7	0·10	35	0·18
G4P-UD	4	0·11	11	0·13	7	0·10	22	0·11
G3P-UD	3	0·08	7	0·08	10	0·15	20	0·10
G12P-UD	2	0·05	2	0·02	6	0·09	10	0·05
G10P-UD	0	0·00	3	0·04	2	0·03	5	0·03
G1+G9P-UD	0	0·00	3	0·04	1	0·01	4	0·02
G1+G4P-UD	1	0·03	1	0·01	2	0·03	4	0·02
G8P-UD	0	0·00	1	0·01	1	0·01	2	0·01
G1+G2+G9P-UD	1	0·03	0	0·00	0	0·00	1	0·01
G1+G2P-UD	0	0·00	1	0·01	0	0·00	1	0·01
G1+G3P-UD	0	0·00	0	0·00	1	0·01	1	0·01
Total	**67**	**1·76**	**138**	**1·61**	**90**	**1·33**	**295**	**1·54**

and Greece. The prevalence of G4P[8] strains was 80% in Lithuania in 2007/2008 and >40% in Bulgaria and Germany in 2008/2009, and was found in all countries in the third season (2008/2009). G9P[8] strains were the most common strain found in Spain in the first season (2006/2007), and the second most common in Bulgaria, France, Italy and Finland (Fig. 3).

G12 rotavirus strains were found to be circulating with an incidence of >1% in nine countries including Finland (6·8%), Denmark (3·2%), Lithuania (2·4%), Hungary (2·3%), Greece (1·6%), The Netherlands (1·8%), Germany (1·7%), UK (1·5%) and Bulgaria (1·2%). With the exception of Sweden, G12 strains were found in all other countries. G12 strains were found in each of the three seasons in Denmark, Hungary, UK, Bulgaria, Italy and France.

G8 strains were found in two countries in the 2006/2007 season, in seven countries in the 2007/2008 season and in nine countries in the 2008/2009 season, with the highest incidence overall seen in UK (2·1%). The incidence was <1% in all other countries (Belgium, Bulgaria, Denmark, France, Germany, Greece, Italy, Lithuania, Spain, Sweden, The Netherlands).

Demographic data

Age distribution

Infection peaked in children aged 1–2 years, but was seen in all age groups (Fig. 4*a*). Minor peaks, possibly associated with contact with infected children or grandchildren and waning immunity in the elderly population could also be seen (Fig. 4*b*).

Distribution according to hospital or community setting

For those cases for whom the setting was provided, 62·4% of samples were collected from hospitalized patients and 37·6% from patients in the community. With the exception of G2P[4] strains there were no significant differences in the distribution of common or emerging genotypes between hospital patients and those in the community (Table 6*a*). G2P[4] was significantly associated ($\chi^2 = 22{\cdot}98$, $P < 0{\cdot}0001$) with hospitalized patients and may be associated with the periodic upsurge of G2P[4] strains, possibly associated with the selection of an antibody escape mutant. Infection can often require hospitalization as symptoms may be severe in the absence of cross-reactive antibodies associated with previous infections (see Age distribution section above).

Table 5. *Distribution of possible emerging strains between 2006 and 2009 as single strain infections or contributing to multiple strain infections*

	2006/2007		2007/2008		2008/2009		Total	
	No.	%	No.	%	No.	%	No.	%
G12P[8]	21	0·55	46	0·54	52	0·77	119	0·62
G12P[6]	1	0·03	4	0·05	41	0·61	46	0·24
G12P-UD	2	0·05	2	0·02	6	0·09	10	0·05
G1 + G12P[8]	2	0·05	2	0·02	5	0·07	9	0·05
G12P[9]	0	0·00	4	0·05	4	0·06	8	0·04
G9 + G12P[8]	0	0·00	1	0·01	4	0·06	5	0·03
G12P[4]	0	0·00	0	0·00	3	0·04	3	0·02
G4 + G12P[8]	0	0·00	2	0·02	1	0·01	3	0·02
G3 + G9 + G12P[8]	0	0·00	2	0·02	0	0·00	2	0·01
G2 + G12P[4]	0	0·00	0	0·00	2	0·03	2	0·01
G12P[4] + P[8]	0	0·00	1	0·01	1	0·01	2	0·01
G2 + G12P[4] + P[8]	1	0·03	1	0·01	0	0·00	2	0·01
G8 + G12P[6] + P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G1 + G12P[6]	1	0·03	0	0·00	0	0·00	1	0·01
G1 + G12P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G8 + G12P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G8 + G12P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G12P[8] + P[9]	0	0·00	0	0·00	1	0·01	1	0·01
G12P[9] + P[11]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G2 + G12P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G2 + G12P[6] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G4 + G12P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G9 + G12P[4] + P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G8 + G12P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G9 + G12P[8] + P[9]	1	0·03	0	0·00	0	0·00	1	0·01
G10 + G12P[6]	0	0·00	0	0·00	1	0·01	1	0·01
Total G12	**29**	**0·76**	**66**	**0·77**	**132**	**1·95**	**227**	**1·19**
G8P[4]	0	0·00	3	0·04	63	0·93	66	0·34
G8P[8]	2	0·05	2	0·02	6	0·09	10	0·05
G8P[6]	4	0·11	1	0·01	1	0·01	6	0·03
G8P[14]	0	0·00	1	0·01	2	0·01	3	0·01
G3 + G8P[8]	0	0·00	0	0·00	6	0·09	6	0·03
G4 + G8P[4]	0	0·00	0	0·00	2	0·03	2	0·01
G1 + G8 + G12P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G8P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G1 + G8P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G3 + G8P[8]	0	0·00	1	0·01	0	0·00	1	0·01
G8 + G12P[4]	0	0·00	0	0·00	1	0·01	1	0·01
G8P[4] + P[6]	0	0·00	0	0·00	5	0·07	5	0·03
G8P[6] + P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G8P[4] + P[8]	0	0·00	0	0·00	1	0·01	1	0·01
G8 + G12P[6] + P[8]	0	0·00	0	0·00	2	0·03	2	0·01
G8 + G12P[4] + P[6]	0	0·00	0	0·00	1	0·01	1	0·01
G8P-UD	0	0·00	1	0·01	1	0·01	2	0·01
Total G8	**6**	**0·16**	**9**	**0·11**	**95**	**1·40**	**110**	**0·57**

Distribution according to rural or urban setting

For those patients for whom the setting was provided, 89·7% samples were collected from urban populations and 10·2% from rural populations. The percentages of the European population living in urban and rural areas are estimated to be 73·1% and 26·9%, respectively (www.cap-lmu.de/fgz/statistics/urban_pop.php). G1P[8] strains ($\chi^2 = 19{\cdot}88$, $P < 0{\cdot}0001$) were found significantly more often in

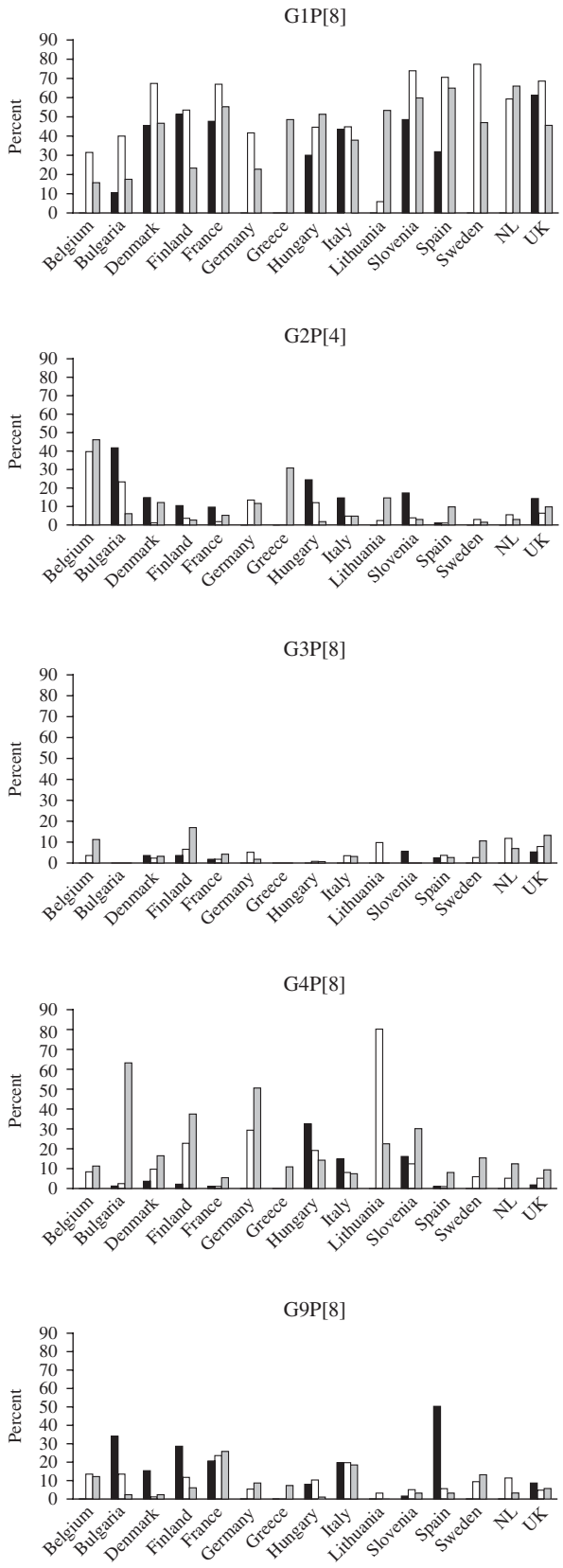

Fig. 3. Changing strain distribution in seasons and countries between 2006 and 2009. ■, 2006/2007; □, 2007/2008; ▨, 2008/2009.

rural populations, whereas G2P[4] ($\chi^2 = 8{\cdot}07$, $P < 0{\cdot}005$) and G9P[8] ($\chi^2 = 19{\cdot}62$, $P < 0{\cdot}0001$) were found more commonly in urban populations (Table 6*b*). The increased incidence of G9P[8] and G2P[4] strains in the urban population may reflect the recent introduction of either an antibody escape mutant, such as seen previously with G2P[4], or an animal human reassortant, such as G9P[8]. The decreased incidence of G1P[8] in the urban population may be a result of the increased activity of G2P[4] and G9P[8] resulting in fewer susceptible individuals.

Distribution according to gender

For those subjects for whom gender was provided, 54·7% were male and 45·3% were female, which reflects the European birth cohort male : female ratio of 1·21 : 1. No significant differences in the distribution of common or emerging rotavirus strains were found between males and females (Table 6*c*).

DISCUSSION

EuroRotaNet, was established in order to determine the diversity of co-circulating rotavirus strains in Europe over three or more rotavirus seasons from 2006/2007. Initially, 11 countries participated in the network and this had expanded to 16 countries by the 2008/2009 season. For analytical purposes, limited epidemiological data including age, sex, symptoms, date of onset, date of sample collection, country, region and settings, including hospital or community and rural or urban were collected.

The data on seasonality confirm the previously reported trend that rotavirus infections spread in Europe from South to North and West to East. This is similar to that observed in North America [20].

It might be expected that genotypes with a relatively low incidence are at a disadvantage in terms of transmission opportunities, and this is likely to be reflected by an increase in infections in older children. Data from Table 7, with the exception of G2P[4], do not support this, and the rationale for common strains such as G1P[8] and uncommon strains such as G8P[4] to significantly infect children between ages 14 and 15 months, is unexplained. The significant peak of infection in older children associated with G2P[4] may reflect poor cross-protection generated through previous infections with other common human rotavirus strains. Interestingly, this may reflect a replicative disadvantage of this strain in a mixture of

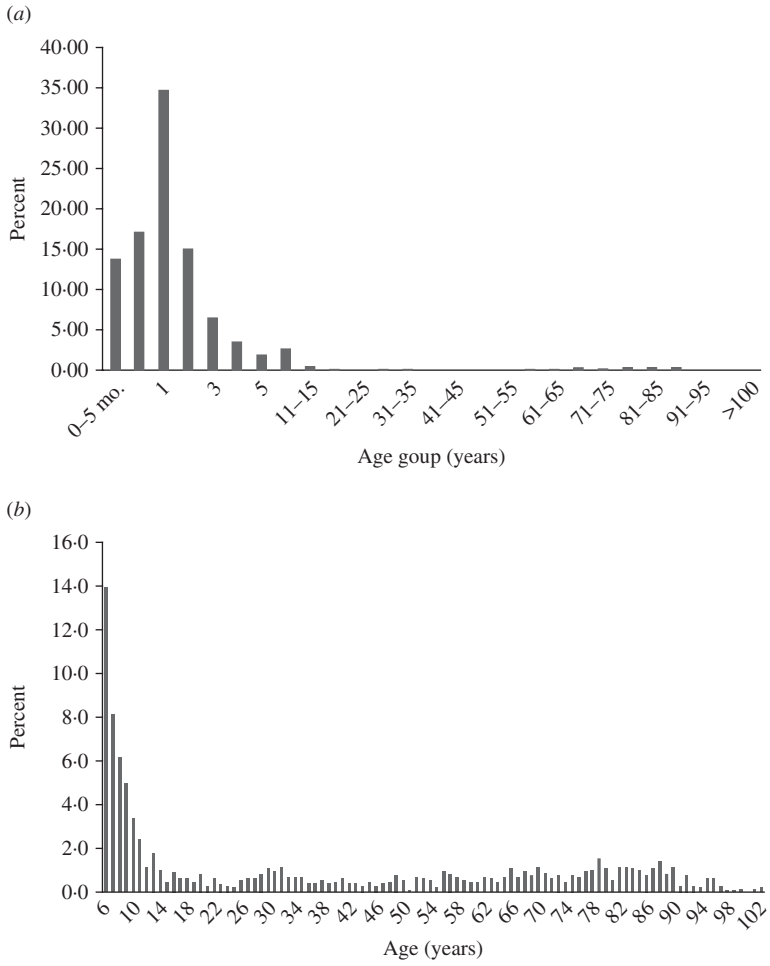

Fig. 4. Age distribution. (*a*) Rotavirus infections by age group in all countries ($n = 17\,510$). (*b*) Rotavirus infections from ages 6 to >100 years ($n = 1283$).

predominantly fit viruses infecting an immunologically naive child. This taken with the development of immunological protection against the commonly circulating strains at a young age increases the probability that any new infection in an older child is likely to be with a less common, possibly less fit strain, such as G2P[4].

This report highlights the tremendous diversity of rotavirus strains co-circulating in the European population and points to the many origins of these strains including genetic reassortment and interspecies transmission. Mixed infections and zoonotic introductions are common, but many will lead to evolutionary dead-ends with only those virus strains demonstrating fitness for transmission within the human population emerging to become significant human pathogens.

The G- or P-types associated with partially typed strains were representative of those genotypes seen in fully characterized strains. Therefore, failure to type is most likely to be associated with technical issues and/or low viral load, although occasionally they may represent usual genotypes, particularly in those associated with an animal rotavirus G- or P- types [21–26]. The failure to identify G-types associated with P[6], P[9], P[10], P[11] and P[14] may be associated with the presence of unusual G-types for which G genotype-specific primers were not included in the study protocol. Similarly, the inability to determine the P-type of strains with G12 or G10 may be due to a lack of P genotype-specific primers. Interestingly, other strains associated with these G- or P-types were fully characterized during the study.

With the ability of the network to identify strains circulating with an incidence of 1%, possible emerging strains such as G8 and G12 have been identified during the first 3 years of the study and the analysis of recent data indicates their increased incidence.

G12P[6] and G12P[14] are likely to represent zoonotic spread into the human population, whereas,

Table 6. *Distribution of common and emerging rotavirus genotypes. The total number of strains shown refers to the total number of rotavirus strains in each of the settings/gender groups, and includes mixed infections and human rotavirus reassortant strains* (*not shown in the table breakdown*)

(*a*) Hospitalized and community cases

Genotype	Hospital		Community	
	No.	%	No.	%
G1P[8]	5008	48·24	2900	46·27
G2P[4]	1106	10·65	524	8·36
G3P[8]	383	3·69	336	5·36
G4P[8]	1673	16·11	924	14·74
G9P[8]	1228	11·83	801	12·78
G12 with any	137	1·32	77	1·23
G8 with any	66	0·64	42	0·67
Total typed	**10 382**		**6267**	

(*b*) Urban and rural settings

Genotype	Urban		Rural	
	No.	%	No.	%
G1P[8]	2677	39·13	371	47·44
G2P[4]	976	14·26	82	10·49
G3P[8]	231	3·38	29	3·71
G4P[8]	1258	18·39	168	21·48
G9P[8]	792	11·58	49	6·27
G12 with any	101	1·48	11	1·41
G8 with any	24	0·35	5	0·64
Total typed	**6842**		**782**	

(*c*) Males and females

Genotype	Male		Female	
	No.	%	No.	%
G1P[8]	4529	47·63	3710	47·17
G2P[4]	953	10·02	809	10·29
G3P[8]	412	4·33	336	4·27
G4P[8]	1473	15·49	1286	16·35
G9P[8]	1142	12·01	933	11·86
G12 with any	95	1·00	107	1·36
G8 with any	56	0·59	44	0·56
Total typed	**9508**		**7865**	

G12P[4] and G12P[8] are more likely to be the result of reassortment in human and animal strains. The plethora of mixtures of rotaviruses containing G12 strains may suggest environmental or food-/water-borne transmission events. Mixtures can also be seen to contain potential zoonotic strains other than G12 such as G8, G10, P[6], P[9] and P[11] and so may represent an environmental reservoir of rotavirus strains associated with a mixture of potentially contaminating human and animal faeces. Similarly, G8P[14] and G8P[6] may be zoonotic, whereas, G8P[4] and G8P[8] may result from reassortment of an animal strain carrying G8, with human strains. Twenty-four mixtures made up of 12 different combinations of G- and/or P-types were found, suggesting infection in some instances through contact with a mixture of human and animal strains.

Table 7. *Median age of infection compared with overall incidence by genotype*

Genotype	Median age of infections (months)	Incidence (%)
G1P[8]	15	48·43
G2P[4]	22	10·07
G3P[8]	15	4·27
G4P[8]	18	15·06
G9P[8]	15	11·56
G8P[4]	14	0·34
G12 with any P	19	1·19
Mixed genotypes	18	4·22

The genes encoding VP7 of the G8 strains from the UK were sequenced. The temporal and geographical distribution and diversity of G8 strains seen before 2009 may suggest multiple zoonotic introductions [13, 27–33] whereas the similarity of the strains described in the current paper is likely to be the result of sustained person-to-person transmission of a strain adapted to the human host (Fig. 5). Interestingly, when G12 strains from UK, Hungary and Bulgaria were sequenced, detailed phylogenetic analysis indicated multiple, unrelated, introductions and may represent a series of evolutionary dead-ends associated with an inability of these strains to sustain human-to-human transmission (Fig. 6). It is reasonable to suggest that because of the frequent introductions of G12 strains into the human population it may be only a matter of time before a reassortment event provides a G12 animal/human hybrid strain of comparable fitness to those current stains circulating commonly in the human population.

The introduction of universal rotavirus vaccination in at least two of the participating countries, and partial vaccine coverage in some others may provide data on diversity driven by vaccine introduction and possible strain replacement. Discussions on changes to the current EuroRotaNet surveillance protocols in

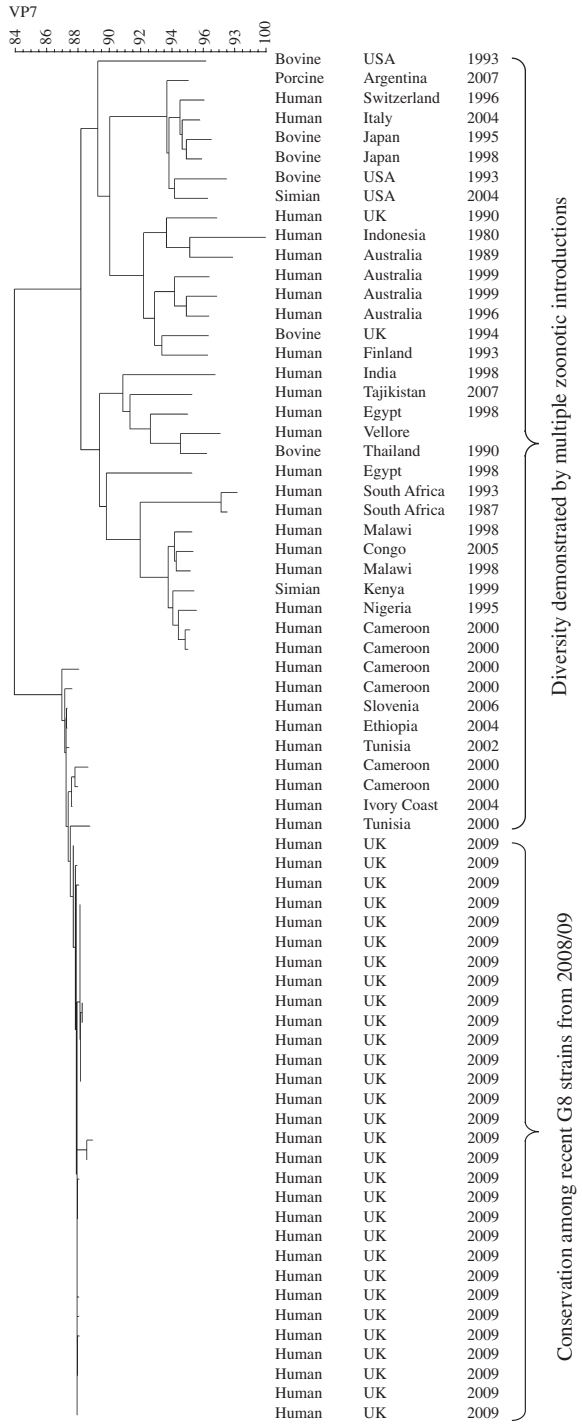

Fig. 5. Dendrogram (Neighbour Joining) of sequences of the gene encoding VP7 of G8 strains. Host, country and year of identification are indicated after each strain.

light of rotavirus vaccine introduction will be held during the course of the next two seasons and will involve participants, funders and the European Centre for Diseases Control (ECDC). In light of these developments, it should now be possible for

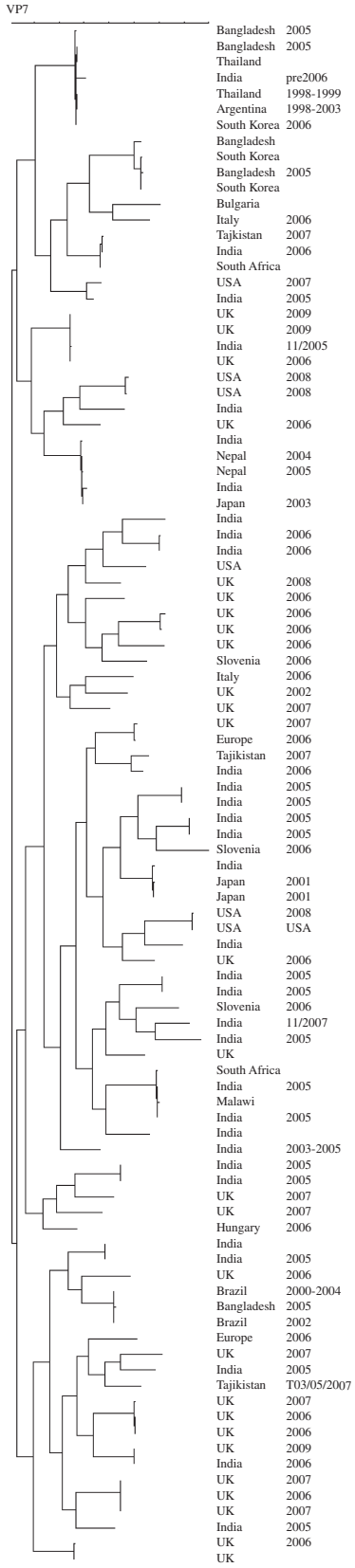

Fig. 6. Dendrogram (Maximum Parsimony) of sequences of the gene encoding VP7 of G12 strains. Strains included are global and represent introductions between 1991 and 2009.

EuroRotaNet, with others, to evolve into a network capable of performing detailed rotavirus surveillance activities. These activities would provide data on the effectiveness of rotavirus vaccination programmes and the impact of these programmes on the virus populations co-circulating in Europe, and the changing patterns of infections by age, season, setting, etc.

DECLARATION OF INTEREST

EuroRotaNet receives funding from GSK and SPMSD in equal parts through a non-restrictive collaborative grant.

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