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Molecular surveillance of norovirus, 2005-16 : an epidemiological analysis of data collected from the NoroNet network

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1 **Analysis of norovirus molecular surveillance data collected through the NoroNet**
2 **network, 2005 – 2016**

3

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21

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23

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82 **Abstract**

83

84 **Background**

85 Noroviruses are a common aetiology of acute gastroenteritis worldwide. Development
86 of vaccines requires detailed understanding of global genetic diversity of noroviruses.
87 This study describes trends in epidemiology and diversity based on global NoroNet
88 surveillance data, and gives a future perspective on the global surveillance needs in
89 light of these developments.

90

91 **Methods**

92 The study analysed n=16635 norovirus sequences with associated epidemiological
93 metadata, shared between 2005 and 2016 through NoroNet by partners from Europe,
94 Asia, Oceania, and Africa. Sequences and epidemiological data were obtained from
95 samples collected for outbreak investigations and diagnosis of sporadic gastroenteritis
96 cases by clinical-, public health-, and food microbiology laboratories.

97

98 **Findings**

99 During the study period, 26 different norovirus capsid genotypes circulated and 22
100 different recombinant genomes were found. The previously observed 2-3-year
101 periodicity of emergence of genogroup II genotype 4 (GII.4) drift variants was not
102 observed since 2012. Instead, the GII.4 Sydney capsid seems to persist through
103 recombination, and we report a novel recombinant of GII.P16-GII.4 Sydney 2012
104 variant in Asia and Europe. The novel GII.P17-GII.17, first reported in Asia in 2014,
105 has circulated widely in Europe. GII.4 viruses were more common in outbreaks in
106 healthcare settings compared to other genotypes.

107

108 **Interpretation**

109 Continuous changes in the global norovirus genetic diversity highlight the need for
110 sustained global norovirus surveillance, including assessment of possible immune
111 escape and evolution by recombination to provide a full overview of norovirus
112 epidemiology for future vaccine policy decisions.

113

114 **Funding**

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116 the Virgo Consortium funded by Dutch government, and by the Hungarian Scientific
117 Research Fund.
118

119 **[BOX] Research in context**

120

121 **Evidence before this study**

122 We searched Pubmed for articles published before 9th of July 2017 using keywords
123 (worldwide OR global) AND norovirus AND genetic AND diversity in the title or
124 abstract, and found 109 original research articles. The majority of studies reported on
125 norovirus genetic diversity in a limited geographic area, timeframe, or focused on a
126 single genotype. None of the studies presented long-term global norovirus diversity
127 trends combined with epidemiological metadata, except one study focusing on the
128 global norovirus diversity among oyster outbreaks.

129

130 **Added value of this study**

131 This study reports long-term global trends in norovirus genetic diversity combined
132 with epidemiological metadata, obtained from reports from 19 countries across four
133 continents/regions shared through a jointly owned database. It shows that multiple
134 norovirus genotypes are co-circulating simultaneously with continuous and rapid
135 changes in the norovirus genetic diversity worldwide, and with substantial regional
136 differences, possibly reflecting differences in epidemiology, susceptibility, or both.
137 We show differences in the preferred transmission route, preferred outbreak setting,
138 and seasonal variation between norovirus genotypes. Finally, we discuss gaps in the
139 norovirus surveillance and give recommendation for improvements to fulfil
140 surveillance needs in light of vaccine development and other future interventions.

141

142 **Implications of all the available evidence**

143 Norovirus candidate vaccines are currently tested in clinical trials. This study shows
144 that a future norovirus vaccine needs to induce broad protective immunity, or would
145 need to be updated on a regular basis due to continuous and rapid changes in the
146 norovirus genetic diversity. This study highlights the need for a global norovirus
147 surveillance system using optimized sequencing protocols to monitor possible
148 immune escape and evolution by recombination to provide data for vaccine updates.
149 Future studies need to address the underlying factors for preferences in transmission
150 routes, preferences in outbreak setting, and differences in seasonality among
151 noroviruses.

152

153 **Background**

154 Acute gastroenteritis is the second greatest burden of all infectious diseases and
155 norovirus is responsible for almost one fifth of all cases worldwide¹. For healthy
156 individuals, norovirus illness is typically self-limiting and of short duration, but risk
157 groups like young children, elderly, and immunocompromised patients can suffer
158 from prolonged symptoms². In order to better understand the epidemiology and
159 impact of norovirus and to identify (international) outbreaks, surveillance networks
160 have been set up in some countries in the last two decades. These efforts have been
161 challenging as norovirus surveillance is not mandatory in many countries, and if
162 available does not always include genetic data. Despite these challenges, collaborative
163 studies have identified international food-borne outbreaks, and substantially increased
164 our knowledge on the norovirus diversity and antigenic evolution with the voluntary
165 adoption of sequence-based typing^{3,4}. The genus *Norovirus* is highly diverse and
166 divided in seven genogroups (G) of which GI, GII, and GIV have been found among
167 humans. Genogroups are further subdivided in more than 40 genotypes⁵. The
168 epidemiology and human health impact are strongly shaped by norovirus evolution
169 through recombination or accumulation of mutations, known as genetic drift⁶. To
170 capture this diversity, norovirus nomenclature is based on two parameters describing
171 the genetic lineages of the gene encoding the viral polymerase (ORF1) and the capsid
172 protein (ORF2). Polymerase genotypes are distinguished from capsid genotypes by a
173 P in their name (e.g. GII.P4). This dual typing approach allows for tracking of
174 noroviruses, including recombinant forms⁷. In 2002, an informal international data
175 sharing network was established to study noroviruses and their diversity in relation to
176 human health impact⁸. The work from NoroNet has contributed to the understanding
177 that noroviruses from different genetic lineages may behave differently. Genogroup II
178 genotype 4 (GII.4) has been the predominant strain globally and responsible for
179 approximately 70% of outbreaks since the start of NoroNet⁹⁻¹¹. The antigenicity of the
180 capsid surface alters in a stepwise manner by selection of variants under the pressure
181 of population immunity – a process called epochal evolution³. In addition, frequent
182 exchanging of genes (recombination) results in emergence of novel noroviruses.
183 There is currently no licensed norovirus vaccine on the market, but potential
184 candidates have been tested in phase I and II clinical trials^{12,13}. Vaccine design is
185 complicated by the large antigenic variation within the genus, and is currently
186 targeting most commonly found genotypes. In view of the above, most likely, a future

187 vaccine would need to be updated on a regular basis given the flexibility of norovirus
188 to escape natural infection-derived population immunity, hence requiring improved
189 coverage of surveillance¹⁴. We analysed whether and how data obtained via the
190 NoroNet surveillance network can be used to address the following outstanding
191 questions regarding norovirus molecular epidemiology:

- 192 1. What are the trends in genomic diversity, recombination, and norovirus
193 reporting?
- 194 2. Is there evidence for differences by genogroup / genotype in region, setting,
195 and mode of transmission?
- 196 3. Where do new variants of norovirus emerge and can emerging variants be
197 predicted from globally linked surveillance data?

198
199

200 **Methods**

201

202 *NoroNet surveillance network*

203 NoroNet links clinical-, public health-, and food microbiology laboratories willing to
204 share norovirus molecular and epidemiological data on outbreaks and sporadic cases,
205 and has been in existence since the mid-1990s^{8,10,15}. The network started as EU
206 funded network in 1999, continuing since 2002 as global NoroNet⁸. A jointly owned
207 web-based database with online analysis tools was developed in which participants
208 share and compare their data. Participation is on a give and take basis and partners
209 have signed a code of conduct on uses of the data, after which they are granted full
210 access to the data. Partners are expected to contribute to joint reports, and the joint
211 database has been used for in depth studies following approval of partners.

212

213 *Samples and study area*

214 Specimens were obtained for the purpose of outbreak investigations and diagnosis of
215 sporadic gastroenteritis cases. All RT-PCR positive cases confirmed by sequencing
216 can be shared via NoroNet. Data from partners with less than 50 submitted sequences
217 during the study period were excluded. Based on these criteria, the study included
218 norovirus sequences obtained from samples collected in 19 countries: Austria,
219 Belgium, China, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Japan,
220 the Netherlands, New Zealand, Russia, Slovenia, South Africa, Spain, Sweden, and

221 the United Kingdom. Less than 50 entries had been obtained from partners in
222 Australia, Chile and Norway.

223

224 *Data analysis*

225 All entries submitted from January 1st 2005 to November 17th 2016 were downloaded
226 on November 18th 2016. Records from non-human origin, without sample date or with
227 a sample date prior to 2005 were removed from the analysis. Norovirus sequences
228 were genotyped by the online norovirus typing tool¹⁶. Sequences overlapping the
229 ORF1/ORF2 for which ORF1 and ORF2 genotypes could be assigned were analysed
230 separately. All available sequences in the NoroNet database, including those before
231 2005, were used for the analysis of first reports of emerging GII.4 variants. The
232 Maximum likelihood trees were inferred with PhyML version 3.1, using the general
233 time reversible (GTR) nucleotide substitution model with a proportion of invariant
234 sites and a Γ distribution of among-site rate variation¹⁷.

235

236 *Role of the funding source*

237 The funders had no role in designing the study, data collection, data analysis or
238 interpretation of data, writing the report, or in the decision to submit the paper for
239 publication. The corresponding author had full access to all data in the study and had
240 full responsibility for decision to submit for publication.

241

242 **Results**

243

244 *Surveillance coverage*

245 Sixteen countries (Austria, Belgium, Denmark, Finland, France, Germany, Hungary,
246 Italy, the Netherlands, Spain, China, Japan, South Africa, Sweden, United Kingdom,
247 Russia) submitted norovirus sequences in five or more successive years of which six
248 countries submitted sequences during the entire study period (Finland, France,
249 Germany, Hungary, Italy, and the Netherlands). The NoroNet surveillance network is
250 well represented in Europe and has a smaller number of collaborators in Asia,
251 Oceania, and Africa (Table S1).

252

253 *Number of reported sequences, sequence length and genome position*

254 A median of 870 (IQR 345) ORF1 sequences and a median of 577 (IQR 594) ORF2
255 sequences was reported per year. Sequence reads had an average length of 351 bases
256 and the majority of sequences were located in the RNA-dependent RNA polymerase
257 region of ORF1 or 5' side of ORF2 (Figure 1). Only 2.7% of sequences covered the
258 main antigenic sites located at the P2 domain of VP1. During the study period, 154
259 full VP1 sequences were reported including three full genome sequences (KC175323,
260 KC631827, and KP998539). An increased number of reported ORF1 sequences was
261 observed in years of or post introduction of new GII.4 variants (Den Haag 2006b in
262 2006, New Orleans 2009 in 2009, and Sydney 2012 in 2012) which could be
263 primarily attributed to GII.P4 and GII.Pe (Figure 2A). The apparent decline in number
264 of reported sequences in 2016 is an artefact due to the selection of sequences until
265 November 18th 2016 and a submission delay.

266

267 *Norovirus diversity at the genotype level*

268 The number of reported sequences and GI versus GII ratio per country was analysed
269 to get a better understanding of the genogroup coverage and diversity (Table S1).
270 Overall, 1372 of 16635 (8.2%) sequences belonged to norovirus GI, 15256 of 16635
271 (91.7%) sequences belonged to GII, and 7 of 16635 (0.0%) sequences belonged to
272 GIV.1. Austria reported the lowest GI proportion (3.2%) and Sweden the highest
273 (22.3%) among European countries, while countries in Asia and South Africa only
274 reported GII strains. Trends per genotype per year for GI and GII are shown in
275 Figures 2A and 2B. The most consistently and commonly detected genotype was
276 GII.P4 with 6125 of 11252 (54.8%) ORF1 sequences and 4184 of 6423 (65.1%)
277 ORF2 sequences listed as GII.4 by the phylogeny based typing tool. The remaining
278 ~40% is a diverse mixture of 31 ORF1 and 25 ORF2 genotypes with some genotypes
279 only detected incidentally, while other genotypes were detected more often in some
280 years.

281

282 *Emergence of novel GII.17 genotype*

283 NoroNet detected a sharp increase in the number of GII.P17 and GII.17 strains in
284 2015 – 2016 compared to previous years (Figure 2A and 2B). GII.P17 and / or GII.17
285 were widely detected among European countries (Belgium, Finland, France,
286 Germany, Hungary, Italy, the Netherlands, Russia, and Slovenia) in 2015 – 2016, but
287 not in all (Ireland, Spain, and United Kingdom) (Table S2A and S2B). The GII.P17

288 and GII.17 proportion of total number of sequences per country showed large
289 variation among European countries (range 4·2 - 53·9% and 5·3 - 44·5%,
290 respectively). GII.P17 and GII.17 were co-circulating with GII.P4, GII.Pe, and GII.4
291 strains in Europe, and were only more prevalent than GII.P4, GII.Pe, or GII.4 in
292 France (ORF1) and Russia (ORF1 and ORF2). China and Japan submitted in total
293 n=10 ORF1 and n=73 ORF2 sequences to NoroNet in 2015 - 2016, and China
294 reported n=1 GII.17 strain.

295

296 *Trends in GII.4 variants*

297 The NoroNet GII.4 variant distribution time trends are shown in Figure 3. In 2006,
298 GII.4 Hunter 2004 was replaced by GII.4 Den Haag 2006b, succeeded by GII.4 New
299 Orleans 2009 and GII.4 Sydney 2012 in the Northern hemisphere winter seasons of
300 2009/2010 and 2012/2013, respectively. The GII.4 Sydney ORF2 variant circulated as
301 recombinant with GII.Pe or GII.P4 New Orleans 2009 since it emerged in 2012, and
302 has not (yet) developed a new ORF1 variant. The GII.4 New Orleans 2009 ORF2
303 variant almost disappeared as of 2013, while the corresponding GII.P4 New Orleans
304 ORF1 variant was still widely detected due to recombination with the GII.4 Sydney
305 2012 ORF2 variant. The GII.4 variant group ‘other’ represents variants that were only
306 detected with limited geographic distribution and at low level incidence or sequences
307 that could not be typed to the variant level by the norovirus genotyping tool i.e. due to
308 a short sequence length. Variants that were detected infrequently during the study
309 period are: Camberwell 1994, Farmington Hills 2002, Asia 2003, Kaiso 2003,
310 Yerseke 2006a, Apeldoorn 2007, and Osaka 2007. A novel GII.P16-GII.4 Sydney
311 2012 recombinant was detected in 2014 (n=2) (Germany and the Netherlands), not
312 detected in 2015, and detected in Japan, China, and the Netherlands (n=13) in 2016
313 (see paragraph recombination for more information on the novel GII.P16-GII.4
314 Sydney 2012 recombinant).

315

316 *Origin of novel GII.4 drift variants*

317 To assess when and where novel drift variants originate, we assessed the sampling
318 date and country of origin of the first reported sequence of global drift variants (Table
319 S3). All assessed variants, except Hunter 2004, were detected 2-5 years before the
320 global predominance of the particular strain, which may indicate that new drift
321 variants were present at low levels in the population before their actual global

322 emergence. Hunter 2004 was firstly detected in the Netherlands in the year of
323 emergence 2004.

324

325 *Recombination*

326 To assess the influence of ORF1/ORF2 recombination on the norovirus diversity, we
327 selected all sequences (n=1047) that were overlapping the ORF1/ORF2 junction and
328 for which both ORF1 and ORF2 sides could be genotyped by the norovirus
329 genotyping tool. 477 of 1047 (45.6%) sequences were assigned as a recombinant
330 strain (Table S4). No between genogroup recombination was observed. Remarkably,
331 some polymerase types are more prone to recombine than others. Recombination
332 within GII was most common: 457 recombinant sequences belong to GII of which
333 GII.Pe–GII.4, GII.P21–GII.3, and GII.P7–GII.P6 are the most commonly detected
334 recombinants. ORF2 GII.4 has been detected in combination with GII.P12, GII.P16,
335 and GII.Pe. The GII.P12 recombinant was detected in 2005 – 2006 in combination
336 with GII.4 Asia 2003. GII.P16 and GII.Pe are both only found in combination with
337 GII.4 Sydney 2012 between 2014 and 2016 (data not shown). GII.P16 was found in
338 combination with five different VP1 genotypes: GII.3, GII.4, GII.10, GII.12, and
339 GII.13 which each form a separate clade in a maximum likelihood tree inferred from
340 partial GII.P16 sequences (Figure S1). Three variants of GII.4 Sydney are currently
341 co-circulating, all resulting from recombination: GII.P4 Orleans 2009-GII.4 Sydney
342 2012, GII.Pe-GII.4 Sydney 2012 and GII.P16-GII.4 Sydney 2012. The antigenic
343 regions in the capsid do not contain any amino acid changes compared to previously
344 circulating GII.4 Sydney strains, although the VP1 sequences of GII.P16-GII.4
345 Sydney 2012 cluster separately from other GII.Pe-GII.4 Sydney strains (Table S5 and
346 Figure S2).

347

348 *Differences by season, region, setting, and mode of transmission*

349 The European norovirus season coincides with the Northern Hemisphere winter
350 season (Figure 4A). GII.Pe/GII.P4-GII.4 sequences show the clearest winter
351 seasonality patterns while GI and GII non GII.Pe/GII.P4-GII.4 strains are more
352 continuously present throughout the year, but never exceed the number of
353 GII.Pe/GII.P4-GII.4 sequences. The rate of norovirus submissions in Africa (all
354 reported by South Africa) shows an elevation in the months September – November
355 which coincides with the Southern Hemisphere spring season (Figure 4B). Asia

356 (reported by China and Japan) shows an elevation of the norovirus incidence in the
357 Northern Hemisphere winter season with the peak in November, two months earlier
358 compared to Europe (Figure 4C). Oceania (reported by New Zealand) shows highest
359 incidence in October and November (spring) (Figure 4D).

360

361 The suspected mode of transmission was reported for n=6446 entries: 77·4% person-
362 to-person transmission (n=4990), 19·9% foodborne transmission (n=1280), 2·1%
363 waterborne transmission, and 0·7% other transmission mode (n=133, n=43,
364 respectively) (Figure 5A). GII.4 is relatively more often transmitted via person-to-
365 person compared to other genotypes.

366

367 The setting of the norovirus outbreak was reported for n=8772 entries: 29·7% hospital
368 setting (n=2603), 36·0% residential institution (n=3154), 9·3% hotel, restaurant or
369 caterer (n=819), 11·8% day care or school (n=1039), 13·2% other (n=1157) (Figure
370 5B). The majority of sequences were derived from samples obtained in health care -
371 or residential institutions. GII.4 was relatively more often detected in healthcare
372 settings (hospitals and residential institutions) compared to non-GII.4 genotypes.

373

374 **Discussion**

375 Despite differences in norovirus surveillance among countries and a lack of it in many
376 others, the current NoroNet system is able to observe global trends and major shifts in
377 the genetic composition of the virus population at the level of genotype and variant, as
378 was shown by this study and by others^{6,10,18,19}.

379

380 The first question addressed in this study is about the trends in norovirus genomic
381 diversity, recombination, and norovirus reporting. During the study period, we
382 observed circulation of at least 26 ORF2 genotypes when looking at diversity of the
383 capsid gene. The viral capsid contains epitopes that are targeted by protective
384 antibody responses, and understanding this diversity is important for evaluation of
385 candidate vaccines²⁰. It was previously noted that increased notification reflect true
386 increases in disease trends^{18,21}. Therefore, the observed increase in reported sequences
387 post emergence of new GII.4 variants is probably related to an increase in norovirus
388 activity. GII.4 Sydney 2012 is the predominantly detected variant worldwide since
389 2012 and, given the replacement cycle of two to three years shown for previous

390 variants, a new antigenic variant has been anticipated for some years. This trend in
391 antigenic evolution, however, was not observed in the period described here. Instead,
392 viruses with GII.4 Sydney capsids, have evolved by recombination, suggesting that
393 recombination somehow favours virus maintenance in the population. For GII.4,
394 recombination has previously only been with the closely related sequence types
395 GII.Pe and GII.P12, which are both suggested to be derived from an ancestor of
396 GII.P4²². The drivers for emergence of recombinant genomes in a population
397 previously exposed to the same capsid sequences remains to be understood. The novel
398 recombinant GII.P16-GII.4 Sydney 2012 may have increased fitness due to changes
399 in the RNA dependent RNA polymerase (RdRp) that alter the polymerase fidelity and
400 interaction with VP1, leading to differences in replication and/or transmission
401 efficiency²³⁻²⁶.

402

403 In addition to the globally prevalent GII.4 viruses, recent studies from Asia reported a
404 major shift in genotype composition from the predominant GII.4 to the novel
405 GII.P17-GII.17 norovirus strain (GII.17 Kawasaki 2014) late 2014 and onwards^{19,27}.
406 The number of detected GII.P17-GII.17 strains among Asian countries within our
407 network was limited and likely caused by a filtered submission of the respective
408 countries. The GII.P17-GII.17 strain was widely detected among most European
409 countries in 2015 and 2016 and showed substantial differences in prevalence among
410 countries. This strain has not (yet) fully replaced GII.4 strains.

411

412 The great genetic diversity of noroviruses is typically not considered in
413 epidemiological or clinical studies, but may translate to differences in the
414 epidemiology. Therefore, we compared distribution of reported modes of transmission
415 and settings for the reported outbreaks by genotype (question 2). The most commonly
416 reported transmission mode for the GII.4 outbreaks reported to NoroNet was person-
417 to-person transmission and the most commonly reported setting was residential
418 institution¹⁰. Underlying driving factors for these differences compared with other
419 genotypes are unknown. We observed substantial regional variation in the norovirus
420 genotype distribution possibly reflecting differences in epidemiology, susceptibility of
421 the population, or both.

422

423 Norovirus surveillance is done on a voluntary basis since funding for the network is
424 unavailable. This is reflected by unstable reporting behaviour of many countries and a
425 potential bias in this study. A limitation of the NoroNet network is that
426 unstandardized convenience sampling and irregular submission affects the ability of
427 the network to robustly identify the effect of introduction of new genotypes and
428 variants on the norovirus impact and severity. Another limitation of the study are the
429 gaps on the surveillance map with missing or limited data from most countries in
430 Africa, Middle East, North – and South America, Oceania, and Asia. The USA and
431 Australia do have norovirus surveillance, but use separate databases to store and
432 analyse their data. Future integration of surveillance databases could help to improve
433 our understanding of the norovirus (molecular) epidemiology.

434

435 A potential use of the NoroNet network is the identification of international
436 outbreaks, which have been observed during periods of sustained funding^{4,28}. The
437 currently provided sequence data can be used to genotype a virus to the level of
438 genotype and variant, but is less suitable for phylogenetic analysis for the purpose of
439 international outbreak investigations due to the lack of standardisation of sequencing
440 protocols. The use of next generation sequencing is explored to allow whole genome
441 sequencing as a new standard to overcome this problem²⁹⁻³¹. Most countries currently
442 upload data to the NoroNet database batch wise, which leads to a submission delay
443 and identification of international outbreaks potentially months after their occurrence.
444 Countries would need to upload data on a weekly basis to be able to set effective
445 public health measures (i.e. withdraw of a contaminated food product from the
446 market).

447

448 Norovirus vaccine candidates are currently in phase I and II trials and although
449 vaccine cross-protection, efficacy, and effectiveness need to be evaluated, especially
450 in vulnerable patient populations, it seems likely that a norovirus vaccine will be
451 available in the near future. Such a vaccine will likely need to be updated on a regular
452 basis due to escape of the virus from population immunity, especially by the
453 predominant GII.4³². Essential data about the antigenic changes, especially those
454 located in the P2 domain of the major capsid of the virus, can be obtained via a global
455 surveillance system. As a minimum, a shared protocol for sequencing is needed,
456 preferably including the ORF1 / ORF2 overlap to genotype both the viral RNA-

457 dependent RNA polymerase and VP1, and to detect recombinant strains. A protocol
458 for sequencing this particular region has been described³³. In addition to this protocol,
459 a subset of specimens could be monitored for changes in the antigenic regions using a
460 protocol spanning the P domain of VP1. Whole genome sequencing via next
461 generation sequencing techniques could replace both protocols and potentially
462 provide a better insight in the evolution of the virus, including the not well studied
463 VP2.

464

465 One of the major questions within the norovirus research field is whether we are
466 capable of predicting emerging variants in the near future, the third and last question
467 addressed in our study. All recent major drift variants were already circulating years
468 before they became dominant as shown by this study and by others, suggesting early
469 warning surveillance for variant emergence would be possible³⁴. If we assume that
470 new variants develop in the human population and could emerge anywhere in the
471 world, as shown by this study and by others, this would require a surveillance system
472 with global coverage including large-scale genomics to capture both capsid diversity
473 and recombination^{35,36}. A next step would be to predict antigenic properties from the
474 genomic diversity, although this is likely to be challenging and requires development
475 of phenotypic assays to assess antigenicity and immunity, similar to the model of the
476 global influenza virus surveillance network. More research and new funding sources
477 are needed to address these issues.

478

479 **Contributors**

480 MK, MG, and JB designed the study. MK, MG and JB analysed and interpreted the
481 data, and MG and JB prepared the tables and figures. MK, MG and JB wrote the
482 manuscript. AK, MC, HV and NI collected data and critically read the manuscript. All
483 other authors contributed by submitting data during the study period.

484

485 **Declaration of interests**

486 We declare that we have no conflicts of interest.

487

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496

497 **Author declaration**

498 DJA is affiliated to the National Institute for Health Research Health Protection
499 Research Unit (NIHR HPRU) in Gastrointestinal Infections at University of Liverpool
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501 East Anglia, University of Oxford and the Institute of Food Research. DJA is based at
502 The London School of Hygiene and Tropical Medicine, and Public Health England.
503 The views expressed are those of the author(s) and not necessarily those of the NHS,
504 the NIHR, the Department of Health or Public Health England.

505

506 **Figure legends**

507

508 **Figure 1** Position of 16628 sequence reads on the norovirus genome. Each sequence
509 represents a line in the figure. Boxes above the graph represent the norovirus open
510 reading frames (ORFs) of reference GII.Pe-GII.4 Sydney 2012 (Genbank accession:
511 JX459908). ORF1 encodes for a polyprotein that is post-translationally cleaved by the
512 virus-encoded protease (Pro) into six non-structural proteins (p48, NTPase, p22, VPg,
513 Pro, and RNA-dependent RNA polymerase (RdRp)). ORF2 encodes for the major
514 capsid protein (VP1) which consists of a shell (S) and protruding domains P1 and P2
515 with antigenic epitopes A, D, and E. ORF3 encodes for the minor capsid protein VP2.
516

517 **Figure 2** Number of reported ORF1 sequences (n=11252) stratified per genotype
518 group, genotype, and year (**A**) and number of reported ORF2 sequences (n=6423)
519 stratified per genotype group, genotype, and year (**B**). Note that n=1047 sequences
520 overlapping ORF1/ORF2 are counted for both ORF1 and ORF2.

521

522 **Figure 3** ORF1 GII.P4 variant trends per year (n=8083, top) and ORF2 GII.4 variant
523 trends per year (n=4184, bottom). The relatively high proportion of viruses/sequences
524 typed as “other” in the oldest category of submissions is an artefact due to the typing
525 tool that was used. This tool performs a phylogeny based assignment of norovirus
526 sequences to genera, genotypes, and variants. For correct assignment of variants, the
527 reference sequences need to be periodically updated, when a new variants arise. By
528 focusing on correct assignment of recent sequences, older strains may then be labelled
529 as “unknown” with the current version of the typing tool.

530

531 **Figure 4** Norovirus seasonality patterns in Europe (n=13935) (**A**), Africa (n=195)
532 (**B**), Asia (n=262) (**C**), and Oceania (n=806) (**D**), stratified per genotype group.
533 Records without sample month were removed for this analysis.

534

535 **Figure 5** Norovirus transmission route (n=8772) (**A**) and suspected outbreak setting
536 (n=6446) (**B**), stratified per genotype group. Records without known transmission
537 route or suspected outbreak setting were removed. Outbreaks with suspected
538 foodborne origin and subsequent person-to-person transmission were recoded as
539 foodborne.

540

541 **Figure S1** Maximum likelihood tree for region B of ORF1 sequences displaying the
542 genetic diversity of GII.P16 sequences that are found in combination with different
543 VP1 sequences (used sequence length 289 nucleotides, n=34). GII.P16-GII.4 Sydney
544 2012 sequences are indicated in red.

545

546 **Figure S2** Maximum likelihood tree inferred from all complete GII.4 VP1 sequences
547 displaying the genetic diversity of GII.4 sequences that are detected in combination
548 with different polymerase genotypes. GII.P16-GII.4 Sydney 2012 sequences are
549 indicated in red.

550

551

552

553 **References**

- 554 1. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in
555 cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*
556 2014; **14**(8): 725-30.
- 557 2. Lindsay L, Wolter J, De Coster I, Van Damme P, Verstraeten T. A decade of
558 norovirus disease risk among older adults in upper-middle and high income
559 countries: a systematic review. *BMC infectious diseases* 2015; **15**: 425.
- 560 3. Siebenga JJ, Vennema H, Renckens B, et al. Epochal evolution of GGII.4
561 norovirus capsid proteins from 1995 to 2006. *J Virol* 2007; **81**(18): 9932-41.
- 562 4. Verhoef L, Kouyos RD, Vennema H, et al. An integrated approach to
563 identifying international foodborne norovirus outbreaks. *Emerg Infect Dis* 2011;
564 **17**(3): 412-8.
- 565 5. de Graaf M, van Beek J, Koopmans MP. Human norovirus transmission
566 and evolution in a changing world. *Nat Rev Microbiol* 2016; **14**(7): 421-33.
- 567 6. Siebenga JJ, Vennema H, Zheng DP, et al. Norovirus illness is a global
568 problem: emergence and spread of norovirus GII.4 variants, 2001-2007. *J Infect*
569 *Dis* 2009; **200**(5): 802-12.
- 570 7. Kroneman A, Vega E, Vennema H, et al. Proposal for a unified norovirus
571 nomenclature and genotyping. *Arch Virol* 2013; **158**(10): 2059-68.
- 572 8. Koopmans M, Vennema H, Heersma H, et al. Early identification of
573 common-source foodborne virus outbreaks in Europe. *Emerg Infect Dis* 2003;
574 **9**(9): 1136-42.
- 575 9. Siebenga JJ, Vennema H, Duizer E, Koopmans MP. Gastroenteritis caused
576 by norovirus GGII.4, The Netherlands, 1994-2005. *Emerg Infect Dis* 2007; **13**(1):
577 144-6.
- 578 10. Kroneman A, Verhoef L, Harris J, et al. Analysis of integrated virological
579 and epidemiological reports of norovirus outbreaks collected within the
580 Foodborne Viruses in Europe network from 1 July 2001 to 30 June 2006. *J Clin*
581 *Microbiol* 2008; **46**(9): 2959-65.
- 582 11. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinje J. Genotypic and
583 epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. *J*
584 *Clin Microbiol* 2014; **52**(1): 147-55.
- 585 12. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against
586 experimental human GII.4 virus illness: a challenge study in healthy adults. *J*
587 *Infect Dis* 2015; **211**(6): 870-8.
- 588 13. Treanor JJ, Atmar RL, Frey SE, et al. A novel intramuscular bivalent
589 norovirus virus-like particle vaccine candidate--reactogenicity, safety, and
590 immunogenicity in a phase 1 trial in healthy adults. *J Infect Dis* 2014; **210**(11):
591 1763-71.
- 592 14. Debbink K, Lindesmith LC, Baric RS. The state of norovirus vaccines. *Clin*
593 *Infect Dis* 2014; **58**(12): 1746-52.
- 594 15. Kroneman A, Harris J, Vennema H, et al. Data quality of 5 years of central
595 norovirus outbreak reporting in the European Network for food-borne viruses. *J*
596 *Public Health (Oxf)* 2008; **30**(1): 82-90.
- 597 16. Kroneman A, Vennema H, Deforche K, et al. An automated genotyping tool
598 for enteroviruses and noroviruses. *J Clin Virol* 2011; **51**(2): 121-5.
- 599 17. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate
600 large phylogenies by maximum likelihood. *Syst Biol* 2003; **52**(5): 696-704.

- 601 18. van Beek J, Ambert-Balay K, Botteldoorn N, et al. Indications for
602 worldwide increased norovirus activity associated with emergence of a new
603 variant of genotype II.4, late 2012. *Euro Surveill* 2013; **18**(1): 8-9.
- 604 19. de Graaf M, van Beek J, Vennema H, et al. Emergence of a novel GII.17
605 norovirus - End of the GII.4 era? *Euro Surveill* 2015; **20**(26).
- 606 20. Lindesmith LC, Costantini V, Swanstrom J, et al. Emergence of a norovirus
607 GII.4 strain correlates with changes in evolving blockade epitopes. *J Virol* 2013;
608 **87**(5): 2803-13.
- 609 21. Verhoef L, Depoortere E, Boxman I, et al. Emergence of new norovirus
610 variants on spring cruise ships and prediction of winter epidemics. *Emerg Infect*
611 *Dis* 2008; **14**(2): 238-43.
- 612 22. Eden JS, Tanaka MM, Boni MF, Rawlinson WD, White PA. Recombination
613 within the pandemic norovirus GII.4 lineage. *J Virol* 2013; **87**(11): 6270-82.
- 614 23. Bull RA, Eden JS, Rawlinson WD, White PA. Rapid evolution of pandemic
615 noroviruses of the GII.4 lineage. *PLoS Pathog* 2010; **6**(3): e1000831.
- 616 24. Arias A, Thorne L, Ghurburrun E, Bailey D, Goodfellow I. Norovirus
617 Polymerase Fidelity Contributes to Viral Transmission In Vivo. *mSphere* 2016;
618 **1**(5).
- 619 25. Ruis C, Roy S, Brown JR, Allen DJ, Goldstein RA, Breuer J. The emerging
620 GII.P16-GII.4 Sydney 2012 norovirus lineage is circulating worldwide, arose by
621 late-2014 and contains polymerase changes that may increase virus
622 transmission. *PloS one* 2017; **12**(6): e0179572.
- 623 26. Subba-Reddy CV, Yunus MA, Goodfellow IG, Kao CC. Norovirus RNA
624 synthesis is modulated by an interaction between the viral RNA-dependent RNA
625 polymerase and the major capsid protein, VP1. *J Virol* 2012; **86**(18): 10138-49.
- 626 27. Chan MC, Lee N, Hung TN, et al. Rapid emergence and predominance of a
627 broadly recognizing and fast-evolving norovirus GII.17 variant in late 2014. *Nat*
628 *Commun* 2015; **6**: 10061.
- 629 28. Verhoef L, Hewitt J, Barclay L, et al. Norovirus genotype profiles
630 associated with foodborne transmission, 1999-2012. *Emerg Infect Dis* 2015;
631 **21**(4): 592-9.
- 632 29. Brown JR, Roy S, Ruis C, et al. Norovirus Whole-Genome Sequencing by
633 SureSelect Target Enrichment: a Robust and Sensitive Method. *J Clin Microbiol*
634 2016; **54**(10): 2530-7.
- 635 30. Bavelaar HH, Rahamat-Langendoen J, Niesters HG, Zoll J, Melchers WJ.
636 Whole genome sequencing of fecal samples as a tool for the diagnosis and
637 genetic characterization of norovirus. *J Clin Virol* 2015; **72**: 122-5.
- 638 31. van Beek J, de Graaf M, Smits SL, et al. Whole genome next generation
639 sequencing to study within-host evolution of chronic norovirus infection among
640 immunocompromised patients. *J Infect Dis* 2017.
- 641 32. Parra GI, Squires RB, Karangwa CK, et al. Static and Evolving Norovirus
642 Genotypes: Implications for Epidemiology and Immunity. *PLoS Pathog* 2017;
643 **13**(1): e1006136.
- 644 33. van Beek J, van der Eijk AA, Fraaij PL, et al. Chronic norovirus infection
645 among solid organ recipients in a tertiary care hospital, the Netherlands, 2006-
646 2014. *Clin Microbiol Infect* 2016.
- 647 34. Allen DJ, Trainor E, Callaghan A, O'Brien SJ, Cunliffe NA, Iturriza-Gomara
648 M. Early Detection of Epidemic GII-4 Norovirus Strains in UK and Malawi: Role of

649 Surveillance of Sporadic Acute Gastroenteritis in Anticipating Global Epidemics.
650 *PloS one* 2016; **11**(4): e0146972.
651 35. Bull RA, Tu ET, McIver CJ, Rawlinson WD, White PA. Emergence of a new
652 norovirus genotype II.4 variant associated with global outbreaks of
653 gastroenteritis. *J Clin Microbiol* 2006; **44**(2): 327-33.
654 36. Eden JS, Hewitt J, Lim KL, et al. The emergence and evolution of the novel
655 epidemic norovirus GII.4 variant Sydney 2012. *Virology* 2014; **450-451**: 106-13.
656

Figure 1

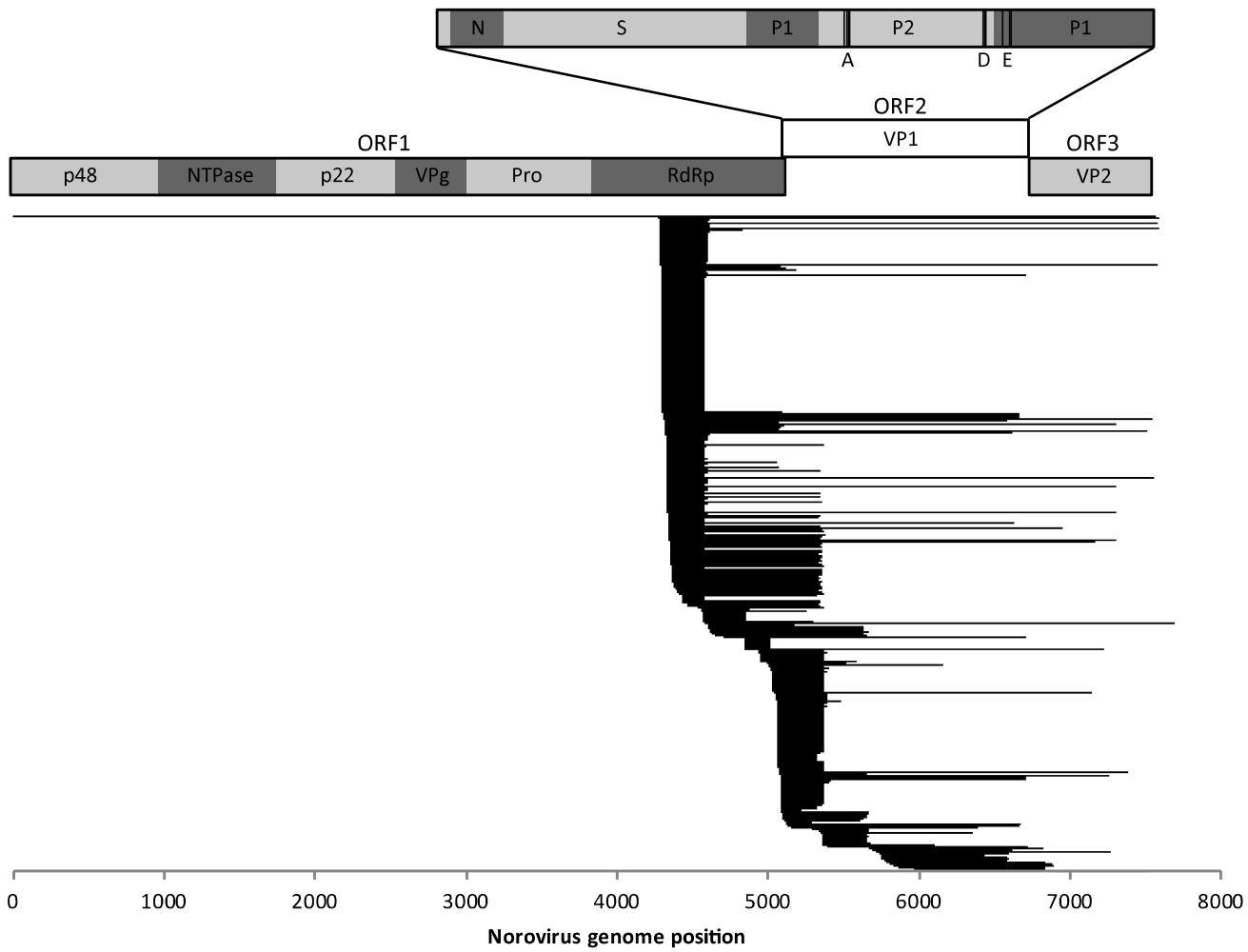
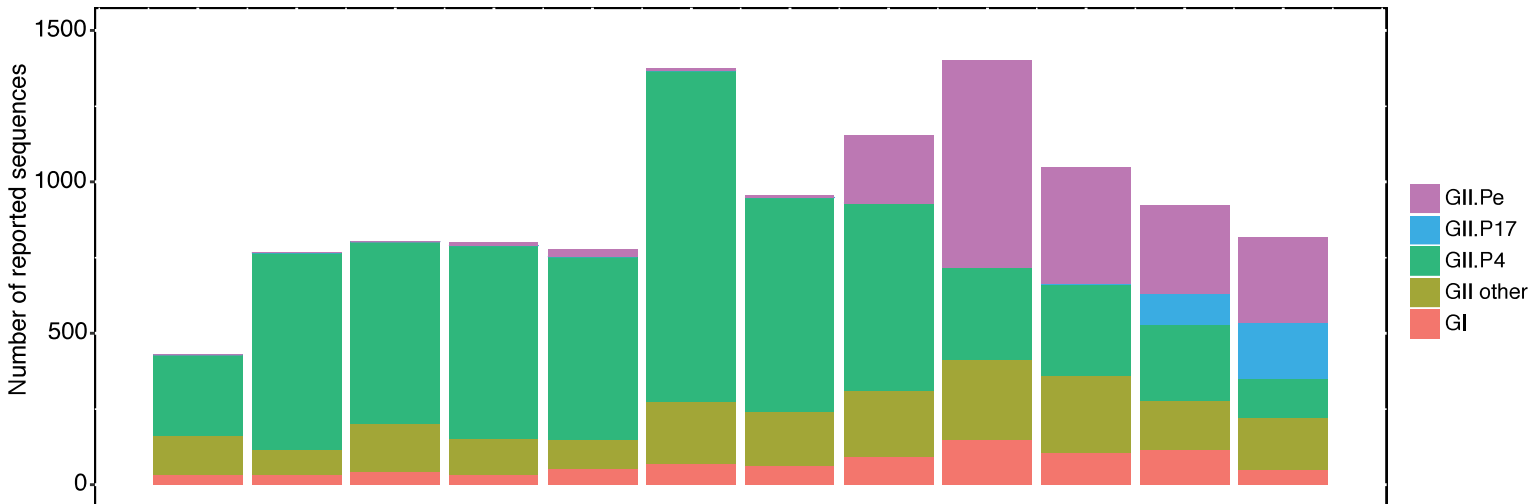
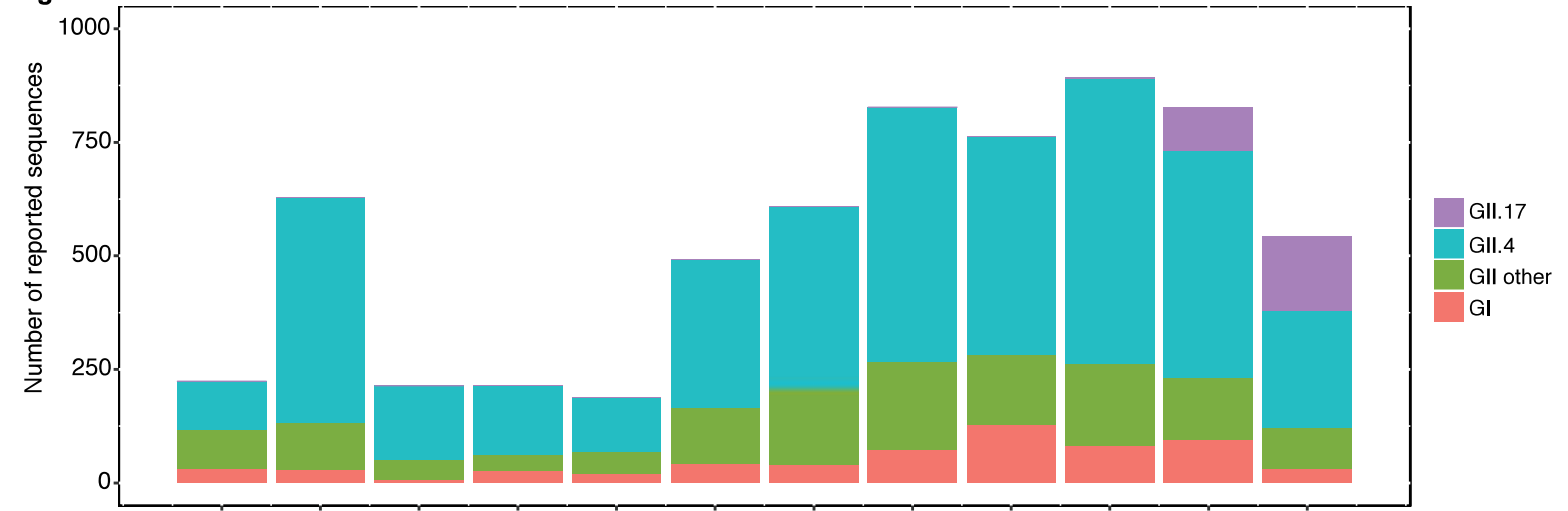


Figure 2A



	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
GI.P1	4 (0,9)	0 (0)	2 (0,2)	0 (0)	0 (0)	1 (0,1)	2 (0,2)	0 (0)	4 (0,3)	3 (0,3)	6 (0,7)	1 (0,1)
GI.P2	3 (0,7)	13 (1,7)	5 (0,6)	3 (0,4)	0 (0)	3 (0,2)	3 (0,3)	5 (0,4)	2 (0,1)	17 (1,6)	10 (1,1)	17 (2,1)
GI.P3	9 (2,1)	7 (0,9)	17 (2,1)	18 (2,2)	16 (2,1)	4 (0,3)	7 (0,7)	10 (0,9)	34 (2,4)	39 (3,7)	51 (5,5)	7 (0,9)
GI.P4	9 (2,1)	7 (0,9)	5 (0,6)	5 (0,6)	33 (4,2)	32 (2,3)	21 (2,2)	14 (1,2)	40 (2,9)	4 (0,4)	10 (1,1)	12 (1,5)
GI.P5	2 (0,5)	1 (0,1)	0 (0)	0 (0)	1 (0,1)	0 (0)	0 (0)	0 (0)	6 (0,4)	4 (0,4)	7 (0,8)	3 (0,4)
GI.P6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0,3)	0 (0)	0 (0)	0 (0)
GI.P7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	20 (1,5)	3 (0,3)	6 (0,5)	7 (0,5)	2 (0,2)	3 (0,3)	0 (0)
GI.P8	2 (0,5)	0 (0)	0 (0)	1 (0,1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	0 (0)	0 (0)	0 (0)
GI.P9	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	0 (0)	0 (0)	0 (0)	4 (0,3)	4 (0,4)	0 (0)	1 (0,1)
GI.Pa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	2 (0,2)	2 (0,1)	0 (0)	0 (0)	0 (0)
GI.Pb	2 (0,5)	4 (0,5)	13 (1,6)	5 (0,6)	2 (0,3)	10 (0,7)	25 (2,6)	50 (4,3)	43 (3,1)	21 (2)	22 (2,4)	4 (0,5)
GI.Pd	3 (0,7)	2 (0,3)	1 (0,1)	0 (0)	1 (0,1)	0 (0)	1 (0,1)	1 (0,1)	3 (0,2)	9 (0,9)	0 (0)	4 (0,5)
GI.Pf	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (0,5)	1 (0,1)	2 (0,2)	5 (0,5)	1 (0,1)
GI.LP2	15 (3,5)	10 (1,3)	9 (1,1)	8 (1)	11 (1,4)	23 (1,7)	11 (1,2)	17 (1,5)	39 (2,8)	35 (3,3)	34 (3,7)	43 (5,3)
GI.LP3	1 (0,2)	2 (0,3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI.LP4	269 (62,7)	649 (84,6)	603 (75,1)	639 (79,7)	603 (77,6)	1094 (79,6)	709 (74,2)	617 (53,4)	302 (21,5)	301 (28,7)	252 (27,3)	127 (15,5)
GI.LP6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	1 (0,1)	0 (0)	0 (0)	0 (0)	0 (0)
GI.LP7	59 (13,8)	18 (2,3)	39 (4,9)	28 (3,5)	28 (3,6)	31 (2,3)	67 (7)	95 (8,2)	93 (6,6)	81 (7,7)	62 (6,7)	33 (4)
GI.LP8	1 (0,2)	0 (0)	0 (0)	1 (0,1)	1 (0,1)	3 (0,2)	0 (0)	1 (0,1)	2 (0,1)	2 (0,2)	0 (0)	0 (0)
GI.LP11	1 (0,2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI.LP12	3 (0,7)	6 (0,8)	2 (0,2)	0 (0)	0 (0)	0 (0)	0 (0)	7 (0,6)	1 (0,1)	0 (0)	0 (0)	3 (0,4)
GI.LP13	0 (0)	7 (0,9)	5 (0,6)	2 (0,2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI.LP15	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	1 (0,1)	0 (0)	1 (0,1)	0 (0)	1 (0,1)	0 (0)	0 (0)
GI.LP16	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	0 (0)	5 (0,5)	17 (1,5)	19 (1,4)	17 (1,6)	5 (0,5)	31 (3,8)
GI.LP17	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	4 (0,4)	102 (11,1)	185 (22,6)
GI.LP20	3 (0,7)	1 (0,1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	0 (0)
GI.LP21	42 (9,8)	39 (5,1)	101 (12,6)	70 (8,7)	46 (5,9)	52 (3,8)	31 (3,2)	30 (2,6)	75 (5,3)	92 (8,8)	41 (4,4)	49 (6)
GI.LP22	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	0 (0)	0 (0)	3 (0,3)	16 (1,1)	2 (0,2)	0 (0)	3 (0,4)
GI.LPc	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0,3)	2 (0,2)	0 (0)	0 (0)
GI.LPe	0 (0)	1 (0,1)	0 (0)	12 (1,5)	24 (3,1)	7 (0,5)	7 (0,7)	225 (19,5)	686 (48,9)	384 (36,6)	291 (31,6)	281 (34,4)
GI.LPg	1 (0,2)	0 (0)	1 (0,1)	7 (0,9)	7 (0,9)	93 (6,8)	61 (6,4)	47 (4,1)	14 (1)	22 (2,1)	20 (2,2)	12 (1,5)
GI.LPm	0 (0)	0 (0)	0 (0)	3 (0,4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	429 (100)	767 (100)	803 (100)	802 (100)	777 (100)	1374 (100)	955 (100)	1155 (100)	1403 (100)	1048 (100)	922 (100)	817 (100)

Figure 2B



	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
GI.1	3 (1,3)	6 (1)	0 (0)	0 (0)	1 (0,5)	4 (0,8)	3 (0,5)	0 (0)	5 (0,7)	2 (0,2)	6 (0,7)	7 (1,3)
GI.2	0 (0)	8 (1,3)	2 (0,9)	4 (1,9)	0 (0)	2 (0,4)	1 (0,2)	7 (0,8)	4 (0,5)	15 (1,7)	18 (2,2)	2 (0,4)
GI.3	11 (4,9)	4 (0,6)	4 (1,9)	11 (5,1)	5 (2,7)	6 (1,2)	8 (1,3)	22 (2,7)	42 (5,5)	36 (4)	40 (4,8)	11 (2)
GI.4	12 (5,4)	8 (1,3)	1 (0,5)	3 (1,4)	14 (7,4)	16 (3,2)	18 (3)	13 (1,6)	34 (4,5)	5 (0,6)	10 (1,2)	4 (0,7)
GI.5	2 (0,9)	2 (0,3)	0 (0)	1 (0,5)	1 (0,5)	1 (0,2)	0 (0)	0 (0)	7 (0,9)	3 (0,3)	5 (0,6)	5 (0,9)
GI.6	3 (1,3)	2 (0,3)	1 (0,5)	5 (2,3)	0 (0)	7 (1,4)	11 (1,8)	22 (2,7)	28 (3,7)	17 (1,9)	13 (1,6)	3 (0,6)
GI.7	0 (0)	1 (0,2)	0 (0)	1 (0,5)	0 (0)	8 (1,6)	2 (0,3)	9 (1,1)	4 (0,5)	1 (0,1)	4 (0,5)	0 (0)
GI.8	0 (0)	0 (0)	0 (0)	1 (0,5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI.9	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	5 (0,7)	3 (0,3)	0 (0)	0 (0)
GII.1	2 (0,9)	0 (0)	0 (0)	4 (1,9)	0 (0)	31 (6,3)	29 (4,8)	35 (4,2)	12 (1,6)	14 (1,6)	15 (1,8)	3 (0,6)
GII.2	6 (2,7)	7 (1,1)	7 (3,3)	7 (3,3)	9 (4,8)	6 (1,2)	15 (2,5)	11 (1,3)	30 (3,9)	29 (3,2)	34 (4,1)	22 (4,1)
GII.3	21 (9,4)	15 (2,4)	22 (10,3)	10 (4,7)	16 (8,5)	15 (3)	45 (7,4)	29 (3,5)	17 (2,2)	53 (5,9)	23 (2,8)	34 (6,3)
GII.4	107 (47,8)	493 (78,5)	163 (76,2)	151 (70,6)	118 (62,8)	327 (66,3)	402 (65,9)	559 (67,6)	479 (62,8)	628 (70,3)	500 (60,5)	257 (47,3)
GII.5	0 (0)	2 (0,3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0,5)	13 (1,7)	3 (0,3)	0 (0)	0 (0)
GII.6	23 (10,3)	25 (4)	9 (4,2)	6 (2,8)	8 (4,3)	10 (2)	42 (6,9)	70 (8,5)	34 (4,5)	63 (7,1)	35 (4,2)	11 (2)
GII.7	28 (12,5)	43 (6,8)	0 (0)	1 (0,5)	2 (1,1)	4 (0,8)	18 (3)	26 (3,1)	28 (3,7)	3 (0,3)	7 (0,8)	8 (1,5)
GII.8	1 (0,4)	3 (0,5)	0 (0)	1 (0,5)	0 (0)	1 (0,2)	0 (0)	1 (0,1)	1 (0,1)	0 (0)	2 (0,2)	0 (0)
GII.10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0,2)	0 (0)	6 (0,7)	1 (0,1)	0 (0)
GII.12	0 (0)	1 (0,2)	3 (1,4)	1 (0,5)	9 (4,8)	37 (7,5)	4 (0,7)	6 (0,7)	1 (0,1)	2 (0,2)	8 (1)	2 (0,4)
GII.13	1 (0,4)	2 (0,3)	0 (0)	1 (0,5)	0 (0)	13 (2,6)	6 (1)	4 (0,5)	10 (1,3)	4 (0,4)	5 (0,6)	4 (0,7)
GII.14	2 (0,9)	0 (0)	1 (0,5)	5 (2,3)	3 (1,6)	1 (0,2)	2 (0,3)	2 (0,2)	7 (0,9)	1 (0,1)	6 (0,7)	6 (1,1)
GII.15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0,1)	1 (0,1)	0 (0)	0 (0)
GII.16	1 (0,4)	2 (0,3)	0 (0)	0 (0)	0 (0)	1 (0,2)	1 (0,2)	1 (0,1)	0 (0)	0 (0)	0 (0)	0 (0)
GII.17	0 (0)	1 (0,2)	0 (0)	1 (0,5)	1 (0,5)	1 (0,2)	1 (0,2)	0 (0)	1 (0,1)	3 (0,3)	94 (11,4)	164 (30,2)
GII.20	1 (0,4)	3 (0,5)	0 (0)	0 (0)	1 (0,5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GII.21	0 (0)	0 (0)	1 (0,5)	0 (0)	0 (0)	2 (0,4)	2 (0,3)	3 (0,4)	0 (0)	1 (0,1)	0 (0)	0 (0)
Total	224 (100)	628 (100)	214 (100)	214 (100)	188 (100)	493 (100)	610 (100)	827 (100)	763 (100)	893 (100)	826 (100)	543 (100)

Figure 3

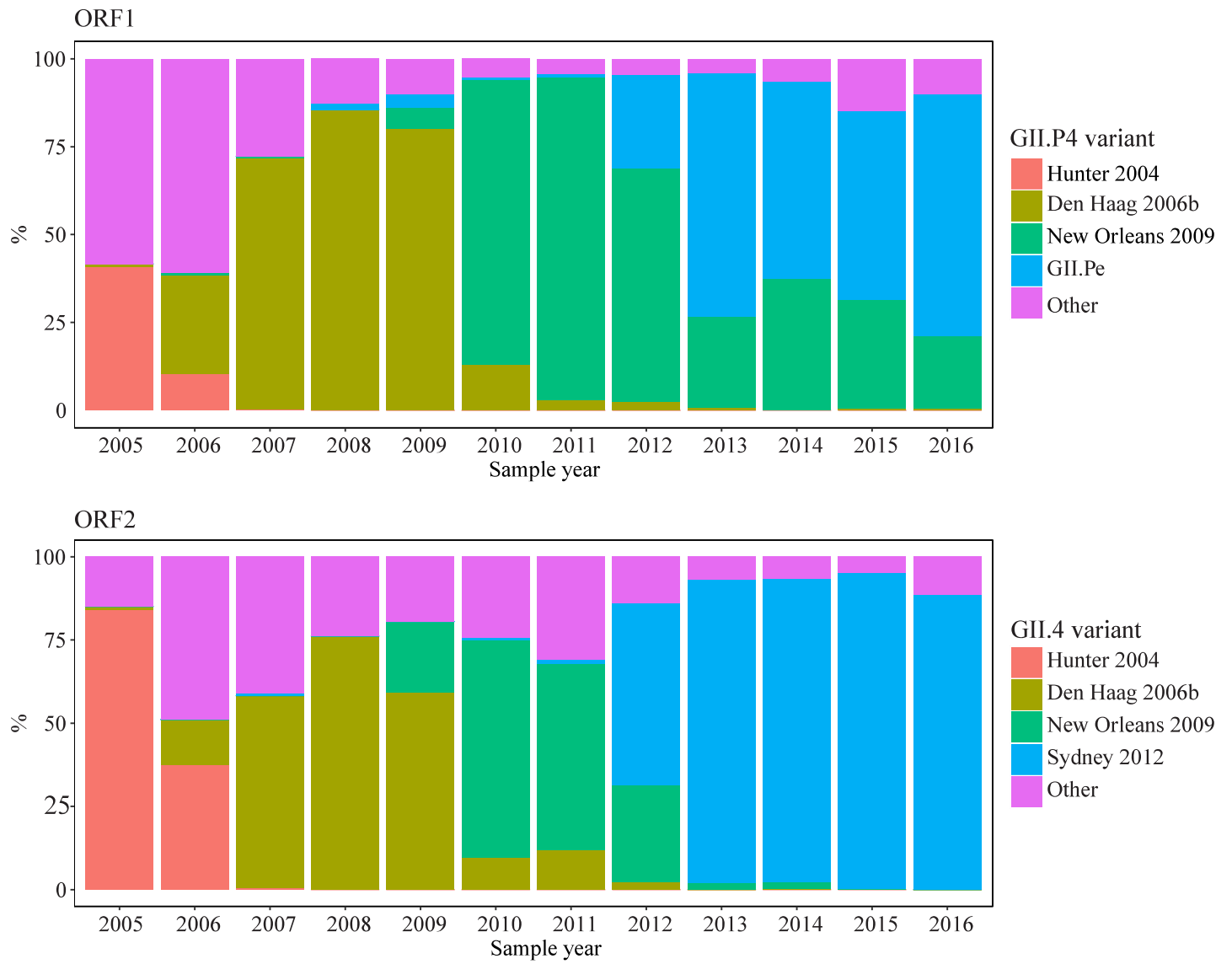


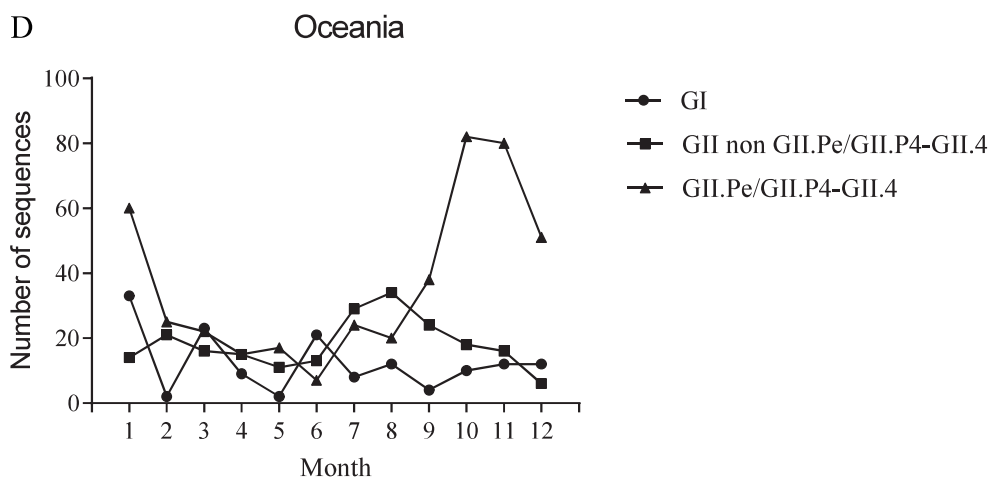
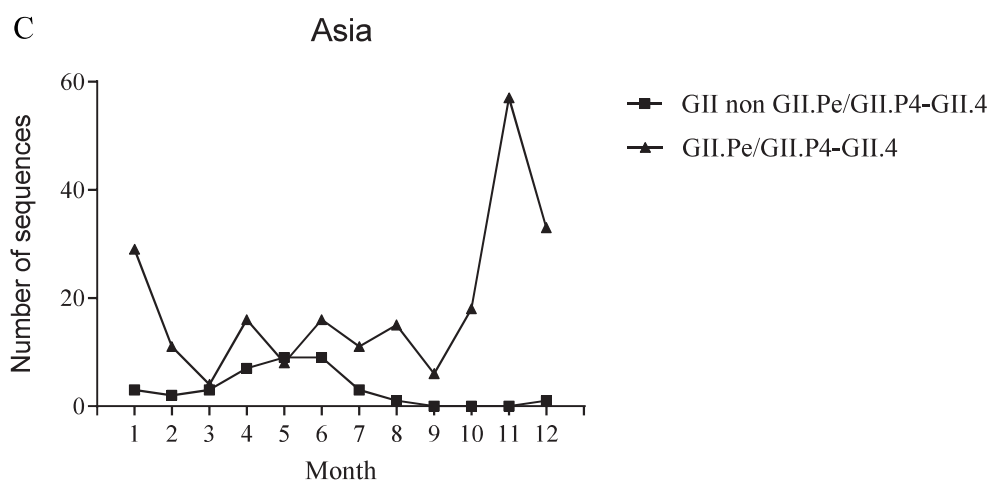
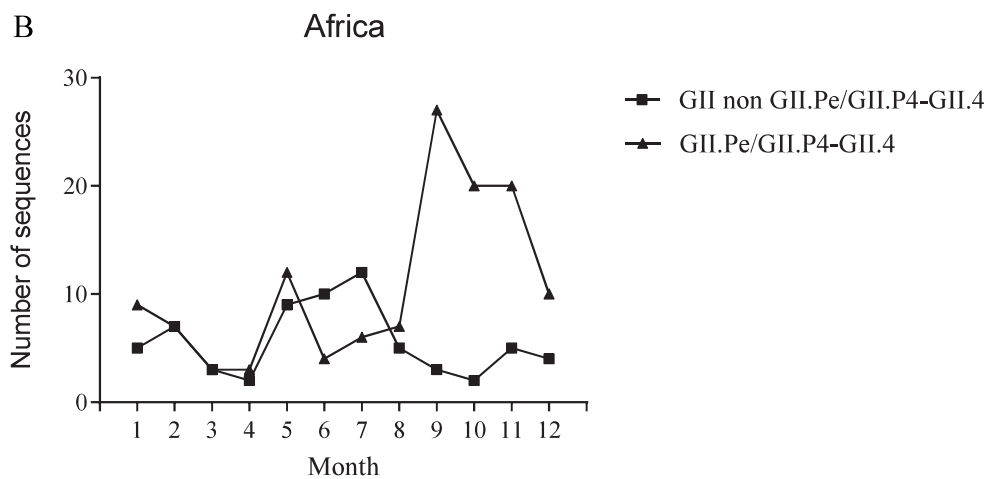
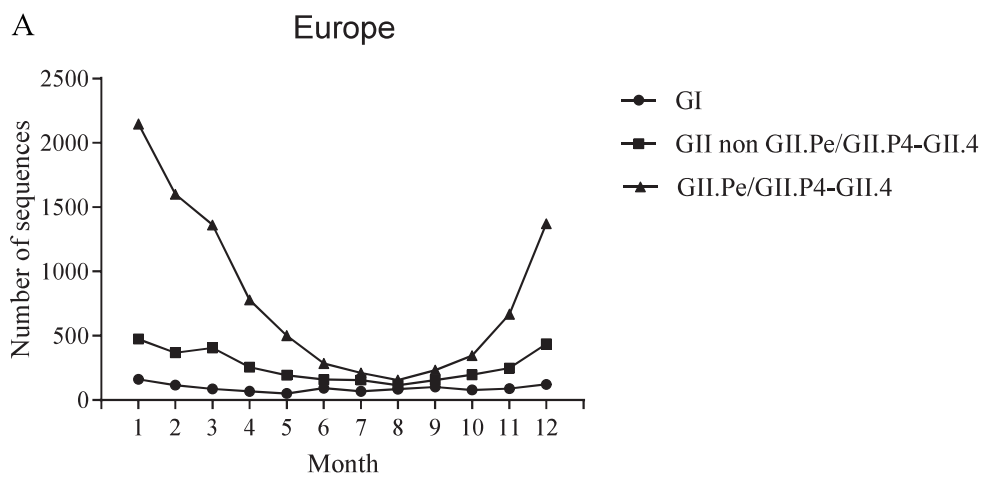
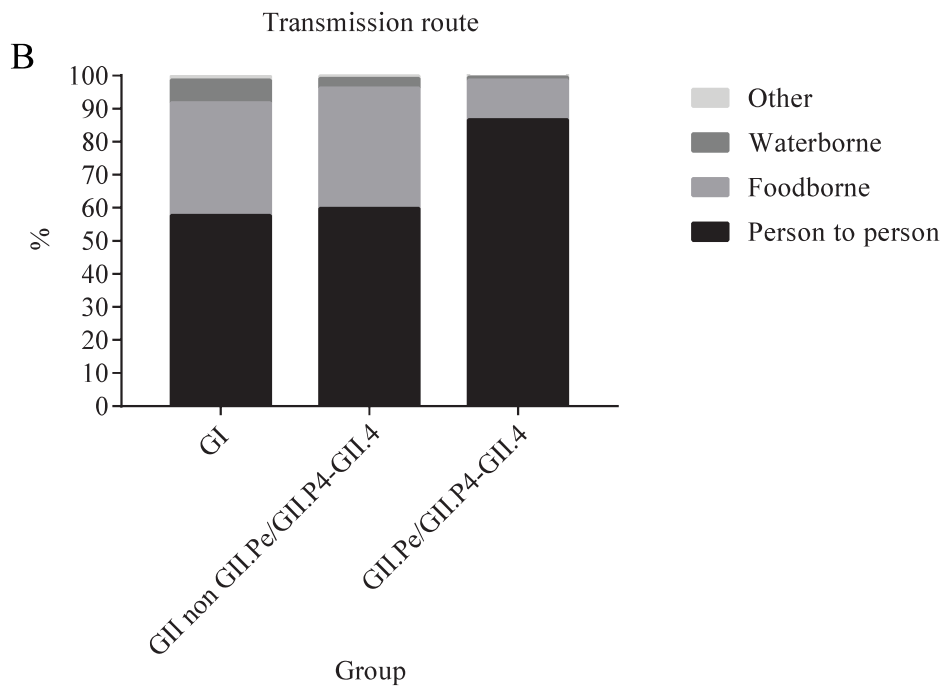
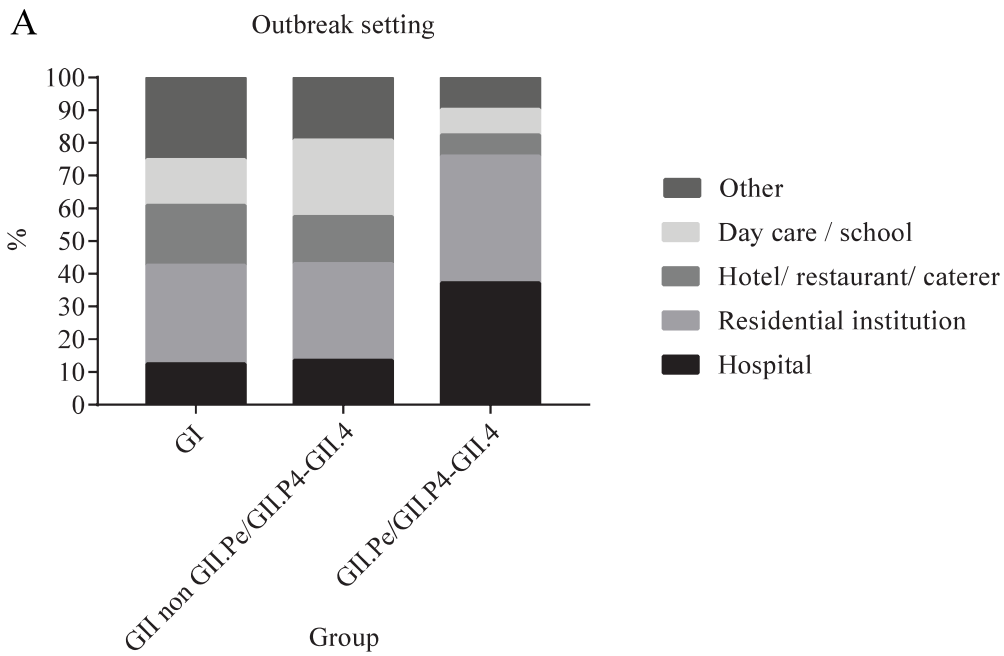
Figure 4

Figure 5



Supplementary Table 1 Number of reported GI and GII sequences per continent/region and country

Continent	Country	GI (%)	GIH (%)	Total
Europe	Austria	6 (3,2)	180 (96,8)	186
Europe	Belgium	41 (11,4)	319 (88,6)	360
Asia	China	0 (0)	142 (100)	142
Europe	Denmark	67 (10,4)	580 (89,6)	647
Europe	Finland	96 (8,5)	1037 (91,5)	1133
Europe	France	267 (8,2)	3004 (91,8)	3271
Europe	Germany	183 (16,4)	932 (83,6)	1115
Europe	Hungary	43 (5,2)	791 (94,8)	834
Europe	Ireland	11 (7)	147 (93)	158
Europe	Italy	23 (7,7)	276 (92,3)	299
Asia	Japan	0 (0)	293 (100)	293
Europe	Netherlands	327 (6)	5100 (94)	5427
Australia	New Zealand	148 (18,4)	658 (81,6)	806
Europe	Russia	23 (7,5)	283 (92,5)	306
Europe	Slovenia	15 (6,7)	209 (93,3)	224
Africa	South Africa	0 (0)	195 (100)	195
Europe	Spain	16 (5,5)	274 (94,5)	290
Europe	Sweden	69 (22,3)	241 (77,7)	310
Europe	United Kingdom	37 (5,9)	595 (94,1)	632

Supplementary Table 2A Number of reported norovirus ORF1 sequences stratified per genogroup/genotype, country, and time

Country	2005-2014						2015-2016					
	GI (%)	GII other (%)	GII.P4 (%)	GII.PI7 (%)	GII.Pe (%)	Total	GI (%)	GII other (%)	GII.P4 (%)	GII.PI7 (%)	GII.Pe (%)	Total
Austria	6 (3,3)	28 (15,2)	145 (78,8)	0 (0,0)	5 (2,7)	184	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
Belgium	14 (8,6)	45 (27,8)	62 (38,3)	0 (0,0)	41 (25,3)	162	3 (6,7)	12 (26,7)	14 (31,1)	5 (11,1)	11 (24,4)	45
China	0 (0,0)	0 (0,0)	0 (0,0)	1 (33,3)	2 (66,7)	3	0 (0,0)	8 (100)	0 (0,0)	0 (0,0)	0 (0,0)	8
Denmark	43 (8,3)	116 (22,4)	351 (67,6)	0 (0,0)	9 (1,7)	519	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
Finland	83 (7,7)	108 (10,0)	862 (80,1)	0 (0,0)	23 (2,1)	1076	8 (30,8)	7 (26,9)	0 (0,0)	3 (11,5)	8 (30,8)	26
France	80 (7,0)	104 (9,1)	682 (59,4)	3 (0,3)	279 (24,3)	1148	17 (5,0)	34 (10,1)	71 (21,1)	143 (42,4)	72 (21,4)	337
Germany	81 (14,7)	167 (30,4)	249 (45,3)	0 (0,0)	53 (9,6)	550	20 (18,7)	31 (29,0)	11 (10,3)	11 (10,3)	34 (31,8)	107
Hungary	33 (4,5)	120 (16,3)	535 (72,9)	0 (0,0)	46 (6,3)	734	9 (11,8)	10 (13,2)	3 (3,9)	25 (32,9)	29 (38,2)	76
Italy	9 (7,0)	9 (7,0)	38 (29,7)	0 (0,0)	72 (56,3)	128	1 (4,2)	7 (29,2)	4 (16,7)	1 (4,2)	11 (45,8)	24
Japan	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	1 (100)	1	0 (0,0)	2 (100)	0 (0,0)	0 (0,0)	0 (0,0)	2
Netherlands	221 (5,3)	829 (19,7)	2516 (59,8)	1 (0,0)	642 (15,3)	4209	106 (10,3)	207 (20,2)	272 (26,5)	51 (5,0)	389 (38,0)	1025
New Zealand	71 (18,3)	102 (26,3)	47 (12,1)	0 (0,0)	168 (43,3)	388	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
Russia	0 (0,0)	12 (92,3)	0 (0,0)	0 (0,0)	1 (7,7)	13	0 (0,0)	19 (21,3)	4 (4,5)	48 (53,9)	18 (20,2)	89
Slovenia	8 (13,3)	11 (18,3)	41 (68,3)	0 (0,0)	0 (0,0)	60	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
South Africa	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
Spain	6 (4,2)	22 (15,5)	114 (80,3)	0 (0,0)	0 (0,0)	142	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
Sweden	4 (16,7)	12 (50)	4 (16,7)	0 (0,0)	4 (16,7)	24	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0
United Kingdom	21 (12,2)	11 (6,4)	140 (81,4)	0 (0,0)	0 (0,0)	172	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0

Supplementary Table 2B Number of reported norovirus ORF2 sequences stratified per genogroup/genotype, country, and time

Country	2005-2014						2015-2016					
	GI (%)	GII other (%)	GII.4 (%)	GII.17 (%)	Total	GI (%)	GII other (%)	GII.17 (%)	GII.4 (%)	Total		
Austria	0 (0,0)	1 (50,0)	1 (50,0)	0 (0,0)	2	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0		
Belgium	14 (12,2)	24 (20,9)	77 (67,0)	0 (0,0)	115	10 (26,3)	10 (26,3)	2 (5,3)	16 (42,1)	38		
China	0 (0,0)	36 (37,1)	60 (61,9)	1 (1,0)	97	0 (0,0)	0 (0,0)	1 (2,2)	44 (97,8)	45		
Denmark	28 (18,8)	73 (49,0)	47 (31,5)	1 (0,7)	149	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0		
Finland	5 (17,2)	3 (10,3)	21 (72,4)	0 (0,0)	29	0 (0,0)	2 (100)	0 (0,0)	0 (0,0)	2		
France	130 (9,5)	230 (16,8)	1007 (73,5)	3 (0,2)	1370	40 (9,6)	67 (16,1)	151 (36,2)	159 (38,1)	417		
Germany	63 (17,9)	118 (33,6)	170 (48,4)	0 (0,0)	351	19 (17,6)	30 (27,8)	11 (10,2)	48 (44,4)	108		
Hungary	2 (3,8)	26 (50,0)	24 (46,2)	0 (0,0)	52	0 (0,0)	0 (0,0)	1 (4,5)	21 (95,5)	22		
Ireland	4 (3,4)	14 (11,8)	101 (84,9)	0 (0,0)	119	7 (17,9)	8 (20,5)	0 (0)	24 (61,5)	39		
Italy	15 (10,4)	23 (16,0)	106 (73,6)	0 (0,0)	144	0 (0,0)	7 (25,9)	3 (11,1)	17 (63,0)	27		
Japan	0 (0,0)	0 (0,0)	265 (100)	0 (0,0)	265	0 (0,0)	0 (0,0)	0 (0,0)	28 (100)	28		
Netherlands	26 (4,8)	84 (15,6)	428 (79,6)	0 (0,0)	538	35 (8,3)	73 (17,4)	37 (8,8)	275 (65,5)	420		
New Zealand	77 (18,4)	115 (27,5)	226 (54,1)	0 (0,0)	418	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0		
Russia	6 (6,4)	58 (61,7)	30 (31,9)	0 (0,0)	94	17 (15,5)	18 (16,4)	49 (44,5)	26 (23,6)	110		
Slovenia	7 (5,7)	20 (16,3)	96 (78,0)	0 (0,0)	123	0 (0,0)	0 (0,0)	3 (7,3)	38 (92,7)	41		
South Africa	0 (0,0)	65 (33,3)	128 (65,6)	2 (1,0)	195	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0		
Spain	10 (7,2)	47 (34,1)	81 (58,7)	0 (0,0)	138	0 (0,0)	0 (0,0)	0 (0,0)	10 (100)	10		
Sweden	65 (22,7)	116 (40,6)	103 (36,0)	2 (0,7)	286	0 (0,0)	0 (0,0)	0 (0,0)	0 (0,0)	0		
United Kingdom	37 (6,5)	76 (13,4)	456 (80,1)	0 (0,0)	569	0 (0,0)	11 (17,7)	0 (0,0)	51 (82,3)	62		

Supplementary Table 3 First detections of global GII.4 drift variants

GII.4 variant	Year of emergence	First record ORF1	First ORF1 country	first record ORF2	First ORF2 country
Hunter 2004	2004	6-Apr-2004	The Netherlands	6-Apr-2004	The Netherlands
Den Haag 2006b	2006	14-Feb-2002	Germany	30-Sep-2003	Japan
New Orleans 2009	2009	12-Dec-2006	France	24-Apr-2009	South Africa
Sydney 2012	2012	-	-	Oct-2007	The Netherlands

Supplementary Table 4 ORF1 / ORF2 combinations (n=1047) detected by NoroNet 2005 - 2016

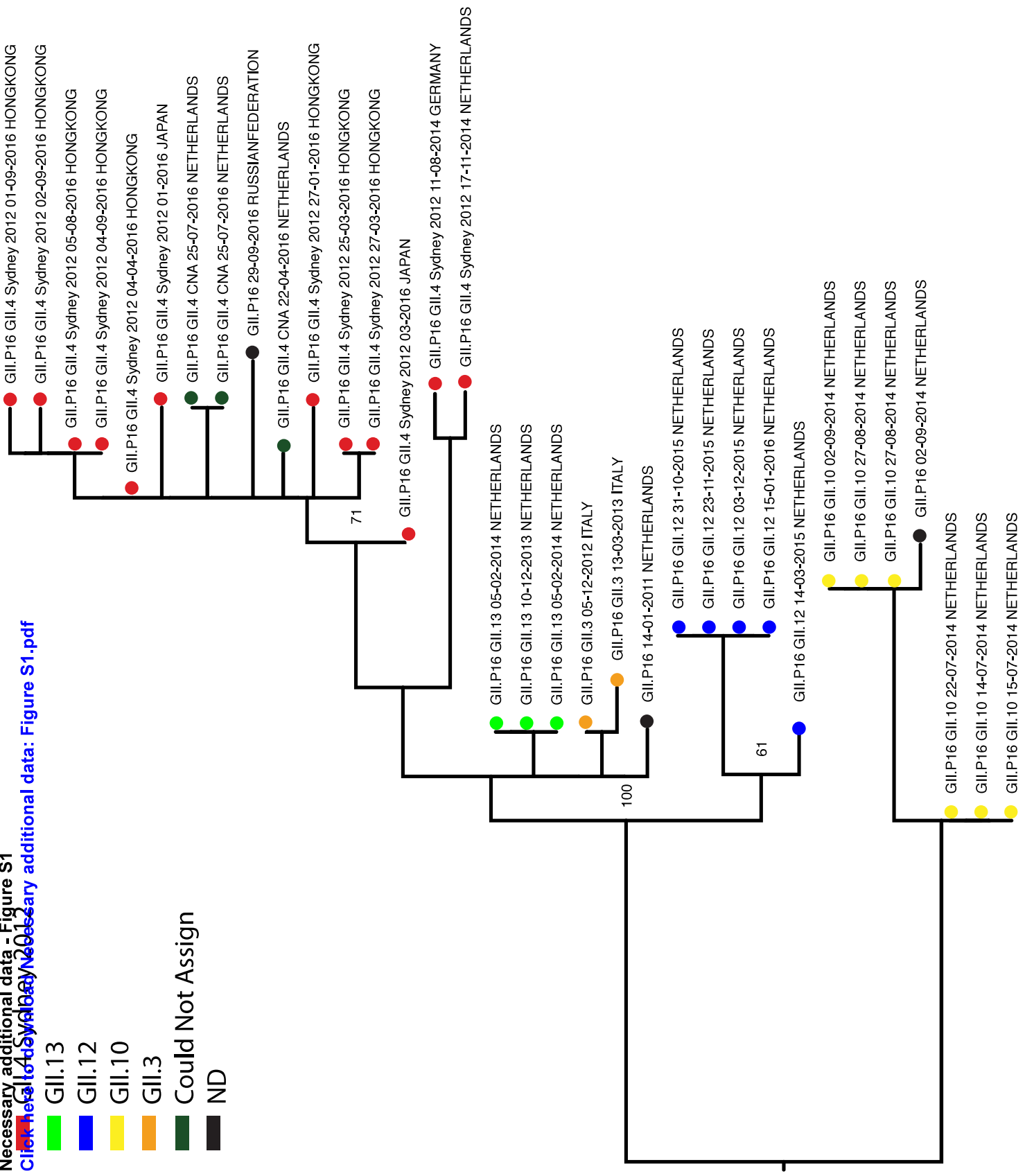
	GI.1	GI.2	GI.3	GI.4	GI.5	GI.6	GI.1	GI.2	GI.3	GI.4	GI.5	GI.6	GI.7	GI.10	GI.12	GI.13	GI.14	GI.17	Total
GI.P1	9																		9
GI.P2		10																	10
GI.P3			26																26
GI.P4				15															15
GI.P5					9														9
GI.P7			1																1
GI.Pb						9													9
GI.Pd			10																10
GI.LP2							12				1								13
GI.LP4									441										441
GI.LP7												27	9				6		42
GI.LP12									1	3									4
GI.LP16								2	15					6	5	3			31
GI.LP17																		39	39
GI.LP21								63							2				65
GI.LP22											2							1	3
GI.LPc							3												3
GI.LPe								2		301									303
GI.LPg							8								6				14
Total	9	10	37	15	9	9	11	14	66	760	3	6	9	6	11	5	6	40	1047

Supplementary Table 5 Amino acid (aa) comparison of the blockade epitopes A, D, and E between reference GII.Pe-GII.4 Sydney 2012 and novel GII.P16-GII.4 Sydney 2012 recombinant strains.

Accession nr	Sample location	Sample date	GII.4 ORF2 variant												
			A	A	A	A	A	A	D	D	D	E	E	E	
JX459908.1	Australia	Mar-12	T	S	R	N	E	D	G	T	T	T	S	N	T
JX459907.1	Australia	May-12	T	S	R	N	E	D	S	T	T	T	S	N	T
Outbreak number	Sample location	Sample date	294	296	297	298	368	372	393	394	395	407	412	413	
	Recombinant														
OHI16002	Japan	Jan-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-886	Hong Kong	Jan-16	T	S	R	N	E	D	S	T	T	S	N	T	
OC16023	Japan	Mar-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-937	Hong Kong	Mar-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-938	Hong Kong	Jul-16	T	S	R	N	E	D	S	T	T	S	N	T	
5061600252	Netherlands	Jul-16	T	S	R	N	E	D	S	T	T	S	N	T	
5061600253	Netherlands	Jul-16	T	S	R	N	E	D	S	T	T	S	N	T	
5061600205	Netherlands	Apr-16	T	S	R	N	E	D	G	T	T	S	N	T	
CUHK-NS-943	Hong Kong	Apr-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-1002	Hong Kong	Aug-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-1037	Hong Kong	Sep-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-1038	Hong Kong	Sep-16	T	S	R	N	E	D	S	T	T	S	N	T	
CUHK-NS-1044	Hong Kong	Sep-16	T	S	R	N	E	D	S	T	T	S	N	T	

Necessary additional data - Figure S1
 Click here for a download of the necessary additional data: Figure S1.pdf

- GII.13
- GII.12
- GII.10
- GII.3
- Could Not Assign
- ND



0.02

