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SHORT COMMUNICATION

Emergence of novel recombinant GII.P16_GII.2 and GII.P16_GII.4 Sydney 2012 norovirus strains in Italy, winter 2016/2017

Maria Cristina Medici[1], Fabio Tummolo[1], Vito Martella[2], Flora De Conto[1], Maria Cristina Arcangeletti[1], Federica Pinardi[1], Francesca Ferraglia[1], Carlo Chezzi[1], Adriana Calderaro[1]

[1]*Department of Medicine and Surgery, University of Parma, Parma, Italy;*
[2]*Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano, Italy*

SUMMARY

In the winter season 2014/15, the GII.P17_GII.17 norovirus strain Kawasaki 2014 emerged in Italy, co-circulating with pandemic GII.4 strains. In March 2016, molecular investigation identified novel GII.P16 recombinant noroviruses in children with gastroenteritis in Italy.
In 43.10% of the genotyped noroviruses GII.P16 strains were identified: 12 were characterized as GII.2 and 13 as GII.4 Sydney 2012 capsid genotypes. The GII.P16 genotype became predominant in January-February 2017 along with an increase in norovirus activity. The capsid gene was characterized as GII.2 or GII.4 Sydney 2012 variant. The emergence of two different recombinant GII.P16 viruses, of which one harboring a pandemic GII.4 capsid sequence, suggests the potential for a future pandemic.

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Norovirus (NoV) strains undergo a continuous process of genetic/antigenic diversification and periodically generate new strains via accumulation of point mutations or recombination, with one novel variant emerging every two to three years and becoming predominant globally.
Observations from Germany reported an increase in NoV cases in winter 2016 and the emergence of a new recombinant strain GII.P16_GII.2 (Niendorf *et al*., 2017). The increase in GII.P16_GII.2 cases was also described in a large gastroenteritis outbreak in Japanese children in mid-2016 (Thongprachum *et al*., 2017). Moreover, during 2016 the NoV GII.P16 was found in combination with a GII.4 Sydney 2012 capsid sequence prevailing over GII.P17_GII.17 Kawasaki strains in South Korea (Choi *et al*., 2017).
Here we report the emergence of both the GII.P16 recombinant forms during the 2016/2017 winter season in Italy, extending information on the geographic spread and clinical relevance of these viruses in Europe.
NoVs are a major cause of acute gastroenteritis in both children and adults, with sporadic cases and outbreaks in various epidemiological settings (Green, 2013). NoVs are small round non-enveloped viruses with a 7.5 kb single stranded positive-sense RNA that contains three open reading frames (ORFs). NoVs are classified into seven genogroups (GI-GVII) (Vinjé, 2015). Each genogroup can be further divided into various genotypes based on either RdRp sequences (ORF1, pol genotype) or VP1 (ORF2, cap genotype). Although more than 30 cap genotypes within genogroups GI, GII, and GIV may infect humans, a single genotype, GII.4, was identified in the vast majority of NoV-associated cases of gastroenteritis worldwide since the mid-1990s. However, since the latter half of 2014, a new GII.17 variant has been reported as the main cause of outbreaks over GII.4 in East Asia and has also occurred in America and Europe, as well as in Italy in 2015 (de Graaf *et al*., 2015; Medici *et al*., 2015; Sanchez *et al*., 2017).
The Italian Study Group for Enteric Viruses (ISGEV; http://isgev.net) monitors the epidemiology of enteric viruses in children through hospital-based surveillance. Monitoring and characterization of NoVs is achieved by sequencing the diagnostic regions A and C of NoV genome and interrogation of the Norovirus Typing Tool database (http://www.rivm.nl/mpf/norovirus/typingtool).
Between January 2016 and February 2017 a total of 114 (11.77%) stool samples out of 968 (74/816, 9.06% in January-December 2016; 40/152, 26.31% in January-February 2017) from children (age range: 4 days-13 years and 6 months, mean age: 3 years and 8 months) suffering from gastroenteritis and attending the University Hospital of Parma, Northern Italy (inpatients: 658, outpatients: 310) tested positive for NoVs. All the stools were investigated for diagnostic purposes by FilmArray Gastrointestinal Panel assay (BioFire Diagnostics, USA) for the simultaneous detection of 22 common diarrhoeal agents, including NoV GI/GII.
Upon multi-target sequence analysis of a selection of about half of the NoV-positive cases (50.87%, 58/114), 25 (43.10%) were GII.P16 RdRp genotype *(Figure 1)*. The capsid gene was characterized as GII.2 in 12 cases and GII.4 Sydney 2012 in 13 cases. The GII.P16_GII.2 Italian strains

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Corresponding author:
Maria Cristina Medici
E-mail: mariacristina.medici@unipr.it

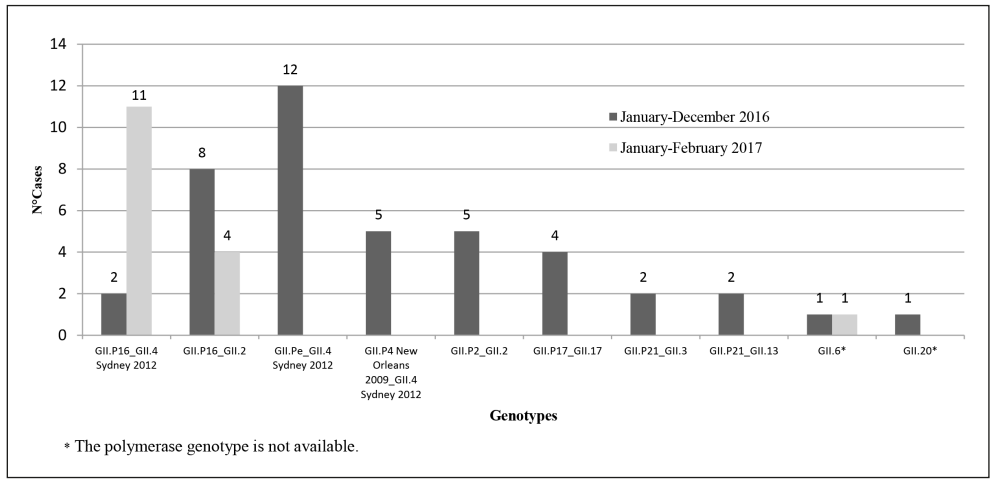

Figure 1 - *Genotypes of norovirus (n°58) detected in children with gastroenteritis, January 2016 - February 2017.*

were genetically closely related to German strains (98-99% id nt) detected in high prevalence in September-December 2016 (Niendorf *et al*., 2017), while GII.P16_GII.4 Sydney 2012 strains are the first recorded in Europe and highly related to GII.P16_GII.4 Sydney 2012 strains (98-99% id nt) reported in dramatic increase in environmental water neighboring human communities in South Korea since December 2015 (Choi *et al*., 2017). The present molecular study detected GII.P16 NoVs from March 2016 and this became the predominant polymerase genotype in January-February 2017 (93.75%, 15/16: 4 GII.P16_GII.2 and 11 GII.P16_GII.4 Sydney 2012), in association with the displacement of the previous GII.4 Sydney 2012 forms. Interestingly, the comparison of the prevalence of NoV infection in the first two months of 2016 with that of the same period of 2017 showed a more than twofold increase in NoV activity (from 12.94% to 26.36%), highlighting that the high genetic variability of NoV can rapidly affect its fitness and causes the emergence and dramatic increase in its circulation.

Molecular investigation identified GII.P16_GII.2 and GII.P16_GII.4 Sydney 2012 recombinant NoVs from the stools of Italian children with gastroenteritis since 2016. Recombinant GII.P16 forms, equipped with GII.3 or GII.13 cap sequence, were first identified in the area of Parma at low levels during 2010-2012 (Medici *et al*., 2014). The emergence of two novel recombinant GII.P16 viruses, of which one harboring a pandemic GII.4 Sydney 2012 cap sequence, suggests the potential for a future pandemic, mirroring what was seen globally for the epidemic GII.4 variants, that arose from pre-existing GII.4 viruses through evolutionary intermediate forms (Hoa Tran *et al*., 2013). Interestingly, also GII.2 cap sequence may have an epidemiological role as three different GII.2 recombinant forms (GII.P16, GII.Pe and GII.P17), one of which combined with the GII.P16 sequence, emerged as major cause of gastroenteritis outbreak in Japanese children in mid-2016 (Thongprachum *et al*., 2017).

The emergence of the novel GII.P16 and the displacement of previous GII.4 forms could represent a challenge for the efficacy of the candidate NoV vaccines (Bernstein *et al*., 2015) which target the globally predominant NoV GII.4. Continued surveillance for NoV infection and further data on clinical and molecular epidemiological features will enable accurate evaluation of the public health repercussions of the new GII.P16 recombinant forms. Adequate hand hygiene remains the most effective control measure against NoV transmission. It could be useful to prevent NoV foodborne diffusion with stringent new guidelines for the management of food workers.

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