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1 **Title: Clinical severity and molecular characteristics of circulating and**
2 **emerging rotaviruses in young children attending hospital emergency**
3 **departments in France**

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45 ABSTRACT**46 Objectives**

47 Group A rotavirus (RVA) is the leading cause of acute gastroenteritis in young children
48 worldwide. A prospective surveillance network has been set up to investigate the virological
49 and clinical features of RVA infections and to detect the emergence of potentially epidemic
50 strains in France.

51 Methods

52 From 2009 to 2014, RVA-positive stool samples were collected from 4800 children under 5
53 years old attending the paediatric emergency units of 16 large hospitals. Rotaviruses were
54 then genotyped by RT-PCR with regard to their outer capsid proteins VP4 and VP7.

55 Results

56 Genotyping of 4708 RVA showed that G1P[8] strains (62.2%) were predominant. The
57 incidence of G9P[8] (11.5%), G3P[8] (10.4%) and G2P[4] (6.6%) strains varied considerably,
58 whilst G4P[8] (2.7%) strains were circulating mostly locally. Of note, G12P[8] (1.6%) strains
59 emerged during the seasons 2011-12 and 2012-13 with 4.1% and 3.0% of prevalence,
60 respectively. Overall, 40 possible zoonotic reassortants, such as G6 (33.3%) and G8 (15.4%)
61 strains, were detected, and were mostly associated with P[6] (67.5%). Analysis of clinical
62 records of 624 hospitalized children and severity scores from 282 of them showed no
63 difference in clinical manifestations or severity in relation to the genotype.

64 Conclusions

65 The relative stability of RVA genotypes currently co-circulating and the large predominance
66 of P[8] type strains may ensure vaccine effectiveness in France. The surveillance will
67 continue to monitor the emergence of new reassortants that might not respond to current
68 vaccines, all the more so as all genotypes can cause severe infections in infants.

69 **INTRODUCTION**

70 Group A rotavirus (RVA) is the leading cause of acute gastroenteritis (AGE) in young
71 children worldwide, and is estimated to cause around 453,000 deaths every year in children
72 under 5 years old, mostly in developing countries (1). Although fast and appropriate care has
73 considerably reduced mortality in industrialised countries, rotaviruses are still responsible for
74 considerable morbidity in infants and generate significant health costs.

75 RVAs belong to the *Reoviridae* family and possess a genome composed of 11 dsRNA
76 genomic segments encoding six structural (VPs) and five/six non-structural (NSPs) proteins.

77 On the basis of the outer capsid proteins VP7 and spikes VP4, RVA can be classified into G
78 and P genotypes, respectively. Nucleotide differences in these two genes currently allow the
79 classification of RVA in 27 G- and 37 P-genotypes, among which 12 G and 15 P are

80 associated with infections in humans, thus showing a considerable diversity of strains (2, 3) .

81 Both viral outer layer proteins elicit the production of neutralizing antibodies in the host, but
82 no precise G or P type clearly correlates with the severity of the disease. The five RVA
83 genotype combinations G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are responsible for
84 approximately 90% of RVA infections in children (4). Of note, uncommon G types such as
85 G5, G8, G10 and G12 have emerged in various areas of the world, notably in tropical regions
86 (5, 6).

87 Local data on the current burden of rotavirus disease, including both virological and clinical
88 aspects, are important for decision-making and optimization regarding immunization
89 strategies (7). Hence, knowledge of the molecular epidemiology and antigenic diversity of co-
90 circulating rotaviruses is necessary to ensure the suitability and the efficacy of vaccines.

91 Indeed, RVA diversity is constantly generated by positive selection of single amino acid
92 mutations in defined epitopes, and particularly in highly divergent regions of the outer capsid
93 protein VP7 (8). Programs to monitor rotavirus antigenic drifts that might be caused by

94 specific immunologic pressures and to monitor potential reassortments between human or
95 human and animal strains have to be carefully developed.

96 In France, rotavirus infections occur mostly during the winter and spring seasons. Although
97 deaths remain exceptional, RVA are responsible for about 300,000 acute diarrhoea episodes,
98 half of which are severe, 140,000 consultations and 18,000 hospitalisations each year (9). In
99 spite of the introduction of RVA vaccines in 2006, coverage remains particularly low in
100 France and was estimated at around 8.9% in French infants under 12 months old in 2011 (10).
101 Since the establishment of the French rotavirus surveillance network by the National
102 Reference Centre for Enteric Viruses (Dijon, France), it has become possible to assess the
103 nationwide circulation of RVA strains, especially in infants. This prospective study was
104 designed to monitor and characterize rotavirus infections, including both viral and clinical
105 data, in children under 5 years old suffering from community-acquired acute gastroenteritis
106 and attending paediatric emergency units during the rotavirus vaccine era in France. Special
107 attention was paid to the detection of uncommon strains and emerging reassortants.

108 **METHODS**

109 After its approval by the local ethical committee (Comité Consultatif de Protection des
110 Personnes dans la Recherche Biomédicale de Bourgogne, Dijon, France, on the 24th of
111 November 2005), the surveillance study was conducted during five consecutive seasons from
112 July 2009 to June 2014, and involved 4800 children under 5 years old suffering from
113 rotavirus-induced acute gastroenteritis and attending the paediatric emergency units of 16
114 French Hospitals in 13 regions, including Paris.

115 Acute gastroenteritis was defined by at least three soft or liquid stools or three bouts of
116 vomiting in 24h. Children presenting with chronic diarrhoea, immune deficiency,
117 inflammatory disease of the digestive tract, or nosocomial infections were excluded.
118 Clinical data, including personal identification and clinical symptoms, were collected from
119 children presenting to paediatrics departments. Informed consent was obtained for all
120 subjects. Disease severity was calculated using the Vesikari scale, a 0-20 point numerical
121 score which assesses the clinical severity of rotavirus infections (where higher scores indicate
122 greater severity), as previously described (11). An episode of gastroenteritis with a score ≥ 11
123 is considered a severe episode.

124 The stool samples were routinely screened for RVA using mainly immunochromatographic
125 (ICG) tests or enzyme immuno-assays (EIA). All rotavirus-positive samples were stored at -
126 20°C until genotyping by the National Reference Centre for Enteric Viruses, University
127 Hospital of Dijon, France. The rotavirus strains were genotyped using RT-PCR according to
128 the EuroRotaNet methods (www.eurorota.net/docs.php) and using the Qiagen OneStep RT-
129 PCR kit (Qiagen, Hilden, Germany). The VP7, VP4, VP6 and NSP4 PCR products from
130 strains of interest were sequenced with the same primers as for amplifications. All the
131 sequencing reactions were performed using the ABI[®] PRISM[®] Terminator Cycle Sequencing
132 Kit on a 3130XL DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The

133 nucleotide sequences were edited and genotyped using BioNumerics software (Applied Maths
134 NV, Sint-Martens-Latem, Belgium) and a selection of sequences from rotavirus reference
135 strains available from the GenBank database. Phylogenetic analysis was performed using
136 MEGA6 software (12). After sequence alignment using the MUSCLE programme (13),
137 phylogenetic tree was inferred using the Maximum Likelihood method based on the Tamura
138 3-parameter model, which was the best-fit DNA substitution model for the nucleotide dataset
139 submitted. Bootstrap values were calculated for 1000 replicates. The nucleotide sequences of
140 representative G12 strains from this study have been deposited in the GenBank database
141 (<http://www.ncbi.nlm.nih.gov/genbank/>) under the following accession numbers: KU291317
142 to KU291349.

143 Statistical analyses were performed using STATA[®] v11.0 software from StataCorp (College
144 Station, TX, USA). Comparisons of clinical data and severity scores between G1 and G12
145 RVA, and between all RVA genotypes were performed using the Fisher-exact test for
146 categorical data and the Kruskal-Wallis test for quantitative data. Fractional polynomials were
147 used to model the relationship between children's ages and severity scores using a linear
148 regression model including a non-zero intercept. P values of ≤ 0.05 were considered
149 significant.

150 RESULTS

151 From July 2009 to June 2014, 4800 stools specimens were collected with a mean rate of 73.8
152 samples per centre and per season (i.e. a 12-month period that begins in July and ends in June
153 of the year after). The mean and median ages of the young patients were 13.7 and 10.5
154 months, respectively (range, 0.2 to 59.9 months) and the male/female ratio was 1.34. Most
155 rotavirus infections occurred in children under 2 years old (83.9%). From the 4800 rotavirus-
156 positive stool samples, 92 (1.9%) could not be genotyped by RT-PCR, and 4708 were
157 successfully genotyped. Of these, 4578 (97.2%) faecal specimens contained only one RVA
158 strain, 64 (1.4%) were mixed infections of G and P types, and 66 (1.4%) were partially
159 genotyped.

160 RVA infections occurred in France throughout the year with a peak between January and
161 April (**Figure 1A**). However, in most of the provincial cities, RVA infections were peaking 2
162 months later (i.e between February and March) than in the largest cities in our network (i.e.
163 between January and February in Paris and Lyon) (**Figure 1B**).

164 165 Molecular distribution of circulating rotavirus strains

166 G1 strains were the most predominant during the course of the study and in most cities with a
167 mean prevalence of 63.5% [53.5-73.8]. The four other major G genotypes (G2, G3, G4 and
168 G9) accounted for 34.0% [17.9-44.7] of infections with wide individual amplitude according
169 to the season (**Tables S1** and **S2**). Of note, G12 strains accounted for 1.7% [0.0-3.3]. Mixed
170 infections involved associations of G1 strains with one of the other major G genotypes in
171 75.5% [57.1-100.0]. In addition, the distribution of P genotypes showed a clear predominance
172 of P[8] strains with a mean detection rate of 91.2% [76.6-97.9] followed by P[4] strains with
173 8.0% [1.4-22.1]. The study of the distribution of G genotype prevalence per month showed
174 that G2 and G9 RVA infections were more frequently detected during the first part of the

175 epidemic season (i.e. from September to January) whereas G3, G4, G12 and to a lesser extent
176 G1 RVA infections were more frequent during the second part and at the peak of the epidemic
177 (from January to June) (**Figures S1A and S1B**). Moreover, no predominant RVA genotype
178 was detected according to the age group.

179
180 Distribution of the genotype combinations showed a clear predominance of G1P[8] strains
181 (62.2% [54.3-73.1]) associated with heterogeneous circulation of secondary major strains, i.e.
182 G9P[8] (11.5% [7.3-22.0]), G3P[8] (10.4% [1.5-19.3]), G2P[4] strains (6.6% [1.1-17.5]) and
183 G4P[8] (2.7% [0.8-7.3]), depending on seasons (**Table 1**). The five major genotype
184 combinations accounted for 93.3% [92.3-96.2] of strains, showing a relatively stable detection
185 rate from season to season. Of note, G2P[4] strain circulation rapidly decreased from 17.5%
186 during the 2009-2010 season to reach 1.1% during the last season, whilst G9P[8] strains,
187 which remained stable at 8.9% [6.3-11.5] during the first four seasons, increased to 22.0%
188 during the 2013-2014 season. The circulation of G3P[8] strains increased significantly during
189 the last three seasons to reach a mean detection rate of 15.5% [13.2-19.3] (**Figure S2**).

190 Combinations of major human RVA genotypes (i.e. P[4] with either G1, G3, G4 and G9; and
191 P[8] with G2) were regularly detected during the study with a mean prevalence of 1.7% [0.6-
192 3.2] but were decreasing over time (**Table 1**).

193

194 **Emergence of G12P[8] rotavirus strains**

195 During the study period, 78 G12P[8] strains were detected with a mean prevalence of 1.6%
196 [0.0-4.1], of which 68 were detected during the 2011-12 and 2012-13 seasons with 4.1%
197 (n=35) and 3.0% (n=33) of prevalence, respectively. At the regional level, G12P[8] strains
198 emerged in 2011-2012 at various rates in all regions except Dijon and Charleville-Mézières
199 (**Figure S3**). They were still detected during the subsequent season in only five cities (Brest,

200 Lille, Paris, Poitiers and Saint-Etienne). VP7 phylogenetic analysis showed that all these
201 strains belonged to lineage III and segregated into two relatively distinct main clusters that
202 differed from those of the older G12P[8] strains circulating in France between 2005 and 2009,
203 albeit, only three strains detected in 2014 (R8391, R8422 and R8964) were more closely
204 related to these older G12P[8] strains (**Figure S4**). All G12P[8] strains harboured VP6 genes
205 of human origin (genotype I1). However, among these, two strains harboured NSP4 genes of
206 animal origin (genotype E2), whilst all of the others were of human origin (genotype E1).

207

208 **Human and animal reassortant rotaviruses**

209 Forty (0.8%) atypical RVA strains, of which 27 (67.5%) were P[6] strains, were detected
210 during the surveillance and further characterized according to their VP6 and NSP4 coding
211 genes (**Table S3**). Among these, 20 (51.3%) strains bore genotypes commonly found in cattle:
212 13 (33.3%) G6, six (15.4%) G8 and one (2.6%) G10 strains. Among the G8 strains, three
213 were combined with a major human P type: two with P[8] and one with P[4] in three different
214 locations and seasons. The single G10 strain was combined with P[8] and harboured VP6 and
215 NSP4 genes of human origin. Other strains were unusual combinations of common G types
216 with common human or potential zoonotic P types such as P[3], P[6], P[9] and P[14].

217

218 **Clinical characteristics and severity of rotavirus infections**

219 Clinical records could be collected from 624 children who were thereafter hospitalized in
220 paediatrics departments. Comparisons of clinical records in relation to genotypes are reported
221 in **Table 2**. No difference was found between RVA genotypes for the clinical presentation.
222 None of the patients had been vaccinated. No deaths were reported during the study period.
223 Vesikari severity scores could be calculated for 282 children from the same cohort. In all, 148
224 (51.7%) infants, most of whom were more than 6 months old (91.9%), hospitalized with

225 rotavirus gastroenteritis were classified as severe according to the Vesikari scale (i.e. a score
226 ≥ 11 points). Severity was significantly lower in children aged 0-6 months with a mean score
227 of 8.9 ± 2.7 ($P < 0.0001$) than in the three other age groups. A linear regression model using
228 fractional polynomials was used to study this relationship, i.e. between the children's age in
229 months and the severity score, and no linear relation was found as shown in **Figure S5**. The
230 mean Vesikari severity score of the 282 rotavirus infections was calculated at 10.30 ± 3.3
231 where G1P[8] score was 10.24 ± 3.9 and G12P[8] score was 10.13 ± 2.8 . No difference in
232 Vesikari severity score was found between G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and
233 G12P[8] ($P = 0.604$), between G1P[8] and G12P[8] ($P = 0.813$) or between P[4] (9.93 ± 2.9) and
234 P[8] (10.38 ± 3.4) RVA infections ($P = 0.386$).

235 **DISCUSSION**

236 During this 5-year study, we have showed that G12P[8] RVA strains have slightly emerged in
237 France during two consecutive seasons, and confirmed that RVA infection severity is not
238 related to genotype.

239 Although rotavirus infections in Europe have been reported to occur with a geographical
240 gradient of increasing incidence from the south-west towards the north (14), at the country
241 level, they seem to spread more according to centrifugal progression from areas where the
242 population density is the highest towards the lowest. In France, the winter rotavirus epidemics
243 occurred globally 2 months earlier in large cities than in smaller provincial cities, as
244 previously reported (15). Indeed, high population densities in the large cities facilitate contact
245 between individuals and may thus play a substantial role in shifting the epidemic peak.

246 During the study, the circulation of the G1P[8] strain in France was overall predominant and
247 similar to that in the rest of the world despite the unexpected transient emergence of new
248 major genotypes. This predominance may be due to the emergence of new antigenic G1
249 variants appearing or disappearing alternately under the influence of natural immune-pressure
250 mechanisms (16).

251 Following their emergence in France, G9P[8] strains maintained a relatively high incidence
252 level (15), before strongly decreasing during the current study period. Interestingly, this
253 decline occurred much later in France than in other European countries, where, after
254 emergence, the prevalence of G9P[8] strains progressively decreased season after season (17).
255 However, during the last season in France, high G9P[8] prevalence was observed again. To
256 date, there is no evidence of any particular environmental or immune factors that could
257 explain the long circulation of G9 strains or their renewed circulation in French infants.
258 During the study, G2P[4] prevalence was markedly high during the 2009-2010 season, whilst
259 it was negligible in the rest of the study period. These fluctuations reflect here the normal

260 interseasonal diversity of strains driven by the reemergence of VP7 antigenic mutants, or
261 potentially due to reassortment among co-circulating strains or an introduction of new
262 lineages of G2 strains or antigen combinations (**18**).

263 While the detection rate for G4P[8] was relatively negligible during the study, the circulation
264 of G3P[8] strains increased and then stabilized at around 14%. This increased frequency was
265 supposedly at the expense of the decrease in the frequencies of G1P[8] and G9P[8] (**19, 20**),
266 but could just be related to the re-emergence of G3 antigenic mutants.

267 G12 strains, first detected in France in 2004, were the most frequent among the uncommon
268 strains detected in Europe and Australia (**17, 21**). Recently, G12P[8] have emerged during the
269 2011-12 season in 11 of the 13 monitored regions but were only persistent in 5 regions during
270 the subsequent season. This particularly mild emergence may be probably explained by both a
271 herd immunity against P[8] types and an acquired cross-reactive immunity of the population.
272 Indeed, it differs from the intense emergence reported in the North of Spain during the 2010-
273 2011 season (**22**), although it could be related the more so as the two countries share the same
274 borders and the Spanish G12 emergence preceded only by 1 season the French one.

275 Phylogenetically, these G12 strains belong to the lineage III but diverge from the older strains
276 previously detected, suggesting that an evolution of VP7 antigens or an introduction of new
277 strains in the country have occurred.

278 Infections with viruses of possible zoonotic origin was regularly observed during the 5
279 seasons, notably G6 and G8 strains associated with P[6] or P[14], which harboured NSP4 and
280 VP6 genes from porcine and bovine hosts, and might have resulted from natural reassortment
281 during animal-human transmissions or mixed human rotavirus infections from environmental
282 reservoirs (**23, 24**). These observations demonstrate the interspecies transmission of RVAs,
283 notably from animals living in close contact with humans, resulting in the introduction of new
284 rotavirus genotypes in humans. Interestingly, two G8P[8] and one G8P[4] strains were

285 detected in three different places showing that some G8 strains might initiate their adaption to
286 humans, as previously observed for G4, G9 and G12 strains. While the G8P[4] strain was
287 associated with animal VP6 and NSP4 and was seemingly related to African strains (25), both
288 G8P[8] strains harboured human VP6 and NSP4 genes, thus showing active recombination
289 with human P[8] strains, as previously reported (26). These human G8P[8] strains require
290 further investigations to evaluate their potential emergence; their fitness is currently
291 insufficient to allow easy transmission to humans.

292

293 Analysis of the clinical records showed no difference in clinical manifestations or severity in
294 relation to genotype. These results are consistent with our previous findings (15, 27). More
295 than half of the rotavirus infections in these children were considered severe, which is
296 consistent with previous studies (28, 29). RVA infections in children under 6 months old
297 were, however, less severe than those in any other age group, probably thanks to the
298 protection provided by maternal antibodies. Commonly, differences in severity may be
299 explained by variations in virulence between strains and by the immune status of the
300 population towards a serotype which is partially dependent upon the infecting strain.
301 However, globally, most studies on rotavirus infection severity have been either inconclusive
302 or contradictory (27, 30-32). Nevertheless, both VP7 and VP4 antigens induce antibodies, and
303 most of the genotypes compared had the same P[8] type, thus abrogating the differences
304 between G types. As the severity of the disease does not depend on age but on the infecting
305 virus and previous exposure (33), severe infections may be found mostly during primary
306 infection or exposure to new antigens, whilst less severe infections occur after second
307 exposure, during which heterotypic protection may reduce the symptoms and shorten the
308 infection. These data therefore suggest that VP4 and VP7 antigens are not the only factors
309 responsible for the severity of rotavirus infections. Other strain-specific viral factors and

310 immune status, whether naive or after previous infections, influence the intensity and the
311 severity of the clinical symptoms. The data also strongly suggest that all RVA genotypes may
312 cause acute gastroenteritis, as their G and P types cannot be dissociated, and thus reinforce the
313 need for vaccines that ensure effective protection, whether directly or by cross-reaction,
314 against all rotavirus infections.

315 Today, rotavirus vaccination is still too low in France to have any impact on annual epidemics
316 other than locally. The relative stability of RVA genotypes currently co-circulating and the
317 large predominance of P[8] type strains may ensure vaccine effectiveness, and, given the good
318 outcomes of vaccination in countries with sufficient coverage (34), extensive vaccination
319 should be encouraged in France. The surveillance of rotavirus infections during ongoing and
320 future vaccination programs will ensure the detection of emergent new reassortants and will
321 help the health authorities to optimize appropriate vaccination strategies against rotavirus
322 diseases, all the more so as all genotypes can cause severe infections in infants.

323 **TRANSPARENCY DECLARATION**

324 The authors declare no competing financial interests in the conduct of the study. This work
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327

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328 **REFERENCES**

- 329 1. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, et al. 2008
330 estimate of worldwide rotavirus-associated mortality in children younger than 5 years
331 before the introduction of universal rotavirus vaccination programmes: a systematic
332 review and meta-analysis. *Lancet Infect Dis.* 2012;12(2):136-41.
- 333 2. Trojnar E, Sachsenroder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an
334 avian group A rotavirus containing a novel VP4 gene with a close relationship to those of
335 mammalian rotaviruses. *J Gen Virol.* 2013;94(Pt 1):136-42.
- 336 3. Matthijssens J, De Grazia S, Piessens J, Heylen E, Zeller M, Giammanco GM, et al.
337 Multiple reassortment and interspecies transmission events contribute to the diversity of
338 feline, canine and feline/canine-like human group A rotavirus strains. *Infect Genet Evol.*
339 2011;11(6):1396-406.
- 340 4. Iturriza-Gomara M, Dallman T, Banyai K, Bottiger B, Buesa J, Diedrich S, et al.
341 Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by
342 EuroRotaNet, a pan-European collaborative strain surveillance network. *Epidemiol
343 Infect.* 2011;139(6):895-909.
- 344 5. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its
345 implication for the development and implementation of an effective rotavirus vaccine.
346 *Rev Med Virol.* 2005;15(1):29-56.
- 347 6. Todd S, Page NA, Duncan Steele A, Peenze I, Cunliffe NA. Rotavirus strain types
348 circulating in Africa: Review of studies published during 1997-2006. *J Infect Dis.*
349 2010;202 Suppl:S34-42.
- 350 7. Matthijssens J, Bilcke J, Ciarlet M, Martella V, Banyai K, Rahman M, et al. Rotavirus
351 disease and vaccination: impact on genotype diversity. *Future Microbiol.*
352 2009;4(10):1303-16.
- 353 8. Ciarlet M, Hoshino Y, Liprandi F. Single point mutations may affect the serotype
354 reactivity of serotype G11 porcine rotavirus strains: a widening spectrum? *J Virol.*
355 1997;71(11):8213-20.
- 356 9. Melliez H, Boelle PY, Baron S, Mouton Y, Yazdanpanah Y. [Morbidity and cost of
357 rotavirus infections in France]. *Med Mal Infect.* 2005;35(10):492-9.
- 358 10. Martinot A, Cohen R, Denis F, Gaudelus J, Lery T, Lepetit H, et al. [Assessing early
359 childhood vaccination coverage in France after 2013 vaccination schedule
360 implementation]. *Arch Pediatr.* 2014;21(12):1389-90.
- 361 11. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for
362 clinical severity of diarrhoeal episodes. *Scand J Infect Dis.* 1990;22(3):259-67.
- 363 12. Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S. MEGA6: Molecular
364 Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* 2013;30(12):2725-9.
- 365 13. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high
366 throughput. *Nucleic Acids Res.* 2004;32(5):1792-7.

- 367 14. Koopmans M, Brown D. Seasonality and diversity of Group A rotaviruses in Europe.
368 *Acta Paediatr Suppl.* 1999;88(426):14-9.
- 369 15. de Rougemont A, Kaplon J, Pillet S, Mory O, Gagneur A, Minoui-Tran A, et al.
370 Molecular and clinical characterization of rotavirus from diarrheal infants admitted to
371 pediatric emergency units in France. *Pediatr Infect Dis J.* 2011;30(2):118-24.
- 372 16. Arista S, Giammanco GM, De Grazia S, Ramirez S, Lo Biundo C, Colomba C, et al.
373 Heterogeneity and temporal dynamics of evolution of G1 human rotaviruses in a settled
374 population. *J Virol.* 2006;80(21):10724-33.
- 375 17. Iturriza-Gomara M, Dallman T, Banyai K, Bottiger B, Buesa J, Diedrich S, et al.
376 Rotavirus surveillance in Europe, 2005-2008: web-enabled reporting and real-time
377 analysis of genotyping and epidemiological data. *J Infect Dis.* 2009;200 Suppl 1:S215-21.
- 378 18. Wu FT, Banyai K, Jiang B, Wu CY, Chen HC, Feher E, et al. Molecular epidemiology of
379 human G2P[4] rotaviruses in Taiwan, 2004-2011. *Infect Genet Evol.* 2014;28:530-6.
- 380 19. Stupka JA, Degiuseppe JI, Parra GI, Argentinean National Rotavirus Surveillance N.
381 Increased frequency of rotavirus G3P[8] and G12P[8] in Argentina during 2008-2009:
382 whole-genome characterization of emerging G12P[8] strains. *J Clin Virol.*
383 2012;54(2):162-7.
- 384 20. Mitui MT, Chan PK, Nelson EA, Leung TF, Nishizono A, Ahmed K. Co-dominance of
385 G1 and emerging G3 rotaviruses in Hong Kong: a three-year surveillance in three major
386 hospitals. *J Clin Virol.* 2011;50(4):325-33.
- 387 21. Kirkwood CD, Boniface K, Bogdanovic-Sakran N, Masendycz P, Barnes GL, Bishop RF.
388 Rotavirus strain surveillance--an Australian perspective of strains causing disease in
389 hospitalised children from 1997 to 2007. *Vaccine.* 2009;27 Suppl 5:F102-7.
- 390 22. Cilla G, Montes M, Gomariz M, Alkorta M, Iturzaeta A, Perez-Yarza EG, et al. Rotavirus
391 genotypes in children in the Basque Country (North of Spain): rapid and intense
392 emergence of the G12[P8] genotype. *Epidemiol Infect.* 2013;141(4):868-74.
- 393 23. Rahman M, De Leener K, Goegebuer T, Wollants E, Van der Donck I, Van Hoovels L, et
394 al. Genetic characterization of a novel, naturally occurring recombinant human G6P[6]
395 rotavirus. *J Clin Microbiol.* 2003;41(5):2088-95.
- 396 24. Cunliffe NA, Gentsch JR, Kirkwood CD, Gondwe JS, Dove W, Nakagomi O, et al.
397 Molecular and serologic characterization of novel serotype G8 human rotavirus strains
398 detected in Blantyre, Malawi. *Virology.* 2000;274(2):309-20.
- 399 25. Nyaga MM, Stucker KM, Esona MD, Jere KC, Mwinyi B, Shonhai A, et al. Whole-
400 genome analyses of DS-1-like human G2P[4] and G8P[4] rotavirus strains from Eastern,
401 Western and Southern Africa. *Virus Genes.* 2014;49(2):196-207.
- 402 26. Delogu R, Lo Presti A, Ruggeri FM, Cella E, Giovanetti M, Ciccozzi M, et al. Full-
403 genome characterization of a G8P[8] rotavirus that emerged among children with
404 diarrhea in Croatia in 2006. *J Clin Microbiol.* 2013;51(5):1583-8.

- 405 27. Aupiais C, de Rougemont A, Menager C, Vallet C, Brasme JF, Kaplon J, et al. Severity
406 of acute gastroenteritis in infants infected by G1 or G9 rotaviruses. *J Clin Virol*.
407 2009;46(3):282-5.
- 408 28. Matthijssens J, Zeller M, Heylen E, De Coster S, Vercauteren J, Braeckman T, et al.
409 Higher proportion of G2P[4] rotaviruses in vaccinated hospitalized cases compared with
410 unvaccinated hospitalized cases, despite high vaccine effectiveness against heterotypic
411 G2P[4] rotaviruses. *Clin Microbiol Infect*. 2014;20(10):O702-10.
- 412 29. Lewis KD, Dallas MJ, Victor JC, Ciarlet M, Mast TC, Ji M, et al. Comparison of two
413 clinical severity scoring systems in two multi-center, developing country rotavirus
414 vaccine trials in Africa and Asia. *Vaccine*. 2012;30 Suppl 1:A159-66.
- 415 30. Cascio A, Vizzi E, Alaimo C, Arista S. Rotavirus gastroenteritis in Italian children: can
416 severity of symptoms be related to the infecting virus? *Clin Infect Dis*. 2001;32(8):1126-
417 32.
- 418 31. Linhares AC, Verstraeten T, Wolleswinkel-van den Bosch J, Clemens R, Breuer T.
419 Rotavirus serotype G9 is associated with more-severe disease in Latin America. *Clin*
420 *Infect Dis*. 2006;43(3):312-4.
- 421 32. Clark HF, Lawley DA, Schaffer A, Patacsil JM, Marcello AE, Glass RI, et al. Assessment
422 of the epidemic potential of a new strain of rotavirus associated with the novel G9
423 serotype which caused an outbreak in the United States for the first time in the 1995-1996
424 season. *J Clin Microbiol*. 2004;42(4):1434-8.
- 425 33. Albano F, Bruzzese E, Bella A, Cascio A, Titone L, Arista S, et al. Rotavirus and not age
426 determines gastroenteritis severity in children: a hospital-based study. *Eur J Pediatr*.
427 2007;166(3):241-7.
- 428 34. Leshem E, Moritz RE, Curns AT, Zhou F, Tate JE, Lopman BA, et al. Rotavirus vaccines
429 and health care utilization for diarrhea in the United States (2007-2011). *Pediatrics*.
430 2014;134(1):15-23.
- 431 35. de Rougemont A, Kaplon J, Lebon P, Huet F, Denis F, Alain S, et al. Unexpected
432 substitution of dominant rotavirus G genotypes in French hospitalized children over five
433 consecutive seasons. *Eur J Clin Microbiol Infect Dis*. 2009;28(4):403-7.
- 434

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 438 in Dijon, France.

439

440 **AUTHOR CONTRIBUTIONS STATEMENTS**

441 **AdR, JK and PP** conceived and designed this study and revised the manuscripts. **JK and CF**
 442 performed the experiments. **MCLG, AMT, CP, AV, LMM, MC, RM, FH, FD, DH, ML,**
 443 **CB, AD, SA, JL, YG, BL, YM, FMS, AG, JG, VF, MR, VAF, SB, AGC, DG, PL, ML,**
 444 **PM, JFM, AS, GA, ABD, DO, RC, GL, OM, SP, BP, JL** collected the stool samples and
 445 the clinical data. **SA** performed the statistical analysis and modelling of the data. **AdR**
 446 analyzed the data and wrote the paper. All authors reviewed the manuscript.

447

448 **ADDITIONAL INFORMATION**

449 **GenBank accession numbers:** RVA/Human-wt/FRA/Lyon-R5957/2012/G12P[8]:
 450 KU291317, RVA/Human-wt/FRA/Poitiers-R5485/2012/G12P[8]: KU291318, RVA/Human-
 451 wt/FRA/Limoges-R6183/2012/G12P[8]: KU291319, RVA/Human-wt/FRA/Saint-Etienne-
 452 R6834/2013/G12P[8]: KU291320, RVA/Human-wt/FRA/Saint-Etienne-
 453 R6784/2013/G12P[8]: KU291321, RVA/Human-wt/FRA/Montpellier-R5396/2012/G12P[8]:
 454 KU291322, RVA/Human-wt/FRA/Orleans-R7653/2013/G12P[8]: KU291323, RVA/Human-
 455 wt/FRA/Montpellier-R7934/2014/G12P[8]: KU291324, RVA/Human-wt/FRA/Paris-
 456 R7590/2013/G12P[8]: KU291325, RVA/Human-wt/FRA/Caen-R6761/2013/G12P[8]:
 457 KU291326, RVA/Human-wt/FRA/Paris-R7128/2013/G12P[6]: KU291327, RVA/Human-
 458 wt/FRA/Paris-R6766/2013/G12P[8]: KU291328, RVA/Human-wt/FRA/Paris-
 459 R8857/2014/G12P[8]: KU291329, RVA/Human-wt/FRA/Orleans-R6110/2012/G12P[8]:
 460 KU291330, RVA/Human-wt/FRA/Paris-R6296/2012/G12P[8]: KU291331, RVA/Human-
 461 wt/FRA/Caen-R6679/2012/G12P[8]: KU291332, RVA/Human-wt/FRA/Paris-
 462 R7151/2013/G12P[8]: KU291333, RVA/Human-wt/FRA/Poitiers-R6940/2013/G12P[8]:
 463 KU291334, RVA/Human-wt/FRA/Brest-R6975/2013/G12P[8]: KU291335, RVA/Human-
 464 wt/FRA/Saint-Etienne-R8964/2014/G12P[8]: KU291336, RVA/Human-wt/FRA/Saint-
 465 Etienne-R1725/2007/G12P[6]: KU291337, RVA/Human-wt/FRA/Brest-
 466 R8391/2014/G12P[8]: KU291338, RVA/Human-wt/FRA/Brest-R8422/2014/G12P[8]:
 467 KU291339, RVA/Human-wt/FRA/Brest-R5875/2012/G12P[8]: KU291340, RVA/Human-
 468 wt/FRA/Lyon-R5790/2012/G12P[8]: KU291341, RVA/Human-wt/FRA/Lille-

469 R5374/2012/G12P[8]: KU291342, RVA/Human-wt/FRA/Lille-R6147/2012/G12P[8]:
470 KU291343, RVA/Human-wt/FRA/Lille-R5373/2012/G12P[8]: KU291344, RVA/Human-
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472 R6642/2012/G12P[8]: KU291346, RVA/Human-wt/FRA/Rennes-R5533/2012/G12P[8]:
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474 wt/FRA/Rennes-R5510/2012/G12P[8]: KU291349, RVA/Human-wt/FRA/Lyon-
475 R5615/2012/G12P[8]: HF952916, RVA/Human-wt/FRA/Saint-Etienne-
476 R4925/2011/G12P[8]: HF952915, RVA/Human-wt/FRA/Paris-R4380/2010/G12P[6]:
477 HF952913 and RVA/Human-wt/FRA/Dijon-R4969/2011/G12P[8]: HF952914.
478

479 **FIGURE LEGENDS**

480 **Figure 1. Temporal and geographical distribution of rotavirus infections in France from**
481 **July 2009 to June 2014.**

482 **A.** 5-year cumulative distribution of rotavirus infections per month in 7 major centres; **B.**
483 Annual distribution of rotavirus infections per month of 7 major centres.

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484 **ONLINE APPENDIX FIGURE LEGENDS**

485 **Figure S1. Temporal distribution of group A rotavirus according to G genotype in**
486 **France from 2009 to 2014.**

487 A. 5-year cumulative distribution of G genotypes according to detection month ; B. 5-year
488 cumulative prevalence of G genotypes according to detection month.

489

490 **Figure S2. Distribution of RVA genotype combination over the last 13 epidemic seasons**
491 **from 2001 to 2014.**

492 Data from 2001 to 2009 were extracted from our previous studies for comparison ^(15, 35).

493 Detailed numbers of G12 strains for each season are indicated in the bottom table. * first
494 detection of G12P[8]; ** emergence of G12P[8] strains in France.

495

496 **Figure S3. Regional G12P[8] detection rate from 2009 to 2014.**

497 Bars in red: G12 detection rates during emergence; bars in blue: G12 detection rates before
498 and after emergence.

499

500 **Figure S4. Maximum Likelihood phylogenetic tree based on the partial nucleotide**
501 **sequences (575 nt) of the rotavirus G12 VP7 coding gene.**

502 The tree is drawn to scale, with branch lengths measured in the number of substitutions per
503 site. Reference strains retrieved from GenBank database are in bold-face. The French

504 rotavirus G12 strains from this study are coloured according to the season of sample

505 collection: brown for the 2009-2010 season, green for the 2010-2011 season, red for the 2011-

506 2012 season, blue for the 2012-2013 season and pink for the 2013-2014 season. The

507 following representative French rotavirus G12 strains from this study were submitted to the

508 GenBank database.

509 **Figure S5. Fractional polynomial analysis of the relation between child age in month and**
510 **severity scores using a linear regression model including a non-zero intercept.**
511 IC95 is shown in grey

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TABLE 1. Distribution and detection rates of G and P genotype combinations of group A rotavirus detected in France from 2009 to 2014

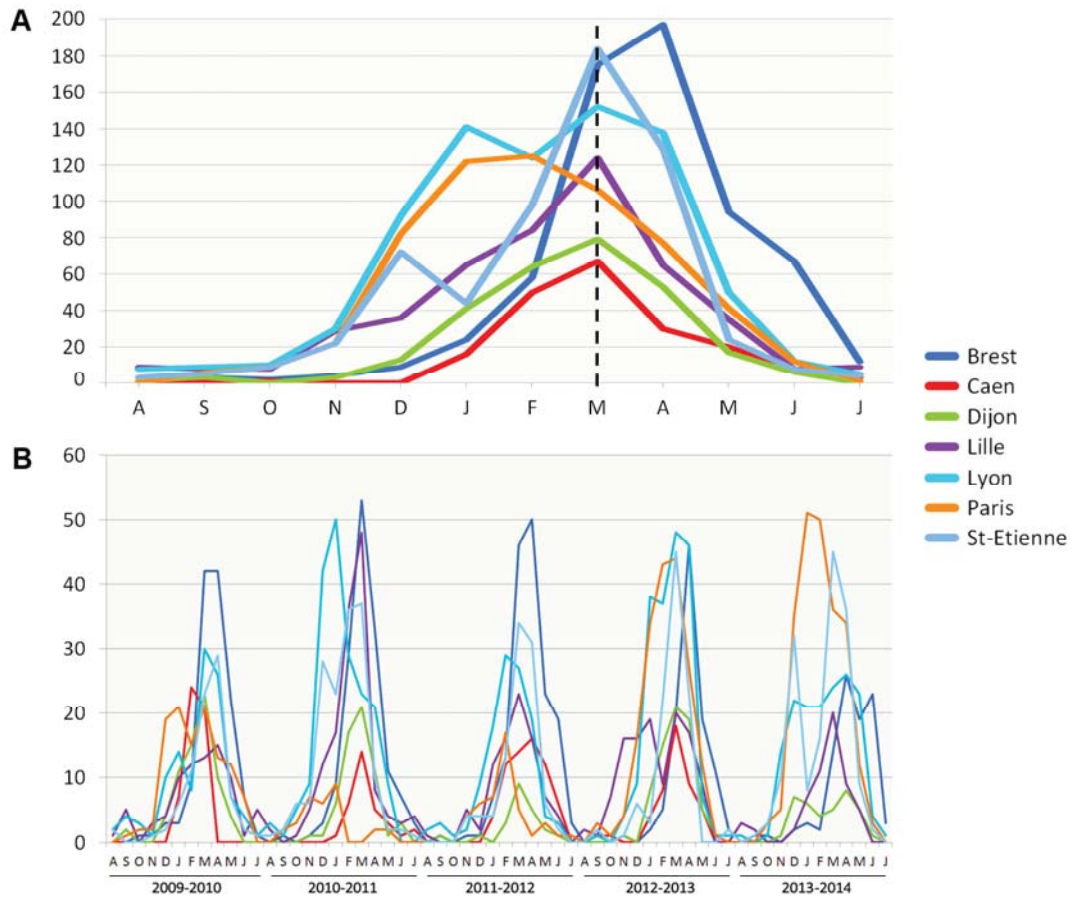
	No. of strains detected during the 5 consecutive seasons (%)					Total strains (%)
	2009-2010 <i>n</i> =812	2010-2011 <i>n</i> =859	2011-2012 <i>n</i> =849	2012-2013 <i>n</i> =1095	2013-2014 <i>n</i> =1093	
Common strains	757 (93.2)	826 (96.2)	784 (92.3)	1011 (92.3)	1010 (92.4)	4388 (93.2)
G1P[8]	503 (61.9)	628 (73.1)	465 (54.8)	731 (66.8)	593 (54.3)	2920 (62.0)
G2P[4]	143 (17.5)	37 (4.3)	50 (5.9)	43 (3.9)	12 (1.1)	285 (6.1)
G3P[8]	12 (1.5)	35 (4.1)	164 (19.3)	144 (13.2)	153 (14.0)	508 (10.8)
G4P[8]	16 (2.0)	63 (7.3)	7 (0.8)	24 (2.2)	12 (1.1)	122 (2.6)
G9P[8]	83 (10.2)	63 (7.3)	98 (11.5)	69 (6.3)	240 (22.0)	553 (11.7)
Emerging strains	0	3 (0.3)	35 (4.1)	33 (3.0)	7 (0.6)	78 (1.7)
G12P[8]						
Human reassortants	28 (3.2)	11 (1.3)	17 (2.0)	11 (1.0)	7 (0.6)	74 (1.6)
G1P[4]	5 (0.6)	4 (0.5)	4 (0.5)	0	0	13 (0.3)
G2P[8]	1 (0.1)	4 (0.5)	8 (0.9)	7 (0.6)	5 (0.5)	25 (0.5)
G3P[4]	21 (2.4)	1 (0.1)	1 (0.1)	0	1 (0.1)	24 (0.5)
G4P[4]	0	0	1 (0.1)	2 (0.2)	0	3 (0.1)
G9P[4]	1 (0.1)	2 (0.2)	3 (0.4)	2 (0.2)	1 (0.1)	9 (0.2)
Unusual strains	11 (1.4)	5 (0.6)	4 (0.5)	12 (1.1)	8 (0.7)	40 (0.8)
G1P[6]	1 (0.1)	0	0	5 (0.5)	0	6 (0.1)
G3P[3]	0	0	0	1 (0.1)	0	1 (<0.1)
G3P[6]	1 (0.1)	0	0	0	1 (0.1)	2 (<0.1)
G3/G9P[6] ^a	0	2 (0.2)	0	0	0	2 (<0.1)
G3P[9]	0	0	0	0	3 (0.3)	3 (0.1)
G4P[6]	2 (0.2)	0	0	0	0	2 (<0.1)
G6P[6]	1 (0.1)	1 (0.1)	2 (0.2)	2 (0.2)	4 (0.4)	10 (0.2)
G6P[14]	2 (0.2)	1 (0.1)	0	0	0	3 (0.1)
G8P[4]	1 (0.1)	0	0	0	0	1 (<0.1)
G8P[6]	1 (0.1)	0	0	0	0	1 (<0.1)
G8P[8]	0 (0.1)	1 (0.1)	0	1 (0.1)	0	2 (<0.1)
G8P[14]	1	0 (0.1)	1 (0.1)	0	0	2 (<0.1)
G10P[8]	0	0 (0.1)	1 (0.1)	0	0	1 (<0.1)
G12P[6]	1 (0.1)	0	0	3 (0.3)	0	4 (0.1)
Mixed infections^b	16 (2.0)	12 (1.4)	5 (0.6)	10 (0.9)	21 (1.9)	64 (1.4)
Partially typed	0	4 (0.5)	4 (0.5)	18 (1.6)	40 (3.7)	66 (1.4)

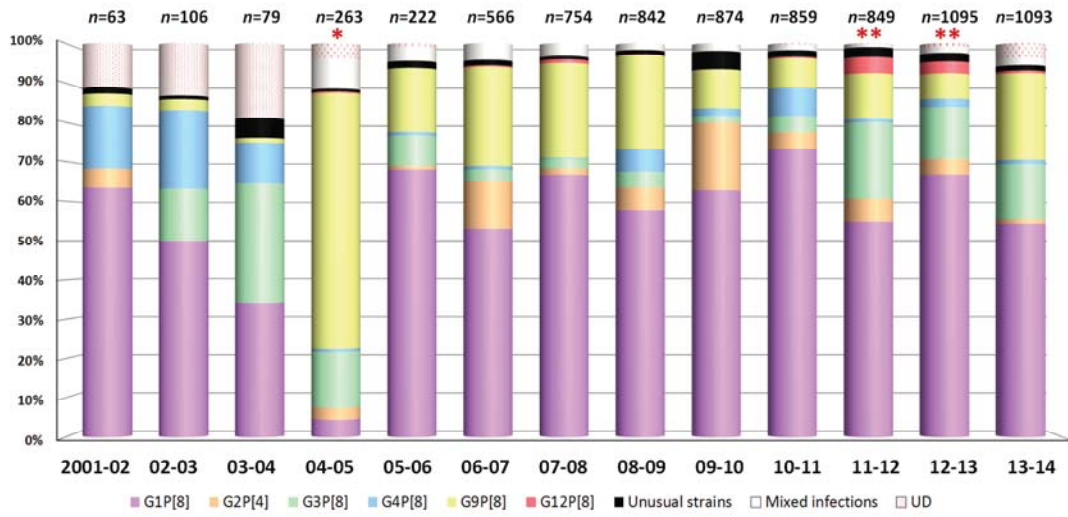
^a These strains are also included in mixed infections; ^b Details on mixed infection strains are reported in Table S1.

TABLE 2. Clinical characteristics according to rotavirus genotypes in 624 hospitalized diarrheal children between 2009 and 2014

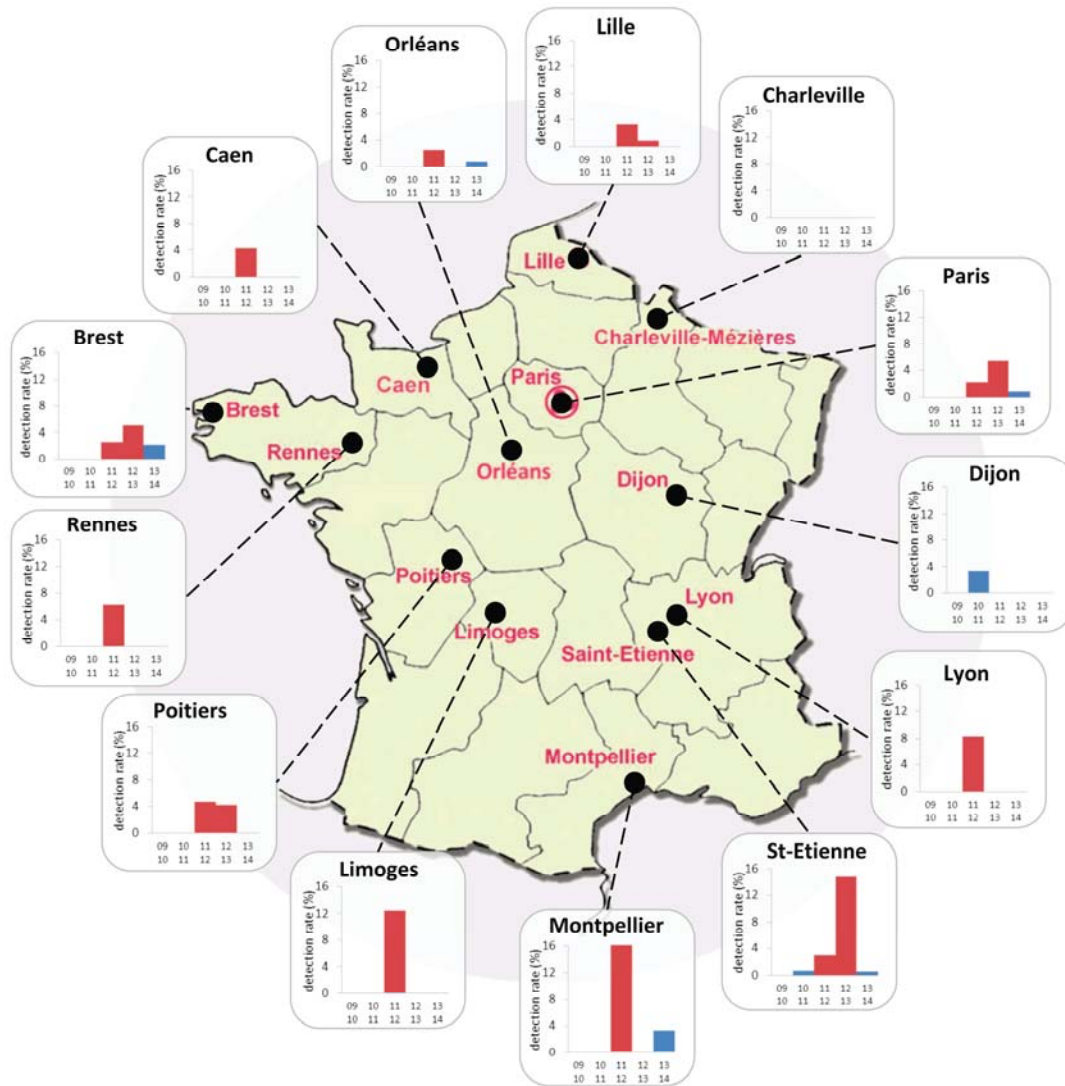
	G1P[8]	G2P[4]	G3P[8]	G4P[8]	G9P[8]	G12P[8]	Total	P^a	P^b
Prevalence	n=407 64.2%	n=59 9.3%	n=74 11.7%	n=5 0.8%	n=52 8.2%	n=27 4.3%	n=624 100%	-	-
Mean age, months	14.2 ±11.0	15.6 ±13.4	15.6 ±11.9	17.5 ±15.6	11.9 ±8.8	15.5 ±11.2	14.3 ±11.0	0.512 ^d	0.675 ^d
Sex ratio	1.6	1.3	1.3	0.7	2.5	1.8	1.6	0.520 ^e	0.495 ^e
Diarrhea	93.9%	94.9%	97.3%	100.0%	96.2%	96.0%	94.7%	0.607 ^e	0.962 ^e
Mean number of diarrhea bouts per day	6.0 ±3.9	4.5 ±3.0	8.3 ±5.9	6.4 ±5.9	7.5 ±3.3	5.9 ±5.8	6.4 ±4.3	0.433 ^d	0.078 ^d
Vomiting	80.0%	84.7%	89.0%	100.0%	78.8%	80.0%	81.4%	0.575 ^e	0.498 ^e
Mean number of vomiting bouts per day	4.6 ±3.4	5.3 ±5.5	6.1 ±4.0	2.5 ±0.7	5.8 ±5.2	7.7 ±6.3	5.1 ±4.2	0.114 ^d	0.516 ^d
Blood in stool	1.2%	0.0%	0.0%	0.0%	0.0%	4.0%	1.0%	0.306 ^e	0.464 ^e
Fever >38.5°C	72.3%	73.9%	61.0%	75.0%	64.3%	77.8%	70.5%	0.349 ^e	0.361 ^e
Temperature, °C	38.5 ±1.0	38.6 ±0.9	38.3 ±1.0	38.4 ±0.1	38.3 ±0.8	38.5 ±0.9	38.5 ±0.9	0.719 ^d	0.308 ^d
Dehydration	54.7%	53.4%	75.7%	40.0%	65.4%	56.0%	57.7%	0.139 ^e	0.131 ^e
Dehydration degree, %	7.2 ±3.5	6.0 ±2.5	7.0 ±3.4	3.5 ±2.1	6.6 ±3.6	6.4 ±2.8	6.9 ±3.4	0.545 ^d	0.223 ^d
Hypovolemia	37.9%	25.9%	64.8%	20.0%	46.0%	54.2%	41.0%	0.345 ^e	0.158 ^e
Intravenous rehydration	61.2%	65.5%	74.3%	40.0%	60.8%	68.0%	63.2%	0.337 ^e	0.265 ^e
Oral rehydration	13.6%	19.0%	12.2%	20.0%	21.6%	12.0%	14.4%	0.553 ^e	0.402 ^e
Intensive care	1.2%	1.7%	2.7%	0.0%	1.9%	0.0%	1.4%	0.740 ^e	0.628 ^e
Hospitalization duration, days	2.8 ±1.7	2.8 ±1.6	2.7 ±1.4	1.8 ±1.1	2.5 ±1.5	3.2 ±1.7	2.8 ±1.6	0.372 ^d	0.550 ^d

^a Comparison between G1P[8] and G12P[8] groups; ^b Comparison between all genotype groups; ^c Comparison within all genotype groups; ^d using Kruskal-Wallis test; ^e using Fisher's exact-test; Statistically significant if P<0.05.





G12P[8]	-	-	-	1	1	2	7	1	0	3	35	33	7
G12P[6]	-	-	-	-	-	-	1	-	1	-	-	3	-



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